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Preface

Dear readers,

This special issue of the "Bulgarian Chemical Communications" puts together most of the studies, presented during the XI International Conference of Food Physicists (ICFP), which took place on June 10-12, 2014 in Plovdiv, Bulgaria. The Conference was organized by the International Society of Food Physicists and the Faculty of Physics at Plovdiv University "Paisii Hilendarski". The ICFP started in 1994 in Budapest, Hungary and it has been hold every two years in some of the famous universities in Central and Eastern Europe, which carried out training and research in the field of food science - Bucharest, Romania (1996), Lublin, Poland (1998), Istanbul, Turkey (2000), Brno, Czech Republic (2002), Pécs, Hungary (2004), Senta Serbia (2006), Plovdiv, Bulgaria (2008), Nitra, Slovakia (2010) and Budapest, Hungary (2012).

The conference was attended by 47 scientists from 9 countries - Bulgaria, Turkey, Hungary, Romania, Slovakia, the Czech Republic, Estonia, Russian Federation and Kosovo. There were presented 15 oral presentations and 33 posters. The participation of some invited lectors and lot of young scientists and PhD students contributed to the attractiveness of this scientific forum.

The main topics discussed during the conference were:

- Physical parameters of foodstuff that can be used for quality control and safety.
- Physical and organoleptic parameters of reformulated foods - healthy foods low in salt, sugar and fat.
- Verification of destructive and non-destructive physical methods for analyzing the quality of food products.
- Physical principles of innovative technologies for food processing.

The presentations demonstrated the interdisciplinarity of the food science and the relationships between food technology, physical parameter of foodstuff and the quality of final products.

The Organization Committee of the conference thanks this journal for the help in publishing the full text articles and for the chance given to the participants to meet the wide audience.

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Effect of composition and microwave radiation on electrical impedance spectrum of cow milk

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Electrical impedance spectrum of cow milk with 1.5%, 2.8%, 3.5% and full fat content was determined in frequency range from 30 Hz up to 1 MHz. The impedance magnitude and the phase angle were measured before and after microwave irradiation – with power 900 W and with radiation time of 10, 20, 30, 40, 50 and 60 s. The temperature of milk samples after radiation was measured. The impedance spectrum of samples with various fat contents was determined after conventional heating process at temperature reached under microwave radiation. The microwave radiation decreased the impedance magnitude in the whole investigated frequency range when radiation time increased up to 30 s, but further increase of radiation time did not caused further decrease. The phase angle in low frequency range (30 Hz – 10 kHz) also decreased after radiation and this change raised as the radiation time became longer. The conventional heating at low temperature range – 20 °C – 40 °C – resulted in increasing of impedance magnitude but at higher temperatures the decrease of impedance magnitude was observed. The effect of conventional heating on the phase angle was similar to the effect of microwave radiation. Electrical impedance spectroscopy can be used to distinguish milk sample heated by microwave radiation from milk heated by conventional method.

Key words: milk, microwave radiation, heating, electrical impedance spectrum.

INTRODUCTION

The milk undergoes several - among others some heating - processes before consumption. The radiation of microwave oven used in millions household for reheating and processing foods can cause various physical and chemical changes in processed object. In scientific literature there are few articles in which the effect of microwave radiation on structure of milk is discussed [1-3]. Generally there is stated, that microwave heating is very effective for destruction of microorganisms and the structure changes caused by it is similar to the changes after conventional heating [1,3].

The microwave of 2.45 GHz frequency excites the dipole of water molecules and the high energy of dipoles can cause a local temperature increase. The temperature gradient results in a heat flow and so the temperature of whole food in oven increases. Microwave exposure of milk can decrease the averages of fat, protein, dry substance and lactose concentrations while the density averages can increase [3]. Some structure changes can be observed in casein stabilized by microwave energy on gold-nanoparticles, too [4].

The electrical impedance spectrum of biological material contains four bands characteristic for ion concentration, membrane state, macromolecular organization, bound and free water [5]. The electrical impedance spectrum of milk can be described with a model circuit consisting of a parallel connection of serial RC element with a capacitance [6]. The admittance values can be used for detection of added water to the full fat milk [7].

The aim of our work was to investigate, whether the electrical impedance spectroscopy is sensitive enough to detect the structural changes caused by microwave radiation in milk.

EXPERIMENTAL

Full fat cow milk was purchased from a local farm and milk of 3.5 %, 2.8 % and 1.5 % fat content were bought in a local shop. 80 ml of milk in glassware was put into the domestic microwave oven of a Whirlpool VIP 20, with double emission system, and was radiated 10, 20, 30, 40, 50 and 60 s with 900 W powers. The temperature of milk was measured after microwave radiation. For conventional heating the glassware with milk was in water bath and the water temperature was controlled with a Hake DC10 P5 thermostat. Temperature value was set for the same value reached after microwave radiation.

The magnitude, Z, and phase angle, φ, of electrical impedance were measured with a HP 4284A precision LCR meter in frequency range

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from 30 Hz up to 1 MHz. Two ECG Ag/AgCl electrodes (Fiab Spa) in 1 cm distances from each other were put into milk. The measuring voltage was 1 V, which is too low to cause changes in biological material. The measured spectra were open-short corrected to eliminate the stray capacitance and inductance. The real part, $R=Z\cos\varphi$, and imaginary part, $X=Z\sin\varphi$ were calculated and represented as a function of frequencies. The difference spectra ($R(t)$-$R(22\ °C)$) obtained by subtraction of real part spectrum determined at room temperature (22 °C) from real part spectrum determined after microwave radiation at higher temperature, (t), were calculated. The same difference spectra were evaluated for imaginary parts ($X(t)$-$X(22°C)$), too. These difference spectra were also determined for conventionally heated milk samples.

RESULTS AND DISCUSSION

The electrical impedance spectrum of milk depends on its composition. Decreasing fat content increased the magnitude of impedance (Figure 1A) in the whole investigated frequency range. The phase angle values (Figure 1B) at low frequencies also increased with decreasing fat content. The impedance magnitude of milk with 3.5 % fat content was slightly lower, than the impedance magnitude of sample with 2.8 % fat. Similar anomalous change was observed if 2 - 3 % water was added to full fat milk [7]. This can be explained by the hydrolysis of milk fat [7].

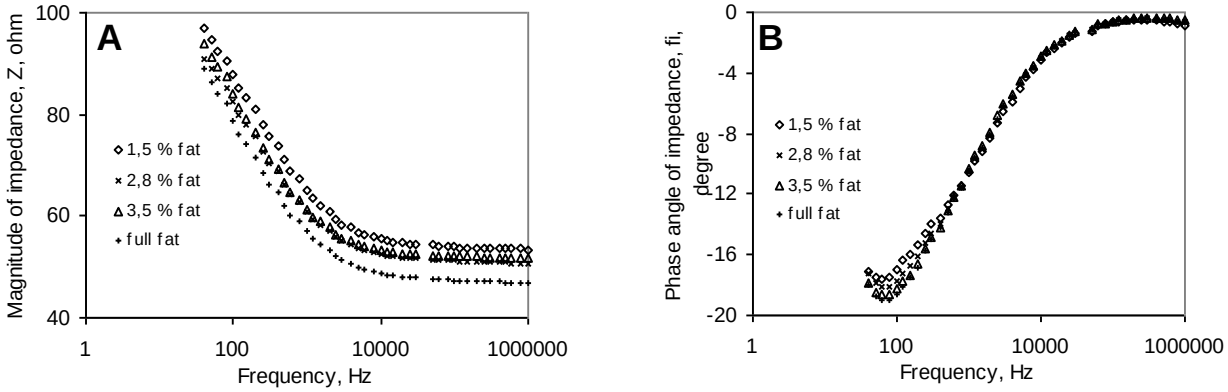

Fig. 1. Magnitude (A) and phase angle (B) of electrical impedance measured in milk with 1.5 %, 2.8%, 3.5% and full fat content at 22 °C temperature.

Microwave radiation (Figure 2A) of increasing time durations decreased the real part of impedance in full fat milk. 10 s and 20 s radiation caused only little decrease, but 30 s and longer radiation remarkably reduced the real part. The extent of real part decrease remained practically constant even the time of radiation was raised from 30 s up to 60 s. The imaginary part increased after microwave radiation and the measure of change was similar to the changes in real part. After 10 s, 20 s and 30 s microwave radiation the imaginary part became higher and higher and the increase was practically constants after 30 s and longer radiation. There was similar tendency in changes both in real and imaginary parts of milk samples with 3.5 %, 2.8 % and 1.5 % fat content (not shown).

After the conventional heating of full fat milk on water bath (Figure 2B) the real part of impedance increased and only at temperature higher than 43 °C decreased. The imaginary part of electrical impedance decreased as the temperature increased. There was similar tendency in changes both in real and imaginary parts of milk samples with 3.5 %, 2.8 % and 1.5 % fat content (not shown).

The difference spectra ($R(t)$-$R(22\ °C)$;$X(t)$-$X(22\ °C)$) of microwave radiated milk of 2.8 % fat content (Figure 3. A and B) are remarkably differ from difference spectra of no radiated, conventionally heated milk of 2.8 % fat content (Figure 4A and B). Differences increased while radiation time increased from 0 up to 30 s, and remained constant when the radiation time was further increased. It seems that during 30 s radiation the changes caused by microwave radiation were completed. Dumuta et al. [3] found that there is a critical radiation time about 30 s. The decrease of lipid, protein, lactose concentration and increase of density depended on radiation time from 0 s up to 30 s, but these changes not have further increased after 30 - 120 s radiation time [3]. The difference spectra of milk with 1.5 %, 3.5 % and full fat content were similar to difference spectra of 2.8 % fat containing milk (not shown).

Difference spectra for radiated milk samples can be explained by the several chemical processes observed after microwave radiation: auto oxidation milk lipid, induction of reactive oxygen species, resulting of free radicals, changes in whey protein

structure, change in β-lactoglobulin folding process and changes in water structure [3].

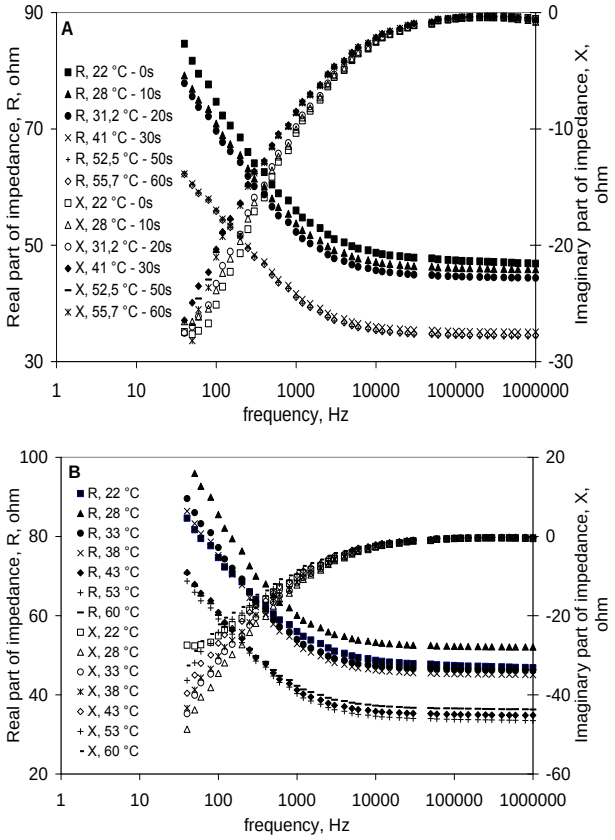

Fig. 2. Real part and imaginary part of electrical impedance of full fat milk after microwave radiation (A) and after conventional heating (B)

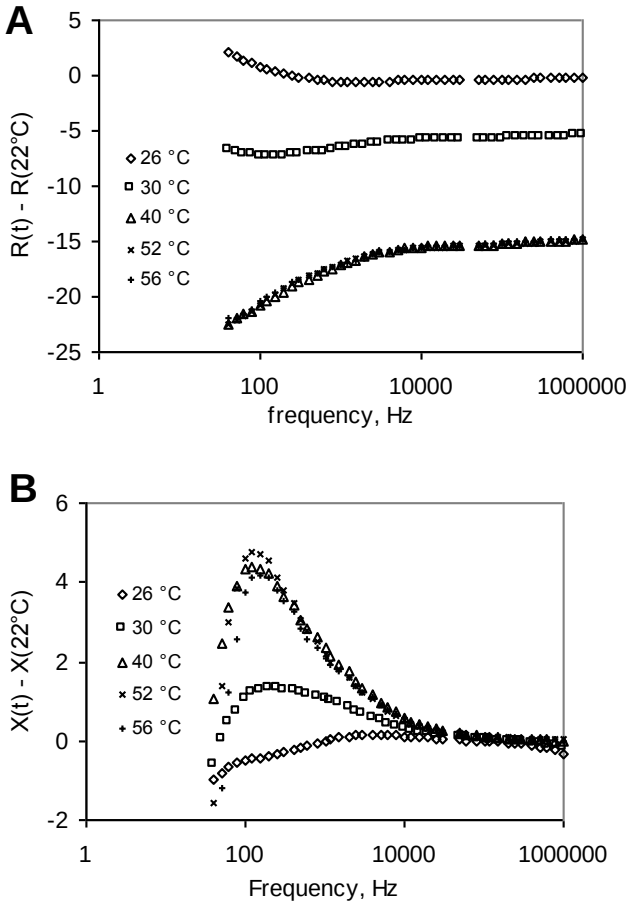

Fig. 3. Difference of real part (A) and imaginary part (B) of impedance spectrum of microwave radiated milk with 2.8 % fat at the reached average temperature according to the real and imaginary part of impedance spectrum of 2.8 % fat containing no radiated milk at 22 °C temperature

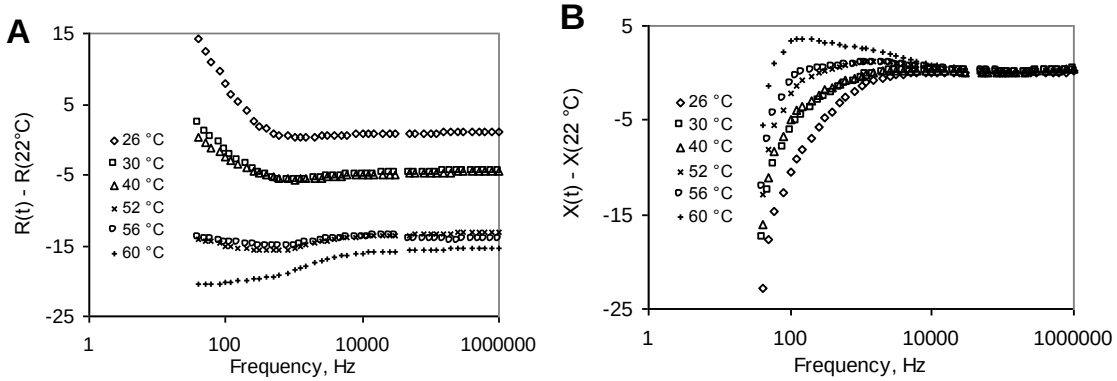

Fig. 4. Difference of real part (A) and imaginary part (B) of impedance spectrum of conventionally heated - up to temperature reached with microwave radiation - milk with 2.8 % fat according to the real and imaginary part of impedance spectrum of 2.8 % fat containing milk at 22 °C temperature.

CONCLUSION

The impedance magnitude and the real part of impedance of milk was decreased by microwave radiation when radiation time increased up to 30 s, but further increase of radiation time did not caused further decrease. The conventional heating at low temperature range resulted in increasing of impedance magnitude but at higher temperatures (> 40 °C) the decrease of impedance magnitude was observed. While the imaginary part of milk

impedance increased after microwave radiation the conventionally heating decreased the imaginary part of impedance. Electrical impedance spectroscopy is enough sensitive to show that the structure of microwave radiated milk differs from the structure of conventionally heated milk. This result can be used in the future in practice for detecting the microwave radiation of milk
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ВЛИЯНИЕ НА СЪСТАВА И МИКРОВЪЛНОВОТО ОБЛЪЧВАНЕ ВЪРХУ ЕЛЕКТРИЧНИЯ ИМПЕДАНСЕН СПЕКТЪР НА КРАВЕ МЛЯКО

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(Резюме)

В настоящата работа беше определен електричният импедансен спектър в диапазона от 30 Hz до 1 MHz на краве мляко с масленост 1.5%, 2.8%, 3.5%, пълномаслено. Големината на импеданса и фазовият ъгъл са измерени преди и след микровълново облъчване с мощност 900 W и продължителност 10, 20, 30, 40, 50 и 60 s. Измерена беше температурата на образците от мляко след облъчването. Импедансният спектър на образците с различно маслено съдържание беше определен след процес на конвенционално нагряване при температурата, която се достига при микровълновото облъчване. Микровълновото облъчване до 30 s доведе до намаляване големината на импеданса в целия изследван честотен диапазон, а по-нататъшното увеличаване на времето на облъчване не предизвика допълнително намаляване. Фазовият ъгъл, измерен в нискочестотния диапазон (30 Hz – 10 kHz), също показа намаление след облъчването, като тази промяна се увеличи при увеличаване времето на облъчването. Конвенционалното нагряване доведе до увеличаване големината на импеданса в нискотемпературния диапазон 20 °C – 40 °C и до намаляването му при по-високи температури. Ефектът на конвенционалното нагряване върху фазовия ъгъл беше подобен на ефекта от микровълновото облъчване. Електричната импедансна спектроскопия може да бъде използвана за различаване на образци от мляко, загрявани чрез микровълново облъчване, от образци, загрявани чрез конвенционален метод.

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Detection of wax coatings on plums by rapid physical methods

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Plums epicuticular wax provides fruit surface water repellency and reduces water loss through the skin. In order to enhance its effect and to prolong the shelf-life of the plums, commercial EU allowed waxes thin coatings are made. Unfortunately in the real practice, some not permitted artificial waxes are also applied. The present study aims at presenting three rapid physical methods for detection and recognition of wax type coatings. Optical microscopy reveals quite different surface morphology of all 10 used waxes, making it possible to detect the wax-treatment existence but not always to identify the type of the wax. Contact angle measurements with polar and nonpolar liquids display clear differences between the two values combinations but this method should be used with caution because of the difficulty in contact angle estimation onto the curved plum surface. Differential scanning calorimetry appears to be the most precise technique since the plum bounded water and the epicuticular wax, both have evaporation and melting peaks far enough from the melting peaks of all investigated waxes, allowing one to detect and identify the type of the wax treatment.

Keywords*: plum, wax fruit coatings, DSC, optical microscopy, contact angle*

INTRODUCTION

The main aim of all fruits producers is to reduce the crop losses and to maintain the quality of the fresh fruits over extended periods of time. From the other side, consumer interest brings together health, nutrition, and food safety combined with environmental concerns. Both points of view meet at protecting fruits with environmentally friendly, nontoxic coatings, which preserve the flavour, nutrition value and visual appearance. Even though many scientific attempts are put on formulation of different kind of coatings [1-3], the most often used coatings are those from the four EU allowed as food additives waxes – beeswax, candelilla wax, carnauba wax and shellac [4]. Such harmless coatings are proved to reduce decay, reduce moisture and weight loss, enhance external appearance, regulate the respiration rate and have antifungal effect [5]. Unfortunately in some cases, because of their lower prizes, some synthetic waxes, not allowed for use in the food products, are also applied for plum coatings. The standard control of the wax coatings is performed by Gas chromatography combined with Mass spectrometry [6]. In the gas chromatograph the evaporated wax components are transported with a carrier gas flow through a capillary column and separated by their different transportation speeds (retention time). However, the components must be vaporisable without decomposition. Wax components with hydroxyl-groups (alcohols, fatty acids) need to be derivatised, which means, the active H-atoms have to be masked and inactivated in order to avoid decomposition or peak broadening. Hence, this method is time consuming, requires specific sample preparation and is not always very effective, for instance if the epicuticular wax and the applied wax have very similar chemical composition.

The present study aims at presenting three rapid physical methods, mainly light microscopy, static contact angle measurements of a small sessile drop, and differential scanning calorimetry (DSC), for detection of plum wax coatings and recognition of wax type. For these purpose 10 different waxes from four categories were used – paraffins, ceresins, palm wax, and beeswax.

EXPERIMENTAL

For the present investigation "Black diamond" plums were bought from the local market. The plums were selected for uniform maturity, size, colour and absence of physical damage or grey. The artificial commercial waxes were supplied by Evricom LTD, Bulgaria. They belong to three different categories – paraffin (E1, E4, E6, E21, E53), ceresin (ECP, EC2, EC3), and palm wax (ES4, ES5). Beeswax was delivered from the local apiarists.

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Two types of samples were prepared: planar wax films and wax plum coatings. The neat wax planar films were prepared by casting the molten wax onto the microscope glass. The plum coatings were prepared at first by fruit dipping into the molten wax and subsequent thinning the coatings in hot air flow. The thinning was performed until the coating became invisible to the naked eye.

The wax morphology was investigated by means of optical (light) microscope type Biorex 3 (Konus, Italy), offering magnification up to 1000x, equipped with CCD camera Microview (Konus, Italy). For the contact angle measurements droplets of 5 μl (for the neat wax films) or 2 μl (for the plum coatings) were inserted onto the surface by means of precise 10 μl micro syringe (Innovative Labor System GmbH, Germany) supplied with steel needle. All measurements were performed at ambient conditions. The final value for the contact angle was obtained as an average of at least 5 drops for each surface. The drops were recorded with CS01-200 Digital microscope (CoolingTech, China) and the contact angle was obtained by image processing with the microscope software. Thermal behavior of the waxes and wax coated plums was examined via Differential scanning calorimeter DSC SETARAM 141. The samples were prepared by fine pilling the plums so that a thin slice consisting of wax and small amount to plum skin was yielded. The samples with masses of several milligrams were first cooled down to 0 °C at cooling rate 5 K/min and then heated at the same rate up to about 150 °C. For the calibration Indium standard was used, having melting temperature of 157 °C and melting enthalpy 28.4 J/g.

RESULTS AND DISCUSSION

As a preliminary study, the planar wax films were investigated under microscope. The images reveal that for all 10 different waxes different types of morphologies occur (see the examples given in Figure 1) as a result of self-assembly of the wax molecules depending on chain-length distribution, functional groups, crystallization conditions and concentration of the major components of the wax [7]. The solid wax films, similar to the epicuticular wax itself, may consist of partially crystalline and partially amorphous or disordered regions [6].

The specific wax films texture is preserved to a great extent even when the waxes were made in the form of plum coatings. The coatings change the

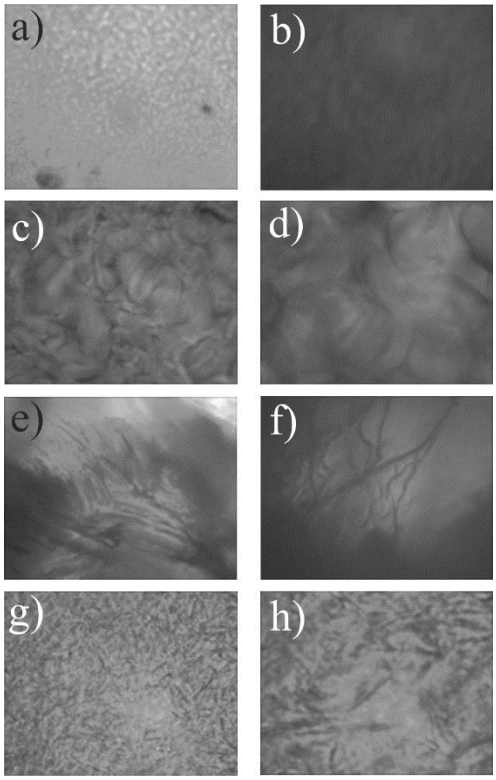

Fig. 1. Microscopic images of different planar wax films and different magnifications: a) beeswax, 40x; b) beeswax, 100x; c) E53, 40x; d) E53, 100x; e) ES5 40x; f) ES5, 100x; g) ECP 40x; h) ECP 100x.

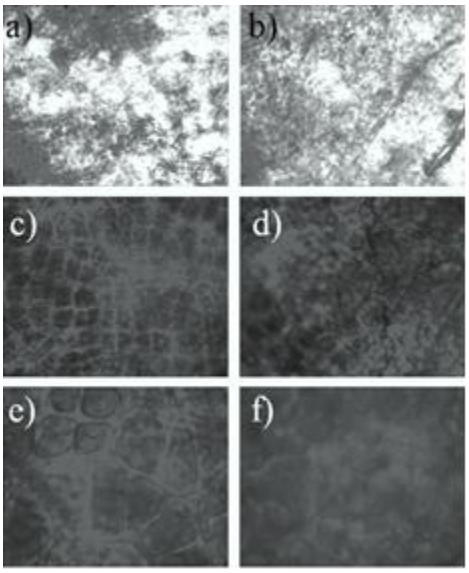

Fig. 2. Microscopic images of plums at different magnifications: a) 10x, natural plum; b) 10x, plum covered with beeswax; c) 40x, natural plum; d) 40x plum covered with beeswax; e) 100x, natural plum; d) 100x, plum covered with beeswax.

initial plum surface morphology, which can be easily established under microscope with appropriate magnification. In Figure 2, as an example, the texture of the natural plum and the

Table 1. Results for the contact angle measurements of different waxes with distilled water, θ_{DW}, and methylene iodide, θ_{MI}, wax melting enthalpy, ΔH_m, melting temperatures T_m^W of the neat wax and T_m^{PC} of the plum coating, and temperature shifts $\Delta T=T_m^{PC}-T_m^W$. The values in bold are the peaks of the bounded-water evaporation.

Sample	Type of the surface	θ_{DW} [deg]	Θ_{MI} [deg]	ΔH_m [J/g]	T_m^W [°C]	T_m^{PC} [°C]	ΔT [°C]
Natural plum	Smooth	66	30	-	-	86 **118**	-
Beeswax	Smooth	106	60	215	66.5	65.1 **106.0**	-1.4
ES 4	Rough	65	51	149	- 55.3 67.5	53.8 58.9 68.5 **121.0**	- 3.6 1
ES 5	Rough	92	52	259	66.3 -	58.5 **120.8**	7.8
E 6	Smooth	106	59	270	47.1 65	- 63.6 **129.8**	- - 1.4
EC 3	Smooth	106	64	254	49.3 73.3	44.4 60.9 **113.1**	5.3 8.1
E 53	Smooth	116	65	252	45.4 63.8	44.4 60.9 **113.1**	-1.0 -2.9
ECP	Smooth	108	67	247	59.7	61.5 **118.9**	2.5
E 1	Smooth	120	70	219	38.7 61.4	59.6 **122.0**	-1.5
EC 2	Smooth	122	72	242	57.2	54.4 **123.4**	-2.8
E 21	Smooth	74	72	256	36.2 57.2	- 57.1 77.4 **116.1**	- -0.1 -

beeswax coated plum is presented. The beeswax was chosen since its surface was the smoothest one and it was expected to be the most difficult to be recognized as the plums were coated with it. At magnification 10×, both textures look quite similar (Figure 2a, 2b) but going to magnification 40× the dissimilarities start to appear (Figure 2c, 2d). At such magnification the cuticle layer with separate cells is clearly seen in the images without wax coverage, whereas smeared images without clear features are seen for the coated plums. The magnification 100× offers the best resolution for the requested distinction.

As it is clearly visible (Figure 2e, 2f), the separate cells of the natural plum are very well seen, whereas for the treated plum mostly the wax coating without any specific texture is seen. According to the results, light microscopy, as one of the mostly available and simplest experimental methods in the laboratories, can provide

information about the wax treatment of the fruits. Although one should keep in mind, that the final morphology of the coating depends not only on the type of the wax but also on the surface roughness of the plums and hence, the microscopy can give reliable information mostly about the existence of wax-treatment but not on the type of the wax.

Contact angles have been used for a long time as an indicator of surface wettability in the food and plant science [2, 8-11]. The well-known Young equation describes the balance at the three-phase contact of solid, liquid and vapor: $\gamma_{sv} = \gamma_{sl}+\gamma_{lv}.\cos\theta_Y$, where the interfacial tensions, γ_{sv}, γ_{sl} and γ_{lv}, form the equilibrium contact angle of wetting, often referred to as the Young contact angle, θ_Y. Because of the existing difference in the structure of all artificial waxes, one could expect that the equilibrium contact angle will be different, depending on the liquid used, wax type and the surface roughness. The results for a contact angles, measured onto the planar wax films surfaces are listed in Table 1. As it is seen, the combination of the two contact angles – that for distilled water (polar liquid), θ_{DW}, and that for methylene iodide (nonpolar liquid), θ_{MI}, appear to be unique for each wax coating. When the contact angle was measured onto the curved wax plum coatings, the results differ in most of some cases with $\pm 1^o$. The largest difference of about 6^o was observed for ES5 wax coating, and it would be attributed to the significant roughness of the wax surface morphology (see Figure1). The apparent contact angle in this case is very sensitive to that morphology, because the size of the drop (less than 1 mm) is comparable with the size of the roughness.

DSC is a standard method for investigation of the thermal behaviour of many materials and it is also applied in the field of food science [12, 13]. In the present investigation, two sets of DSC curves were recorded – waxes only and the second one plum with coatings. The results are shown in Table 1 and in Figure 3. As it was pointed out by some authors [6] the term ‘‘wax’’ is used for a variety of natural or artificial commercial products that contain fatty materials of various kinds. According to the chemical definition, waxes consist of aliphatic compounds with discrete molecules (no polymers), are solid and meltable without decomposition normally below 100 °C. Most of the plant waxes fit to these criteria but they contain other components as well, which lead to higher melting temperatures of those waxes [6]. This is the main reason one can distinguish via DSC the artificial wax coatings from the natural ones. The results show, that the wax coatings melting temperatures shift with respect to that of the bulk waxes, but the main features of the DSC endotherms remain unchanged. In some cases, the enthalpy of the multicomponent peaks changes, which can be attributed to the polymorphism of those waxes, which does not affect the number of the melting peaks. The highest temperature peaks in each DSC run of the plums with coatings is attributed to the bounded water evaporation. Additional experiments (not shown) prove that the melting temperature of the epicuticular plum wax was 86 °C. The obtained results show that DSC analysis of the melting enthalpies and melting temperatures is able to reliably distinguish not only the wax-treatment existence but the type of the wax used as well.

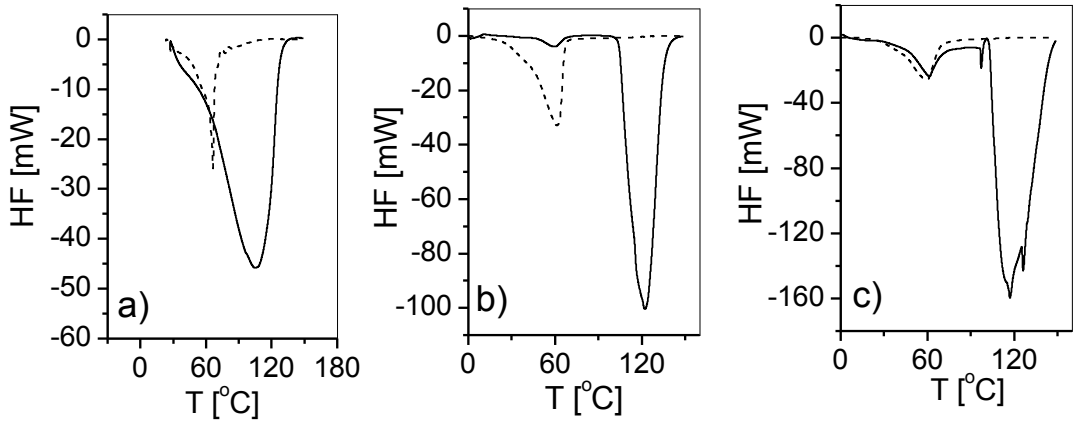

Fig. 3. DSC thermograms of bulk waxes (dashed line) and wax plum coatings and plums (solid line) with: a) Beeswax; b) E1 wax; c) ECP wax.

CONCLUSION

The optical microscopy reveals quite different surface morphology of all waxes used, so it is possible to detect the wax-treatment existence but not always to identify the type of the wax. Contact angle measurements of planar wax films as well as plum wax-coatings, measured with polar (distilled water) and nonpolar (diiodomethane) liquids display clear differences between the two values combinations but this method should be used with caution because of the difficulty in contact angle estimation onto the curved plum surface. Differential scanning calorimetry appears to be the most precise technique. The bounded water in the plums and the plum epicuticular wax, both have evaporation and melting peaks far enough from the melting peaks of all investigated waxes. This allows one to observe the peculiarities of the melting peaks for all different waxes and to identify the type of the wax employed.

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ОТКРИВАНЕ НА ВОСЪЧНИ ПОКРИТИЯ ВЪРХУ СЛИВИ ЧРЕЗ БЪРЗИ ФИЗИЧНИ МЕТОДИ

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(Резюме)

Епикутикулярният восък при сливите осигурява отблъскващ нежеланите вещества ефект и намалява загубите на вода през повърхността им. В практиката, за подсилване на този ефект, както и за удължаване на времето за съхранение в магазинната мрежа, се изработват покрития от разрешени от Европейската общност восъци/парафини. Целта на настоящата публикация е да представи три бързи физични метода за откриване на съществуването на такива покрития и за разпознаване на типа им. Оптичната микроскопия разкрива различаващата се морфология за всички 10 изследвани покрития, което прави възможно откриването на третиране, но не винаги е възможно идентифицирането на типа на покритията. Измервания на контактния на ъгъл на омокряне, с полярна и неполярна течности, показва явни различния между двойките ъгли за всички изследвани покрития, но методът следва да се използва внимателно, като се има предвид възникващата от закривената повърхност на сливите възможна неточност в определянето на ъглите. Диференциалната сканираща калориметрия се оказва най-прецизният метод, тъй като пиковете на изпарение на свързаната вода, както и този на топене на естествения епикутикулярен восък са достатъчно отдалечени от пиковете на топене на всички изследвани восъци/парафини, което позволява откриването на съществуващо покритие и идентифицирането на типа му.

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Characterization of extra virgin olive oils adulterated with sunflower oil using different physical methods

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This paper describes a study of the usefulness of some physical methods in the detection of adulteration of extra virgin olive oil with controlled concentration of relatively cheap sunflower oil. We have tested three physical methods measuring refractive indices (RI) and their dispersion curves, fluorescence spectra and color parameters that are related to the chemical structure and content of the olive oils. The RI values of the samples were measured with a total experimental uncertainty of less than 3×10^{-4} by the method of the disappearing diffraction pattern for two wavelengths – 405 nm and 532 nm at a temperature 23°C. Fluorescence spectra were measured using a fiber optic spectrometer (AvaSpec-2038, Avantes) and the samples were excited by light emitting diodes at 370 nm, 395 nm, 425 nm and 450 nm using the set up. The spectrometer's sensitivity is in the (200 – 1100) nm range with a resolution of about 8 nm. The color parameters (index of lightness L^*, a^*, b^*, chroma C^* and hue angle h_ab) corresponding to the uniform color space CIELab, were determined on a Lovibond PFX 880. The color parameters were used for determining the β – carotene and chlorophyll content in the investigated samples. All of the obtained experimental results suggest that the three optical methods presented are correlated and could be useful for a fast detection of sunflower adulteration of extra virgin olive oils. These techniques are sensitive and rapid and also do not require any additional chemical agents.

Keywords: *Refractive index, fluorescence, extra virgin olive oil, sunflower oil, chlorophyll, β – carotene.*

INTRODUCTION

Olive oil is a valuable food product as compared with other vegetable oil products. As a result, the adulteration of olive oil with cheaper vegetable oil becomes a real concern. For this reason, the analysis of edible oils for possible adulterants is very important for food safety and protection of consumers. Various physical and chemical tests have been used to establish the authenticity of olive oil and to detect the level of adulterants in it [1-2]. The most useful are chromatographic methods [4-6]. They offer high sensibility and accuracy, but are also time consuming and expensive. For this reason in our study we suggest three fast and cheap optical methods – refractometry, fluorescence and color measuring - that can be used for determining the adulterants in olive oils. The aim of this paper is the investigation of the extra virgin olive oil with controlled sunflower oil content by proposed three optical methods.

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EXPERIMENTAL

Samples

In this work we have investigated three samples with different characteristics. Samples of extra virgin olive oil (EVOO) were provided by Greece Company and its botanical origin and quality were guaranteed by the supplier. Samples of sunflower oil (SFO) were obtained from local Bulgarian superstore. Binary samples of 50 % EVOO and 50 % SFO were prepared using a KERN ABJ 80 - 4M analytical balance (precision 0.1 mg). All the samples were stored in fully filled and closed glass vessels and kept in the dark in an incubator at a fixed temperature of 25 °C.

Refractive index measurements

The refractive indices (RI) for all the samples were measured by the method of the disappearing diffraction pattern using a laser refractometer at wavelengths of 405 nm and 532 nm. The method and the laboratory device were reported earlier [7]. The several microliters of the samples were put between a reflecting grating and a glass prism with $RI = N$. When the light beam from one of the lasers

falls at an angle smaller than the critical angle α_C the beam penetrates through the sample, reaches the reflecting grating and creates a diffraction pattern. In the case when the incidence angle is equal to or higher than the critical angle α_C only one total reflected beam is observed. Therefore, measuring the critical angle α_C as the angle of disappearance of the diffraction pattern we could calculate the RI by the following formula:

$$n = N \sin\left[A \pm \arcsin\left(\frac{\sin \alpha_C}{N} \right) \right] \qquad (1)$$

where A and N were the prism refractive angle and the refractive index, respectively. In our experiment A = 65ºC, N (405 *nm*) = 1.7880, N (532 *nm*) = 1.7480. The experimental uncertainty was Δn = ±0.0003.

Fluorescence spectra measurements

The sources used to measure the fluorescence spectra are 370 nm, 395 nm, 425 nm light emitting diodes (LEDs). A fiber optic spectrometer (AvaSpec-2038, Avantes) with sensitivity in the (200-1100) nm range and a resolution of about 8 nm was used to measure the fluorescence spectra. The oil samples were placed in a cuvette 10 mm x 10 mm and irradiated by LEDs.

Color measuring

Using a Lovibond PFX 880 (UK) colorimeter and a cuvette of a 10 mm length (Recommendations on uniform color spaces, 1971), the color parameters in CIELab colorimetric system have been obtained. All measurements have been carried out at room temperature immediately after opening the oil bottle. , Color coordinates, color parameters *a*, *b* and brightness *L* of tested samples have been measured. The chlorophyll and β – carotene are calculated by using the transmission spectra in the visible region and values for color parameters by software program developed specially for Lovibond PFX 880 from the producer. Parameters such as chroma $\left(C^*_{ab}\right)$ and hue h_{ab} were defined by Eqns (2) and (3):

$$C^*_{ab} = \sqrt{\left(a^*\right)^2 + \left(b^*\right)^2} \qquad (2)$$

$$h_{ab} = \arctan\left(\frac{b^*}{a^*} \right) \qquad (3)$$

RESULTS AND DISCUSSION

The fluorescence spectra in the visible region of the investigated samples are obtained for excitation wavelength respectively λ_e = 370 nm, 395 nm, 425 nm and 450 nm. The best ration for fluorescence emission vs. excitation intensity is found at λ_e = 395 nm (Figure 1).

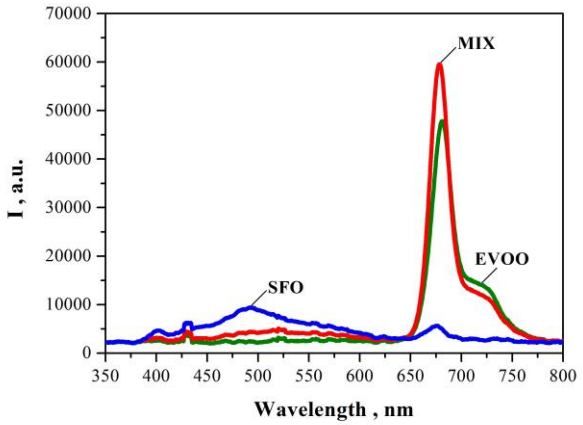

Fig. 1. Fluorescence spectra of the olive oil samples.

There are four fluorescence peaks for investigated samples related to β – carotene at λ = 430 nm; oxidation products at about λ= (500-520) nm; chlorophyll at λ = (675-678) nm; non-determined pigments at λ = 700 nm.

Color parameters for investigated samples are measured in CIELab colorimetric system. The results are present in Table 1.

Table 1. Color parameters for olive oil and its double mixture with sunflower oil.

Samples	**SFO**	**MIX**	**EVOO**
Olive oil concentration, %	**0**	**50**	**100**
L	92.51	90	81.1
a	-1.73	-12.1	-11.7
b	6.93	55.6	81.1
C_{ab}	7.14	56.9	81.9
ΔE	75.68	27	0
h_{ab}	-76	-77.8	-81.8
β–carotene, ppm	2	18.4	34.7
Chlorophyll, ppm	0.011	20.7	5.18

The brightness *L* decreases with increasing of concentration of extra virgin olive oil and content of β – carotene and chlorophyll increases. The influence of green component increases when the content of olive oil is raised. The linear dependence exists between values of *b** and concentration of olive oil. The greatest color difference is between pure olive oil and sunflower oil.

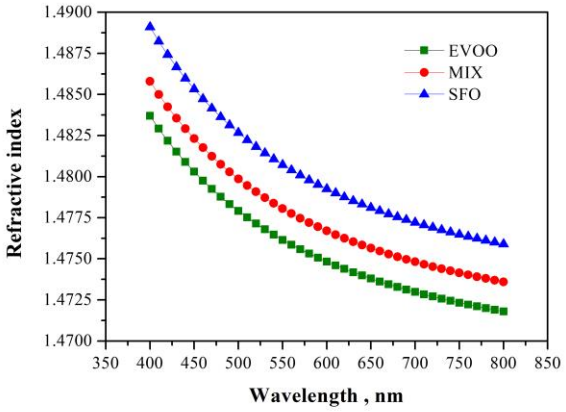

Fig. 2. RI dispersion curves of the olive oil samples.

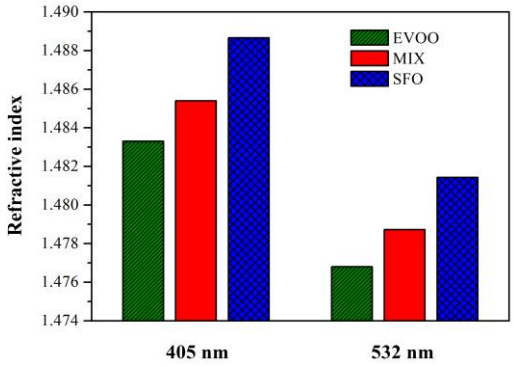

Fig. 3. RI of the olive oil samples for two wavelengths.

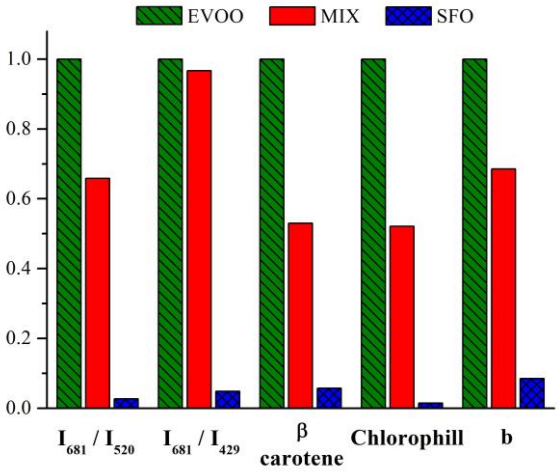

Fig. 4. Normalized experimental data for the olive oil samples.

The data obtained from the two wavelengths RI measurements were used for the construction of dispersion curves using the one-term Sellmeier equation far from the fundamental absorption band[8]. By the one-oscillator Sellmeier's model we can determine the Sellmeier's coefficients s and λ_s from the systems:

$$n_{s_i}^2 - 1 = \frac{s\lambda_i^2}{\lambda_i^2 - \lambda_s^2}, \qquad i = 1,2 \tag{4}$$

Then we can calculate the RI dispersion curves in the visible spectral range for all samples using the already determined coefficients. Figure 2 presents the obtained dependences for RI.

The refractive indices of the EVOO measured at room temperature are in very good agreement with the results announced in work[9]. The comparison of dispersion curves shows that the RI is the lowest for the EVOO and the RI value increases in direct proportional to adding of SFO as it is shown in Figure 3 for two wavelengths – 405 nm and 532 nm. It is shown that the reduction of the RI is associated with a greater amount of EVOO.

In Figure 4 the normalized changes of the fluorescence peak, the normalized content of chlorophyll and β – carotene and the normalized color characteristics with the increase of the SFO content are presented. In Figure 4 each parameter value is normalized by using the EVOO value as reference.

The increase of the two fluorescence peaks (I_{681}/I_{520} and I_{681}/I_{429}) and the color parameter b associated with a greater amount of EVOO could be related to the increase of chlorophyll and β – carotene too.

In view of the results of the RI and the chemical analysis presented in Figure 3 and Figure 4 the RI reduction could be related to the increase of the chlorophyll content and of β – carotene content.

In future investigations the authors plan to study in more detail the relation between fatty acid content of the samples and the intensity of the fluorescence peaks as well as the relation between chlorophyll and β – carotene contents and RI.

Hence, three independent optical methods – measuring of refractive indices, fluorescence spectra, and color parameters, can be used for identify a pure EVOO and determining the presence of SFO adulterant. The results obtained demonstrate that the measured physical parameters are related to chemical structure and content of the olive oils. So, these methods are useful of the detection of adulteration of extra virgin olive oil with cheap sunflower oil.

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ХАРАКТЕРИЗИРАНЕ НА СТУДЕНО ПРЕСОВАН ЗЕХТИН, ФАЛШИФИЦИРАН С ОЛИО, ЧРЕЗ РАЗЛИЧНИ ФИЗИЧНИ МЕТОДИ

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(Резюме)

В тази статия се изследва полезността на някои физични методи за откриването на фалшификация на студено пресован зехтин с контролирана концентрация на сравнително евтино слънчогледово масло. Тествани са три физични методи за измерване съответно на показателите на пречупване (ПП) и получаване на дисперсионните криви; на флуоресцентните спектри и на цветовите характеристики, които са свързани с химичната структура и съдържанието на зехтините. Стойностите на ПП на образците са измерени с експериминтална неопределеност по-малка от 3×10^{-4} по метода на изчезващата дифракционна картина за две дължини на вълната – 405 nm и 532 nm при температура 23°C.. Флуорисцентните спектри са измерени с влакнестооптичен спектрометър AvaSpec-2038, Avantes, като образците бяха възбуждани със светодиоди, излъчващи съответно на 370 nm, 395 nm, 425 nm и 450 nm. Чуствителността на спектрометъра в областта (200 – 1100) nm е около 8 nm. Изучени бяха цветовите характеристики (L^*, a^*, b^*,C^* и h_{ab}) на олиото в SIELab колориметрична система чрез Lovibond PFX 880. Цветови характеристики бяха използвани за определяне на β - каротин и на съдържанието на хлорофил в изследваните проби. Всички получени експериментални резултати показваха, че трите представени оптически методи са взаимосвързани и биха могли да бъдат полезни за бързо откриване на фалшификация на студено пресован зехтин със слънчогледово олио. Тези техники са бързи и чувствителни, а освен това не изискват използването на никакви допълнителни химически агенти.

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Physical studies of plant wax from watermelon

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The plant wax isolated from the cuticle of watermelon *(Citrullus lanatus),* American variety *Crimson Sweet* is investigated in this study. The isolated plant wax is characterized by means of infrared spectroscopy (IR), X-ray diffraction (XRD), differential thermal analysis (DTA) and scanning electron microscopy (SEM). The obtained results for the composition, structure and the thermal stability of the studied plant wax are necessary for defining the exact composition, properties and quality of edible films, containing the studied plant wax as a hydrophobic component.

Key words: plant wax, watermelon, cuticle, edible films and coatings

INTRODUCTION

An alternative of the synthetic packages is the preparation and use of edible films from natural biomaterials. This is a problem of the present day, which involves a high number of scientists from all over the world [1-3]. The limited use of these packaging materials, in which composition the main components are proteins and polysaccharides, is due to the high prime cost and to the bad moisture protection of the covered foods. One of the possibilities to improve their hydrophobic properties is to add lipids and waxes in their composition.

Plant waxes are situated on the surface of higher plants (leafs, flowers, fruits) and are directly influenced by the environment. They reduced the transpiration (evaporation of water) through the cuticle increase the stability of plants towards different diseases and frost, made the surface of plants more stable towards moisture [4-6]. The main components in the composition of plant waxes are hydrocarbons, esters (mono-, di-, hydroxyl-), free primary alcohols, aldehydes, free acids, secondary alcohols, ketones and hydroxyl ketones, free diols, glycerides, triterpenes. There are lipids in the composition of plant waxes which prevent the plants from the unsuitable action of the factors of the environment. The lipids are used in edible films as a barrier towards gases, moisture (the cuticle of fresh fruits contains waxes) and to improve the sensor properties of foods (appearance, vain show). The thick edible films must be removed before consumption of the food, when are used thin layer of edible films, they are suitable for consumption [7].

The composition of edible films usually contain beeswax and some plant waxes, which are obtained in high quantity - candelilla wax, carnauba wax. There are good results obtained by using of beeswax [8,9], candelilla wax [10,11], carnauba wax [12] which increase the hydrophobic properties of the food emulsion films [13,14]. More perspective from an economic point of view at the present moment is the possibility to replace them with a plant wax, obtained from waste materials of the food and of the agricultural technology [15]. In connection with this it is interested to investigate the possibility of using the peels of watermelons, which are waste materials from the places of public resort and a raw material from which is extracted the plant wax. The plant wax obtained from the watermelon is not studied up to this moment.

The scope of this study is to investigate the physical characteristics of the plant wax isolated from the cuticle of watermelon *(Citrullus lanatus),* American variety *Crimson Sweet*, cultivated and offered in Bulgaria. They determined the properties of the plant wax and the possibility to be used as a component in the composition of emulsion edible films, increasing its hydrophobic properties.

EXPERIMENTAL

The object of research is the most popular and cultivated in Bulgaria American variety (*Crimson Sweet*) watermelon (*Citrullus lanatus).*

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The methods used for the physical study of the plant wax, extracted from the cuticle of watermelon (*Citrullus lanatus)* are as follows**:**

- The plant wax was extracted from the raw material with a heated up to 50°C chloroform for 3 minutes. The obtained warm extract was filtrated and concentrated. The concentrating has been done by evaporation of the solvent. The obtained plant wax was dried and stored at temperature 3°C. The method was first applied and described from Casado and Heridia [16].
- IR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrophotometer in the range of 4000-450 cm^{-1}. The samples were pressed in KBr tablets.
- XRD were collected at room temperature 25 °C on a Bruker D8 Advance instrument with CuKα radiation and a LynxEye detector within the 2θ range from 5.3 to 80°, with a constant step 0.02°, 2θ degrees at counting time 1 s/step. Data evaluation was made with the use of a Software package EVA phase identification was made with the use of data base ICDD-PDF2.
- DTA – TG studies were made according to the method, described from Wendlandt [17]. The principle of the DTA - TG method is based on measuring of the temperature difference between the reference and the studied samples. Depending on the ongoing processes in the studied material (exothermic or endothermic) positive or negative difference in the temperature can be registered. The method has been carried out on LABSYS TM EVO apparatus, SETARAM, France in the temperature region of 10 - 300 °C, heating rate 5 °C/min and gas carrier - synthetic air passing out through the workspace with rate of 20 ml/min. The studied sample with weight 10 - 20 mg has been placed in corundum crucible. The numerical data of the mass changes of the sample have been collected and presented in xls or dat files. TG-resolution - 0.02 μg, dynamic drift of the baseline (1 hour) - 10 μg.
- Scanning electron microscope (SEM) Jeol T-200 Japan, was used to study the surface morphology of the obtained wax samples.

RESULTS AND DISCUSSION

The difference between the mineral waxes and those containing lipids and butyric acids can be observed by means of IR spectroscopy (Dudley and Fleming [18]. The wax from *Citrullus lanatus* belongs to the second type and its spectra seens much more complicated compared to those of the mineral waxes. From the frequencies of the absorption peaks, it is impossible to determine whether various functional groups are present or absent [18].

The obtained results by the means of IR spectroscopy of the studied plant wax isolated from *Citrullus lanatus* are shown in Figure 1.

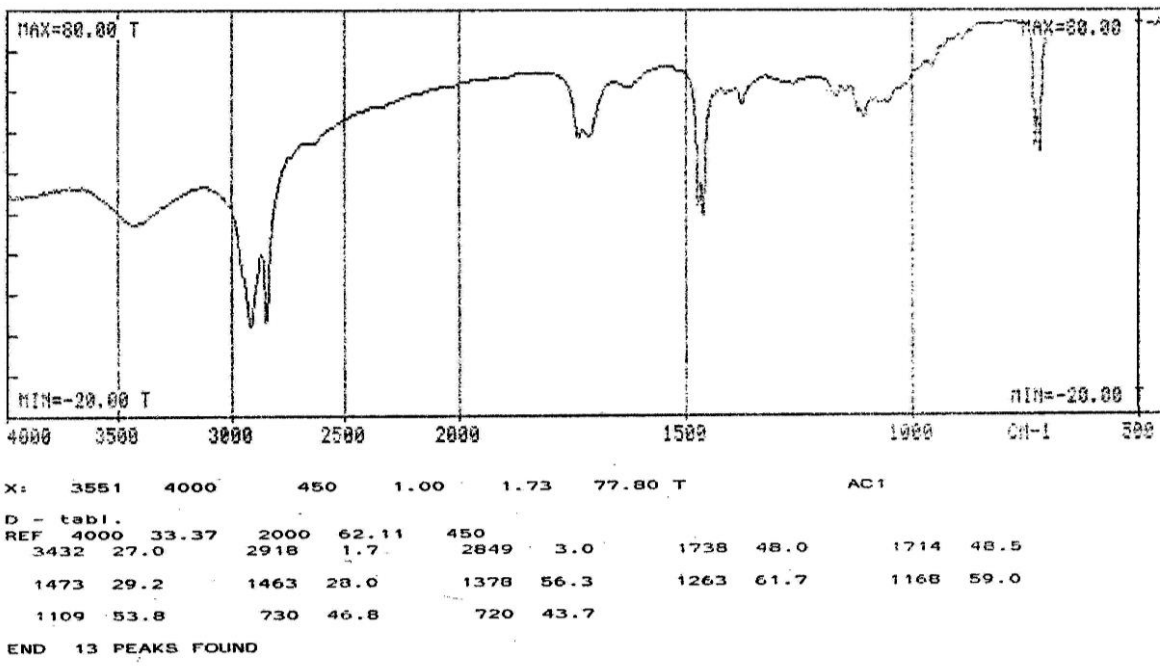


Fig. 1. The IR spectra of the wax from *Citrullus lanatus*

On Figure 1, the IR spectrum of the wax from *Citrullus lanatus* shows that clear peak points at about 720 cm^{-1} corresponding to $–CH_2$ groups, and the presence of the $– CH_3$ group at about 1463 cm^{-1}. The following peaks have been also found: 730 cm^{-1}, 1109 cm^{-1}, 1168 cm^{-1}, 1263 cm^{-1}, 1378 cm^{-1}, 1473 cm^{-1}, 1714 cm^{-1}, 1738 cm^{-1}. 2849 cm^{-1}, 2918 cm^{-1}, 3432 cm^{-1}. More detailed identification of these peaks and the corresponding compounds will be done in a following study, by means of gas chromatography. As it can be seen, the IR spectra show the bands characteristic for wax at 3432 cm^{-1} – the stretching vibrations of intermolecular bound hydroxyl groups and/or water molecules. Doublet at 2849 cm^{-1} and 2919 cm^{-1} – stretching vibrations of CH groups, 1714 cm^{-1} and 1738 cm^{-1} – stretching vibrations of the carbonyls of esters and CO groups of un-ionized carboxyls of terpenoids or organic acids; 1473 cm^{-1} – planar deformation vibrations of CH groups (-CH_2 scissors) and doublet at 720 cm^{-1} – nonplanar skeletal deformation vibrations of long-chain hydrocarbons; 1168 cm^{-1} stretching vibrations of C–O–C groups. The following peaks have been also detected: A cluster of features for the CH_2 symmetric stretches at 2849 cm^{-1} and 2918 cm^{-1}; 1378 cm^{-1} – corresponds to CH_3 – symmetric deformation. It should be mentioned that the features for the methylene C–H stretches are in agreement with the traditional explanation of the spectra of long-chain aliphatic molecules[19] (Gibbs 2002). However some authors have pointed out that the absorption band at 2849 cm^{-1} is due to the absorption of neighboring methylene groups in trans-configuration and the mentioned band can be taken as evidence of all trans configurations, as it is expected for a wax in crystalline state. The band at 1378 cm^{-1} can be related to the "O–H deformation" i.e. C–O–H angle bend, as reported by Gibbs[19] (Gibbs 2002). This C–O–H in-plane angle-bending mode may account for the shoulder at 1453 cm^{-1} found on the lower side of the strong CH_2 scissors feature at 1473 cm^{-1}. This band can also be assigned to the CH_3 symmetric deformation, as was mentioned above. According the data obtained from the FT-IR analysis we can conclude that the adjacent methylene groups are predominantly in the trans configuration and absorb strongly at 2849 cm^{-1} and also the watermelon wax contains long-chain aliphatic molecules or n-paraffins (mixture of different hydrocarbons) coexisting with terpenoids, organic acids and water molecules.

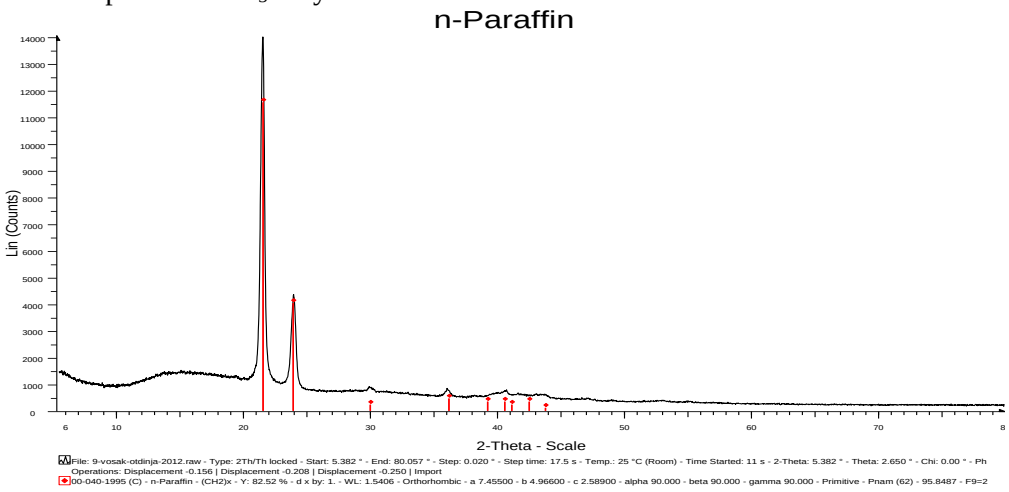


Fig. 2. X-ray structure analysis of the wax from *Citrullus lanatus*

Figure 2 shows powder XRD pattern of the samples with well-defined peaks corresponding to n-Paraffin, described in orthorhombic Space Group P*nam* with unit cell parameters *a*=7.455Å, *b*=4.966Å and *c*=2.589Å. From the study of the wax from *Citrullus lanatus* by means of X-ray structure analysis, it was ascertained that the dominating component of the wax studied is normal paraffin containing straight chains of -(CH2)n- (ICDD-PDF2 # 40-1995). It should be mentioned that 2.589Å is the minimal distance corresponding to one carbon in the alkane chain and the cell described above should be considered as a sub cell. Usually n-alkanes have c-chain length varying between 5 and 60 carbon atoms. The melting point increases with the increase of the chain length.

The results of the DTA –TG studies of the plant wax from (*Citrullus lanatus)* were presented on Fig. 3.

DTA-TG thermograms (e. g. Figure 3) present two endotherm peaks of the studied plant wax. The first one is in the temperature interval 45-93.4 °C, for

which one is clearly expressed a deep peak maximum 79 °C and temperatures of onset 75.8 °C and offset 82.4 °C. The second one is quite more stretched from the first one in the temperature interval 96-233 °C and with a peak maximum at 169 °C and temperatures of onset 169 °C and offset 230 °C. The term gravimetric curve of the mass changes shows that the main loss of the mass started after 200 °C. The main conclusion from the carried out research is, for the preparation of edible films the studied plant wax must be heated at least up to 80 °C, in order to achieve its dispersed in water emulsion at the same temperature, which will spare the thermal sensible biopolymer macromolecules.

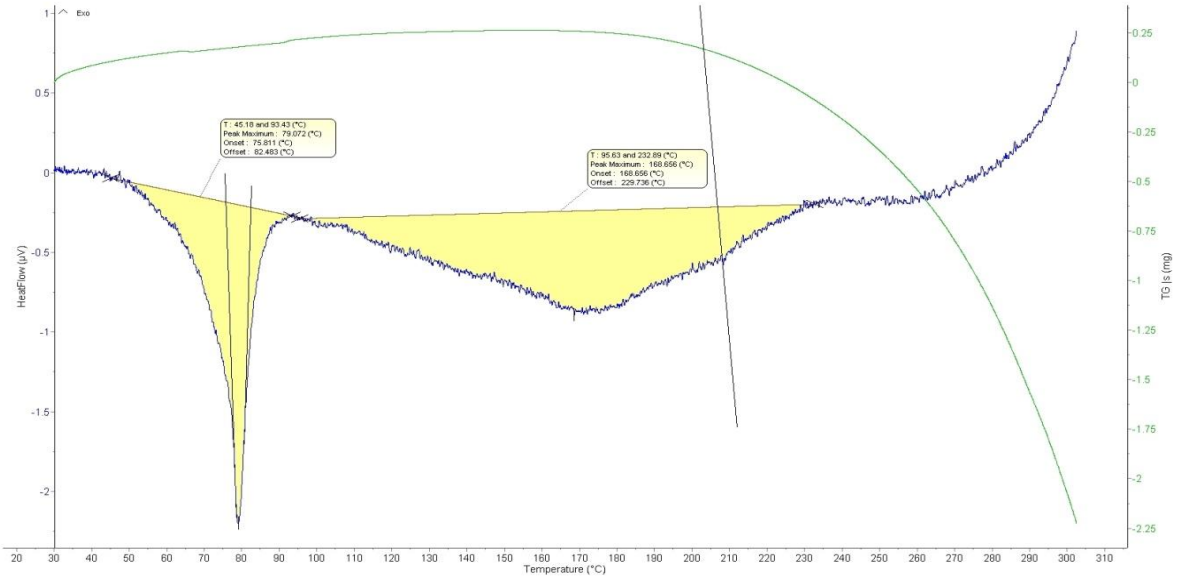


Fig. 3. DTA –TG analysis of the wax from *Citrullus lanatus*

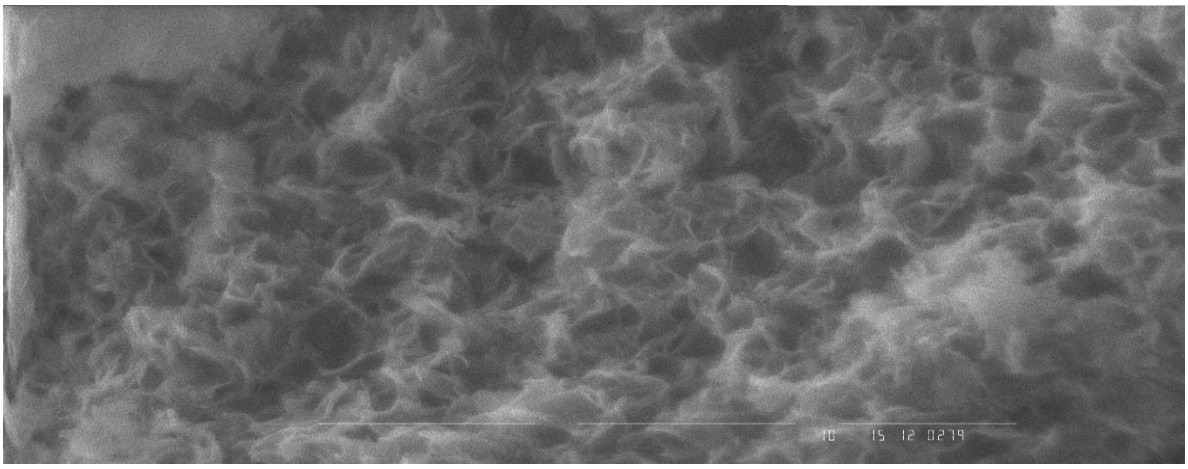


Fig. 4. SEM analysis of the wax from *Citrullus lanatus*

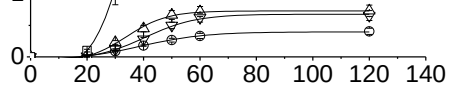
The results of the SEM studies of the plant wax from (*Citrullus lanatus)* were presented on Fig. 4. The structural image obtained from the SEM (Fig. 4) proved that the plant wax extracted from watermelon (*Citrullus lanatus)* is a complicate capillary-pore system.

CONCLUSION

FT-IR analysis indicate that the adjacent methylene groups in the studied wax are predominantly in the trans configuration and absorb strongly at 2849 cm^{-1}. The watermelon wax contains long-chain aliphatic molecules or n-paraffins coexisting with water molecules, terpenoids and organic acids.

It can be concluded that the dominating component in the composition of the studied wax is n-Paraffin. The DTA-TG analysis shows that the main loss of the mass started after 200 °C. The capillary-pore system of the studied was has been confirmed.

In this study we also found that for the preparation of edible films using the studied plant wax is necessary to heat it at least up to 80 °C, in



order to achieve its disperse in the water emulsion at the same temperature.

The obtained results for the composition, structure and thermo stability of the studies plant wax are of great value. They are necessary for the determination of the optimal composition, properties and quality of the edible films which contained as a hydrophobic component the studied wax.

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ФИЗИЧНИ ИЗСЛЕДВАНИЯ ВЪРХУ РАСТИТЕЛЕН ВОСЪК ОТ ДИНЯ

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(Резюме)

В настоящата работа е изследван растителен восък, изолиран от кутикулата на диня (*Citrullus lanatus*), американски сорт *Crimson Sweet*. Изолираният растителен восък е изследван посредством инфрачервена спектроскопия (ИЧ), рентгенова дифракция (XRD), диференциално термичен анализ (DTA) и сканираща електронна микроскопия (SEM). Получените резултати за състава, структурата и термичната стабилност на изследвания растителен восък са необходими за дефиниране на точния химичен състав, свойства и качество на ядливи филми, съдържащи като хидрофобна компонента изучавания восък.

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The effect of extrusion variables on the color of apple pomace - wheat semolina extrudates

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Apple pomace - wheat semolina blends were extruded in a laboratory single screw extruder (Brabender 20 DN, Germany) with screw diameter 19 mm and die diameter 5 mm. Effects of feed composition, moisture content, screw speed, and barrel temperature on color of the extruded products were studied. Response surface methodology with combinations of feed composition (10, 30, 50, 70, 90%), moisture content (17, 20, 23, 26, 29%), screw speed (120, 150, 180, 210, 240 rpm), and barrel temperature (130, 140, 150, 160, 170°C) was applied. Feed screw speed was fixed at 70 rpm. The compression ratio of the screw was 3:1. The temperatures of the feed and II-nd zone were 150 and 160ºC, respectively. The color changes of apple pomace - wheat semolina blends during extrusion were measured in CIE Lab color system using a colorimeter Colorgard 2000 (BYK – Gardner Inc., USA). The total color differences between the extruded and non-extruded samples were expressed by ΔE. The average ΔE values ranged from 6.52 to 10.94. Statistical analysis showed that feed composition, feed moisture content, and barrel temperature had an effect on the total color differences ($P<0.05$) whereas the screw speed had no effect on the color.

***Keywords*:** color, extrusion, apple pomace

INTRODUCTION

In many areas of the food industry, extrusion is an important manufacturing method. The processing conditions used in extrusion cooking - high temperature, pressure, and low moisture content of the feed - often give rise to a colored product or even a change in the color of the raw feed even though the residence time is low. It is logical to assume that the conditions of extrusion processing directly affect the color of the product although post-extrusion treatment also has a role to play [1-3]. Harper [4] mentioned that fading of color components is a common occurrence in extruded foods.

Color is perceived three dimensionally, based on responses of three different receptors (red, green, and blue) in the human eye [5]. The Judd–Hunter L, a, b and CIE Lab L^*, a^*, b^* are alternative color scales used to measure the degree of lightness (L), the degree of redness (+a) or greenness (-a), and the degree of yellowness (+b) or blueness (-b), with the CIE Lab scale being most commonly used for the evaluation of color in foods [6]. Conversion of a^* and b^* readings to hue and chroma values gives results more closely associated with human perception [7].

The aim of this investigation was to study the effect of extrusion variables on the color of extruded apple pomace - wheat semolina blends.

EXPERIMENTAL

Materials

Apple pomace is a by-product obtained during juice processing. Commercial apples (Granny Smith variety) are refrigerated and stored until the juice processing. The apple pomaces are dried a laboratory heat dryer at 60°C. The dried pomaces were ground using a hammer mill then mixed with commercial wheat semolina and distilled water to be obtained the desired ratios (Table 1). The prepared wet samples were placed and kept in sealed plastic bags for 12 h in a refrigerator at 5°C. The samples were tempered for 2 h at room temperature prior to extrusion.

Extrusion

The samples were extruded in a laboratory single screw extruder (Brabender 20 DN, Germany). The extruder barrel (476.5 mm in length and 20 mm in diameter) contained three sections and independently controlled die assembly electric heaters. The feed screw speed was fixed at 70 rpm. The screw speed was 120, 150, 180, 210, 240 rpm according to the experimental design (Table 1). The compression ratio of the screw was 3:1. The temperatures of the feed and kneading zone were 150 and 160ºC, respectively. The temperature of

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Table 1. Independent variable values and corresponding levels

Independent variables	Levels				
	- 2	**- 1**	**0**	**+ 1**	**+ 2**
Apple pomace / wheat semolina (C_{pom}), % - X_1	10	30	50	70	90
Feed moisture content (W), % - X_2	17	20	23	26	29
Screw speed (n), rpm – X_3	120	150	180	210	240
Final cooking zone temperature (Tm), °C – X_4	130	140	150	160	170

Table 2. Color parameters (L^*, a^*, and b^* values) of non-extruded apple pomace – wheat semolina blends

Apple pomace content (%)	L^*	a^*	b^*
10	89.28	1.99	24.54
30	83.24	3.36	28.74
50	80.31	4.00	30.09
70	80.45	3.98	30.46
90	81.26	3.79	29.76

the final cooking zone was 130, 140, 150, 160, 170°C. The die diameter was 5 mm.

Total color difference (ΔE)

The extrudates were finely ground using a laboratory hammer mill. The color parameters determined for the raw blends (non-extruded) and extruded samples included L^*, a^* and b^* values (CIE Lab system) using a colorimeter Colorgard 2000, BYK – Gardner Inc., USA. Total color difference (ΔE) was calculated applying the equation

$$\Delta E = \sqrt{(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2} \quad (1)$$

where L, a, and b are the values for the extruded samples; L_o, a_o, and b_o are the values for the raw blends (Table 2).

The color parameters are the mean values of ten observations.

Experimental design and data analysis

The effect of feed composition (apple pomace/wheat semolina) - X_1, feed moisture content - X_2, screw speed – X_3, and temperature of final cooking zone – X_4 on color (response, y) of the extruded products was investigated using response surface methodology (Table 1). A central composite rotatable design was used: $2k+2.k+n_o$, where k is the number of the independent variables, n_o the replicates of the center point (n_o=5).

A regression model is the following:

$$y = b_0 + \sum_{i=1}^{n} b_i . x_i + \sum_{i=1}^{n} b_{ii} . x_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} . x_i . x_j \quad (2)$$

where b_0, b_i, b_{ii}, and b_{ij} are constant coefficients.

SYSTAT statistical software (SPSS Inc., Chicago, USA, version 7.1) and Excel were used to analyze the data results.

RESULTS AND DISCUSSION

The total color differences between the extruded and non-extruded samples expressed by ΔE are given in Table 3. The extrudates were darker in color compared to their raw blends. L^* values of the extruded samples (from 72.06 to 83.03) were lower than Lo values of the raw blends (from 80.31 to 89.28). This may be due to the formation of brown pigments through non-enzymatic reactions that occur during the product processing.

Our results show that the total color difference of the extruded apple pomace - wheat semolina blends increases from 8.24 to 10.41 (L^* value decreases from 77.14 to 72.11) with raising the feed moisture content from 17 to 29% at apple pomace content 50%, temperature of final cooking zone 150ºC, and screw speed 180 rpm. Gujska and Khan[8] have extruded high starch fractions of navy, pinto, and garbanzo beans with different feed moisture contents. They have reported that increasing moisture content resulted in decreased L^* values of the extruded beans.

L^* value of the extruded apple pomace - wheat semolina blends increases from 75.61 to 82.96 with raising the screw speed from 120 to 240 rpm at apple pomace content 50%, feed moisture content 23%, and temperature of final cooking zone 150ºC. Similar finding was reported by Kannadhason et al. [9].

The results of the statistical analysis of variance (ANOVA) for the color show that 6 effects have P-values less than 0.05 indicating that they are significantly different from zero at the 95.0% confidence level. The R-squared statistic is 0.80; the standard error of the estimate - 0.89, the mean absolute error - 0.45. The regression equation describing the effect of extrusion variables on the total color difference (ΔE) of extruded apple pomace - wheat semolina blends is given in Table 4. The coefficients in the regression equation can be

used to examine the significance of each term relative to each other when used with coded values. Statistical analysis showed that feed composition, feed moisture content, and temperature of final cooking zone had an effect on the total color differences ($P<0.05$), whereas screw speed had no effect on the color. Each of the estimated effects and interactions are shown in the standardized diagram (Figure 1). The linear effect due to the feed composition of the apple pomace - wheat semolina blend had mostly influence on the total color difference followed by squared and linear effects due to the feed moisture content.

The effect of changes in feed moisture content and feed composition on the total color differences of the samples is given in Figure 2. ΔE values increased with an increase in moisture content and apple pomace content in the blends.

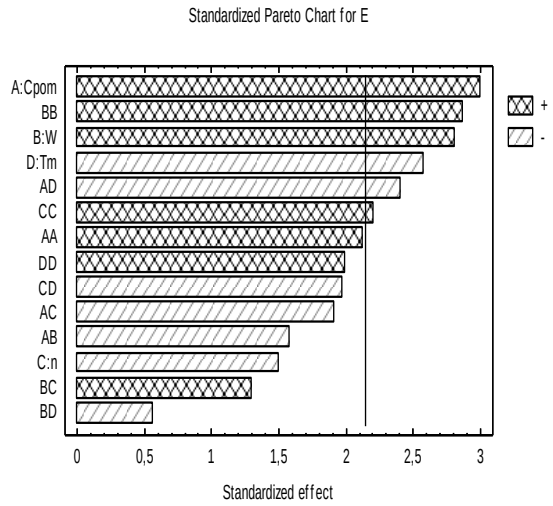

Fig. 1. Estimated effects of regression model coefficients on the total color differences.

Table 3. Total color differences of extruded apple pomace – wheat semolina blends

№	L^*	a^*	b^*	ΔE
1.	81.02	3.47	22.54	6.59
2.	72.40	4.66	23.91	10.40
3.	78.18	3.94	23.67	7.19
4.	73.67	5.41	22.07	10.88
5.	79.46	3.94	22.86	7.01
6.	73.34	4.48	24.03	9.60
7.	75.34	4.35	23.41	9.58
8.	72.06	5.97	23.73	10.94
9.	83.03	3.36	22.22	6.52
10.	73.91	4.84	22.53	10.31
11.	76.53	4.20	22.70	9.07
12.	78.77	3.72	22.30	8.34
13.	79.08	4.04	23.38	6.82
14.	77.38	4.48	24.64	6.60
15.	80.57	3.53	21.30	7.91
16.	77.07	4.12	23.31	7.91
17.	80.66	4.00	22.45	9.09
18.	76.84	5.14	22.61	8.51
19.	77.14	4.57	22.51	8.24
20.	72.11	5.19	23.79	10.41
21.	75.61	4.01	21.52	9.77
22.	82.96	3.92	22.59	7.95
23.	73.02	5.62	24.46	9.35
24.	82.54	4.22	22.35	8.06
25.	81.98	4.00	22.49	7.18
26.	80.53	4.53	23.12	6.99
27.	80.18	3.76	23.28	6.79
28.	80.06	3.46	22.89	7.23
29.	80.13	3.88	23.19	6.88

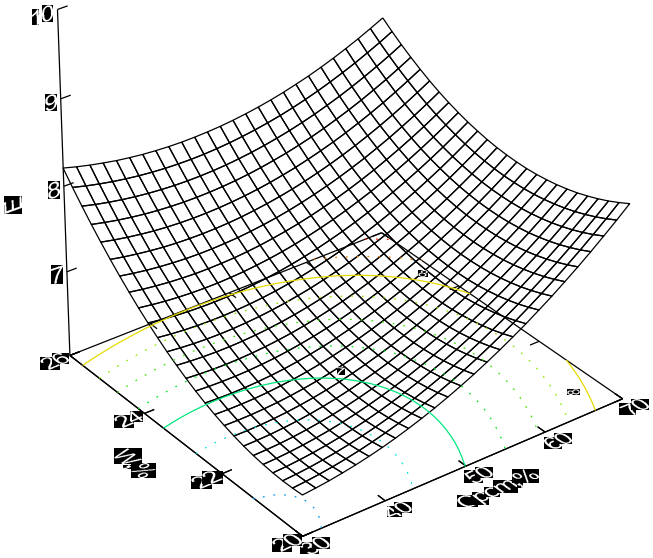

Fig. 2. Effect of feed moisture and feed composition on the total color difference (ΔE) of extruded apple pomace - wheat semolina blends.

Table 4. Regression equation coefficients for total color differences of extruded apple pomace – wheat semolina blends in terms of coded variables (correlation coefficient, $R^2 = 0.80$)

Variables	Coefficients
Constant	+60.6185
X_1	+0.6012*
X_2	- 2.0634*
X_3	+0.0181
X_4	- 0.5978*
X_1X_1	+0.0009
X_2X_2	+0.0560*
X_3X_3	+0.0004*
X_4X_4	+0.0035
X_1X_2	- 0.0059
X_1X_3	- 0.0007
X_1X_4	- 0.0027*
X_2X_3	+0.0032
X_2X_4	- 0.0042
X_3X_4	- 0.0015

X_1 - feed composition (%), X_2 - feed moisture (%), X_3 - screw speed (rpm), X_4 - barrel temperature (°C).
*Significant at 95% CI.

CONCLUSION

The effect of extrusion variables on the color of apple pomace - wheat semolina extrudates was studied. The color changes of apple pomace - wheat semolina blends during extrusion were measured in CIE Lab color system using a colorimeter Colorgard 2000 (BYK – Gardner Inc., USA). The total color differences between the extruded and non-extruded samples were expressed by ΔE. The average ΔE values ranged from 6.52 to 10.94. Statistical analysis showed that feed composition, feed moisture content, and temperature of final cooking zone had an effect on the total color differences ($P<0.05$), whereas screw speed had no effect on the color.

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ВЛИЯНИЕ НА НЯКОИ ПАРАМЕТРИ НА ЕКСТРУДИРАНЕ ВЪРХУ ЦВЕТА НА ЕКСТРУДАТИ ОТ ЯБЪЛКОВИ ПРЕСОВКИ И ПШЕНИЧЕН ГРИС

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(Резюме)

Смеси от ябълкови пресовки и пшеничен грис са екструдирани на едношнеков лабораторен екструдер (Brabender 20 DN, Германия) с диаметър на шнека 19 mm и диаметър на дюзата на матрицата 5 mm. Изследвано е влиянието на съдържанието на ябълкови пресовки, влажността, честотата на въртене на шнека и температурата на матрицата върху цвета на екструдираните продукти. Приложен е метода на повърхността на отражението със следните комбинации: съдържание на ябълкови пресовки (10, 30, 50, 70, 90%), влажност (17, 20, 23, 26, 29%), честота на въртене на шнека (120, 150, 180, 210, 240 min^{-1}) и температура на матрицата (130, 140, 150, 160, 170°C). Честотата на въртене на дозиращия шнек се фиксира на 70 min^{-1}. Степента на компресия на шнека е 3:1. Температурите на първа и втора зона са фиксирани съответно на 150 и 160°C. Цветът на екструдатите е измерен с колориметър Colorgard 2000, BYK – Gardner Inc., USA. Цветовите разлики между екструдираните и неекструдираните проби са изразени от ΔE. Средните стойности на ΔE варират от 6,52 до 10,94. Статистическият анализ показва, че съдържанието на ябълкови пресовки, влажността и температурата на матрицата оказват влияние върху цветовите разлики ($P < 0.05$), докато честотата на въртене на шнека не влияе върху цвета.

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Oxidative stability and stabilization of grape seed oil

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Oxidative stability of vegetable oil recovered from grape seeds was investigated. The Induction period (IP) of the oil determined by Rancimat method at 100ºC was found to be 7 h. Different natural antioxidant mixtures and individual pure compounds were examined for stabilization of the oil. The use of vegetable extracts, in concentration 0.3% has insignificant effect on the stabilization (1.1 - 1.3 times). The addition of some individual pure antioxidants in concentration 0.05% has a better effect. The best results were established using butyl gallate – Induction period was 26.2 h and rosemary – Ip was 18.7 h. The oxidative stability increased gradually with the increasing of concentration of butyl gallate and was highest at 0.2%- Induction period was found to be 42.1 h.

Key words: Grape seed oil, oxidative stability, stabilization, antioxidants

INTRODUCTION

The fruits of grape (Vitis vinifera L.) find application as food for direct consumption, as well as source for the production of wine. Grape seeds are waste product after separation of the wine and have recently been utilized for obtaining of glyceride oil [1-3]. The grape seed oil presents interest as functional food with therapeutical effect because it contains a high quantity of polyunsaturated fatty acids in particular linoleic acid [4]. Moreover glyceride oil is used as salad oil and for preparing cosmetic products. Besides triacylglycerols, the glyceride oil contains micro components valuable biologically active substances as tocopherols, phytosterols, carotenoids which play a significant role for the estimation of food value and increasing oxidative stability [5]. On the other hand, the presence of high content of unsaturated fatty acids makes the oil very unstable towards oxidation, the term of storage decreases and the oil deteriorated of food value.

Lipid oxidation of grape seed oil is an important problem and leads to a decrease of quality, safety and nutritional value. For stabilization and prolongation of the term for conservation of the oil many different antioxidants in food chemistry are used for stabilization and prolongation safety of the oil. Natural antioxidants come from plant leafs; stems or seeds are in high demand for food application because of their safety compared to synthetic antioxidants [6, 7].

In Bulgaria, there are has also significant quantities of grape seeds as waste products which are used for obtaining of glyceride oil. So far, the composition and oxidative stability of the oil have not been investigated. In this connection the aim of this work is to investigate the composition of the oil, its oxidative stability and possibilities for stabilization of the oil by different natural and synthetic antioxidants for prolongation of the term for preservation of its food value.

EXPERIMENTAL

All solvents and reagents were with analytical grade of purity and were used without additional purification. Reference phospholipids and fatty acid methyl esters were purchased from Fluka (Chemie Gmbh, Switzerland). Reference tocopherols isomers and individual sterols were purchased from Merck (Darmstadt, Germany).

Sample: Grape seed oil. The oil was purchased from a local market and was used directly for the investigation.

Determination of bioactive compounds

*Phospholipids.*The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 by volume. Etalon – 10 μl/cm^3 water solution of KH_2PO_4 as phosphorus [8].

Sterols. The oil was hydrolized with ethanolic KOH [9], sterols were extracted with light

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petroleum ether and purified by thin layer chromatography.

Total sterol content was determined spectrophotometrically [10] at 597 nm.

Tocopherols. Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) [11] on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50 – 5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000.

*Fatty acids.*The fatty acid composition was determined by gas chromatography after transmethylation of the respective sample with 2% methanolic H_2SO_4 at 50°C according to Christie [9]. GC was performed on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 60 m x 0.25 mm (I.D.) capillary DB – 23 column (Hewlett Packard GmbH, Austria) and a FID [12].

Antioxidants. Antioxidants were purchased from Fluka (Chemie Gmbh, Switzerland): butyl gallate, Merck (Darmstadt, Germany) - α-tocopherol, oxynex, Hofman-La Roshe (Switzerland) – ascorbylpalmitat; Koch-Light Laboratories Ltd (Germany) – quercetin and rosemary. The natural antioxidant mixtures are with undefined composition and were obtained by "Ikarov" Ltd.

Stabilization. To the samples (25 g grape seed oil) were added natural and synthetic antioxidants as pure compounds or as mixture of two compounds. The compositions of antioxidants were prepared as follows: The compounds were precisely weighed and solubilized in pure ethanol as 20 g/100 ml solution. Then the solution was added to the oil in order to obtain desired concentration of the antioxidants. The obtained solution was vigorously mixed and than the ethanol was removed by flush with nitrogen. Then the oil was stored in dark bottles at 20°C.

Oxidative stability. The stability of the oil was examined by measurement the change of Induction period using conductometric detection of volatile products of oil degradation-Rancimat method [5]. The oxidative test was used Rancimat apparatus Methrom 679 (Methrom, Herisau, Switzerland). Three milliliters of each sample were weighted into reaction vessel glassware. The heat temperature was set a 100°C; the rate of air flow through the sample was about 20 l/h; the volume of bidistilled water into the trap was 60 ml. All determinations of the oxidative stability were performed in three replicates.

Antioxidative effect (AOE) was calculated by the formula:

$$AOE = \frac{\text{Introducti on period (IP) with additive}}{\text{IP without additive}}.$$

*Statistics.*All data are presented as a mean value of three separate measurements ± standard deviation (SD, at P = 0.05).

RESULTS AND DISCUSSION

General characteristics of the oil were determined, such as: content of total phospholipids, sterols and tocopherols and fatty acid composition. The results are shown in Table 1.

Table 1. Content of bioactive compounds in grape seed oil.

Compounds	Content
Sterols, %	0.2 ± 0.06
Phospholipids, %	1.5 ± 0.6
Tocopherols, mg/kg	46 ± 0.9
Fatty acids, %	
$C_{14:0}$ Myristic	0.1 ± 0.2
$C_{16:0}$ Palmitic	9.4 ± 3.8
$C_{16:1}$ Palmitoleic	0.1 ± 0.5
$C_{17:0}$ Margaric	0.1 ± 0.5
$C_{18:0}$ Stearic	3.7 ± 1.5
$C_{18:1}$ Oleic	17.9 ± 3.6
$C_{18:2}$ Linoleic	67.9 ± 13.6

In comparison with other vegetable oils as sunflower, olive, rapeseed [13], grape seed oil is characterized by a low content of sterols and tocopherols but the quantity of phospholipids is relatively high. Linoleic acid predominates in the triacylglycerols followed by oleic acid. This composition of grape seed oil is close to data reported earlier by other researchers [3, 6, 14-17].

Natural antioxidants and synthetic analogs of natural antioxidants were used for stabilization of the oils. They have some advantages in comparison with synthetic antioxidants as follows: readily accepted by consumers; they are safe additives with nutraceutical value [18].

Antioxidant activity of different natural plant antioxidant mixtures in concentration 0.3% was examined for stabilization of grape seed oil. The results are presented in Table 2.

Table 2. Antioxidant activity of natural plant antioxidant mixtures.

Antioxidant mixture	Induction period, h	Antioxidative effect, times
1. Control sample	7.0 ± 0.3	-
2. Comomile	8.3 ± 0.3	1.2
3. Nettle	7.0 ± 0.1	1.0
4. Marigold	7.0 ± 0.1	1.0
5. Yellow tutsan	5.7 ± 0.2	0.8
6. White milfoil	7.0 ± 0.2	1.0
7. Unsaponifiable of grape seed oil (extract of resveratrol)	8.1 ± 0.3	1.2

The oxidative stability of investigated grape seed oil was found to be higher than values reported earlier (4.8 h), but significantly lower in comparison with other vegetable oils as olive (22.0 h), corn (11.0 h) [19]. These data are result of different content of unsaturated fatty acids and respectively tocopherols and sterols – the main antioxidant and synergist in the oil.

Rancimat test showed the insignificant increasing of the stability (0 – 20%) regardless of relatively high concentration of the added antioxidant mixtures.

The effect of the individual pure natural and synthetic antioxidants put in oil in concentration (0.05%) is presented in Table 3.

The highest extension of the stability was established using butyl gallate. Induction period as index for stability was found to be 26.2 h. The other antioxidants increase the stability significant by less (from 7.0 h for control sample to 9.9 – 18.7 h (1.4 – 2.7 times). In this connection the next investigations were performed by butyl gallate only. Since as salad oil grape seed oil is used as a component for manufacturing of cosmetic products where it is possible to put a large quantity of antioxidants (about 0.2%), the investigation was carried out with concentrations of butyl gallate 0.02 – 0.2%.

Table 3. Antioxidant activity of individual pure natural and synthetic antioxidant mixtures.

Antioxidant mixtures	Induction period, h	Antioxidative effect, times
1. Control sample	7.0 ± 0.3	-
2. β-carotene + α-tocopherol	9.9 ± 0.2	1.4
3. Oxinex	11.1 ± 0.4	1.6
4. Rosemary	18.7 ± 0.4	2.7
5. Quercetine	14.1 ± 0.3	2.0
6. Ascorbyl palmitat	11.3 ± 0.5	1.6
7. Butyl gallate	26.2 ± 0.5	3.7

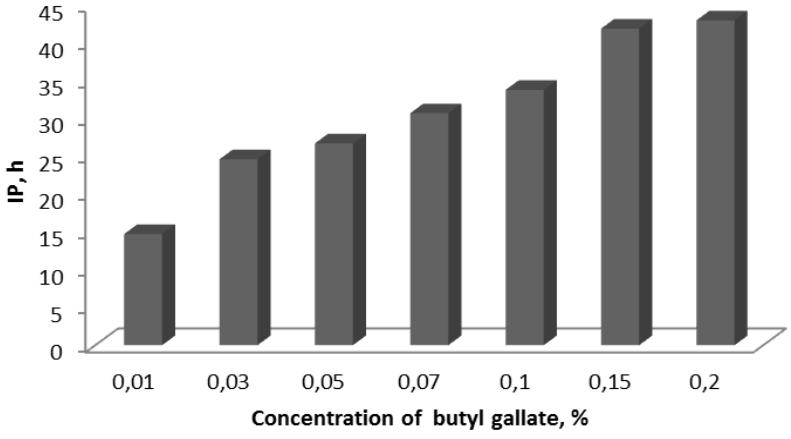

Fig. 1. Antioxidant activity of butyl gallate with different concentrations.

The influence of concentration of butyl gallate over oxidant stability of the oil is presented in Fig. 1.

It was observed good correlation between concentration of the added butyl gallate and increasing of the Rancimat test Induction time. The oxidative stability increased gradually and was highest at 0.2% - Induction period was found to be 43.0 h.

Grape seed oil has very low oxidative stability as a result of the high content of unsaturated fatty acids mainly linoleic. The uses of natural plant antioxidant mixtures do not increase significantly stability of the oil. The best results were obtained using butyl gallate. Quantities about 0.05% added into oil for food purposes increase Induction period more than 2 times. When grape seed oil is used in cosmetics, 0.2% butyl gallate can be put in the oil and Induction period was 6 times more than that of unstabilized oil.

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ОКСИДАНТНА СТАБИЛНОСТ И СТАБИЛИЗИРАНЕ НА ГРОЗДОВО МАСЛО

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(Резюме)

Изследвана е оксидантната стабилност на масло, получено от гроздови семена. Индукционният период, определен чрез метода на Rancimat при 100^0C, е 7 часа. За стабилизирането на маслото са използвани различни природни екстракти и индивидуално чисти антиоксиданти. Употребата на растителни екстракти в концентрация 0,3% има незначителен ефект върху стабилизирането (1,1-1,3 пъти). По-добър ефект показаха някои индивидуално чисти антиоксиданти в концентрация 0,05%. Най-добри резултати са постигнати при употребата на бутил галат, при който индукционният период е 26,2 часа и екстракт от розмарин с индукционен период 18,7 часа. Установено е, че оксидантната стабилност нараства значително с увеличаването на концентрацията на бутил галат, като най-добри резултати се постигат при концентрация 0,2%, при която индукционният период е 42,1 часа.

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Comparative characteristics of sunflower oil with supplement of traditional Bulgarian herbs

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Sunflower oil produced by Pearl Ltd Veliko Tarnovo, with addition of Bulgarian herbs (oregano, thyme and pine cones) has been studied. The starting sunflower oil is a linoleic type containing predominantly linoleic acid ($C_{18:2}$ = 521 g.kg^{-1}), followed by oleic ($C_{18:1}$ = 344 g.kg^{-1}) and palmitic ($C_{16:0}$ = 115 g.kg^{-1}) acid. Upon the examination of fatty acid composition of sunflower oil with various herbs supplements an increase of the oleic acid from 344 g.kg^{-1} in the control to 422 ÷ 441 g.kg^{-1} in the extracts has been found. Oleic / linoleic acid ratio varies between 0.95 and 1.10 in the extracts, whereas in pure sunflower oil it is about 0.66. This ratio indicates a better balanced composition in terms of the nutritional value of the tested samples. Adding herbs to the oil reduces the content of tocopherols (from 721 mg.kg^{-1} to 388-459 mg.kg^{-1}), which has an impact on its oxidative stability. Adding pine cones to sunflower oil reduces its oxidative stability about 3 times (from 10.8 h to 3.5 h), while the addition of oregano and thyme to the oil leads to minor change in oxidative stability (from 10.8 h to 7.6h). Therefore, sunflower oil with addition of herbs is inappropriate for heat treatment, but can be used for sauces, dressings, mayonnaise, and creams with exception of the sample with addition of the pine cones. Color parameters of oils in SIELab colorimetric system have been studied. It was found that the addition of oregano and thyme does not influence significantly the brightness of the samples and leads to an increase in their green components, which is associated with an increase in chlorophyll content from 0.003 ppm for pure sunflower oil to 0.094 ppm - 0.117 ppm for samples with oregano and thyme. The addition of both recent herbs result in double increase the content of β- carotene, respectively from 2.76 ppm for the control to 4.92÷ 5.77 ppm for the oil samples with oregano and thyme.

Keywords: thyme, oregano, pine cones, fatty acid composition, tocopherols, oxidative stability, color

List of abbreviation:
TLC-Thin-layer chromatography;
FAME –Fatty acid methyl esters;
GC-Gas chromatography;
HPLC- High performance liquid chromatography;
FA - Fatty acids;
IP-Induction period;
1 – Oil extract of oregano; 2 - Oil extract of pine cones; 3 - Oil extract of thyme ; 4 -Sunflower oil

INTRODUCTION

Sunflower oil is lipid product typical for Bulgaria with large application in cookery and food industry. Its consumer qualities depend mainly on its fatty acid composition, the content of tocopherols (vitamin E), and of the possibilities to be stable during long term storage and thermal treatment. The main component of triacylglycerol fraction in conventional sunflower varieties is linoleic acid - 500 – 800 g.kg^{-1}, which belongs to the essential fatty acids that are vital for the human body. Due to its unsaturated nature, it is easily amenable to oxidation processes under the influence of light and oxygen from the air, a result of which the sunflower oil relatively quickly loses its consumer properties. Numerous attempts to increase its oxidative stability by the addition thereto of various natural and synthetic antioxidants have been made.

Bulgaria is reach in great variety of herbs that contain a high percentage of biologically active substances. They are rich in various compounds: alkaloids, glycosides, saponins, polysaccharides, tannins, flavonoids, lignans, coumarins, essential oils, vitamins, trace elements etc. In this regard, it is interesting to carried out investigations on the composition and stability of sunflower oil when adding thereto of various kinds of herbs Bulgar.

The main objective of presented study is the examination of fatty acid and tocopherol

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composition, oxidative stability and color parameters of oil extracts from Bulgarian herbs (oregano, thyme and pine cones) with a view to their application in salads, sauces and other food products.

EXPERIMENTAL

Sunflower oil, production of Pearl Ltd Veliko Tarnovo, is used for conducting surveys. The oil extracts were prepared in a ratio of 1:5 (herb / sunflower oil), and were kept under refrigerated conditions (0°C-4°C) for 6 months.

Analysis of fatty acids. The fatty acid composition of oils was determined by gas chromatography (GC) after transmethylation of the respective sample with 20 $g.kg^{-1}$ H_2SO_4 in absolute CH_3OH at 50°C[1]. FAME were purified by TLC on 20x20 cm plates covered with 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with mobile phase n-hexane:diethyl ether (97:3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 60 m x 0.25 mm x 25 μm capillary DB - 23 column (Agilent J&W advanced, Agilent Technology, USA) and a flame ionization detector. The column temperature was programmed from 130 °C (1 min), at 6.5 °C/min to 170°C, at 3.0 °C/min to 215°C (9 min), at 40°C/min to 230°C (1 min); injector and detector temperatures were kept at 270 °C and 280 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL/min; split was 1:50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions[2].

Analysis of tocopherols. Tocopherols were determined directly in the oil by HPLC on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000. The operating conditions were as follows: mobile phase of n-hexane: dioxan 96:4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, and emission 330 nm[3] 20 μL 10 $g.kg^{-1}$ solution of oil in hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. The tocopherol content was calculated on the basis of tocopherol peak areas in the sample versus tocopherol peak area of standard α-tocopherol solution.

Oxidative stability. Oxidative stability of oils was determined by measuring of IP, using conductometric detection of volatile acids. Rancimat apparatus Methrom 679 (Methrom, Herisau, Switzerland) was used at 100°C and an air flow rate 20 l/h[4].

***Color parameters*:** SIELab coordinates have been measured directly with spectrophotometer (Trintometer Lovibond PFX 195, UK). In mentioned colorimetric system L* is the brightness and it takes values from 0 (black) to 100 (white), a* is red-green axis, and b* is yellow-green axis[5]. The content of β-carotene and chlorophyll is defined using special software.

RESULTS AND DISCUSSION

Fatty acid composition of the studied oil extracts is listed in Table 1. Fatty acid composition is one of the main indicators characterizing the nutritional value of the oils and their oxidative stability during storage and heat treatment. The used sunflower oil has predominantly linoleic acid content (521$g.kg^{-1}$), followed by oleic (344$g.kg^{-1}$) and palmitic (115$g.kg^{-1}$), which associates it with the oils of linoleic acid in the range of, where the linoleic acid[6] quantity varies in range 480-740$g.kg^{-1}$, and the oleic one – 140-390$g.kg^{-1}$. It does not differ significantly from previously studied linoleic type oils in Bulgaria, and its composition is closest to that already obtained from Bulgarian sunflower variety "Musala"[6]. The ratio oleic/linoleic acid for oil extracts varies between 0.95 and 1.10, while for control sample it is around 0.66. Last ratio evidences better balance of fatty acid content, which is determining for the nutritive value of the tested samples. Comparatively high content of linoleic acid indicates good nutritive value of oil extracts, because this acid belongs to human body essential fatty acids. The content of oleic acid in oil extracts is higher with 80 – 100$g.kg^{-1}$ compared to the control.

Data about total tocopherol content and composition of oils are presented in Table 2.

Control sample has the highest content of tocopherols (721 $mg.kg^{-1}$), while in the oil extracts its content decreases (388 - 459 $mg.kg^{-1}$**)**. In the oil extracts mainly saturated derivates α- and β - tocopherols are identified. Basic representative of tocopherols in these oil extracts is α- tocopherol (945 – 961$g.kg^{-1}$).

The results from the investigation of oxidative stability of oil extracts are presented on Figure 1.

Table 1. Fatty acid composition of oil extracts with Bulgarian herbs

FA, g.kg^{-1}		1	2	3	4
C 12:0	lauric	3	4	3	2
C 14:0	myristic	3	2	2	2
C 16:0	palmitic	138	118	132	115
C 16:1	palmitoleic	2	1	2	2
C 17:0	margaric	1	1	1	1
C 18:0	stearic	6	6	8	10
C 18:1	oleic	441	422	435	344
C 18:2	linoleic	402	442	410	521
C 20:0	arachidic	2	2	3	1
C 20:1	gadoleic	1	1	3	1
C 22:0	behenic	1	1	1	1
saturated FA		**154**	**134**	**150**	**132**
unsaturated FA		**846**	**866**	**850**	**868**
monounsaturated FA		**444**	**424**	**440**	**347**
polyunsaturated FA		**402**	**442**	**410**	**521**

Table 2. Total tocopherol content and composition oil extracts with Bulgarian herbs

Tocopherols	*1*	*2*	*3*	*4*
α - tocopherol, g.kg^{-1}	946	961	961	945
β - tocopherol, g.kg^{-1}	54	39	39	55
Total tocopherol content, mg.kg^{-1}	458	388	459	721

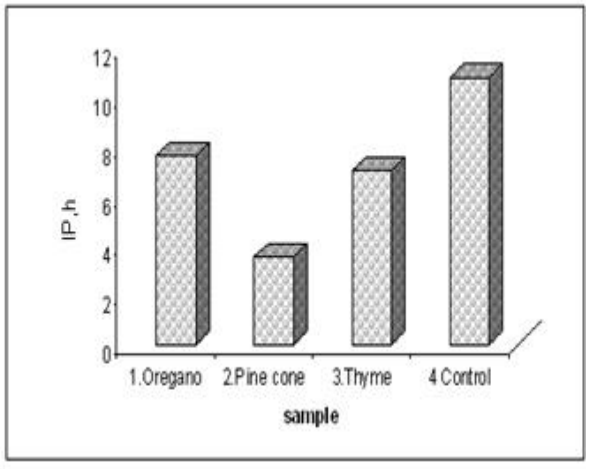

Fig 1. Oxidative stability of oil extracts

Oil extracts of oregano and thyme have lower oxidative stability (with about 3 hours) compared to the control, while the stability of the extract of pine cones is about three times lower than this of the control sample. Therefore, the last extract is not suitable for flavoring sunflower oil, as it leads to rapid oxidation and the oil is unsuitable for use in salads, sauces and more culinary products. Sunflower oil with added oregano and thyme is unsuitable for heat treatment, but due to its well-balanced nutritional value is useful for consumption in salads and sauces. The oil with oregano and thyme oxidative stability is comparable with that of cold-pressed walnut oil, which is used as a delicacy oil dressings for salads, sauces and meals[7].

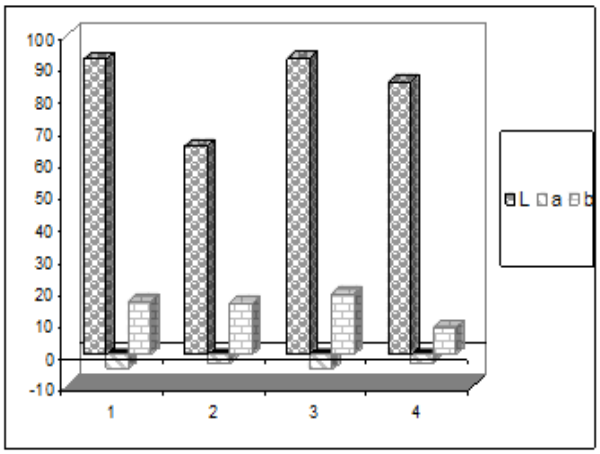

Fig. 2. Basic color coordinates of oil extracts from Bulgarian herbs in SIELab colorimetric system

The data for color parameters in SIELab colorimetric system allows calculating the color difference (ΔE) with respect the basic sample (sunflower oil). The biggest color difference is obtained for the extract of pine cones (ΔE = 21.6), while the color differences for those of oregano and thyme with control samples are compatible, being

respectively 10.9 and 13.0. The brightness of the extract of pine cone (65.14) has highest difference with the one of control sample (85.30). For the rest of the samples the change is not significant. Basic color coordinates are presented on Figure 2.

Oil extracts with oregano and thyme have high content of β-carotene and chlorophyll. The extract of pine cone also demonstrates essential rise of these parameters, but it worsens the gustatory qualities of the sunflower oil and nevertheless it enriches the oil, it makes the oil inapplicable for using in salads, sauces and other food products. The result is shown on Figure 3.

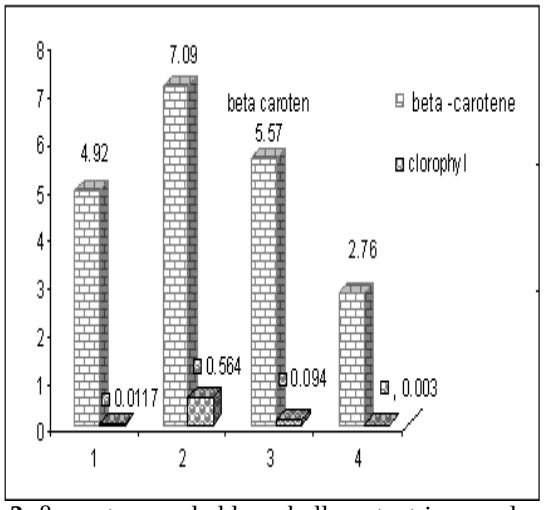

Fig. 3. β-carotene and chlorophyll content in samples of sunflower oil with addition of herbs.

CONCLUSION

Comparatively high content of linoleic acid and tocopherols in studied oil extracts shows their balanced nutritional value. Bulgarian herbs (oregano, thyme and pine corns) added to sunflower oil have prooxidative effect and the obtained oil extracts are inconvenient for thermal treatment. Due to the higher ratio oleic/linoleic acid the oil extracts of oregano and thyme can be used as delicacy dressings for salads, sauces and meals. Oil extracts of oregano and thyme have unchanged brightness and increased contents of chlorophyll and β - carotene, while the extract of pine corn has decreased brightness and increased color difference to the control.

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СРАВНИТЕЛНИ ХАРАКТЕРИСТИКИ НА СЛЪНЧОГЛЕДОВО ОЛИО С ДОБАВКА НА БЪЛГАРСКИ БИЛКИ

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(Резюме)

Слънчогледово олио с добавка на Български билки (риган, мащерка и шишарка),произведено от Pearl Ltd Veliko Tarnovo е изследвано. Посоченото олио е линоленов тип с доминиращо съдържание линоленова киселина ($C_{18:\ 2}$ = 521 $g.kg^{-1}$), следвана от олеинова ($C_{18:\ 1}$ = 344 $g.kg^{-1}$) и палмитинова ($C_{18:\ 2}$ = 521 $g.kg^{-1}$), киселини.Отношението на олеинова към линоленова варира между 0.95 и 1.10 в екстрактите, докато в чистото слънчогледово олио е около 0.66. Това отношение показва добре балансиран състав откъм хранителна стойност в тестваните образци. Добавянето на билки в слънчогледовото олио намалява съдържанието на токофероли (от 721 $mg.kg^{-1}$ до 388-459 $mg.kg^{-1}$), което влияе върху оксидантната стабилност. Добавянето на шишарка в слънчогледовото олио намалява оксидантната стабилност 3 пъти (от 10.8 часа до 3.5 часа), докато добавянето на риган и мащерка водят до минимална промяна в оксидантната стабилност (от 10.8 до 7.6 часа). Следователно слънчогледовото олио с добавка на билки е неподходящо за термична обработка, но може да бъде използвано за сосове, дресинги, майонези и други, с изключение на образеца с добавянето на шишарка.

Изучени са цветовите характеристики на олиото в SIELab колориметрична система. Добавянето на риган и мащерка не влияят на светлостта на образците и водят до нарастване на тяхната зелена компонента, което се свързва с нарастването на хлорофилното съдържание от 0.003 ppm за чисто слънчогледово олио до 0.094 ppm-0.117 ppm за образци с риган и мащерка. Добавката на посочените две билки води до двойно нарастване на β- carotene от 2.76 ppm до 4.92-5.77 ppm за образците с риган и мащерка.

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Scattering and fluorescence spectra of cow milk

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The purpose of this study is to investigate the influence of fat content on the scattering and fluorescence spectra of milk. The composition analyses (content of proteins, fat, carbohydrates and minerals, caloricity), are obtained by Ekomilk-M Milkana KAM 98-2A milk analyzer. For all studied samples we have found out well expressed fluorescence pikes around of 335 nm and around of 373 nm what may be attributed to the presence of aromatic amino acids, nucleic acids and tryptophan residues. One of the samples has shown weak peak around of 500 nm probably due to the riboflavin fluorescence. Plots of the ratio: fluorescence intensity toward scattering intensity as a function of the pumping wavelength, have shown certain maxima with different values for samples of different fat content and different producers. The experimental results suggest that fluorescence and scattering spectra of milk can be used for the identification of different producers of milk and for obtaining information about milk chemical composition.

Keywords: Food control, milk, optical scattering, fluorescence

INTRODUCTION

Production of milk and milk derivates directly depends on the raw milk quality which is defined by the European Committee for Standardization (CEN) in EN ISO 8420:2005 [1]. The large variety of milk based nutrition is rapidly increasing and, in the same time, its components consciously get replaced by improper constituents. For example, milk fat gets replaced by vegetable fat, milk proteins - by other kinds of proteins, and carbohydrates - by certain products improving the texture and test qualities. In accordance with that, rises the necessity to find out adequate methods and equipment for quality control of milk products.

The classical microbiological and chemical analysis of milk and its derivates gives objective, precise and comparable results. However, it requests highly qualified specialists and needs expansive consumables. On the other side, these kinds of analyses are time consuming, and, most often, they use destructive methods. The use of optical devices is an alternative based on different non-destructive physical principles. Food and agriculture industries use mainly optical instruments in the ultraviolet (185-210 nm) and near infrared range (750-2500 nm) where typical chemical groups present in nutrients (C-H, N-H and O-H) get absorbed. In this study we have combined in an experimental setup measurement of transmission, scattering and fluorescence spectra of milk in the optical range of 200-1100 nm.

During the last decades optical transmission and scattering in the Far Ultraviolet (FUV) and Middle Ultraviolet (MUV) range (185-210 nm) and in the Near Infrared (NIR) range (750-2500 nm) have been largely applied in cases where the typical C-H, N-H and O-H chemical groups contained in food products show absorption. Due to the high water absorption, Infrared (IR) range is not appropriate for studying samples with high content of an advantage of optical transmission and scattering in comparison with the others spectral methods is that there is no need for preliminary chemical treatment, dilution or components separation. This permits the use of the same samples for further analysis. Because of different spectral absorption of the main milk components (water, fat, proteins and sugar), they can be studied contemporaneously using Near Inftared Spectroscopy (NIRS) [2].

Milk fat is composed of about 96% triglycerides (1 molecule glycerin and three fatty acids), 2-3% of diglycerides, 1% of phospholipids, essential polyunsaturated fatty acids such as Linoleic acid (LA) and Linolenic acid, as well fat-soluble vitamins (A, B, D, E and K), cholesterol, carotenoids [3]. The other contained molecules are smaller than the wavelength in the visible and near infrared range. When the fat is removed from the milk, the main particles that scatter the shorter wavelength light are the casein micelles and this causes the milk bluish colour. Casein micelles are

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the biggest particles in the milk liquid phase. They represent protein molecules linked through calcium phosphate nanoparticles. Casein micelles have spherical form. The size distribution of casein micelles is very broad (20–250 nm in diameter) [4]. There are four different kinds of casein phosphoproteins (αS1, αS2, β, κ) taking almost 76-86% of all milk proteins [2,5]. The biggest part of the casein proteins are linked in micelles. Milk contains also some other kinds of proteins and enzymes, which have higher solubility and smaller sizes than the casein molecules.

Light scattering effects are significant due to the milk turbidity. Therefore, Mie scattering theory may be applied [6]. Thus, the scattering cross section $\sigma_g = \pi R^2$ (where R is the scattering particle radius) is maximal at smaller wavelength. That allows evaluating the protein molecules sizes according to the formula:

$$\lambda_p = 2\pi R, \quad (1)$$

where λ_p is the wavelength at the pick scattered intensity.

Another non-destructive technique giving additional information for milk composition is fluorescence. However, data interpretation of fluorescence spectra of milk is ambiguous due to absorption by other molecular groups [3]. During the manufacturing process of milk occur biochemical reactions, for example: Maillard reaction, riboflavin degradation, tryptophan modification. For this reason, we consider more appropriate the use of comparative studies by changing one only parameter of the sample at time.

EXPERIMENTAL

In this study we have combined in an experimental setup measurement of transmission, scattering and fluorescence spectra of milk [7,8]. Milk is turbid liquid with significant optical absorption what makes difficult the use of standard spectrophotometer cuvettes. For this reason we have opted for a fiber optic setup. The optical range of 200-1100 nm is covered by two light sources: a halogen and a deuterium lamp. Light is directed through the sample (milk drop) placed on an aluminum base via optical fiber. The transmitted light is conducted by another fiber to the spectrometer AvaSpec 2048 with resolution of 8 nm. The two fibers are fixed on the board at a distance of 200 μm, equal to the fiber core diameter. The scattered light is caught by a third fiber along the direction orthogonal to the transmitted light. In this case the fluorescence spectra, even less intensive because of the low incident light intensity, cannot be separated from the scattered spectra. For evaluation of the fluorescence phenomena we have used a number of narrow waveband light sources UVTOP deep ultraviolet LEDs by Roithner Lasertechnik GmbH [9] with the next wavelengths: 245 nm, 255 nm, 275 nm, 285 nm, 295 nm, 305 nm.

Here we report some experimental data on the influence of fat contents on the scattering and fluorescence spectra of milk. We have chosen three different kinds of commercial cow milk labeled as Sample1, Sample2 and Sample3, and from each of them - samples with of 0.1%, 1.5% and 3.0% fat content. The commercial marks are not named here with the aim to avoid conflict of interest with the producers even more, for our study is important to demonstrate that the fluorescence provoked in the chosen optical range is able to give useful information for distinguishing the present of fat independently of the milk origin. The composition analysis (content of proteins, fat, carbohydrates and minerals, caloricity), has been obtained by Ekomilk-M Milkana KAM 98-2A, Bulteh 2000 Ltd, Stara Zagora, Bulgaria. Some more important parameters are presented in Table1.

Table 1. Composition analysis of milk with different content of fat.

Samples	Milk fat %	Milk-solids-nonfat (MSNF) %	Protein %	Density kg/dm^3
1	0.1	8.02	3.00	1.0295
2	1.5	8.43	3.16	1.0298
3	3.0	7.93	3.00	1.0265

RESULTS AND DISCUSSION

Turbidimetry is based on measuring the loss of intensity of the transmitted light due to scattering from the suspended in the studied medium particles. In this sense, the fiber-optical setup described above can be used for evaluating the absorption properties of milk. In Figure 1a are presented transmission spectra of Samples1 with fat content respectively of 0.1%, 1.5% and 3.0%. In the optical range of 200-1100 nm and in Figure 1b are shown the corresponding absorption curves calculated in the ideal case of lack of scattering light. The well expressed absorption maxima may be explained by Mie scattering from spherical particles with sizes ~ 0.1λ where λ is the wavelength of the absorbed light.

Scattering particles sizes are evaluated by defining the wavelength at the peak of the

absorption intensity of Sample1 with 0.1% of fat (oiliness milk). The experimental value, obtained

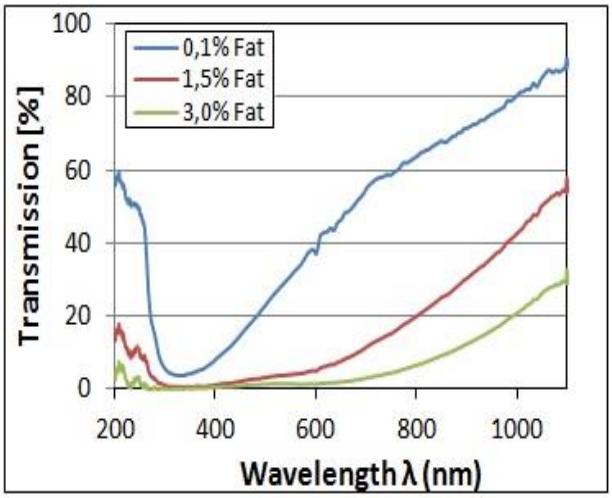

Fig. 1a. Transmission spectra of milk Samples 1 with 0.1%, 1.5% and 3.0% fat content.

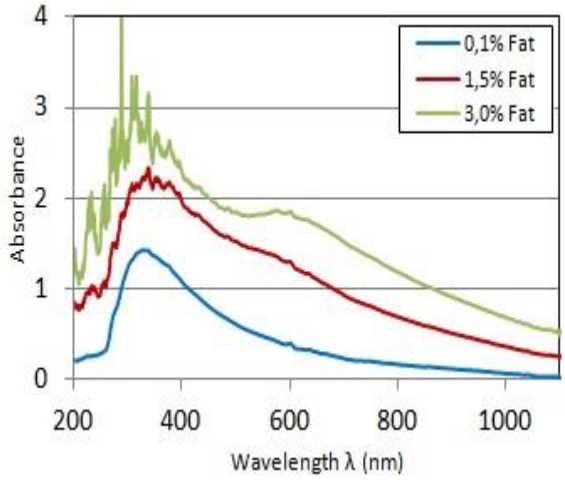

Fig. 1b. Absorption spectra of the same samples.

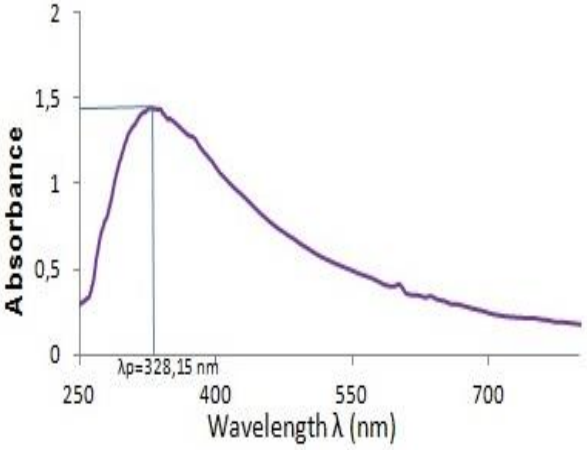

Fig. 2 Absorption spectra of milk Sample 1 with 0,1% of fat (oiliness milk).

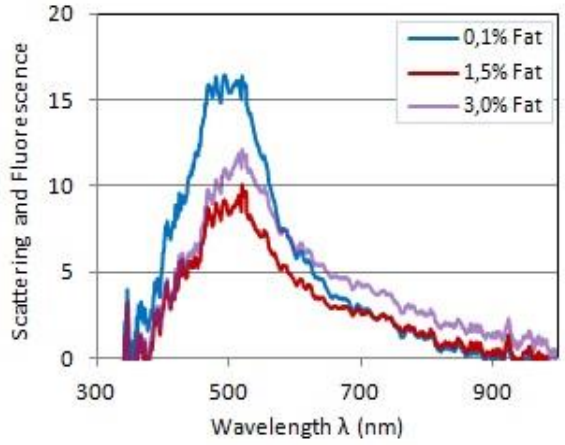

Fig. 3. Scattering and fluorescence spectra of milk Samples1 with 0,1%, 1,5% and 3,0% fat content.

from Fig. 2 is $\lambda_p \approx 328$ nm. The approximate diameter $d = 2R$ of the scattering particles obtained by substitution of this value in formula (1) is:

$$2R = \frac{\lambda_p}{\pi} = \frac{328}{3{,}14} \approx 104nm \qquad (2)$$

With reference to the literature [6] we suppose that the experimental value may be attributed to the scattering from casein micelles. As the protein content is the same for all samples, represented in Figure 1, one can consider that the increase of the absorption with the fat content is due only to its quantity and, eventually, may be used for evaluating the fat percentage in milk. The fat content influences also the scattered light, as it can be seen from Figure 3 where the spectra of scattered light for milk Samples1 (0.1%, 1.5% and 3.0% of fat) are presented.

However, considering the multi-component structure of milk and taking in account the fact that the studied optical region is not well explored for analysis of this nutrient, it's hard to claim obtaining precise information about its composition and structure. Despite of that, this study is promising when looking for simplicity, low price and easy practical control of one component (for example fat or protein content).

In Fig. 4a the fluorescence spectra of milk Sample1 with 15 % of fat content are presented. As expected, different pumping wavelengths provoke different emission of specific fluorescence spectra. For all studied samples we find out well expressed fluorescence pikes around of 335 nm and around of 373 nm which may be attributed to the presence of aromatic amino acids, nucleic acids and tryptophan residues. Sample1 has shown weak peak around of 500 nm probably due to the riboflavin fluorescence [10,11]. One can notice that the quantum efficiency is higher when exiting with LEDs emitting at 275 nm, 285 nm and 305 nm.

Plots of the ratio fluorescence intensity toward scattering intensity I_F/I at 375 nm pumping wavelength for milk Samples1 with fat contents of 0.%, 1.5% and 3.0% are presented in Figure 4b.

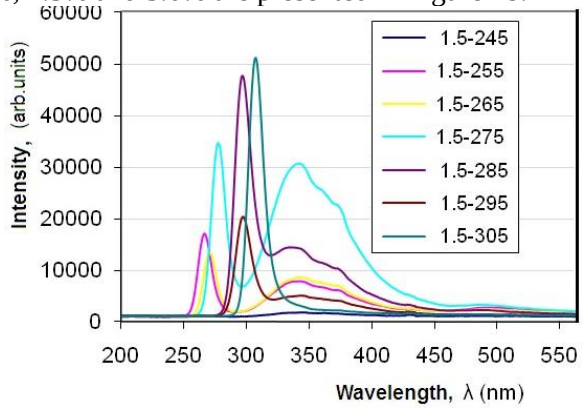

Fig. 4a) Fluorescence spectra of milk Samples1 with 1.5% fat content.

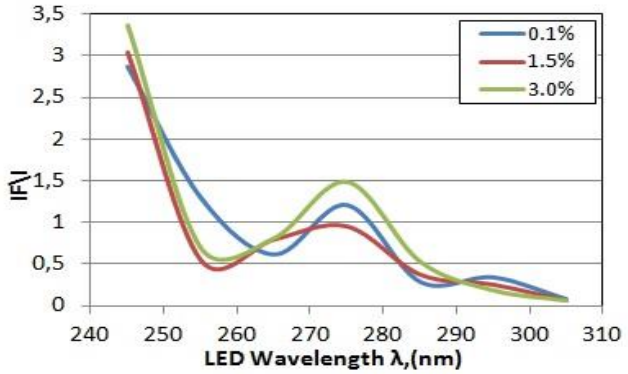

Fig. 4b) Plots of the ratio fluorescence intensity toward scattering intensity at 375 nm pumping wavelength for Sample1 with fat content of 0.1%, 1.5% and 3.0% are presented in Figure 2b.

The results for Samples2 and Samples3 (not presented here) are similar. The ratio I_F/I is highest for excitation at 245 nm. There are maxima of smaller values for 275 nm and for 295 nm excitation. For Samples 2 and Samples 3 the second maximum is at excitation wavelenght of 265 nm. The values of the maxima are different for the samples with different fat content.

CONCLUSIONS

In this work are presented some experimental results concerning the influence of fat content in milk on the scattering and fluorescence spectra in the optical range from 200 nm to 1100 nm. A compact fiber-optic setup and a spectrometer AvaSpec 2048 with resolution of 8 nm are used. The sizes of the scattering particles evaluated from the absorption pick correspond to the sizes of the casein micelles retrieved from the literature. The experimental results show that the intensity of the scattering spectra changes with the fat content. Fluorescence spectra have well expressed picks with maximum intensity at pumping wavelengths of 275 nm, 285 nm and 305 nm. The ration fluorescence intensity toward scattering intensity is also influenced from the fat content. The obtained experimental results suggest that fluorescence and scattering spectra for excitation of milk with ultraviolet light can be used for identification of different production of milk and for obtaining information about its chemical composition.

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СПЕКТРИ НА РАЗСЕЙВАНЕ И ФЛУОРЕСЦЕНЦИЯ НА КРАВЕ МЛЯКО

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(Резюме)

Целта на това проучване е да се изследва влиянието на съдържанието на мазнини върху спектрите на разсейване и флуоресценция на краве мляко. Анализът на състава (съдържание на протеини, мазнини, въглехидрати и минерали, както и калоричност), са получени с помощта на млекоанализатор Екомилк-М Милкана КАМ 98-2А. За всички изследвани проби са установени добре изразени флуоресцентни пикове около дължини на вълните 335 nm и 373 nm, което може да се дължи на присъствието на ароматни аминокиселини, нуклеинови киселини и триптофанови остатъци. Една от пробите показва слаб пик около 500 nm, което вероятно се дължи на флуоресценцията на рибофлавин. На графиките, показващи отношението на интензитета на флуоресценция към интензитета на разсейване като функция от дължината на вълната на възбуждане, се наблюдават максимуми за млечни проби от отделните производители, съдържащи мазнини с различна концентрация. Експерименталните резултати показват, че спектрите на флуоресценция и разсейване на млякото могат да бъдат използвани получаване на информация за химичния състав на млякото, както и за идентифициране на производителя.

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Behaviour of eggshell membranes at tensile loading

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The aim of this paper was to study the mechanical behaviour of the eggshell membrane using tensile tests at different loading rates. The eggshell membrane was obtained from commercial breeding lines of Japanese quails (*Coturnix japonica*). Samples were cut out of the membrane in latitudinal direction. TIRAtest 27025 tensile testing machine equipped with a 200 N load-cell was used. Tensile deformation exhibits both non-linear as well as linear region. The dependence of the stress on the strain in non-linear region can be described using of the Mooney-Rivlin equation. Linear region corresponds to the elastic strain. Parameters of the used equation are dependent on the strain rate. Generally, the strength of the eggshell membrane increases with the strain rate.

***Key words**:* Eggshell membrane, tensile loading, loading rate, stress, strain strength

INTRODUCTION

The eggshell membrane is a tissue found between the calcified eggshell and the albumen of eggs. This structure is a thin, highly collagenized fibrous membrane comprising inner (in contact with the albumen) and outer layers. It is mainly formed by types I, V and X collagen, making up 88 – 96 % of its dry weight. The presence of other proteins, such as osteopontin, sialoprotein and keratin, has also been reported [1]. The biologically active of the eggshell membrane is essential for the formation of the egg, retaining the albumen and preventing the penetration of bacteria [2]. The eggshell membrane also affects the eggshell strength [3]. Even if there are many reports on the use of the eggshell membrane, e.g. in the recovery of gold from waste water [4,5] not much information is known about its physical and structural properties, such as the pore and mechanical characteristics of the membrane. The only exception represents the paper of Torres et al. [6] which is focused on the study of hen`s egg membranes under tensile loading and nanoindentation.

The present paper deals with the mechanical behaviour of the eggshell membrane of quail's eggs using tensile tests at different loading rates. The knowledge of these properties is very useful namely at the study of the egg changes during its storage and at the numerical simulation of the egg loading [7].

EXPERIMENTAL

The eggshell membrane was obtained from commercial breeding lines of *Japanese quails (Coturnix japonica)*. The outer membranes were carefully removed using clamps and washed with distilled water. The membranes were then stored in physiologic saline solution in order to avoid dehydration. Samples were cut out of the membrane in latitudinal direction. TIRAtest 27025 tensile testing machine equipped with a 200 N load-cell was used. Rectangular samples (15 mm x 15 mm) were used for the measurements. It means the initial length of the specimen $l_o = 15$ mm. The thicknesses of the membranes (around 50 μm) were obtained from digital micrometer. Specimens were glued to thin metallic plates – see Figure 1.

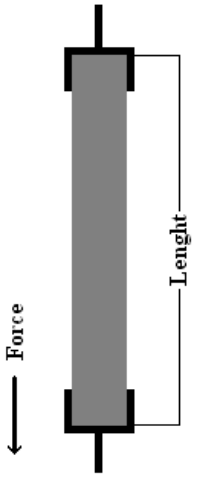

Fig. 1. Schematic of tensile test experiment and attached specimen.

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The deformation of the sample was assumed to be equal to the separation of the crossheads. The force F and the deformation $\Delta l = l - l_o$, where l is the instantaneous specimen length at the time t, are measured during tension and both quantities are recorded. The force-deformation data may easily be transformed into normalized quantities such as stress and strain. The Cauchy strain and Hencky's natural or true strain are of common use in representing compression curves. The Cauchy strain measure gives the relative deformation with respect to the initial sample length

$$\varepsilon_C = \frac{l - l_o}{l_o} = \frac{\Delta l}{l_o}.$$

Hencky's strain (often denoted as 'true' strain) derives from the integration of the infinitesimal strain and is given by

$$\varepsilon_H = \ln\left(\frac{l_o + \Delta l}{l_o}\right) = \ln(1 + \varepsilon_C).$$

The conversion of the force F into engineering stress is simple given by

$$\sigma_u = \frac{F}{A_0},$$

where A_o is the cross section of the undeformed specimen. In order to obtain some information on the true stress an assumption on the material incompressibility is often used. The true stress is than given by

$$\sigma_t = \sigma_u\left[(1 + \varepsilon_c)\right].$$

The specimen deformation is also described using the stretching parameter, λ, which is defined as

$$\lambda = \frac{l}{l_o} = 1 + \varepsilon_C.$$

The transformation force-deformation data into quantities given above have been performed using of MATLAB software. Four speeds, v, were used: 1, 10, 100 and 800 mm.min^{-1}. Loading rate can be converted to the strain rate:

$$\frac{d\varepsilon}{dt} \equiv \dot{\varepsilon} = \frac{v}{l_o}.$$

The corresponding values of strain rates are: 0.00111; 0.0111; 0.111 and 0.888 s^{-1}. Experiments were performed at the room temperature.

RESULTS AND DISCUSSION

It is shown in Figure 2 an example of the dependence of stress on the strain. The qualitative features of this dependence are the same for all used speeds. These curves are similar to those of other membranes as reported e.g. in Torres et al. [6], in which three different regions were found. In the first region (the toe region), little stress is needed to elongate the membrane. The second region is called the hill, and the stiffness of the membrane increases with elongation. Finally, a linear dependency is shown in the third region. The nonlinear dependence can be explained in terms of eggshell membrane microstructure [6].

The behaviour of the toe and hill regions of the eggshell membrane was modelled by using the Mooney-Rivlin equation [8]. According to this theory the strain-dependent behaviour can be represented by the Mooney-Rivlin hyperelastic potential, U_{MR},

$$U_{MR} = C_1(I_1 - 3) + C_2(I_2 - 3),$$

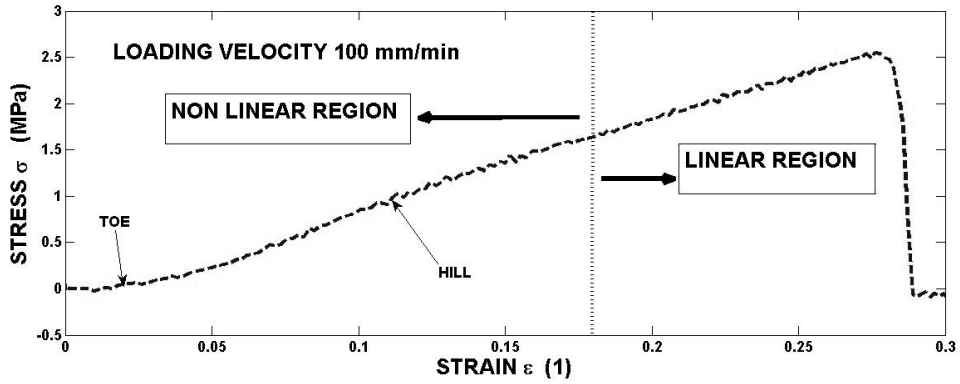

Fig. 2. Example of stress-strain dependence.

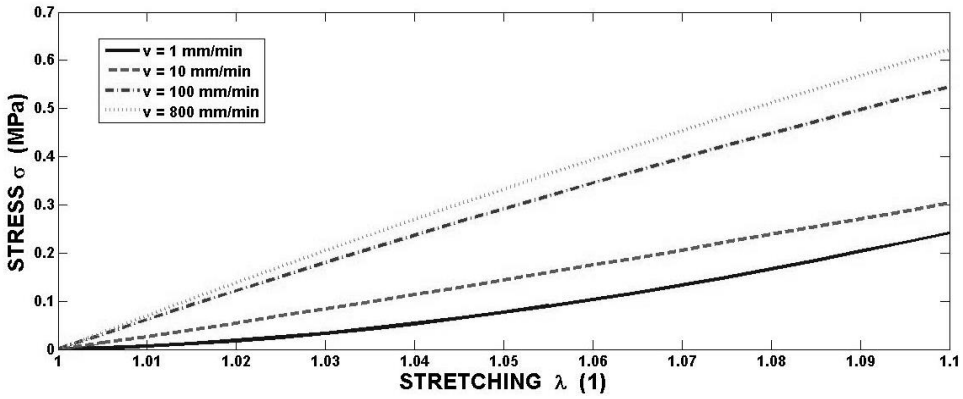

Fig. 3. Mooney-Rivlin model of the stress-strain dependence.

where C_1 and C_2 are material constants with dimensions of stress and:

$I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$ $I_2 = \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_1^2\lambda_3^2\lambda_1$,

λ_2, λ_3 are the stretch ratios in the three principal axes. For uniaxial loading the stress is expressed as:

$$\sigma = \left(\lambda - \frac{1}{\lambda^2}\right)\left(2C_1 + \frac{2C_2}{\lambda}\right), \quad (1)$$

where λ is the stretch ratio in the direction of load. Values of the constants C_1 and C_2 are given in the Table 1. The dependence of stress on the stretching is displayed in the Figure 3. It is evident that these parameters are dependent on the loading velocity. At low strain levels the behaviour is modelled with Equation (1) until the strain is about 10 %. Beyond that point, the sample behaves as a Hookean material. Linear part of the stress – strain dependence enables to evaluate of the Young modulus, *E*. Values of this material parameter are given in the Table 2. The values of this parameter which describes the elastic properties of membranes are independent on the loading rate. This is in agreement with results reported in Bing et al. [9] but in disagreement with the conclusions found by Torres et al. [6] for the eggshell membrane also affects the eggshell strength of the hen`s eggs. The Mooney-Rivlin relation is typically used for the study of rubber elasticity. Rubber is an elastomer formed by a network of cross-linked polymer chains and the deformation of its chains has an entropic origin. The evidence shown here might indicate that, as in the case of collagen molecules and fibrils, the initial deformation of the eggshell membrane has an entropic origin.

Table 1. Parameters of Mooney-Rivlin model.

Loading rate	$2C_1$, MPa	$2C_2$, MPa	R^{2*}
1 mm/min	13.320	-13.1200	0.9933
10 mm/min	5.966	-4.6370	0.9840
100 mm/min	3.787	-0.2228	0.9896
800 mm/min	3.632	-0.1364	0.9929

*R^2 is the correlation.

Table 2. Young modulus of elasticity E.

Loading rate	Min E, MPa	Mean E, MPa	Max E, MPa	Standard deviation
1 mm/min	9.23	9.618	9.89	0.27105
10 mm/min	9.67	9.804	9.93	0.10164
100 mm/min	9.35	9.728	9.91	0.23499
800 mm/min	9.63	9.718	9.82	0.09039

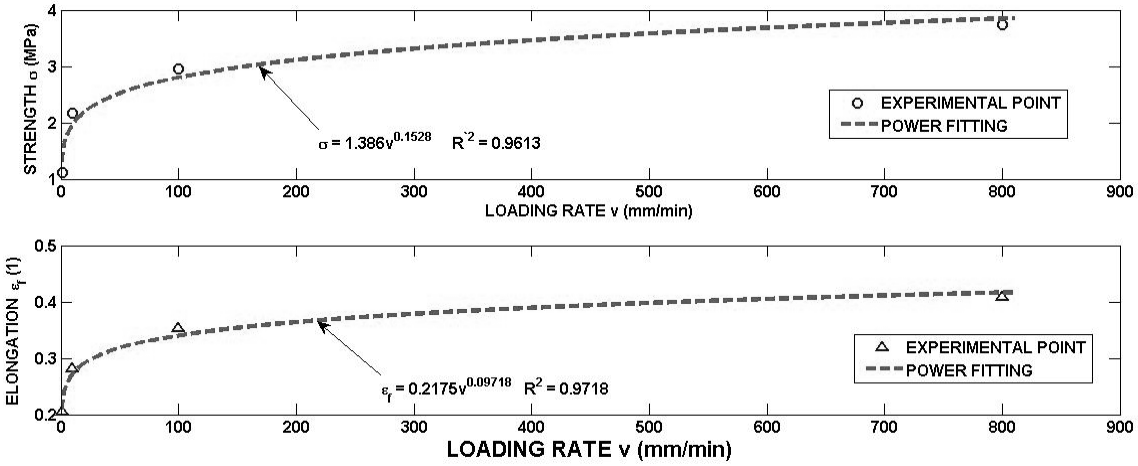

Fig. 4. Effect of loading rate on the fracture parameters of the eggshell membranes.

Table 3. Fracture parameters – ultimate tensile strength, maximum of elongation, strain energy density.

Loading rate	Ultimate tensile strength, MPa			
	Min	**Mean**	**Max**	**Standard deviation**
1 mm/min	1.05	1.11	1.15	0.040
10 mm/min	2.09	2.16	2.24	0.055
100 mm/min	2.80	2.95	3.08	0.106
800 mm/min	3.57	3.74	3.91	0.140
Loading rate	**Elongation [1]**			
	Min	**Mean**	**Max**	**Standard deviation**
1 mm/min	0.184	0.204	0.247	0.0248
10 mm/min	0.242	0.281	0.311	0.0255
100 mm/min	0.339	0.352	0.373	0.0139
800 mm/min	0.387	0.408	0.421	0.0130
Loading rate	**Strain energy density, J/m^3**			
	Min	**Mean**	**Max**	**Standard deviation**
1 mm/min	76874.22	91869.40	128544.590	20844.65
10 mm/min	340874.07	362883.64	376454.469	13293.23
100 mm/min	389065.19	462359.97	486152.422	41308.33
800 mm/min	664204.87	686757.33	704406.124	15044.17

In the next step the values of ultimate tensile strength has been evaluated together with maximum elongation ε_f. The volume density of work, *W*, up to the membrane fracture has been also determined using of the relation:

$$W = \int_0^{\varepsilon_{ff}} \sigma d\varepsilon.$$

The values of these properties are given in the Table 3. The ultimate tensile strength increases with the loading rate as well as the value of the maximum of the elongation – see Figure 4. The dependence of these parameters on the loading rate can be fitted by a power function. Parameters of this function are presented in the Table 1 together with the correlation coefficient R^2.

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ОТНАСЯНИЯ НА МЕМБРАНАТА В ЯЙЧЕНАТА ЧЕРУПКА ПРИ ЕДНООСНА ДЕФОРМАЦИЯ НА ОПЪН

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(Резюме)

Целта на настоящата работа беше да се изучи механичното поведение на мембраната в яйчена черупка при деформация на опън с различни скорости. Мембраната беше получена от търговски породи на японски пъдпъдъци (Coturnix japonica). Образците бяха нарязани по ширината на мембраната. За експериментите беше използван динамометър TIRAtest 27025, снабден с клетка за натоварване 200 N. Деформацията на опън се характеризира с линеен и нелинеен участък. Зависимостта на напрежението от относителната деформация в нелинейния участък може да се опиши посредством уравнението на Мууни-Ривлин. Линейният участък съответства на еластична деформация. Параметрите на използваното уравнение зависят от скоростта на деформация. Като правило силата на разрушаване се увеличава при увеличаване на скоростта на деформация.

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Healthy lipid combination. Effect of thermal processing on the quality characteristics of meat products

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The objective of this study was to investigate the effect of replacing animal fat in meatloaf with walnuts and various vegetable oils (sunflower, sea buckthorn and walnut). The chemical composition, cooking loss, lipid oxidation by thiobarbituric acid-reactive substances method (TBARs), total antioxidant capacity (TAC) of meatloaf was analysed. In the study the values of TBARs for meatloaf with walnuts and vegetable oils were lower than the control sample, revealing that the added materials acted like antioxidants. The sample containing walnuts and sea buckthorn oil had the highest TAC followed by the sample containing walnuts and sunflower oil. The incorporation of vegetable oils and walnuts successfully reduced the animal fat content in the final products while improving other characteristics.

Key words: walnuts, vegetable oils, cooking loss, meatloaf, antioxidant activity.

INTRODUCTION

Fat is an important constituent of processed meat products because it affects the stability of meat emulsions, reduces cooking losses, improves water holding capacity, provides flavor, texture, tenderness, juiciness, mouthfeel [1,2]. Fats have considerable effects on the binding, rheological and structural properties of meat products. Reducing the fat content in meat products and the substitution of animal fat with vegetable oils and walnuts should result in a healthier product [3]. The substitution of animal fat with vegetable oils has been suggested to improve the fatty acid profile and to decrease the cholesterol levels of meat products. Several vegetable oils have already been used as fat substitutes, such as olive, flaxseed, corn, soybean, and canola oil. It has been reported that walnuts, as part of a cardiohealth diet, may reduce the risk of coronary heart disease. This effect has been associated with the blend of nutrients and phytochemicals found in walnuts [4]. The objective of this study was to evaluate the replacing of animal fat with various vegetable oils and walnuts by studying proximate composition, cooking loss, lipid oxidation and total antioxidant capacity of meatloaf.

EXPERIMENTAL

Material

Fresh pork meat and pork back fat were obtained from a local processor at 48 h postmortem. Sea buckthorn oil was obtained from S.C. Hofigal Export Import S.A. Bucharest, Romania. Sunflower oil, walnut oil and all other additives (powder milk, sodium chloride and pepper) were purchased from a local supermarket in Galati (Romania). Walnuts (S.C. Romtransilvan S.R.L, Oradea, Romania) were ground down to a particle size of approximately 12 μm.

Method preparation.

Four different meatloaf formulations were prepared (Table 1). Control meatloaf (M) was made from fresh pork meat, pork back fat, sodium chloride and pepper, while LPFS, LPC and LPN samples contained walnuts, vegetable oil and powder milk.

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Table 1. Formulation (g) of experimental products.

Sample	Meat	Fat	Walnuts	Vegetable oil	Powder milk	Salt	Pepper	Water
M	450	150	-	-	-	3	2	45
LPFS	450	117	30	3	30	3	2	15
LPC	450	117	30	3	30	3	2	15
LPN	450	117	30	3	30	3	2	15

M – Control meatloaf, LPFS – meatloaf with walnuts and sunflower oil, LPC – meatloaf with walnuts and sea buckthorn oil, LPN – meatloaf with walnuts and walnut oil.

Table 2. Proximate composition, energy value and cooking loss of meatloaf containing various vegetable oils and walnuts.

Parameters	Meatloaf			
	M	LPFS	LPC	LPN
Moisture (g/100g)	51.79 ± 0.040	55.22 ± 0.022	52.43 ± 0.017	53.85 ± 0.015
Protein (g/100g)	19.68 ± 0.035	19.89 ± 0.031	20.83 ± 0.040	19.21 ± 0.031
Fat (g/100g)	29.28 ± 0.021	20.88 ± 0.025	20.01 ± 0.040	19.54 ± 0.023
Ash (g/100g)	0.89 ± 0.036	1.36 ± 0.025	1.32 ± 0.012	1.42 ± 0.017
Carbohydrate (g/100g)	-	2.66 ± 0.021	5.41 ± 0.006	5.87 ± 0.023
Energy value (kcal/100g)	342.25 ± 0.015	278.17 ± 0.017	285.07 ± 0.025	276.18 ± 0.021
Cooking loss (%)	13.06 ± 0.058	10.41 ± 0.015	11.93 ± 0.057	10.63 ± 0.032

All values are mean ± standard deviation.

Proximate analysis and cooking loss

The chemical compositions of meatloaf were determined using procedures prescribed by the official methods of analysis [5] for moisture, protein, fat and ash determinations. Carbohydrates were estimated by difference. Energy value was estimated from protein (× 4 kcal/g), carbohydrate (× 4 kcal/g) and fat (× 9 kcal/g) contents for each product. After cooking, the samples were cooled at room temperature for 30 min and the percentage of cooking loss was recorded as described by Franco and coworkers [6]. The analyses were made in triplicate.

Lipid oxidation and total antioxidant capacity

Oxidative stability of all samples was evaluated by measuring the formation of thiobarbituric acid-reactive substances (TBARs) following a modification of the method used by Serrano and coworkers [7]. The results were expressed as mg of malonaldehyde (MDA)/kg of meatloaf sample. Total antioxidant activity was determined based on the 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay developed by Miller and coworkers [8], and modified by Re and coworkers [9]. The results were expressed in μmol of Trolox/g of sample. Each sample was run in triplicate.

RESULTS AND DISCUSSION

Proximate analysis, energy values and cooking loss of meatloaf

The proximate composition, energy value and cooking loss of meatloaf samples are shown in Table 2. The moisture content was higher in the samples with vegetable oil and walnuts than the control because these samples were formulated with added walnuts which had higher water retention and improved emulsion stability. The highest moisture content was found in the sample LPFS. Similar trends in moisture content were observed when different amounts of vegetable oil and dietary fiber were added to meat emulsions Choi and coworkers [1]. The fat content was lower in the samples formulated with vegetable oil and walnuts (replacing pork fat) than the control (29.28 % fat) containing animal fat. The addition of walnuts and vegetable oils significantly raised the ash level of the meatloaf. Meatloaf had a carbohydrate content ranging from 2.66 to 5.87%, where the highest carbohydrate content was found in the sample with walnut oil and walnuts. The differences in energy value of meatloaf formulated with vegetable oils and walnuts were significant. The higher energy value was in the control (342.25 kcal/100 g) with animal fat compared to the other samples. The energy values of the meatloaf containing vegetable oil and walnuts ranged from 276.18 kcal/100 g (LPN) to 285.07 kcal/100 g (LPC). Cooking loss is affected by cooking method,

additives, the type of fat and the amount of fat in meat product. The effects of the replacement of pork back fat with vegetable oils and walnuts on the cooking loss of the meatloaf are shown in Table 2. The cooking loss was lower in meatloaf formulated with vegetable oils and walnuts than in the control sample. Some researchers Choi and coworkers [1] and Choi and coworkers [2] reported that cooking losses for low-fat meat emulsion systems were affected by the type of vegetable oil, dietary fiber used and reducing the animal fat content.

Effect of vegetable oils and walnuts addition on lipid oxidation and total antioxidant capacity of meatloaf

Results for TBARs index (mg MDA/kg of sample) are summarized in Figure 1. The determination of TBARs in samples with vegetable oils and walnuts showed a very low oxidation compared with control sample. TBARs ranged from 0.66 to 3.18 mg MDA/kg of sample. The highest TBARs value is for the control sample and might be due to high fat content in control meatloaf. The replacing of animal fat with vegetable oils and walnuts resulted in inhibition of the lipid oxidation in meatloaf.

TEAC of the samples determined by the ABTS•+ radical probes are presented in Figure 2 and expressed in μM Trolox/g of meatloaf. The total ABTS•+scavenging capacity of the meatloaf samples was in the range 16.26 ± 0.03 μM Trolox/g for control sample to 22.65 ± 0.015 μM Trolox/g for LPC.

Comparing the ABTS•+ results of the meatloaf samples, the TEAC of the samples with vegetable oils and walnuts were significantly higher than the control sample. Different components from vegetable oils and walnuts are responsible of this TEAC.

CONCLUSIONS

Commercially available vegetable oils, such as sunflower, walnut and sea buckthorn oils and walnuts were successfully used as substitutes for pork back fat in the production of meatloaf. Reduced-fat meatloaf would be beneficial for health since they have lower total fat and energy. The addition of various vegetable oils and walnuts can contribute to the development of meatloaf with desirable quality characteristics.

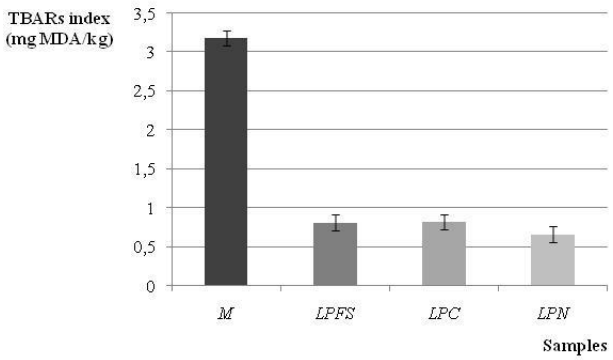

Figure 1. Effect of vegetable oils and walnuts on lipid oxidation of meatloaf.

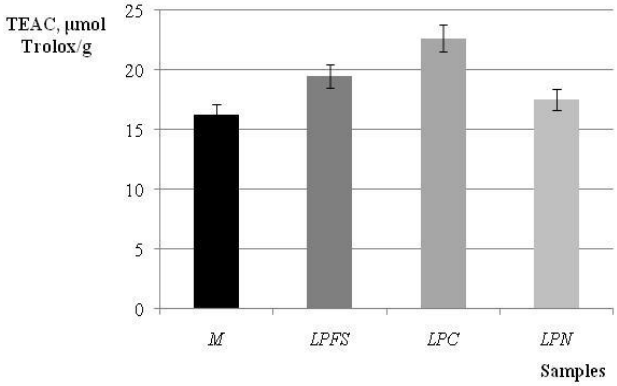

Figure 2. TEAC of meatloaf determined using ABTS as antioxidant probes.

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КОМБИНАЦИЯ НА ЗДРАВОСЛОВНИ ЛИПИДИ. ВЛИЯНИЕ НА ТЕРМИЧНАТА ОБРАБОТКА ВЪРХУ КАЧЕСТВЕНИТЕ ХАРАКТЕРИСТИКИ НА МЕСНИ ПРОДУКТИ

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(Резюме)

Целта на настоящото проучване беше да се изследва ефекта на заместване на животинската мазнина в руло „Стефани" с орехи и различни растителни мазнини (слънчогледова, орехова и от морски зърнастец). Изследвани са химичния състав, загубите при готвене, липидното окисление по метода на реакцията с тиобарбитурова киселина, общия антиокидантен капацитет (ОАК). Беше установено, че за руло „Стефани" с орехи и различни растителни мазнини продуктите от реакцията с тиобарбитурова киселина са по-малко в сравнение с контролата, което разкрива възможността на добавените материали да действат като антиоксиданти. Образците, мазнина от орехи и морски зърнастец, притежават най-висок ОАК, последвани от образците, съдържащи орехи или слънчогледово масло. Включването на растителни масла и орехи успешно намалява съдържанието на животинска мазнина в крайния продукт, като подобрява техните характеристики.

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Thermodynamic properties of mixed origin fat blends

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The role of fats is to provide the desired textural properties of foodstuffs. The effect on the modification of texture is based on the characteristics of solidification and melting. In order to fulfil the desired effect, the blending of different types of fats is the simplest way. The aim of the present work is to investigate the melting and solidifying properties of poultry fats mixed with different vegetable fats. Rendered poultry fat was blended with cocoa butter and palm mid fraction (PMF). Mixing ratios were as poultry fat% - vegetable fat% by 100/0, 75/25, 50/50, 25/75 and 0/100.

Melting and solidification were detected by differential scanning calorimetry (Setaram evo 3 DSC apparatus). Exotherm and endotherm peaks were detected as well as enthalpies were calculated.

During solidification of poultry fat-cocoa butter mixtures the effect of cocoa butter was observable. Increasing the amount of cocoa butter in the blends resulted in higher releasing of heat. The crystallization pattern was dominated by the cocoa butter. On the other hand, the effect of palm mid fraction in the fat mixtures prevailed gradually.

The melting of poultry fat-cocoa butter mixtures followed the type of the cocoa butter. 50% cocoa butter in the blend resulted almost the same crystal structure than the pure cocoa butter. In case of poultry fat-palm mid fraction blends two endotherm peaks were present in the blends similarly to the pure palm mid fraction. Total enthalpy increased according to the increased ratio of palm mid fraction.

Keywords: solidification and melting of fat mixtures, mixtures of poultry and vegetable fats, textural properties of fat mixtures, melting enthalpy of fat mixtures.

INTRODUCTION

Goose fat is a valuable food material due to its smooth texture and pleasant sensory attributions [1-4]. In some food products the fat component is a mixture of vegetable and animal fats especially in complex systems e.g.: patés, spreads, fillings of bakery products etc.

Interaction between fats of different origin in fat blends can modify physical properties of foods and can be in the interest in food technologists [2, 5]. The aim of the present work was to study the physical characteristics of goose fat – cocoa butter and goose fat-palm mid fraction (PMF) blends. Cocoa butter was chosen as a model for fats which consist of symmetrical triglycerides and perform sharp melting behavior; PMF represented fats that show a wide melting temperature interval.

EXPERIMENTAL

Materials

Rendered poultry fat was blended with cocoa butter and palm mid fraction (PMF). Materials were provided by local food factories. Sample preparation followed the method of David Perez-Martinez (2007). Fats were melted in order to destroy crystal memory and following that the different fats were mixed. After homogenization the samples cooled down spontaneously to 5°C and kept under this temperature for at least five days until the measurements. Mixing ratios were as poultry fat% – vegetable fat% by 100/0, 75/25, 50/50, 25/75 and 0/100. Materials were collected from a local market and a local confectionery firm.

Methods

Fatty acid composition of pure fats was analyzed by Gas Cromatograph (GC) according to the methods based on MSZ ISO 5508:1992. Type of the apparatus was HP 5890 GC System, with SGE BPX 70 column with parameters 50 m 0.22 mm 0.25 µm. Heating was from 150 °C to 210 °C (with 1.3 °C min^{-1} heating rate). Pressure: 14 psi, injector: 250 C split, split ratio: 100:1. Detector: 250 C, FID. Carrier gas was hydrogen, the flow was 0.6 cm^3 min^{-1}, injection pressure was 0.965 bars. Identification of fatty acids was based on retention times using fatty acid methyl ester standards.

Melting and solidification were detected by differential scanning calorimetry (Setaram evo 3

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DSC apparatus). Details of the measurements were: 20-25 mg of each sample was put into the 100 µl alumina sample holder. Samples were cooled to 0 °C by 1°C min^{-1} and kept at this temperature for 10 minutes. Heating was performed from 0 °C up to +80°C by 1°C min^{-1}. Samples were kept at this temperature for 30 min. and the cooling program was applied by 1°C min^{-1} to -20°C and kept under this condition for 10 min. Finally, the samples were heated up to ambient temperature. Measurements were done at constant speed during the heating and cooling processes. Results were elaborated by Callisto Processing 1.076 computer program using linear base line. Heat flow and enthalpy of exotherm and endotherm peaks were recorded and calculated.

RESULTS AND DISCUSSION

Composition

Pure fats were characterized by their fatty acid composition. Results are summarized in Table 1.

Table 1. Fatty acid composition (%) of the investigated pure fats

Fatty acid	Fatty acid composition (%)		
	Goose fat	CB	PMF
SAFA	30.5	65.1	56.6
MUFA	58.6	31.9	40.4
PUFA	10.3	2.8	2.6
Not identified	0.6	0.2	0.4

Abbreviations: CB: Cocoa Butter; PMF: Palm Mid Fraction; SAFA: saturated fatty acid; MUFA: monounsaturated fatty acid PUFA: polyunsaturated fatty acid.

Results show that CB contains the highest amount of saturates and Goose fat the least. Goose fat is rich in polyunsaturated fatty acids. Total unsaturates in Goose fat aproach 70%. PMF contained saturated and unsaturated fatty acids in a balanced ratio.

Solidification thermograms

In Figure 1. thermograms of Goose fat, Cocoa butter and their blends are shown. Figure 2. demonstrates thermograms of Goose fat – PMF.

Abbreviations: A: vegetable fat, B: 25-75% blend, C: 50-50% blend, D: 75-25% blend, E: Goose fat

Figure 1 shows that goose fat has small exotherm peaks (at 2°C and 16°C) indicating weak crystal structure. A definite effect of cocoa butter on crystallization is observable. Solidification based on the cocoa butter has a pattern.

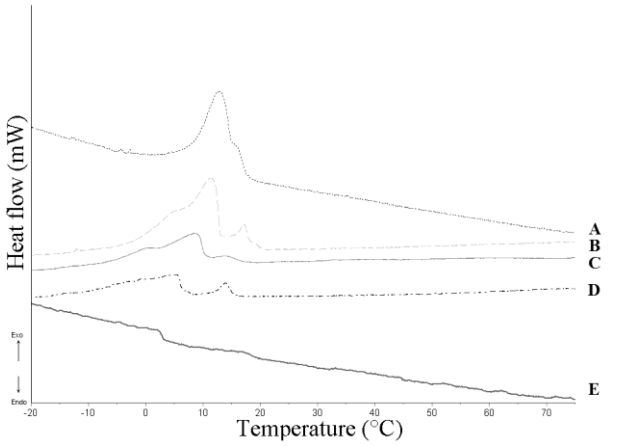

Fig. 1. Cooling thermogram of Goose fat, Cocoa Butter and their blend.
A: Cocoa Butter, **B:** 25-75% G/CB, **C:** 50-50% G/CB, **D:** 75-25% G/CB, **E:** Goose fat

Figure 2 demonstrates that during solidification PMF dominated the whole process. Another characteristic exotherm peak of PMF appeared at 75-25% (Goose fat:PMF) blend and developed according to the increasing amount of PMF

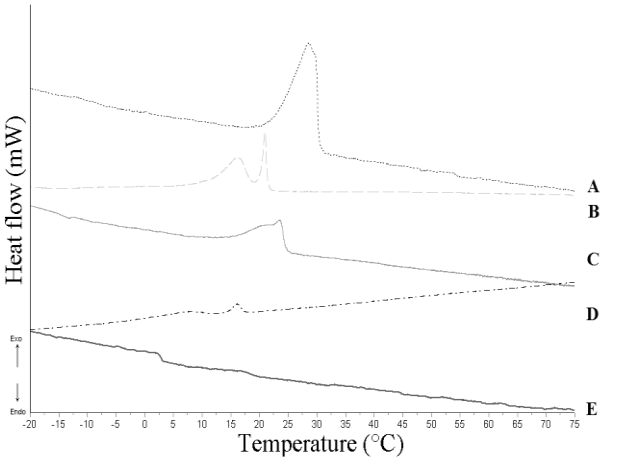

Fig.. 2. Cooling thermogram of Goose fat, PMF and their blend. **A:** PMF, **B:** 25-75% G/PMF, **C:** 50-50% G/PMF, **D:** 75-25% G/PMF, **E:** Goose fat

Average values of the solidification enthalpies of Goose fat-cocoa butter blends are shown in Figure 3. Small total enthalpy of Goose fat implies weak structure. Presence of Cocoa butter in the blend results in uniform increase in enthalpy values.

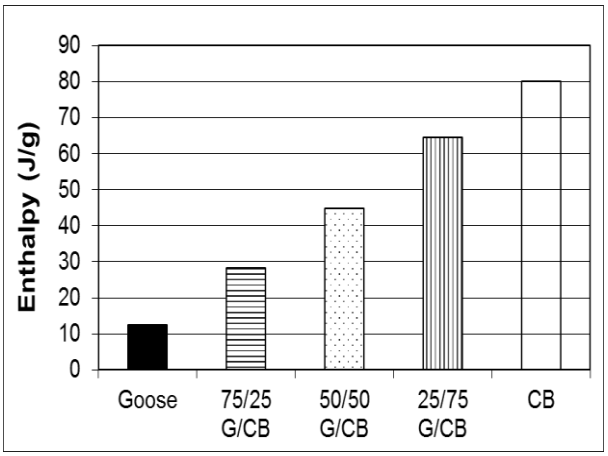

Fig. 3. Total enthalpy during solidification: Goose/Cocoa Butter.

In Figure 4 average of the total enthalpy values of solidification are summarized. PMF displays almost seven times higher enthalpy than Goose fat. Enthalpies increase stepwise by increasing the amount of PMF in the blend.

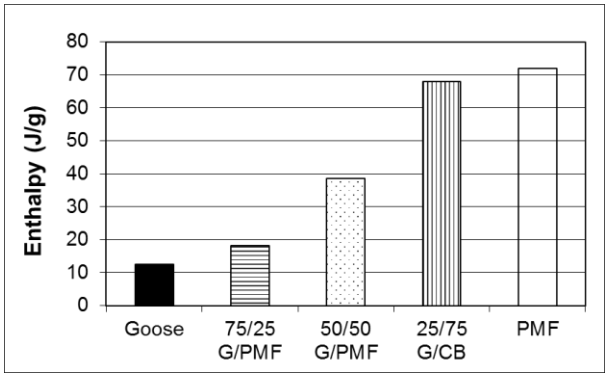

Fig. 4. Total enthalpy during solidification: Goose/PMF.

Melting thermograms

Figure 5 shows the melting thermograms of Goose fat - Cocoa butter blends. From the figure it is clear that goose fat has a weak crystal structure with two small endotherm peaks, Cocoa butter performs one big peak. Melting of the blends became gradually similar to the cocoa butter.Blends containing 50% or more Cocoa butter show only one big endotherm peak.

Figure 6 summarizes the melting thermograms of the Goose fat – PMF blends. Results prove that when the amount of PMF in the blend increases, the shape of the thermogram approaches the pure PMF. This phenomenon indicates that the whole process is dominated by PMF.

In Figure 7 is seen that Goose fat had the lowest and cocoa butter the highest total enthalpy during melting. Increasing amount of cocoa butter in the blend raised the enthalpies in a slightly exponential manner.

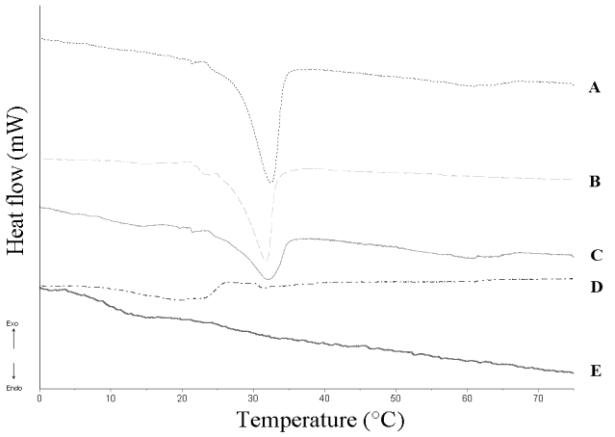

Fig. 5. Melting thermograms of Goose fat, Cocoa Butter and their blend.
A: Cocoa Butter, **B:** 25-75% G/CB, **C:** 50-50% G/CB, **D:** 75-25% G/CB, **E:** Goose fat.

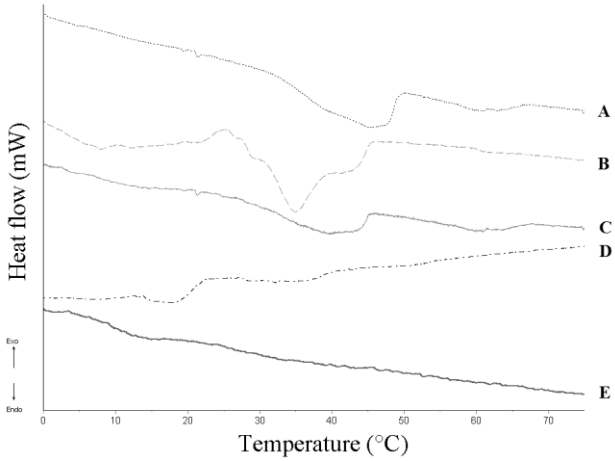

Fig. 6. Melting thermograms of Goose fat, PMF and their blend. **A:** PMF, **B:** 25-75% G/PMF, **C:** 50-50% G/PMF, **D:** 75-25% G/PMF, **E:** Goose fat

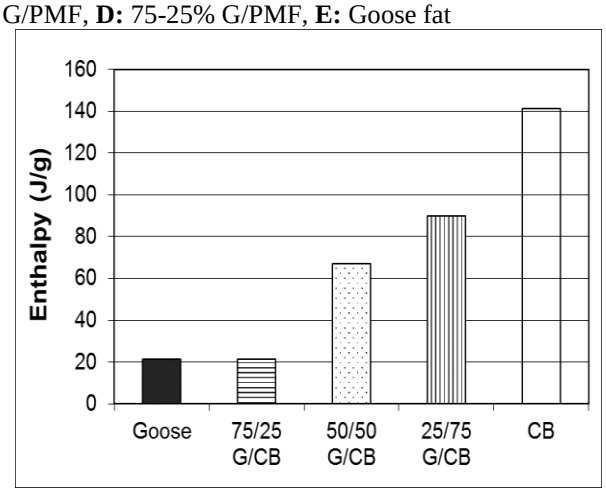

Fig. 7. Total enthalpy during melting: Goose/Cocoa Butter.

Figure 8 demonstrates that PMF caused a remarkable increase in the average of total enthalpy in the blends. Changes were exponential-like.

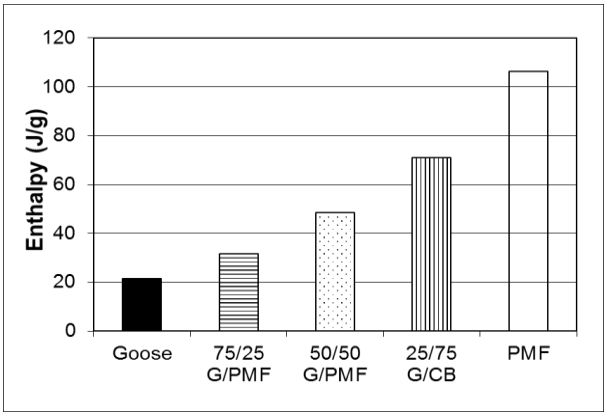

Fig. 8. Total enthalpy during melting: Goose/PMF

CONCLUSIONS

Our results proved that Goose fat has weak crystal structure, characterized as an easy-melt material. The softening effect of goose fat is rather high even if only a small amount is present in the blend. Solidification and melting process of mixed system of goose fat and cocoa butter or palm fat is dominated by the vegetable fat. Results are comparable with the other reported findings [4,5].

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ТЕРМОДИНАМИЧНИ СВОЙСТВА НА СМЕСИ ОТ МАЗНИНИ С РАЗЛИЧЕН ПРОИЗХОД

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(Резюме)

Ролята на мазнините е да осигуряват желани текстурни свойства на хранителните продукти.

Ефектът на модифициране на текстурата се дължи на характеристиките на втвърдяване и топене. Смесването на различни видове мазнини е най-простият начин за постигане на желания ефект. Целта на настоящата работа е изследване на свойствата топене и втвърдяване на птичи мазнини, смесени с различни растителни масла. Разтопена птича мазнина беше смесена с какаово масло и средна фракция на палмово масло (ПСФ). Отношенията на смесване птича мазнина % - растително масло % бяха както следва: 100/0, 75/25, 50/50, 25/75 и 0/100.

Топенето и втвърдяването бяха изследвани чрез диференциално сканираща калориметрия (апарат Setaram evo 3 DSC). За целта бяха установени екзотермичните и ендотермичните пикове и бяха изчислени енталпиите на преходите. Беше наблюдаван ефект на какаовото масло при втвърдяването на смеси птича мазнина – какаово масло. Увеличаването на съдържанието на какаово масло в сместа доведе до по-интензивно освобождаване на топлина. Процесът на кристализация беше доминиран от какаовото масло. От друга ефектът на ПСФ в смесите на мазнини се проявяваше постепенно.

Топенето на смесите от птича мазнина и какаово масло следваха фазовия преход на какаовото масло. 50% какаово масло в сместа доведе до почти същата кристална структура като тази на чистото какаово масло. В случая на смеси от птича мазнина и ПСФ бяха наблюдавани два ендотермични пика, подобни на тези на чистото ПСФ. Общата енталпия нарасна при увеличаване частта на ПСФ.

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Physicochemical characteristic of seed oils of Bulgarian species pumpkin and melon

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Tocopherol, carotenoid and chlorophyll content of three pumpkin species (*Cucurbita moschata, Cucurbita pepo, Cucurbita maxima*) and three melon species (*Honeydew, Dessert 5* and *Hybrid 1*) grown in southern part in Bulgaria were investigated. Highest content of tocopherols was found to be in *Cucurbita moschata* and in *Honeydew* glyceride oils - 417 mg kg^{-1} and 828 mg kg^{-1}, respectively. Chlorophyll content was detected in melon species but this pigment was not observed in pumpkin species. In *Cucurbita maxima* and melon species *Hybrid 1* oils were established higher quantities of β-carotene - 1222.33 ppm and 35.97 ppm, respectively. The green components predominates in the oils of all melon varieties, while there are differences in pumpkin seed oils - from highly dominant red component in *Cucurbita pepo* to less pronounced red color in *Cucurbita moschata* and to a clearly dominant green component in *Cucurbita maxima*. Fluorescence spectra of three wavelength in visible region (λ=370 nm, λ=395 nm, λ=425 nm) and in ultraviolet region (λ=305 nm) were obtained. Correlation between intensity of fluorescence maximum at λ=384 nm and γ - tocotrienol content was found in different species from melon seed oils. There was correlation between intensity of fluorescence maximum at λ=675 nm and chlorophyll content, too. Other fluorescence maxima were found at λ=634 nm, λ=688 nm, λ=480 nm and λ=500 nm, which were connected with presence of different pigments. On the other hand, these fluorescence maximums were indicators of passing process of oxidation.

Key words: *pumpkin and melon seed oils, tocopherols, carotenoids, chlorophyll, fluorescence, colour parameters.*

INTRODUCTION

The *Cucurbitaceae* is a family of annual and perennial herbaceous vines, distributed in tropical, subtropical and temperate areas. It includes about 800 species in 125 genera. The *Cucurbitaceae* family includes gourds, melons, watermelons, pumpkins and squashes. They are characterized by their fleshy fruits.

The chemical content of the pumpkin family has been the object of a number of studies and it has been established that the content of the basic chemical components of the fruit vary in large ranges depending on the sort, the climatic conditions and the way of cultivation [1]. The dry substance of the fruit is within the limits from 6.0 % to 20.0 % out of which between 2.0 % and 14.0 % are sugars. Relatively high are the contents of starch (from 2.0% to 7.0%), fats and proteins (from 1.3 % to 1.5%) of the total fruit weight. They are a rich source of mineral salts of potassium, phosphorus, sodium, magnesium, etc.

Pumpkin seeds contain proteins from 25.0 % to 51.0 %, crude oils from 34.5 % to 60.0 % and in the fatty acid profile dominate linoleic acid – over 40.5 % and oleic acid – over 46.9 %, fibres – around 13.8 %, mineral substances in the range from 4.45 % to 5.00 % [2,3,4]. In the literature, information about the lipid content of oils from seeds of various sorts of the family *Cucurbitaceae* is based on research on the physico-chemical properties and the fatty acid contents of the oils, and the data obtained varied depending on the type of the seeds and the area of cultivation. Data on the content of tocopherols can be found in the literature in the fruit, in the peel, and in the seeds and the latter is the greatest in pumpkin. The content of γ-tocopherols in the seeds was 66.85 mg kg^{-1} and of the α-tocopherols was 25.74 mg kg^{-1} raw weight. The content of β-carotene is the highest in the peel of pumpkin (68.3 mg kg^{-1}), while in the seeds [5] it is 7.15 mg kg^{-1}. The studies on the lipid content of the seeds of local sorts of pumpkin are rather scanty. Cold pressed pumpkin oil is commercially available but it is still not widely popular due to its dark green color.

Melon seeds are characterized by no smaller values and fat contents is 25.0 % - 45.2 % with a high nutritional value, due to the high level of

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polyunsaturated fatty acids (linoleic from 51.0 % to 69.0 % and oleic 12.1 % to 31.0 % acids) [6-11]. Melon seed oil has still found no use, and there lack data on the lipid content.

The objectives of this work are to lead investigations about tocopherol composition of different pumpkin and melon seeds grown in Bulgaria and to examine the physico-chemical characteristics of the oils as color parameters and fluorescence in UV-VIS range.

EXPERIMENTAL

Samples. The pumpkin species *Cucurbita moschata*, *Cucurbita pepo* and *Cucurbita maxima*, and melon species *Honeydew*, *Dessert 5* and *Hybrid 1* from fam. *Cucurbitaceae*, were grown and obtained from the region of South Bulgaria, crop 2013.

Isolation of glyceride oil and determination of oil content. The seeds (50 g sample) were air-dried and ground to powder and the oil was extracted with n-hexane in *Soxhlet* apparatus for 8 h. The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content [12].

Analysis of tocopherols. *T*ocopherols were determined directly in the oil by HPLC on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000. The operating conditions were as follows: mobile phase of n-hexane:dioxan 96:4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, emission 330 nm [13]. 20 μl 1 % solution of oil in hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. Reference tocopherol homologues were purchased from Merck (Darmstadt, Germany). The tocopherol content was calculated on the basis of tocopherol peak areas in the sample versus tocopherol peak area of standard α-tocopherol solution.

Colour parameters. CieLab colour parameters are measured directly by using spectrophotometer (Lovibond Tintometer PFX 195, UK). The content of β-carotene and chlorophyll are determined using special software.

The fluorescence of the samples was studied by exciting them with light emitting diodes (LEDs) emitting at 370 nm, 395 nm and 425 nm and in UV range at λ=305 nm. A 90 degree geometry of light detection in 10x10 mm cuvette were used. Samples were studied without any preliminary solution. For UV illumination the samples are fixed between two quartz plates. Fluorescence and scattering spectra are recorded using fiber-optic spectrometer *Avantes 2048* with a spectral sensitivity within the 250-1100 nm range.

RESULTS AND DISCUSSION

The investigated pumpkin *Cucurbita moschata, Cucurbita pepo* and *Cucurbita maxima* seeds from Bulgarian origin contain high quantities of glyceride oil (45.1 % - 51.5 %) which is rich in poly- and monounsaturated fatty acids especially linoleic acid (35.6 % - 50.8 %) and oleic acid (21.8 % - 35.9 %) and have low amounts of saturated fatty acids [14]. The oil content in the seeds of melon (*Honeydew, Dessert 5* and *Hybrid 1*) varied from 41.6 % to 44.5 % and the major fatty acid in total lipid was linoleic (51.1 % - 58.5 %), followed by oleic acid (24.8 % - 25.6 %) [15].

Tocopherol content and tocopherol composition of the seed oils are presented in Table 1.

Table 1. Total tocopherol content and tocopherol composition of different pumpkin and melon seed oils

Tocopherols	**Pumpkin**			**Melon**		
	Cucurbita moschata	*Cucurbita pepo*	*Cucurbita maxima*	*Honeydew*	*Dessert 5*	*Hybrid 1*
α – tocopherol, %	1.6±0.1	7.1±0.2	2.4±0.1	2.9±0.2	19.7±0.2	6.2±0.1
β – tocopherol, %	-	-	-	1.7±0.1	-	-
γ-tocopherol, %	88.4±0.4	71.7±0.5	58.1±0.2	91.5±0.5	71.4±0.4	78.5±0.2
γ – tocotrienol, %	10.0±0.1	21.2±0.2	39.5±0.4	3.9±0.1	8.9±0.2	15.3±0.3
Total tocopherol content, mg kg^{-1}	417±15	292±10	233±12	828±20	435±10	731±11

The contents of tocopherols were from 233 to 417 mg kg^{-1} and 435 - 828 mg kg^{-1} for the pumpkins and the melon, respectively. The highest content of tocopherols in pumpkin seed oils was found to be in variety *Cucurbita moschata* (417 mg kg^{-1}); and in melon seed oils - *Honeydew* and *Hybrid 1* (828 and 731 mg kg^{-1}, respectively). The content of tocopherols was found to be in *Cucurbita pepo* and *Cucurbita maxima* seed oils (292 - 233 mg kg^{-1}, respectively). According Ardabili et al. [16] and Gemrot et al. [17] the total tocopherol content in oil of pumpkin seeds (*Cucurbita pepo*) was 882.65 mg kg^{-1} and in the seeds was 107.0 mg 100 g^{-1}. Azhari et al. [18] reported for the total tocopherol content in *Cucumis melo* var. *tibish* seed oil was 43.20 mg 100 g^{-1}, which was similar to tocopherol content of oils from *Dessert 5*.

γ-Tocopherol (from 58.1 % to 88.4 %) was dominated component in *Cucurbita maxima, Cucurbita pepo and Cucurbita moschata* pumpkin seed oils followed by γ-tocotrienol (10.0 % - 39.5 %). In the oil from seeds of *Cucurbita pepo* was found a higher content of α-tocopherol (7.1 %) than the other two varieties (1.6 % - 2.4 %). M.Y. Kim et al. [5] reported that the seeds of *Cucurbita pepo* and *Cucurbita moschata* have significantly higher quantity of γ - tocopherol (61.65 mg kg^{-1} - 66.85 mg kg^{-1} in raw weight) than in *Cucurbita maxima* seeds (28.70 mg kg^{-1} raw weight). According to them the content of γ - tocopherol in oil from seeds of *Cucurbita pepo* and *Cucurbita moschata* was 2-3 times higher than α-tocopherol. In another reports in the oil of twelve pumpkin cultivars (*Cucurbita maxima*) α-tocopherol ranged from 27.1 μg g^{-1} to 75.1 μg g^{-1} in the oil, γ-tocopherol from 74.9 μg g^{-1} to 492.8 μg g^{-1} and δ - tocopherol from 35.3 μg g^{-1} to 1109.7 μg g^{-1} [19].

γ - Tocopherol predominated in all melon seed oils (71.4 % - 91.5 %). α - Tocopherol content in the oil from *Dessert 5* was highest (19.7 %), followed by *Hybrid 1* (6.2 %) and *Honeydew* (2.9 %). The content of γ-tocotrienol was found to be 15.3 % in *Hybrid 1*, 8.9% in *Dessert 5* and 3.9% in *Honeydew*. β-Tocopherol was detected in *Honeydew* oil in small amounts (1.7%).

The colour parameters of the considered oils were studied. The results are presented in Table 2.

Chlorophyll content was detected in oils from melon species (0.02-0.04 ppm) but in oils from pumpkin species this pigment was not observed. In *Cucurbita maxima* and melon species *Hybrid 1* oils were established higher quantities of β-carotene - 1222.33 ppm and 35.97 ppm, respectively.

Of all the studied pumpkin oils *Cucurbita pepo* is characterized by lowest luminosity value while for the rest luminosity is comparable. The observations for melon oils are analogous – lowest luminosity are of the *Hybrid* 1 sort, while the highest luminosity is of the oil from the seeds of *Dessert* 5.

In melon seed oils dominant is the green (*a<0.0*) and yellow (*b>0.0*) components. This is not valid for the pumpkin seed oil - it is red (*a>0.0*) for two sorts, while for *Cucurbita maxima* the green components dominates (*a<0.0*).

The fluorescence spectroscopy in the visible and ultraviolet region gives an opportunity for distinguishing of four main fluorescence peaks connected respectively with: the presence of tocopherols at λ= 346 nm and 384 nm; the presence of oxidation products at λ= 514 - 520 nm; the presence of chlorophyll at λ= 675 - 678 nm; the presence of pigments at λ= 620 nm and 700 nm.

Table 2. Colour parameters of oils from seeds of different sorts of melon and pumpkin

Type of oil	Pumpkin seed oil						Melon seed oil					
	Cucurbita moschata		*Cucurbita pepo*		*Cucurbita maxima*		*Honeydew*		*Dessert 5*		*Hybrid 1*	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Luminosity (L)	66.42	0.02	22.31	0.02	60.8	0.00	76.7	0.94	91.78	0.06	48.29	1.83
a	0.61	0.00	16.49	0.02	-7.31	0.01	-4.04	0.1	-5.21	0.01	-3.51	0.21
b	71.63	0.05	35.69	0.05	48.18	0.02	25.4	0.05	22.05	0.05	49.44	1.22
Chlorophyll, ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.02	0.00	0.02	0.00
β-carotene, ppm	72.91	2.32	62.84	4.78	1222.33	0.72	8.73	0.71	6.58	0.03	35.97	4.31

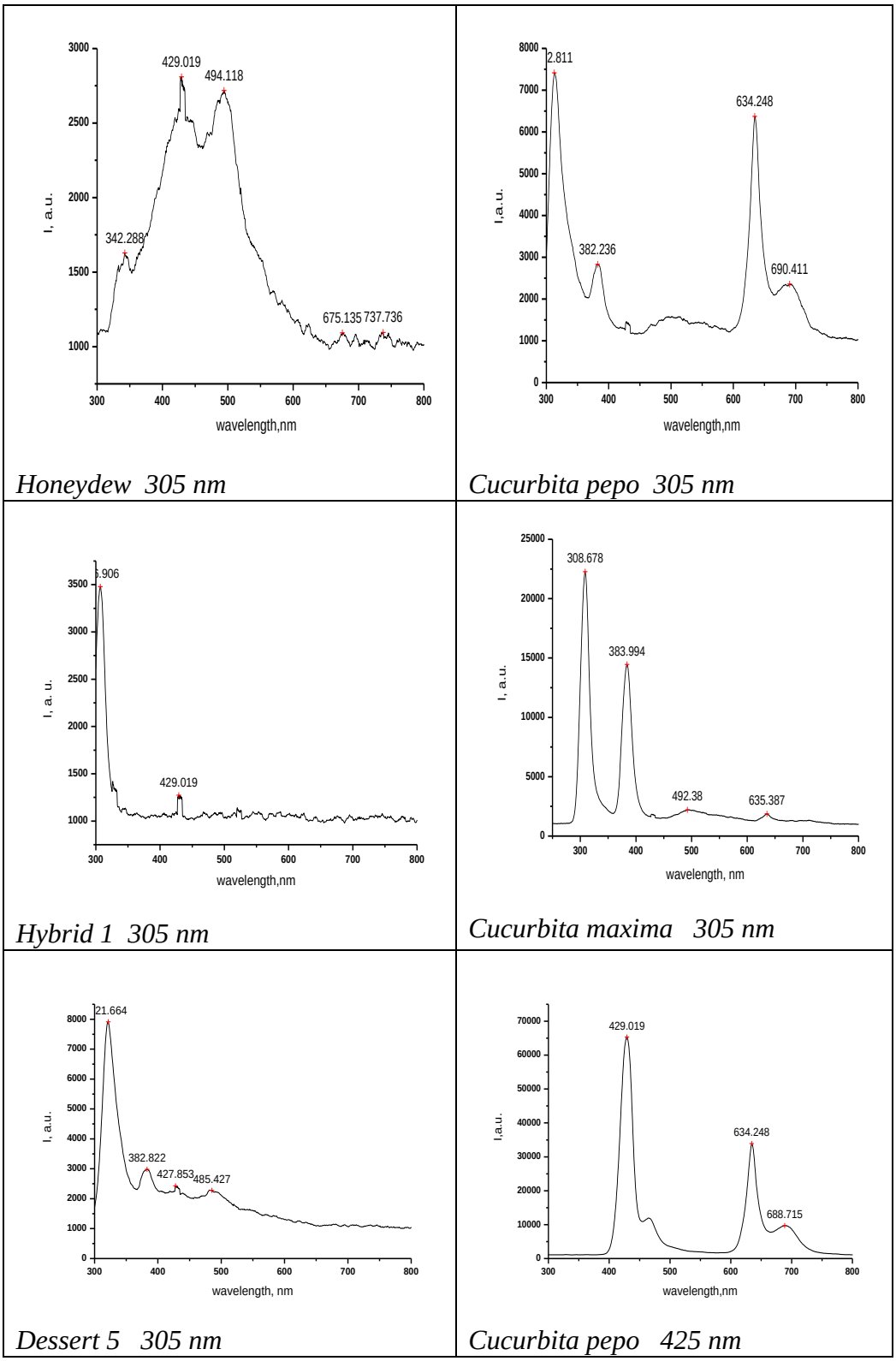

Fig. 1. The fluorescence spectra in the ultraviolet and visible ranges of oils from seeds of Bulgarian sorts of pumpkin and melon.

On analyzing the obtained spectra the best ratio of the fluorescence to the intensity of the excitation source and most fluorescence peaks are obtained at λ = 305 nm and λ = 425 nm. Fig. 1 presents fluorescence spectra of the considered oil samples of pumpkin and melon seeds in the visible and UV ranges at the indicated wavelengths.

The intensive peak at about 342 nm is observed only for oil from melon seeds of the *Honeydew* sort, when it is excited by a LED at λ = 305 nm.

The last fact can be explained with the highest total content of tocopherols from all oils obtained from different sorts of melons.

It is well known that the pigments of the chlorophyll groups are observed with oils extracted directly from seeds of different cultures prior to refining. Since melon oil of the *Honeydew* sort is the richest in chlorophyll (0.04 ppm), a strong fluorescence peak is observed at 675 nm when excited at 305 nm. For the rest of the melon oils no such peaks are observed because their chlorophyll content is comparatively lower (0.02 ppm). Similar fluorescence maxima have been observed for other vegetable oils such as rape seed, soy bean etc [20].

For all sorts of pumpkin oil chlorophyll content is 0.00 ppm and the observed fluorescence maxima around 634 nm and 688 nm are related to the existence of pigments [20] different from chlorophyll.

The maxima of fluorescence radiation are about 480 nm and 500 nm are related to the presence of oxidation processes in oils. From the intensity of this maximum it can be concluded that the presence of the oxidation products is the weakest for melon oil of the type *Hybrid 1*, followed by that of *Dessert 5*. Similar fluorescence maxima are between 500 nm and 520 nm for edible vegetable oils are found in [21]. According to the fluorescence maximum oxidation products exist in the pumpkin seed oil of the type *Cucurbita maxima*.

CONCLUSION

The melon seed oils have higher content of tocopherols than the pumpkin seed oils. The connection between tocopherol content, products of oxidations, chlorophyll, β-carotene and presence of pigments and the fluorescence maxima at different wavelength in UV-VIS were observed. It has been investigated small amounts of β-carotene and chlorophyll in these oils; the exception was the oil from *Cucurbita maxima* in which β-carotene was 1222.33 ppm. Because of the comparatively high content of tocopherols and lower content of oxidation products pumpkin and melon seed oils are very valuable sources of biologically active components and can be use for human consumption, added in foods and cosmetics. This is the first study about physicochemical characteristics of pumpkin and melon seed oils grown in Bulgaria and could take a place in further investigations on these oils and their application in food industries, cosmetics and medicine.

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ФИЗИКОХИМИЧНИ ХАРАКТЕРИСТИКИ НА МАСЛА ОТ СЕМЕНА НА БЪЛГАРСКИ СОРТОВЕ ТИКВА И ПЪПЕШ

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(Резюме)

Изследвани са токоферолния, каротеноидния и хлорофилния състав на семена от три сорта тиква (*Cucurbita moschata, Cucurbita pepo, Cucurbita maxima*) и три сорта пъпеш (*Медена роса*, *Десертен 5* и *Хибрид 1*), отгледани на територията на Южна България. Най-високо съдържание на токофероли е определено в маслата от *Cucurbita moschata* и *Медена роса* - 417 mg/kg и 828 mg/kg, съответно. Съдържание на хлорофил е открито в маслата от изследваните сортове пъпеш, но наличие на този пигмент в маслата от различни сортове тиква не е установено. В тиквеното масло от сорт *Cucurbita maxima* и в пъпешовото масло от сорт *Хибрид 1* са определени високи количества на β-каротен (1222.33 ppm и 35.97 ppm). Зелената компонента преобладава в маслата от всички изследвани сорта пъпеш, докато в маслата от тиква се наблюдават различия – от преобладаваща червена компонента в *Cucurbita pepo*, през по-слабо изразен червен цвят в *Cucurbita moschata* и до ясно доминираща зелена компонента в *Cucurbita maxima*. Получени са и флуоресцентните спектри при различни дължини на вълните във видимия (λ=370 nm, λ=395 nm, λ=425 nm) и в ултравиолетовия спектър (λ=305 nm). Представена е и зависимостта между флуоресцентния максимум при λ=384 nm и съдържанието на γ - токотриенол в маслата от семена на различните сортове тиква и пъпеш. Установено е и съществуването на зависимост между интензитета на флуоресцентния максимум при λ=675 nm и съдържанието на хлорофил. Други флуоресцентни максимуми са получени и при λ=634 nm, λ=688 nm, λ=480 nm и λ=500 nm, които са свързани с наличието на различни пигменти. От друга страна, тези максимуми са и индикатори за протичащи окислителни процеси в маслата.

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Fatty acid composition of lipids in the carp (*Cyprinus carpio* L.) grown in different production systems

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The fatty acid composition of lipids in carps grown in earthen ponds (Fish-Farming Experimental Facility in Trivoditsi village), natural waters (free aquatory of Bistritsa reservoir) and net-cages (cage farm situated in the same reservoir) was analyzed by gas liquid chromatography. The oleic C18:1, palmitic C16:0 and linoleic C18:2, ω -6 acids with percent shares of 27.73 %, 22.97 % and 13.60 %, prevailed in fats extracted from meat of carps grown in Trivoditsi pond, while in triglyceride fraction of carps grown in free waters of Bistritsa reservoir the percentages of the same acids amounted to 33.97 %, 18.70 % and 23.37 % correspondingly. The content of saturated fatty acids varied from 21.63% to 37.33 % and that of unsaturated ones from 62.67 % to 78.37 %. In lipids of carps grown in earthen ponds (Trivoditsi village) the level of polyunsaturated eicosapentaenoic acid (C20:5, ω-3) was higher than in two other investigated carp groups.

The technology of breeding influenced the fatty acid profile of carp lipids mainly by type of applied fodder and abundance of available planktonic organisms.

Key words: *Cyprinus carpio* L., lipids, fatty acid composition.

INTRODUCTION

Fish fats are distinguished by big diversity of fatty acids composing them. The content of unsaturated fatty acids varies from 70 to 83 % and that of saturated ones from 17 to 30 %. As a consequence of this the human body absorbs the fish fats very well. Particularly well presented are the acids with 18, 20 and 22 carbon atoms and the ω-3 fatty acids (FA) – linoleic, linolenic and arachidonic, which, being of highest biological value are marked as essential ones.

According to availability of single fatty acids the lipids extracted from meat of two-year-old carps are either from oleic-linoleic-palmiticoroleic-palmitic-linoleic type where by the content of saturated fatty acids ranged between 22.0 – 34.3 % and of unsaturated ones between 65.7 – 78.0 %. Hadjinikolova [1], Cirkovic and coworkers [2] and Trbovich and coworkers [3] in their comparative studies pointed out the effect of breeding technologies and type of applied fodder on meat quality and FA profile of carp lipids.

In relation to the foregoing the goal of this work is to study the FA composition of meat of carps grown in different production systems by three breeding technologies.

EXPERIMENTAL

The fatty acid composition of lipids in scaly carp (*Cyprinus carpio* L.) of market size bred under conditions of three different technologies, which characteristics are presented on Table 1 is studied.

The fish grown in earthen ponds of the Fish Farming Experimental Facility in Trivoditsi village is fed with sunflower meal and grain, while extruded fish fodder and grain – wheat and screenings in ratio 70:30 are fed to fishes in free aquatory of Bistritsa reservoir. The fishes of the third experimental group held in net - cages in the same reservoir are fed with extruded fish fodder with 25 % protein and 12 % fat content.

During the experiment in the period May-October 2012 the physicochemical characteristics of the aquatic environment are recorded on monthly intervals. The measured water temperature, pH and concentration of dissolved oxygen were within the technological norms for carp ponds ensuring proper gowning conditions.

For investigation a representative number of fishes are taken at random. The weight of single fishes caught in October 2012 varies between 810 – 1159 g. For analysis the individual samples are prepared from fish musculature (the lateral muscle) taken from one and the same position after removal of the skin and subsequent homogenization of the meat.

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Table 1. Characteristics of investigation production systems (sites).

Site/ Characteristics	Tri voditsi 2 Earthen pond No 10	Bistritsa reservoir Free aquatory	Bistritsa reservoir Net-cage farm
Area, dka	45	204	80
Volume, m^3	58 500	714000	320000
Depth , m	1.3	3.5	4.0
Degree of technology intensification	Semi-intensive carp rearing	Semi-intensive carp rearing	Intensive carp rearing

The fatty acid composition of lipids is determined by gas chromatography (GC) after transmethylation of the respective sample with 2 % H_2SO_4 in absolute CH_3OH at 50ºC [4]. Fatty acid methyl esters (FAME) are purified by thin-layer chromatography (TLC) on 20x20 cm plates covered with 0.2 mm silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane: diethyl ether (97:3, v/v). GC is performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatographer equipped with a 60 m x 0.25 mm (I.D.) x 25 μm (film thickness) capillary DB - 23 column (Agilent J&W advanced, Agilent Technology, USA) and a flame ionization detector. The column temperature is programmed from 130°C (1 min), at 6.5°C/min to 170°C, at 3.0°C/min to 215°C (12 min), at 40°C/min to 230°C (3 min); injector and detector temperatures are kept at 270°C and 280°C. Hydrogen is the carrier gas at a flow rate 0.8 mL/min; split was 1:50. Identification of fatty acids is performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [5]. The analytical standard of fatty acid methyl esters (SUPELCO F.A.M.E. Mix C4-C24, purity ~99 %) is from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and reagents are of analytical grade from Merck (Darmstadt, Germany) and are used without additional purification.

RESULTS AND DISCUSSION

The fatty acid composition data of lipids extracted from carp meat are presented in Table 2. The fatty acid composition of carps from earthen ponds in Trivoditsi village shows bigger shares for oleic C18:1 (27.73 %), palmitic C16:0 (22.97 %) and linoleic C 18:2, ω-6 (13.60 %) acids. The percentages of palmitoleic C16:1 (4.23 %), stearic C18:0 (7.37 %), linolenic C18:3, ω -3 (1.60 %), eicosapentaenoic C20:5, ω-3 (4.93 %), behenic C22:0 (3.73 %), and docosahexaenoic C22:6, ω-3 (6.12 %) acids are in the range from 1.60 % to 7.37 %. The percent shares of the remaining fatty acids are around or under 1.0 %. The level of unsaturated acids amounts to 62.67 % and of saturated ones to 37.33 % correspondingly. The content of monounsaturated fatty acids is in the range of 34.06 % and of polyunsaturated fatty acids is about 28.61 % correspondingly. The ratio of ω -6 to ω -3 polyunsaturated FAs is 1.24:1. According to availability of single FAs the lipids extracted from fish meat are from oleic-palmitic-linoleic type.

In the triacylglycerol fraction of fats extracted from meat of carps grown in the free aquatory of Bistritsa reservoir the percentages of oleic C18:1, linoleic C18:2, ω -6 and palmitic C16:0 acids are 33.97 %, 23.37 % and 18.70 %. The percent shares of palmitoleic C16:1 (3.77 %), stearic C 18:0 (5.53 %), linolenic C18:3 w-3 (3.03 %), eicosapentaenoic C20:5 w-3 (2.07 %) and docosahexaenoic C22:6 w-3 (2.40 %) acids vary in the range of 2.07 % to 5.53 %. The percentages of remaining fatty acids are around and under 1.0 %.

The content of unsaturated fatty acids (72.40 %) is 2.5 times more than that of saturated fatty acids (27.60 %) and the ratio between contents of monounsaturated to polyunsaturated fatty acids is 1.2:1. The total percentage of ω -6 polyunsaturated fatty acids is 25.20 %. The linolenic C18:2 acid with 23.37 % takes the biggest share from them. The content of ω -3 fatty acids is 7.50 %. They are presented by approximately equal quantities of linolenic C18:3 (3.03 %), eicosapentaenoic C20:5 (2.07 %) and docosahexaenoic C22:6 (2.40 %) acids. According to the shares of single fatty acids extracted from the fish meat the lipids are of oleic-linoleic-palmitic type.

The triacylglycerol fraction of lipids extracted from meat of carps grown in net-cages positioned in Bistritsa reservoir consists from three basic components - the oleic C18:1 (37.90 %), linoleic C18:2, ω-6 (28.10 %) and palmitic C16:0 (15.03 %) acids. The palmitoleic C16:1 (3.07 %), stearic C18:0 (4.73 %), linolenic C18:3, ω-3 (2.83 %), eicosenoic C20:1 (1.33 %), eicosapentaenoic C20:5, ω-3 (1.10 %) and docosahexaenoic C22:6, ω-3 (1.77 %) acids vary in the range from 1.10 % to 4.73 %. The content of the remaining fatty acids is under 1.0 %. The percentage of unsaturated fatty acids (78.37 %) prevails in the analyzed lipid samples. The percent of monounsaturated fatty

Table2. Fatty acid (FA) composition of analyzed carp samples, (n=3).

Fatty acids, % wt	Breeding facilities and technologies					
	Tri voditsi 2 Earthen pond No 10		Bistritsa reservoir Free aquatory		Bistritsa reservoir Cage farm	
	x ± Sx	Cv	x ± Sx	Cv	x ± Sx	Cv
C 8:0	0.13 ± 0.041	43.301	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
C 12:0	0.13 ± 0.041	43.301	0.10 ± 0.00	0.00	0.10 ± 0.00	0.00
C 14:0	1.40 ± 0.324	32.733	1.17 ± 0.163	19.795	0.90 ± 0.10	0.071
C 14:1	0.20 ± 0.00	0.00	0.10 ± 0.00	0.00	0.10 ± 0.00	0.00
C 15:0	0.57 ± 0.041	10.189	0.33 ± 0.147	62.450	0.17 ± 0.041	36.641
C 16:0	22.97 ± 1.145	7.052	18.70 ± 1.485	11.230	15.03 ± 1.219	11.470
C 16:1	4.23 ± 0.642	21.434	3.77 ± 0.531	19.926	3.07 ± 0.147	6.788
C 17:0	0.33 ± 0.082	34.641	0.23 ± 0.082	49.487	0.10 ± 0.00	0.00
C 17:1	0.47 ± 0.041	12.372	0.33 ± 0.082	34.641	0.17± 0.041	34.641
C 18:0	7.37 ± 0.426	8.182	5.53 ± 0.432	11.042	4.73 ± 0.414	12.379
C 18:1	27.73 ± 2.628	13.401	33.97 ± 1.809	7.531	37.90 ± 1.768	6.596
C 18:2 (ω-6)	13.60 ± 1.098	11.415	23.37 ± 2.780	16.823	28.10 ± 1.377	6.928
C 18:3 (ω-6)	0.23 ± 0.041	24.744	0.37 ± 0.041	15.746	0.47 ± 0.041	12.372
C 18:3 (ω-3)	1.60 ± 0.255	22.535	3.03 ± 0.294	13.725	2.83 ± 0.294	14.694
C 20:0	0.10 ±0.00	0.00	0.13 ± 0.041	43.301	0.10 ± 0.00	0.00
C 20:1	1.23 ± 0.082	9.362	1.30 ± 0.245	26.647	1.33 ± 0.286	30.311
C 20:2 (ω-6)	1.00 ± 0.00	0.00	0.73 ± 0.041	7.873	0.50 ± 0.071	20.000
C 20:3 (ω-3)	0.10 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
C 20:4 (ω-6)	1.03 ± 0.041	5.587	0.73 ± 0.082	15.746	0.73 ± 0.041	7.873
C 20:5 (ω-3)	4.93 ± 0.817	23.406	2.07 ± 0.535	36.638	1.10 ± 0.187	24.052
C 22:0	3.73 ± 0.349	13.213	1.10 ± 0.510	65.556	0.30 ± 0.071	33.333
C 22:1	0.20 ± 0.00	0.00	0.23 ± 0.108	65.465	0.30 ± 0.00	0.00
C 23:0	0.10 ± 0.00	0.00	0.11 ± 0.071	47.141	0.07 ± 0.01	14.286
C 24:0	0.50 ± 0.082	20.377	0.20 ± 0.071	50.000	0.13 ± 0.041	43.301
C 22:6 (ω-3)	6.12 ± 1.824	41.381	2.40 ± 0.534	31.458	1.77 ± 0.634	50.733
Saturated FA	37.33 ± 1.735	6.574	27.60 ± 2.839	14.547	21.63 ± 0.909	5.944
Unsaturated FA	62.67 ± 1.735	3.916	72.40 ± 2.839	5.546	78.37 ± 0.909	1.641
Monounsaturated FA	34.06 ± 3.051	12.702	39.70 ± 1.243	4.428	42.87 ± 1.559	5.145
Polyunsaturated FA	28.61 ± 1.344	6.620	32.70 ± 1.598	6.913	35.50 ± 0.951	3.790
Σω-6	15.86 ± 1.064	9.482	25.20 ± 2.807	15.734	29.80 ± 1.310	6.215
Σω-3	12.75 ± 2.452	28.578	7.50 ± 1.227	23.133	5.70 ± 0.367	9.116
Ratio Σω-6:Σω-3	1.24 ± 0.354	35.714	3.36 ± 1.105	43.390	5.23 ± 0.535	14.377

acids (42.87 %) is higher than of polyunsaturated ones 35.50 %. In the composition of polyunsaturated fatty acids the percent of ω-6 fatty acids (29.80 %) is higher at the expense of the ω-3 fatty acids (5.70 %) whereby the ratio ω-6:ω-3 polyunsaturated fatty acids is 5.23. According to the shares of single fatty acids extracted from the fish meat the lipids are of oleic-linoleic-palmitic type.

The data analysis shows, that three basic components of triacylglycerol fraction of carps grown under conditions of three breeding technologies are palmiticacid C16:0 (from 15.03 % net-cages in Bistritsa reservoir to 22.97 % earthen ponds in Trivoditsi village); oleic acid C18:1 (from 27.73 % earthen ponds in Trivoditsi villageto 37.90 % cages in Bistritsa reservoir) and linoleicacid C18:2 ω -6 (from 13.60 % earthen ponds in Trivoditsi villageto 28.10 % net-cages in Bistritsa reservoir).

It is proved that according to shares of single fatty acids the lipids extracted from the meat of two-year-old carps are of oleic-linoleic-palmitic type (in free aquatory and cages of Bistritsa reservoir) and of oleic-palmitic-linoleic- type (in earthen pond in Trivoditsi village), where by the content of saturated fatty acids varies in the range 21.63 - 37.33 % and of unsaturated ones between 62.67 - 78.37 %. The individual fatty acid profile is affected by applied fodders in different breeding technologies. The obtained results are comparable with those reported by Hadjinikolova [1] in her

investigation of fatty acid composition of the carp. They also are consistent with the investigations of Cirkovic and coworkers [2] and Trbivic and coworkers [3] proving the influence of breeding technologies and type of applied fodder on fatty acid profile of carp lipids.

According to the guidelines of World Health Organization it is assumed that raw materials and natural products with ω-6:ω-3 fatty acid ratios lower than 5.0 are of low risk for human health. To this group belongs the meat of fishes reared in the earthen pond No 10 in Trivoditsi village and in the free aquatory of Bistritsa reservoir, while the ratio of fish group from net-cages of Bistritsa reservoir is 5.23.

The lipids of carp grown in earthen type ponds (Trivoditsi village) have relatively high percentage of polyunsaturated eicosapentaenoic C20:5, ω-3 (4.93 %), which content is 2-12 times higher than in two other investigated carp groups. Most probably thisis due to the good natural food basis of the pond and good trophic level of zooplankton, which are properly supplementing the fish diet of fodder (sunflower meal and grain). This fact proves that breeding technologies including good development of planktonic organisms favor synthesis of eicosapentaenoic acid C20:5 ω-3, what is consistent with the studies of Mráz and coworkers [6].

CONCLUSION

The breeding technology affects the fatty acid profile of carp lipids mainly by type of applied fodder and degree of development of planktonic organisms.

The individual fatty acid profile of carp lipids from investigated groups is of oleic-linoleic-palmitic and oleic-palmitic-linoleic- type. The content of saturated fatty acids is in the range 21.63 - 37.33 % and of unsaturated ones - 62.67 - 78.37 %.

It is proved that the percentage of polyunsaturated eicosapentaenoic acid C20:5, (ω-3) in lipids of carp grown in earthen type ponds (Trivoditsi village) is several times higher than its content in the other investigated carp groups coming from free aquatory and net-cage farm in Bistritsa reservoir.

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МАСТНОКИСЕЛИНЕН СЪСТАВ НА ЛИПИДИ ОТ ШАРАН (*CYPRINUS CARPIO* L.) ОТГЛЕЖДАН В РАЗЛИЧНИ ПРОИЗВОДСТВЕНИ СИСТЕМИ

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(Резюме)

Анализиран е мастнокиселинният състав на липиди, изолирани от шаран, отглеждан в землени басейни (рибно-експериментална база в с. Три водици), в свободна акватория на язовир „Бистрица" и в садковите установки на същия язовир чрез газова хроматография. Олеиновата $C_{18:1}$ (27,73%), палмитиновата $C_{16:0}$ (22,97 %) и линоловата $C_{18:2}$, ω-6 (13,60 %) киселини преобладават в мазнината от месото на шаран, отглеждан в басейна на с. Три водици, докато в триацилглицероловата фракция, изолирана от шаран, отглеждан в язовир „Бистрица" количеството на тези киселини е съответно 33,97 %, 18,70 % и 23,37 %. Количеството на наситените мазнини варира от 21,63% до 37,33 %, а на ненаситените мастни киселини от 62,67 % до 78,37 %. В липидите от шаран, отглеждан в в басейна на с. Три водици количеството на полиненаситената ейкозапентаенова киселина ($C_{20:5}$, ω-3) е по-високо в сравнение с това в липидите на шарани, отглеждани в другите две производствени системи.

Технологията на отглеждане, която включва основно вида на фуража, използван за храна и вида на наличните планктонни организми оказва влияние върху мастнокиселинния състав на липидите, изолирани от месото на шарани, отглеждани в различни производствени системи.

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Physical and physico-chemical parameters of Greek cheeses

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Some physical and physico-chemical parameters of 15 different Greek cheeses were assessed. Cheeses differed as to the origin (cow, sheep and goat), they had different texture (soft, semi-hard and hard) and they were from different regions in Greece. The aw values in observed samples ranged from 0.799 (in semi-hard cheese Kefalotiri) to 0.889 (in soft cheese Katiki Domokou); while the average value of water activity in the samples was 0.851. Water content of the samples was 24.0 to 73.6 %. Dry matter in cheese thus constituted from 26.4 to 76 %. Fat content in dry matter was from 34.27 to 62.11 %. NaCl concentration in cheeses ranged from 0.82 over 5.15 %. Colour data for the monitored cheeses were analyzed colorimetrically and expressed by CIElab colour range as parameters L*, a*, b*.

Keywords: Greek cheese, water activity, pH, dry matter, fat, NaCl, colour.

INTRODUCTION

Cheeses include the traditional Greek foods and their production has a long tradition and describes it already Homer [1]. Greeks in cheese consumption ranks third in the world (behind France and Iceland) with a consumption of 23.4 kg per capita per year (it is more than twice as Slovakia with 10.3 kg per capita) [2]. Greek terrain and climate conditions are suitable for breeding sheep and goats as well as cattle, which also contribute to the wide variety of produced cheeses [3]. The most popular Greek cheese is a soft white cheese feta, which is ripened and stored in brine. According to Greek standards [4] only pure sheep‘s milk or a mixture of sheep‘s and goat‘s milk may be used for the feta cheese production. (usually up to 30 % goat‘s milk in the mixture.) It is well known that as Greek cheese and the name of feta is included in the register of protected denomination of origin (PDO) of the European Union.

In general, the quality of the cheese is affected by a number of parameters and the most primarily evaluates the basic composition: water content, fat content, pH, salt content and water activity. Water in cheese plays a relevant role for the curd consistence and the bacterial metabolism, and consequently for the processes during cheese ripening. The influence of the water content and the water activity on the cheese quality is very complex. Cheese contains beside high molecular proteins also low molecular compounds which are partly produced during ripening or as in the case of NaCl, are added during manufacturing. The low molecular soluble compounds (especially sodium chloride) have the biggest influence on the water activity in cheese [5-7].

Determining the values of water activity, pH and salt content belong to relevant data necessary for assessing the health safety and quality of the cheese [8-11].

The fat content in cheese is an important parameter, because in addition to significantly affect the sensory quality of the cheeses [12] involved also their thermo-physical properties (meltability, flowability, stretchability and oiling-off) especially in semi-hard cheese [13].

Natural colour of fattiest cow’s cheeses, and those from cows grazed on open pasture, tend to be yellower than cheese made from winter milk because of beta carotene. Beta carotene is a fat-soluble yellow pigment contained in grass. Some cheeses made from other animals’ milk are white because they don't store beta carotene in their fat the way cows do, but they convert it to colourless vitamin A [14]. One of validation criterion for milk products made from sheep and goats’ milk can be also colour [15, 16]. Of variations in quality may also draw attention to changes in colour of the cheese.

The aim of presented work was to estimate some physical and physico-chemical properties of typical cheeses from the other countries together with our foreign students and to ascertain what the differences in those properties are.

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EXPERIMENTAL

Assessed were 15 kinds of different cheeses, which differed as to the origin (cow, sheep and goat), they were from different regions in Greece and had a different texture (soft, semi-hard and hard). Cheese samples were purchased commercially in Greece in the months of October-November 2013. Moved to Slovakia in travel refrigerator and kept in the original packaging, respectively, vacuum-packed till the analysis and stored in the refrigerator at appropriate temperatures.

The samples were determined by the value of water activity, pH, water content (moisture), fat content and salt concentration. Colour of cheeses was evaluated by colorimetric method. Two cheeses from each product were sampled, all samples were analyzed in triplicate and results were given as a mean value.

Water activity. Water activity of cheese was estimated by non-destructive method using the LabMaster-a_w apparatus with electro-resistive sensor (from Novasina, Switzerland).

For LabMaster instrument calibration set of originally humidity standards (SAL-T 11, 33, 53, 75, 90 and 97 %) was used. Finely chopped cheese samples were sequentially measured in duplicate in plastic containers (10 ml) along with preheating of the next sample. The resulting a_w value, the selected measurement temperature (25 °C) and time measurements were read from the device LCD display.

pH. pH values were measured with a sharp needle Sen Tix pH-electrode (WTW, Germany) directly in cheese. Combined glass/calomel electrode was thoroughly calibrated with two buffer solutions at pH 4.01 and 7.00. The measurement was repeated consecutively using three different test points. Between each measurement was the electrode wiped to remove cheese, soaked in ethanol/ether (50/50), rinsed with water and wiped.

Water content. The water content (moisture) in cheese was determined by the method of drying to a constant weight at 102 °C (± 2 °C) in the drying oven UFP 500 (Memmert, Germany). Grated cheese (5 g) (m_c) was uniformly distributed at the surface of aluminium dish containing sand (20 g) both being dried in the oven until constant weight (24 hours) at 102 ± 2 °C (m_1). The dish with cheese were then dried and weighed in the same conditions (m_2):

$$\text{Dry mater}\ (\%\ \text{cheese}) = \frac{m_2 - m_1}{m_c} \times 100$$

$$\text{Moisture}\ (\%\ \text{cheese}) = 100 - \text{dry matter}$$

Dry matter was determined in triplicate per sample [17].

*Fat content.*The fat content was determined by simple and rapid Gerber-Van Gulik method. Method is based on the digestion of proteins and non-fat components by concentrated sulfuric acid and the separation of fat from the aqueous phase after centrifugation in special glass butyrometers (Van Gulik). In the butyrometer was charged 3.0 g of grated cheese, than were added 3 ml of distilled water and 10 ml of sulfuric acid (density 1522 kg.m^3). The sample was dissolved in a water bath at 65 °C and 1 ml of amyl alcohol was added to aid the separation of fat and aqueous phases. The sample in butyrometer was homogenized by over-rotation until complete dissolution. Finally, more sulfuric acid was added in order to bring upper fat layer in the measurement zone, again tempered to 65 °C for 5 minutes and centrifuged at Gerber centrifuge for 5 minutes at 1200 rpm and 65 °C. After centrifugation, the weight percentages of fat present in the cheese were read on the butyrometer scale [18].

*NaCl.*The amount of NaCl was determined by Mohr argentometric method [19]. Grated cheese (10.0 g) was stirred in the mortar with 10 ml distillate water, transferred quantitatively into 100 ml volumetric flask and filled up to the mark. Homogenous solution was filtered. In the titrimetric flask was 10 ml of filtrate diluted with 30 ml water and 1 ml of potassium chromate indicator was added. Mixture was titrated with silver nitrate (c = 0.1 mol/litre) till orange colour appeared. The percentage mass fraction of sodium chloride content was calculated from the volume of the silver nitrate solution in millilitres according the formula:

$$\frac{w_{\text{NaCl}}}{\%} = 0.585 \cdot a \cdot f$$

(Where: a = ml of 0.1 mol/l $AgNO_3$; f = factor of 0.1 mol/l $AgNO_3$).

Colour. Colour selection of cheeses was measured by colorimeter CM 410 (Konica Minolta, USA) with C type of illumination and expressed by rectangular CIELab colour range as 3 parameters L*, a*, b* which can accurately characterize various shades of colour and brightness. In the space CIELab L* expresses the brightness values, a* and b* are the chromaticity coordinates. L*

Table 1. Mean value of water activity, pH and sodium chloride content in cheese.

№	Name	a_w	pH	NaCl, %
1	Graviera from Creta	0.821	5.580	2.46
2	Mastelo from Chios	0.871	6.350	2.22
3	Ladotiri from Mytilini	0.831	5.250	2.22
4	Feta from sheep	0.869	4.403	3.63
5	Feta from goat	0.863	4.325	3.63
6	Chaloumi monastiriou	0.881	5.574	1.58
7	Chaloumi from goat and sheep	0.863	5.648	3.04
8	Tiraki Tiniako	0.858	4.355	2.63
9	Kaseri from Mitilini	0.866	5.386	2.01
10	Touloumotyri	0.871	5.565	2.46
11	Dry Mizithra from Creta	0.840	5.216	2.45
12	Pecorino Karali	0.805	5.616	4.83
13	Gaviera from Tinos	0.835	5.665	3.25
14	Kefalotiri Karali from Epirus	0.799	5.366	5.15
15	Katiki Domokou	0.889	4.317	0.82

takes the value from 0 to +100 (from black to white). The values of a* and b* range from –60 to + 60, with –a*direction is greenish, +a* represent red direction, –b* is the blue direction and +b* direction is yellow. H° gives hue angle degree in CIE-LCH colour range [20].

RESULTS AND DISCUSSION

For analyzes have been used two samples of 15 different studied Greek cheeses and each one was estimated in triplicate. Mean values of water activity, pH and sodium chloride are given in Table 1. Determining the values of water activity, pH and salt content belong to relevant data necessary for assessing the health safety and quality of the cheese. Water activity is an important parameter in food technology optimisation to provide microbiological, chemical and physical stability of food. The a_w values in observed samples ranged from 0.799 (in semi-hard cheese Kefalotiri) to 0.889 (in soft cheese Katiki Domokou); while the average value of water activity in the samples was 0.851. The pH of the samples ranged from 4.317 to 6.350. NaCl concentration in cheeses ranged from 0.82 over 5.15 %. Most of the samples (73.3 %) had salt content between 2.0 and 4.0 %. (In two samples was higher salt content in the two lower.) In the sample with the lowest salt content and the highest a_w value was, in contrast, the lowest pH that just provides health safety of product [11].

Results presenting means levels of the water content, dry matter and fat content in the tested samples are shown in Table 2. Water content (moisture) in cheese ranged from 24.0 to 73.6 %. Dry matter in cheese thus constituted from 26.4 to 76 %. Presence of fat in the samples ranged between 12.0 - 43.5 %. Also fat content in cheese dry matter was calculated and expressed in the result table. Fat content in dry matter was from 34.27 to 62.11 %.

Table 2. Mean values of moisture, dry mater and fat content in cheese.

№	Name	Dry matter, %	Moisture, %	Fat, %	Fat in dry matter, %
1	Graviera from Creta	64.2	35.8	22.0	34.27
2	Mastelo from Chios	61.6	38.4	32.0	51.95
3	Ladotiri from Mytilini	76.0	24.0	43.5	57.24
4	Feta from sheep	48.4	51.6	25.0	51.65
5	Feta from goat	48.4	51.6	30.0	61.98
6	Chaloumi monastiriou	58.8	41.2	28.0	47.62
7	Chaloumi from goat and sheep	56.2	43.8	27.0	48.04
8	Tiraki Tiniako	62.6	37.4	37.5	59.90
9	Kaseri from Mitilini	62.0	38.0	30.5	49.19
10	Touloumotyri	56.8	43.2	31.5	55.46
11	Dry Mizithra from Creta	64.4	35.6	40.0	62.11
12	Pecorino Karali	65.0	35.0	31.5	48.46
13	Gaviera from Tinos	66.0	34.0	30.0	45.45
14	Kefalotiri Karali from Epirus	66.2	33.8	32.5	49.09
15	Katiki Domokou	26.4	73.6	12.0	45.45

Table 3. Colour parameters of inner parts in assessed cheese samples.

№	Name	L*	a*	b*	ΔL	H°
1	Graviera from Creta	80.01	-3.46	30.67	0.41	96.44
2	Mastelo from Chios	87.21	-3.71	18.74	0.02	101.20
3	Ladotiri from Mytilini	87.61	-5.76	28.67	0.21	101.36
4	Feta from sheep	91.34	-2.44	14.47	0.32	99.57
5	Feta from goat	94.08	-2.50	11.91	0.25	101.86
6	Chaloumi monastiriou	90.93	-4.09	25.63	0.12	99.07
7	Chaloumi from goat and sheep	88.74	-2.77	18.28	0.08	98.62
8	Tiraki Tiniako	92.76	-1.86	18.79	0.06	95.65
9	Kaseri from Mitilini	79.87	-3.19	25.16	0.25	97.23
10	Touloumotyri	92.36	-4.21	17.23	0.21	102.73
11	Dry Mizithra from Creta	82.45	-2.83	24.06	0.43	96.71
12	Pecorino Karali	84.87	-3.77	22.21	0.39	99.63
13	Gaviera from Tinos	82.27	-1.75	21.72	0.13	94.61
14	Kefalotiri Karali from Epirus	87.57	-1.55	13.63	0.06	96.47
15	Katiki Domokou	93.95	-3.75	14.88	1.64	104.15

The colour characteristics of all samples were monitored in the inner part of cheeses. Mean L*, a*, b* parameters of the cheeses are presented in Table 3. Colour of cheese depends on type of used milk and fat content. L* parameter values ranged in 79.87 - 94.08. Standard deviations (ΔL) ranged in individual samples in 0.02 - 1.64. Intensity of greenish was expressed in a* values from -5.76 to -1.55. Values of the parameter b*, which reflects the intensity of the yellow colour in assessed cheeses is ranged in 11.91- 30.67. Hue angle degree H° ranged in 94.61- 104.15. The measured results can be used when comparing different technologies in cheese production or innovation as it was used, for example by Clareto et al. for comparing cheeses in which the fat was partially substituted with other ingredients [21]. The colour of the cheese surface and of the cheese inner parts is usually characteristic. During cheese ripening L*, a*, b* values did not change significantly [22].

When comparing the measured results with our other work in which we analyzed various Slovak cheeses it can be concluded, that most different were water activities. Greek cheeses a_w value were generally lower compared with similar Slovak cheese samples [23]. The aim of presented work was to estimate some physical and physico-chemical properties of typical cheeses from the other countries together with our foreign students and to ascertain what the differences in those properties are. The first work of this group is now presented, which is dedicated to the most important and specific Greek cheese.

Different 15 kinds of Greek cheese samples were determined by the value of water activity, pH, water content (moisture), fat content and salt concentration. Colour of cheeses was evaluated by colorimetric method. Each determination was carried out in triplicates and results were given as a mean value.

When comparing the measured results with our other work in which we analyzed various Slovak cheeses it can be concluded, that most different were water activities. Greek cheeses a_w value were generally lower compared with similar Slovak cheese samples.

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ФИЗИЧНИ И ФИЗИКОХИМИЧНИ ПАРАМЕТРИ НА ГРЪЦКИ СИРЕНА

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(Резюме)

Оценени бяха някои физични и физико-химични параметри на 15 различни вида гръцки сирена. Сиренатa се различаваха по произход (краве, овче и козе), те се характеризираха с различна текстура (меко, полу-твърдо и твърдо) и бяха от различни райони на Гърция. Стойностите на водната активност в изследваните образци вариpаше от 0,977 (в полутвърдото сирене Кефалотири) до 0,889 (в мекото сирене Катики Домокоу), а средната стойност на водната активност в образците беше 0,851. Водното съдържание на образците беше от 24,0 до 73,6 %. Така сухото съдържание съставлява от 26.4 до 76 %. Масленото съдържание в сухата фаза беше от 34,27 до 62,11 %. Съдържанието на NaCl вариpаше от 0,82 до 5,15 %. Цветните данни за наблюдаваните сирена бяха анализирани чрез колориметрия и изразени в CIElab цветна система чрез параметрите L*, a*, b*.

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Tocopherol composition of lipid in the carp (*Cyprinus Carpio* L.) grown in different production systems

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Tocopherol composition of lipids isolated from carp meat grown in different production systems was investigated by HPLC with fluorescence detection. The total quantity of tocopherols was found to be between 70 - 430 mg/kg. α-Tocopherol predominates in the lipids of carp breeding in earthen ponds (Fish-Farming Experimental Facility in Tri voditsi village-pond No 10; Fish-Farming "Tundzha 79" Ltd - pond No 4 and No 5), in free aquatic environment of Tzarimir 1 reservoir, Tzarimir 2 reservoir, "40 springs" reservoir, Bistritsa reservoir and in net-cages farm situated in the Bistritsa reservoir and in net-cages farm situated in the Kardzhali reservoir. In lipids from carps grown in Bistritsa reservoir and in net-cages situated in the same reservoir a significant amount of γ-tocopherol (65.0% and 38.0% respectively) was established. δ - Tocopherol was detected only in lipids from carp grown in earth pond of Fish-Farming Experimental Facility in Tri voditsi village while β - tocopherol was found in lipids from carp of the Bistritsa reservoir and net-cages situated in the same reservoir.

Keywords: *Cyprinus carpio* L.; fat soluble vitamins; tocopherols, HPLC

INTRODUCTION

The interest in the commercial fish species, subject of the fresh water aquaculture, is determined by their nourishment significance as protein food for people, as well as by the content of the full complex of essential amino acids, fatty acids, vitamins, macro and microelements in their meat. Fish fat is a main supplier of considerable quantities of important for the human organism vitamins, i.e. A, D, E, B_1, B_2, B_{12}, niacin (PP), which are involved in important processes in the human organism.

The lack of enough scientific information about the composition and content of tocopherols in the fats of the carp fish is the reason for this research. Thanks to its antioxidant properties, vitamin E prevents the development of atherosclerosis[1] and the destruction of erythrocytes, and provides the free inflow of oxygen into all cells of the human organism. The biologically active isomer of vitamin E - α-tocopherol acts as antioxidant protection of the membrane structures against to oxidation [2,3]. According to Bramley et al.[1] the antioxidant stability of vitamin E is due to the phenol hydroxyl group and the number of methyl groups of the aromatic ring. Among all isomers and analogues α-tocopherol is characterized with greatest biological activity [2].

In relation to the said by far, the purpose of this research is to determine the quantitative and qualitative composition of tocopherols of fats in the carp (*Cyprinus carpio* L.), grown in different production systems.

EXPERIMENTAL

The quantitative and individual tocopherol composition of fish fat in scaly carp (*Cyprinus carpio* L.) of market size bred in nine production systems, with different level of intensity and feeding, which characteristics are presented on Table1 is studied.

During the investigation in the period May-October 2012-2013 the physicochemical characteristics of the aquatic environment are recorded on monthly intervals. The measured water temperature, pH and concentration of dissolved oxygen were within the technological norms for carp ponds ensuring proper gowning conditions.

For the purposes of the study a representative number of fishes are taken at random principle of selection [3], from each production system, whereas the total processed number consists of 45 samples. The individual samples subject to analysis are

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Table 1. Characteristics of investigation production systems

Type of production systems	**Investigation production systems**								
	Earthen ponds -Fish -Farming			Reservoirs - free aquatory				Net-cages farm situated in reservoirs	
Sites	*Tri voditsi pond No 10*	*„Tundzha 79" pond No 4*	*„Tundzha 79" pond No5*	*"Bistritsa"*	*"Tzarimir 1"*	*"Tzarimir 2"*	*"40 springs"*	*"Bistritsa"*	*„Kardzhali"*
Area, dka	45	750	200	204	500	40	489	80	156
Volume, m^3	58 500	1125000	300000	714000	1250000	80000		320000	936000
Depth, m	1.3	1.5	1.5	3.5	2.5	2.0		4.0	6.0
Degree of technology intensification	Semi-intensive carp rearing	Intensive carp rearing	Intensive carp rearing	Semi-intensive carp rearing	Semi-intensive carp rearing	Intensive carp rearing	Extensive carp rearing	Intensive carp rearing	Intensive carp rearing
Feeds for fish feeding	Sunflower groats and grain	Extruded feed	Pelleted feed	Extruded feed and grain	Extruded feed and grain	Extruded feed	No feed	Extruded feed	Pelleted feed

prepared from the muscular tissue (lateral muscle) of the fish, from one and the same location by separating the skin with the subcutaneous fat and subsequent grinding and homogenization (meat).

The fat content of the fish was determined according to a Schmid-Bondzynski-Ratzlaff method [5]. Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows: mobile phase of n-hexane:dioxan (96:4, v/v), flow rate 1.0 mL/min, excitation 290 nm, emission 330 nm [6]. 20 μL 1% solution of oil in hexane were injected. The individual tocopherols were identified by comparing the retention times with those of standards (reference individual pure tocopherols – DL-α-, DL-β-, DL-γ- and DL-δ-tocopherol with purity ≥98%) purchased from Merck (Darmstadt, Germany). The content of tocopherols in the oils were calculated by comparing the peak areas obtained for the relevant tocopherol in the sample with those obtained for the standard solutions with known concentrations.

RESULTS AND DISCUSSION

The content of lipids in the tested carp fish from different production systems varies from 0.67% (pond No 5 in Fish-Farming "Tundzha 79") to 8.25% (net - cages farm situated in the Bistritsa reservoir) (Table 2). It can be seen that the fat content in the carp fish bred in floating net cages and in dams is higher than that of the carp fish bred in ponds. The obtained results about the fat content of the carp are similar to the data published in earlier researches (3.08 – 6.76%) [7-10], whereas in literature sources there are quoted even higher values for the total lipid content of the carp fish (12.74g/100g) [11].

The average values of the reported quantity of tocopherols (Table 2) in carp fish fat vary significantly from 70 mg/kg (Tzarimir 1 reservoir) to 430 mg/kg (Fish-Farm "Tundzha 79" – pond No 5). The comparison of the data that we have obtained with such by other authors [12,13] indicates that the carp fish fat contains considerably higher quantity of tocopherols, compared to other fish species, both sea and fresh water.

Table 2. Average weight, fats and tocopherols of fat in the carp

Parameters	**Investigation production systems**								
	Earthen ponds- Fish -Farming			Reservoirs - free aquatory				Net-cages farm situated in reservoirs	
	Tri voditsi pond No 10	*„Tundzha 79" pond No 4*	*„Tundzha 79" pond No5*	*"Bistritsa"*	*"Tzarimir 1"*	*"Tzarimir 2"*	*"40 springs"*	*"Bistritsa"*	*„Kardzhali"*
Weight, g	1159	989	1206	909	1469	2217	505	810	1533
Fats, %	0.74	1.03	0.67	2.45	5.43	5.10	1.03	8.25	5.03
Tocopherols, mg/kg	149	227	430	260	70	103	281	98	196

Table 3. Tocopherol composition of lipids in the carp meat

Tocopherols, %	Investigation production systems								
	Earthen ponds –Fish -Farming			Reservoirs - free aquatory				Net-cages farm situated in reservoirs	
	Tri voditsi pond No 10	*„Tundzha 79" pond No 4*	*„Tundzha 79" pond No5*	*"Bistritsa"*	*"Tzari-mir 1"*	*"Tzari-mir 2"*	*"40 springs"*	*"Bistritsa"*	*„Kardzhali"*
α-T	73.7	100.0	96.2	27.0	95.4	84.7	91.4	50.6	99.0
β-T	-	-		8.0	-	-	-	11.4	-
γ-T	10.2	-	3.8	65.0	4.6	15.3	8.6	38.0	1.0
δ-T	16.1	-	-	-	-	-	-	-	-

Legend: α-T – α- Tocopherol; β-T – β-Tocopherol; γ-T – γ- Tocopherol; δ-T – δ- Tocopherol

Stancheva et al.[12] inform that the vitamin E content in pike perch is about 0.5 mg/100g, while in herring is respectively 0.76 mg/100g. Stancheva et al.[13] provide values about the content of tocopherols in sprat fish and gobies about 284.85 ± 44.50 μg/100g, while for the rainbow trout 809.1 μg/100g. According Merdzhanova et al.[8] vitamin E content in bighead carp fillets is 1097.03±44.06 $\mu g.100g^{-1}$ww during the spring and 1051.80±37.11 $\mu g.100g^{-1}$ww during the autumn.

The recorded quantities of tocopherols in the carp fish fat ranging 103 - 430 mg/kg for some production systems give us the grounds to define this fish as a good source of vitamin E.

The content of the indentified classes of tocopherols (α-, β-, γ- и δ- tocopherol) is shown in Table 3.

In the tested production systems α-tocopherol varies from 27.0 % (free aquatory of Bistritsa reservoir) to 100% (Fish-Farming "Tundzha 79" - pond No 4). Content of β- tocopherol is found out only in the samples of fish fat from the free aquatory of Bistritsa reservoir, and net -cages situated in the same reservoir, with quantities from 8.0 % to 11.4 %. Presence of γ-tocopherol is found to be in all the studied production systems, with the exception of Fish-Farming "Tundzha 79", pond No 4. The quantity of γ- tocopherol varies from 1.0 % (net cages situated in the Kardzhali reservoir) to 65.0 % (free aquatory of Bistritsa reservoir). δ-Tocopherol was detected only in the lipids of carp fish bred in earth type pond No 10 of Fish-Farming Experimental Facility in Tri voditsi (16.1%).

The analysis of the data shows that the carp fish fats contain predominantly α-tocopherol and γ-tocopherol. In the seven of the researched fisheries (earthen ponds of Fish-Farming Experimental Facility in Tri voditsi and in Fish-Fatming "Tundzha 79"; Tzarimir 1 reservoir, Tzarimir 2 reservoir, „40 springs" reservoir; net cages in Kardzhali reservoir) α-tocopherol was detected in quantity over 70% of their total content. This shows that the studied fish fat is predominated by the biologically active α- tocopherol, so therefore the fish oil obtained from these fisheries may be classified as α- type. The carp fish lipids, isolated from the fish bred in free aquatory of Bistritsa reservoir are predominated by γ- tocopherol (65.0%) so the oil is classified as γ- type. In the carp fish lipids from fish bred in net cages situated in the same reservoir - the quantity of α-tocopherol is 50.6%, while of γ- tocopherol is 38.0%.

CONCLUSION

It has been found out that the carp fish fat is dominated by the contents of α-tocopherol and γ-tocopherol. For seven of the studied fisheries (earthen ponds of Fish-Farming Experimental Facility in Tri voditsi and in Fish-Fatming "Tundzha 79"; Tzarimir 1 reservoir, Tzarimir 2 reservoir, „40 springs" reservoir; net cages in Kardzhali reservoir) α-tocopherol comprise of over 70% of their total content.

The recorded quantities of tocopherols in the carp fish fat is within the range 103 - 430 mg/kg so it can be defined as good source of vitamin E for the human organism.

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ТОКОФЕРОЛОВ СЪСТАВ НА ЛИПИДИ ОТ ШАРАН (*CYPRINUS CARPIO* L.) ОТГЛЕЖДАН В РАЗЛИЧНИ ПРОИЗВОДСТВЕНИ СИСТЕМИ

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(Резюме)

Изследван е токфероловия състав на липиди, изолирани от месо на шаран (*Cyprinus Carpio* L.), отглеждан в различни производствени системи чрез високоефективна течно-течна хроматография. Общото съдържание на токфероли в липидите, изолирани от шараните варира от 70 до 430 mg/kg. α- Токоферолът доминира в липидите от шаран, отглеждан в земните басейни на рибно-експерименталната база в с. Три водици, басейн №10, рибовъдно стопанство „Тунджа 79", басейни №4 и №5; язовири „Царимир 1", „Царимир 2" и язовир „40 извора", както и в садковите установки на язовир „Бистрица" и язовир „Кърджали". В липидите от шаран, отглеждан в язовир „Бистрица" и в садките на същия язовир е установено значително количество на γ-токоферол (65,0% и 38,0% съответно). δ – Токоферол е установен само в липидите от шаран, отглеждан в басейн № 10 на рибно-експерименталната база в с. Три водици, а β – токоферол е установен в липидите на шаран, отглеждан в язовир „Бистрица" и в садките на същия язовир.

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Antimicrobial effect of encapsulated and non-encapsulated thyme essential oil

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Some plants contain functional compounds with antimicrobial activity. Phenols, polyphenols, micronutrients and essential oils belong to them. These bioactive compounds can be used against pathogenic and food spoilage bacteria. Thyme essential oil belongs to the plant material with a powerful antimicrobial activity. The aim of the study was to test the antimicrobial effect of thyme essential oil and polyethylene foil, which is coated with partially water soluble polymeric film containing encapsulated thyme essential oil on selected microorganisms with or without direct contact. *Escherichia coli*, *Candida tropicalis* and *Penicillium chrysogenum* were used for testing. It was ascertained the impact of encapsulated and non-encapsulated thyme essential oil on tested microorganisms.

Keywords*: Thyme, Essential Oil, Encapsulated Essential Oil, Polyethylene Foil, Antimicrobial Activity*

Abbreviations: EO – essential oil

INTRODUCTION

Some plants are rich in functional compounds as phenols, polyphenols, micronutrients or essential oils [1]. Different biological activities (as antioxidant, antifungal and antibacterial activities) were demonstrated for these compounds. Phenols influence enzyme activity, cause protein denaturation and cell membrane damages of its function or structure, which lead to loss of macromolecules [2]. Polyphenolic compounds have strong antimicrobial activity, which consist in modification of the morphology and disruption of the cell wall. Other phenolic compounds can stimulate DNA degradation [3].

Thyme (*Thymus vulgaris*, L.) belongs to plants with strong antimicrobial activity. The majority of *Thymus* oils are characterized by their rich content of monoterpenes, in particular the phenolic compounds thymol and its isomer carvacrol, accompanied by a range of other more or less biologically active compounds, including eugenol, p-cymen, γ-terpinen, α-pinen, linalool, geraniol and borneol [4,5]. Antimicrobial activity of thyme essential oil was demonstrated in a lot of studies. Thyme suppresses growth of *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli* and *Candida* sp. [5,6]. Thyme essential oil has significant colicid and colistatic properties and damages irreversible the *E. coli* O157:H7 cells within one minute [1,7].

In this study, the antimicrobial effect of encapsulated and non-encapsulated essential oil was tested against selected bacterium, yeast and mould.

EXPERIMENTAL

Microorganisms, which were used for analyses, were *Escherichia coli* CCM 7929, *Candida tropicalis* CCM 8223 and *Penicillium chrysogenum* CCM 8034. 24 hours culture of *E. coli*, which was cultivated in TSB (Biokar Diagnostics, France) at 37 °C, was centrifuged (20 min., 3000 rpm), rinsed with saline and once more centrifuged (20 min., 3000 rpm). A solution of density 1 McF was prepared, which was diluted to density 0.1 McF. 72 hours culture of *Candida tropicalis* and 120 hours culture of *Penicillium chrysogenum*, which were cultivated on Chloramphenicol Glucose Agar (Biokar Diagnostics, France) at 25 °C, were transferred into wells with sterile saline and carefully mixed. Then the solution of density 1 McF was prepared. Solution was diluted to density 0.1 McF.

Sliced cheese (Albert Quality 30 % TVS) was used for analyses with non-encapsulated thyme

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essential oil. One slice was divided into quarters, which were placed in Petri dishes and exposed to UV radiation for 45 minutes. Non-encapsulated thyme essential oil (Manipura, Czech Republic) was used undiluted (concentration 1) and diluted by adding 6 µl into 1 ml of methanol (concentration 2). The sterile paper disc (diameter 9 mm) was saturated with 30 µl of essential oil of appropriate concentration. Disc saturated with methanol was used as a control.

Polyethylene foil, which is coated with partially water soluble polymeric film containing encapsulated thyme essential oil, was used for analyses with encapsulated thyme EO. Samples of this foil with different concentration of EO (variants 1 – 5) were divided into 2x2 cm squares and exposed to UV radiation for 45 minutes. Concentrations of EO are stated in Table 1.

Table 1 Concentration of thyme EO on polyethylene foil

Variant	Weight, %
1	1.6
2	3.5
3	5.8
4	8.6
5	10.3

Volume 0.1 ml of microbial culture of density 0.1 McF was inoculated on VRBL Agar (*E. coli*), on Chloramphenicol Glucose Agar (*C. tropicalis*, *P. chrysogenum*) or on cheese. Squares of tested foil or discs with EO were attached on the inside part of lids of Petri dishes by double-faced tape. In the second variant, squares of foil were placed directly on the surface of nutrient medium. All these variants were prepared in triplicate. Petri dishes were incubated at 37 °C for 24 hours (*E. coli*), at 25 °C for 24 (*C. tropicalis*) or 48 hours (*P. chrysogenum*). After cultivation, the diameters of inhibitory zones were evaluated with a ruler.

RESULTS

There are not any visible changes after 24 hours on slices of cheese when testing thyme essential oil efficacy against *E. coli*. Any zones of inhibition were not observed in Petri dishes with foil without direct contact. In the case of *C. tropicalis* and *P. chrysogenum*, there were not created clear zones of inhibition. The evaluation was therefore reduced on appraisal of growth of yeasts or moulds on the whole cheese surface. Average sizes of diameters of inhibitory zones or intensity of microbial growth are shown in Tables 2, 3 and 4.

Concentrated essential oil had considerable inhibitory effect on *E. coli* on nutrient medium. Encapsulated essential oil was effective at all concentrations with increasing tendency with increasing concentration.

Candida tropicalis was inhibited almost by all EO concentrations in all variants of experiment. Variants of foil 1 and 2 were the exceptions, where there was no reduction in growth of *C. tropicalis*. It was observed an increase in diameters of inhibitory zones or in growth intensity with increasing concentration of essential oil.

Non-encapsulated EO was effective in concentrated form, diluted EO showed no antifungal activity on cheese surface. On nutrient medium, there was significant difference between efficacy of diluted and concentrated EO. Encapsulated EO inhibited growth of *P. chrysogenum* by direct contact in all variants. Essential oil without direct contact suppressed growth of *Penicillium* only in variants 4 and 5, so only at higher concentrations.

Table 2. Impact of thyme EO on *E. coli*

Sample	EO	Encapsulation	Culture Medium	Diameter [mm]
1	Conc.	No	Nutrient Medium	24.67
2	Diluted			0.00
3	Control			0.00
4	V. 1	Yes	Nutrient medium Direct contact	24.33
5	V. 2			30.00
6	V. 3			36.67
7	V. 4			50.00
8	V. 5			48.33

V. 1 – V. 5 – Variants of polyethylene foil with various concentrations of thyme EO
Conc. – Concentrated EO
Diameter – Average size of the identified zones of inhibition

Table 3. Impact of thyme EO on *C. tropicalis*

Sample	EO	Encapsulation	Culture Medium	Growth Intensity / Diameter [mm]
9	Conc.	No	Cheese	+
10	Diluted			++
11	Control			+++
12	Conc.	No	Nutrient Medium	38.33
13	Diluted			10.00
14	Control			0.00
15	V. 1	Yes	Nutrient Medium Direct Contact	20.00
16	V. 2			23.67
17	V. 3			30.00
18	V. 4			35.00
19	V. 5			35.33
20	V. 1	Yes	Nutrient Medium Without Direct Contact	0.00
21	V. 2			0.00
22	V. 3			10.67
23	V. 4			35.00
24	V. 5			36.67

+ Low growth intensity
++ Medium growth intensity
+++ High growth intensity

Table 4. Impact of thyme EO on *P. chrysogenum*

Sample	EO	Encapsulation	Culture Medium	Growth Intensity / Diameter [mm]
25	Conc.	No	Cheese	+
26	Diluted			+++
27	Control			+++
28	Conc.	No	Nutrient Medium	53.33
29	Diluted			18.33
30	Control			0.00
31	V. 1	Yes	Nutrient Medium Direct Contact	20.00
32	V. 2			20.00
33	V. 3			20.00
34	V. 4			27.67
35	V. 5			26.00
36	V. 1	Yes	Nutrient Medium Without Direct Contact	0.00
37	V. 2			0.00
38	V. 3			0.00
39	V. 4			20.00
40	V. 5			20.00

DISCUSSION

Susceptibility of tested microorganisms was dissimilar. In experiment with cheese, *C. tropicalis* was inhibited by EO at most. Reduce in growth was observed even by application of diluted essential oil. Important antifungal activity was proved in [5,8,9]. Non-encapsulated EO was more effective when microorganisms were cultivated on nutrient medium than on cheese. In this case, bigger zones of inhibition were observed. *P. chrysogenum* was the most susceptible; *E. coli* was inhibited at least. All the tested microorganisms were suppressed by encapsulated EO. The greatest diameters of

inhibitory zones were detected in direct contact of encapsulated essential oil with microorganisms. *E. coli* was the most susceptible to the direct contact with EO, inhibition zones measured from 22 to 50 mm. Burt and Reinders (2003) [7] and Imelouane et al. (2009) [10] ascertained that thyme inhibits wide range of microorganisms, including *E. coli*. *P. chrysogenum* was not so much repressed. Inhibition zones were from 20 to 30 mm. Encapsulated EO without direct contact was less effective. Antimicrobial activity was not proved against *E. coli*, there were no inhibitory zones. *P. chrysogenum* was susceptible to EO effects only at higher concentrations (variant 4 and 5). Size of inhibitory zone was 20 mm at both concentrations. *C. tropicalis* was susceptible to EO at variants 3, 4 and 5.

CONCLUSIONS

Thyme EO repressed all the tested microorganisms with different potency. Undiluted essential oil was naturally more effective than diluted EO, but even diluted EO inhibited visibly some tested microorganisms. Encapsulated EO had more considerable activity in direct contact with nutrient medium. The present results indicate that thyme has provable antimicrobial activity and could be used for repression of undesirable microorganisms and their elimination from food.

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АНТИМИКРОБИАЛЕН ЕФЕКТ НА ИНКАПСУЛИРАНО И НЕ-ИНКАПСУЛИРАНО ЕТЕРИЧНО МАСЛО ОТ МАЩЕРКА

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(Резюме)

Някои растения съдържат функционални компоненти с антимикробиална активност. Към тях спадат феноли, полифеноли, микроелементи и етерични масла. Биоактивните компоненти могат да се използват срещу патогени и бактерии, които водят до разваляне на храната. Етеричното масло от мащерка принадлежи към растителните продукти със силна антимикробиална активност. Целта на работата беше да се тества антимикробиалната активност на етерично масло от мащерка и полиетиленово фолио, което е покрито с частично водоразтворим полимерен филм, съдържащ инкапсулирано етерично масло от мащерка, върху подбрани микроорганизми при и без директен контакт. За тестването бяха използвани Еширихия коли (*Escherichia coli*), Кандида тропикалис (*Candida tropicalis*) и Пеницилиум хрисогениум (*Penicillium chrysogenum*). Констатирано е въздействието на инкапсулирано и неинкапсулирано етерично масло от мащерка върху тестваните микроорганизми.

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The reduction of sodium chloride in Telemea cheese. Effect on textural and sensorial properties

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Sodium chloride is traditionally added to cheeses as a preservative and to improve flavor. Physicochemical analysis (sodium chloride, dry matter, fat, proteins, ash, pH and titratable acidity), textural analysis (hardness, adhesiveness, springiness, fracturability, chewiness, gumminess and cohesiveness) and sensorial properties (appearance and color, body and texture, flavor and acceptability) of Telemea cheese samples were investigated during 28 days of ripening. The reduction of sodium chloride had significant influence on cheese hardness, adhesiveness, springiness and gumminess but did not affect the sensorial characteristics of Telemea cheese.

Keywords. Telemea, sodium chloride, sea salt, texture profile analysis.

INTRODUCTION

Nowadays, health authorities recommend decreasing progressively salt content in food products, because an excessive sodium intake may be a cause of pathology. The main sodium source is the sodium chloride (salt) added during food processes or preparation of meals [1]. Salting is an important step in producing cheese, since it determines sensorial properties such as flavour, texture and colour, in addition to modifying microbial activity and producing physical changes in the proteins [2]. Texture is one of the most important characteristics of cheese that determines identity and acceptability. With this property the consumer first identifies and judges the specific variety. The textures of the various types of cheese are clearly very different, but factors that determine changes in texture are basically the same, since the components are the same for all cheese varieties. Only the proportions of the components differ. All the components of a cheese-protein, fat and water (brine) – affect its rheological behavior and therefore its textural properties [3]. Telemea cheese is a white-brined cheese, representing a category of cheeses that owe their characters primarily to a strong acidity and a high salt content. It originated in Romania and spread to other Balkan countries (e.g. Greece, Bulgaria, Turkey) [4]. In the present study, the effect of sodium chloride reduction on the textural and sensorial characteristics of cow milk Telemea cheese during ripening was investigated.

EXPERIMENTAL

Materials

Fresh, cow milk (≈ 20 L) for Telemea cheese production was purchased from a dairy factory from Galati, Romania. The CHOOZITMT1LYO10DC1 – starter culture (Danisco EZAL, France) was used to acidify the milk. This starter consists of: Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. As coagulant, it was used Fromase – Chr. Hansen 22000TL from Rhizomucor miehei with coagulation power Pc = 1:150.000. Calcium chloride was obtained from Chimcomplex S. A. (Bacau, Romania). Sodium chloride and sea salt were purchased from a local supermarket in Galati (Romania).

Cheese manufacture

The Telemea cheese was manufactured according to the classical method (Figure 1). Telemea cheese was ripened in 4 different brine solutions (variant A: 80% water + 20% NaCl; variant B: 80% whey + 20% NaCl; variant C: 80% water + 20% sea salt; variant D: 80% water + 13.2% NaCl + 6.8% KCl). Cheeses were sampled for analysis at the age of 1, 7, 14, 21 and 28 days.

Physicochemical analysis

The physical-chemical analysis was applied to: sodium chloride content (SR EN ISO 5943:2007),

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dry matter (SR EN ISO 5537:2005), fat content (SR EN ISO 1211:2010), proteins (SR ISO/TS 17837:2009), ash (SR EN ISO 707-2009), titratable acidity with NaOH 0.1 n, according SR 143:2008 and pH with a pH-meter InoLAB 730, after calibration with standard solutions of pH 4, 7 and 9. All analyses were performed in duplicate.

Texture profile analysis (TPA)

Texture measurements were performed at room temperature with a CT3 Texture Analyzer (Brookfield, UK). The samples (cylinder with 7 mm length and 12 mm depth) were compressed to 50 % of their original height with a cylindrical probe (TA11/1000) 25.4 mm diameter and a cross-head speed of 2.0 mm/s. The following parameters were determined: hardness, adhesive force, springiness, fracturability, chewiness, gumminess and cohesiveness. All analyses were performed in duplicate.

Sensory evaluation

Sensorial characteristics of the cheese after 28 days of ripening were carried out by five trained panel. The samples were presented to panelists in randomized order after having stood for 2 h at room temperature and were graded between 1 and 10 (1 being very bad and 10 being very good) for flavor, color, texture (hardness, adhesive force, springiness, fracturability, chewiness, gumminess and cohesiveness) and acceptability.

Statistical analysis

Two-way analysis of variance (ANOVA) was used for the comparison of the data obtained behind the sensorial analysis of each type of Telemea cheese. The level of significance was established at $P < 0.05$.

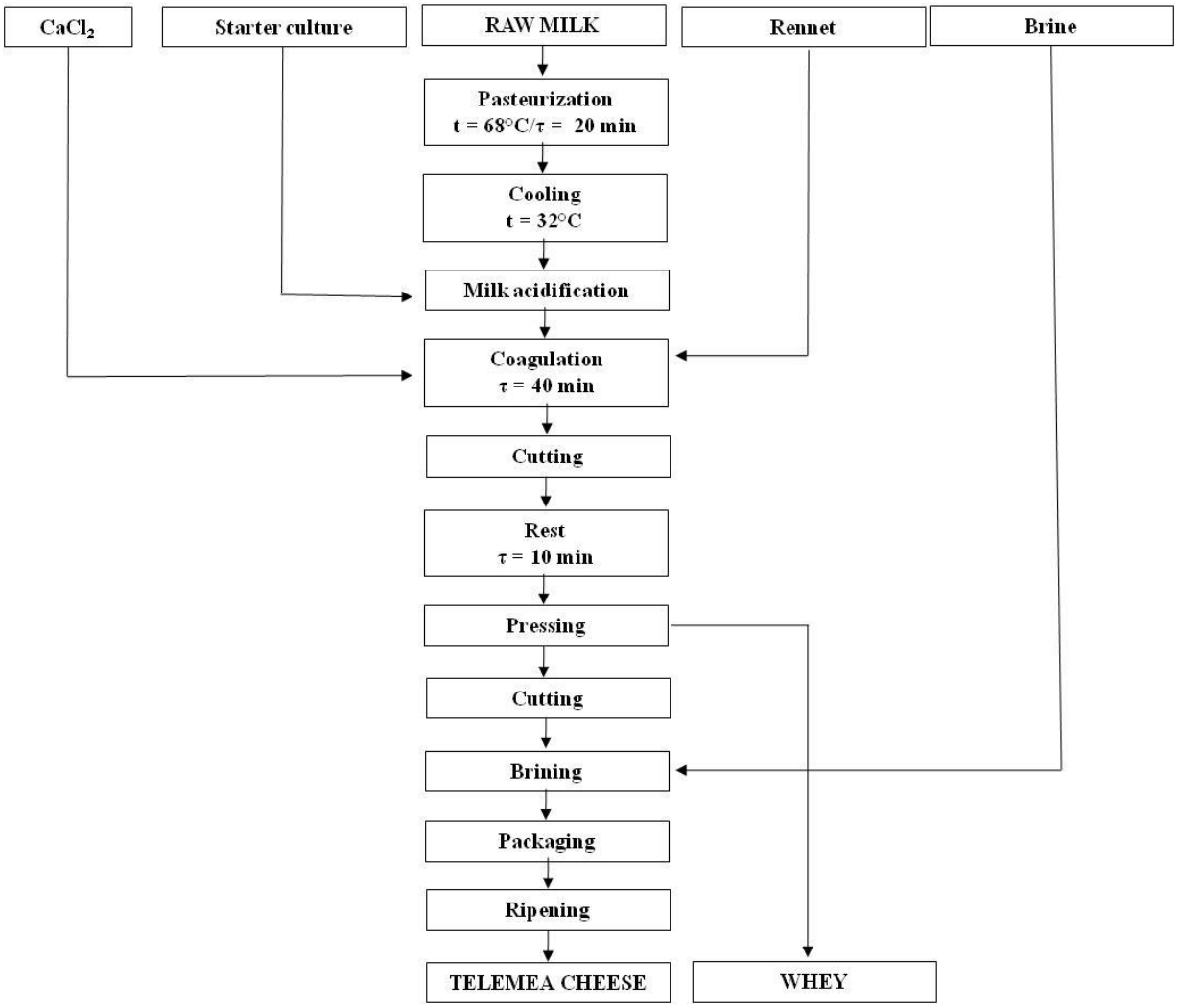

Fig. 1. Techological flowchart for manufacturing the Telemea cheese.

RESULTS AND DISCUSSION

Physicochemical characteristics

The chemical characteristics of cheese samples are reported in (Table 1). Dry matter content of the Telemea cheese samples decreased during ripening and was between 51.88% and 46.88% at the end of 28 days. When cheese is placed in brine, a dynamic mutual diffusion process is established as NaCl molecules move from the brine into the cheese while, water diffuses out through the cheese matrix [5].

Fat content of the Telemea cheese samples at the end of ripening period was between 19.01% and 20.86% and decreased gradually. Changes in fat content could be due to a decrease in dry matter and lipolytic activity. Protein content of the different Telemea cheese samples decreased during ripening and at the end of this period and was between 21.61% and 21.66%.

Decrease in protein content of Telemea cheeses throughout ripening was attributed to proteolysis activities, because proteolysis in Telemea cheese continues during ripening in brine. Titratable acidity of the sample increased throughout ripening period, while pH values decreased. The decrease in pH during the 28 days of curd preservation in brine is due mainly to completion of the lactose fermentation.

Evaluation of texture profile

Parameters revealed by texture profile analysis (TPA) are shown in Table 2. Hardness, that is a measure of the amount of force required to compress the Telemea cheese samples, continuously decreased as storage progressed probably due to breakdown of casein especially αs1 fraction in to lower molecular weight peptide and hydration of the protein matrix. Hardness was higher for cheese samples maintained in variant C of brine solution (80% water + 20% sea salt).

Fracturability values varied from 6.09 to 10.78 (N). These results are similar with the results obtained by Kandarakis [6]. Cohesiveness is the extent to which a cheese can be deformed before it ruptures [7]. Cohesiveness increased gradually during the 28 days of ripening. Finally, the lowest values of gumminess and chewiness were found for cheese sample maintained in brine solution B and D.

Table 1. Physicochemical composition of Telemea cheese samples during storage at 4 °C for 28 days.

Parameters	Telemea cheese			
	A	**B**	**C**	**D**
Dry matter, g/100g	51.88 ± 0.02	49.58 ± 0.05	46.88 ± 0.04	47.28 ± 0.05
Fat, g/100g	20.86 ± 0.05	20.05 ± 0.03	19.01 ± 0.04	19.12 ± 0.03
Protein, g/100g	21.65 ± 0.02	21.66 ± 0.02	21.61 ± 0.03	21.63 ± 0.02
Ash, g/100g	4.73 ± 0.01	6.31 ± 0.03	5.32 ± 0.01	5.68 ± 0.04
NaCl, g/100g	4.23 ± 0.03	4.54 ± 0.04	4.97 ± 0.08	4.01 ± 0.06
pH	4.45 ± 0.03	4.29 ± 0.05	4.35 ± 0.02	4.13 ± 0.02
Titratable acidity, °T	248.2 ± 0.07	249.8 ± 0.07	250.2 ± 0.03	251.4 ± 0.05

All values are mean ± standard deviation.

Table 2. Texture Profile Analysis in Telemea cheese after 28 days of storage.

Sample	Textural parameters						
	Hardness, N	Adhesive force, N	Springiness, mm	Fracturability, N	Chewiness, J	Gumminess, N	Cohesiveness, dimensionless
A	7.79±2.86	0.23±0.2	1.99±0.52	8.15±2.99	0.02±0.0	6.62±1.97	0.83±0.06
B	6.58±0.22	0.1±0.03	2.98±0.70	6.80±0.23	0.015±0.001	5.54±0.25	0.82±0.01
C	10.15±0.12	0.17±0.04	2.65±0.15	10.78±0.17	0.02±0.0	7.69±0.11	0.71±0.0
D	5.90±0.105	0.06±0.003	3.91±0.03	6.09±1.15	0.01±0.0	4.93±0.62	0.81±0.05

All values are mean ± standard deviation.

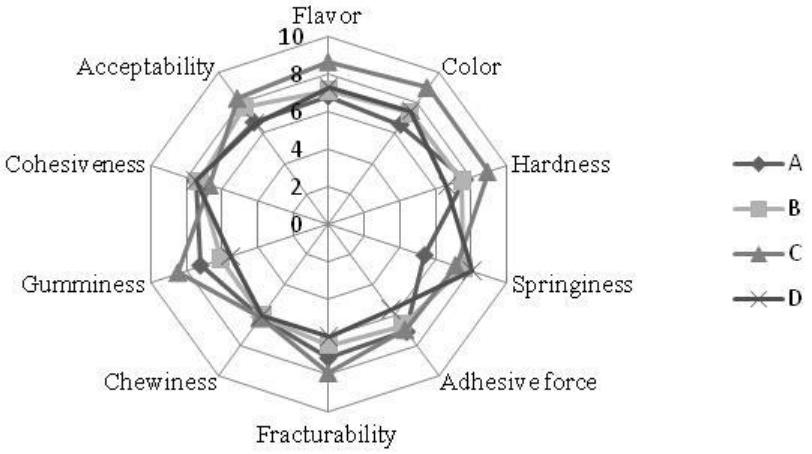

Figure 2. Graphical representation of sensory evaluation of Telemea cheese during storage at 4 °C for 28 days.

Sensory analysis

The results of sensorial analysis of Telemea cheese (Figure 2) showed that scores found for color, hardness, fracturability, chewiness and gumminess were significantly different among the evaluated cheeses. Higher average scores for sensorial characteristics were found for Telemea cheese sample C, which are also in accordance with the results of the instrumental analysis of texture. According to Delgado et al. [8], the flavor of cheeses depends on several reactions, especially the metabolism of lactose and lactate, lipolysis and proteolysis in the cheese matrix.

From the application of the statistical method two-way analysis of variance (ANOVA) (Table 3) it can be observed that between the cheeses samples there are significant differences ($P < 0.05$) expected due to the variability of the auxiliary materials. In the sensorial attributes case, the statistical analysis reveals that the panelists have perceived similar characteristics ($F < Fcr$) without significant differences in the assessment of cheese samples. The good correlation of data can be associated to the panelists experience in Telemea cheese tasting. The selected panelists being trained in this field.

CONCLUSIONS

Sodium reduction can be obtained by using a shorter brine time or a KCl brine. The sea salt adding influenced the textural parameters by increasing the hardness, fracturability, gumminess and cohesiveness values. The main results of the instrumental texture analysis are comparable to those of the sensory analysis. This study indicated that brine concentration had important effects on chemical composition and texture characteristics, but did not affect the sensorial quality of Telemea cheese. The statistical data suggest a good correlation and a correct valuation of the sensorial results.

Table 3. Two-way analysis of variance (ANOVA) applied for sensory attributes of Telemea cheese.

Source of variation	SS	df	MS	F	P-value	F crit
Telemea cheese samples	5.842763	2	2.921381481	5.54238616	0.014830587	3.633723468
Sensorial attributes	9.600652	8	1.200081481	2.276770438	0.026773872	2.59109618
Error	8.43357	16	0.527098148			
Total	23.87699	26				

In this table SS is Sum of Squares, df – degrees of freedom, MS – Mean Squares, F – statistic test and P-value – probability.

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НАМАЛЕНО СЪДЪРЖАНИЕ НА НАТРИЕВ ХЛОРИД В СИРЕНЕ ТЕЛЕМЕА. ВЛИЯНИЕ ВЪРХУ ТЕКСТУРНИЯ И СЕНЗОРНИЯ ПРОФИЛ

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(Резюме)

Натриевият хлорид традиционно се добавя към сирена като консервант и за да подобри аромата им. В настоящата работа са изследвани физико-химични показатели (натриев хлорид, сухо съдържание, мазнини, протеини, пепел, pH титруема киселинност), текстурен профил (твърдост, адхезия, еластичност, чупливост, жилавост и кохезия) и сензорни свойства (външен вид и цвят, текстура, аромат и приемане) на образци от сирене Телемеа в продължение на 28 дни зреене. Намаляването на натриевия хлорид оказва съществено влияние твърдостта, адхезията и еластичността на сиренето, но не влияе върху сензорните характеристики.

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Changes on rheological properties of pomegranate (*Punica granatum* L., cv. Hicaznar) juices during concentration process

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The investigation of the changes on rheological properties during evaporation process could diminish the severe effects of thermal processing at any individual soluble solids content. In this study, the rheological properties of pomegranate juices at different soluble solids content (20, 30, 40 and 50 %) during concentration process applied by using the rotary evaporator were determined. Rheological measurements were conducted in the range of 0 – 264 s^{-1} shear rates by using the concentric cylinder type viscometer. It was found expectedly that apparent viscosity increased as the soluble solids content increased. The apparent viscosity was 0.0024 ± 0.0001 Pa.s for raw pomegranate juices (15.73 ± 0.30 % soluble solids) while it increased to 0.01342 ± 0.0003 Pa.s for the concentrated juice having 50 % soluble solids contents. Four different rheological models were applied to find the suitable model best fitting the experimental data; Newton model, Power Law model, Bingham model and Herschel – Bulkley model. The statistical criteria having highest regression coefficient, lowest root mean square error and lowest chi-square were chosen for selection of best model for fitting. It was determined that the Power law model was best described the experimental shear stress-shear rate relation for pomegranate juices at different concentrations. It was predicted that the consistency coefficient of pomegranate juice increased from 0.005 Pa.s^n to 0.013 Pa.s^n as the soluble solid content was increased from 20 % to 50 %. The rheological data obtained in this study could serve valuable data for calculation of changes in flow behaviors of pomegranate juice in pumping systems depending of their concentration.

Keywords. Rheology, pomegranate juice, model, concentration.

INTRODUCTION

Pomegranate is an important fruit in Middle East, Mediterranean, American, and Arab countries[1-4]. Pomegranate fruit consists of hundreds of seeds and thick reddish skin which covers these seeds, and fruit body is its edible part. Edible parts contain a significant amount of acid, sugar, vitamins, polysaccharides, polyphenols and mineral matters [1,3,5,6].

Rheological measurements are made in order to observe changes in the structure of foodstuffs. Rheology examines changes in viscosity and deformation of a substance that is under the force. It is used for quality control, sensory properties, and development of engineering design in juice industry [3,6]. Several researchers [7-9] have reported that several fruit juices such as tomato juice, liquor extract and grape juice showed Newtonian type fluid properties. Bozkurt and İcier [10] have reported that quince juice have time independent, pseudoplastic and non-Newtonian fluid properties. The rheological properties of pomegranate juice depend on the chemical composition of pomegranate, the pressing method, the operation temperature and the concentration method applied. Yildiz et al. [6] used two different processing methods for obtaining the pomegranate juice regarding pressing with or without peels. They reported that the pressing methods did not affect the rheological properties of raw pomegranate juice. They showed non-newtonian dilatant properties. Altan and Maskan [5] investigated the effects of the concentration method (microwave heating, rotary vacuum evaporation, evaporating at atmospheric pressure) and the temperature which the rheological measurement conducted (10 – 55 °C) on rheological properties of pomegranate concentrate (17.5 – 65 %). They determined that all of the pomegranate juice samples have Newtonian character.

In this study, the changes of apparent viscosity for different soluble solid contents (20, 30, 40 and 50 %) during concentration process were examined. It was also aimed to determine the most appropriate

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rheological model fitting the experimental shear stress and shear rate data best, and to predict the changes of consistency coefficients and flow behavior indexes during concentration process of pomegranate juice.

EXPERIMENTAL

The pomegranates (Punica granatum L., cv. Hicaznar) used in this study were supplied from a local market in İzmir, Turkey. Pomegranates were washed in cold tap water and drained. They were manually cut up and the outer leathery skin was removed. The aril in the sacs was pressed, and fruit juice was extracted. The juice having an initial concentration of 15.73 ± 0.10 % soluble solid contents was concentrated at 60 °C in the vacuum by using a laboratory type rotary vacuum evaporator (Buchi R-3) rotating at 400 rpm.

Total soluble solids content determination. The soluble solids content of the juice samples was measured by refractometer (Hanna Instruments 96801) at 20 °C and expressed in % soluble solid contents.

Rheological measurements. Rheological properties were measured using a concentric cylinder type viscometer (Brookfield LVDV-II, Brookfield Engineering Laboratories, USA). The measurement range of viscometer between 0 and 100 % full scale torques was adjusted by selecting the specific spindle (S-18) and its rotational speed (0.0 – 200 rpm) for pomegranate juice. During the rheological measurement, shear stress (SS), shear rate (SR) and % torque (T) values were recorded for each rotational speed (rpm). Experimental shear stress-shear rate measurements were fitted to selected rheological models to obtain viscous rheological properties of pomegranate juice. Four different rheological models were applied to find the suitable rheological model best fitting the experimental data; Newton model, Power Law Model, Bingham model and Herschel-Bulkley model [10].

Statistical analyses. Compatibility of the model with experimental data was determined by using a non-linear regression analysis of statistical software package (SPSS ver. 20, yıl). Regression coefficient (R^2), root mean square error (RMSE) and chi-square (χ^2) values were calculated. Duncan test was applied as a comparative statistical analysis. The statistical criteria of having highest R^2, lowest RMSE and lowest χ^2 were chosen for selection of best model for fitting [11].

RESULTS AND DISCUSSION

The apparent viscosity of pomegranate juices increased as their water-soluble solids increased ($p < 0.05$) (Figure 1). As the soluble solids content increased from 20 % to 50 %, the apparent viscosity increased approximately 4 folds. Several studies have reported similarly that the water content of juices decreased during concentration process, since the change of viscosity as the increased trend has been expected[5, 7, 8]. However, this result could be only used for overall discussion of the effects of concentration process on rheology of pomegranate juice. For detailed investigation of changes of rheological behavior, the experimental data were fitted to some rheological models, which were generally described the changes of rheological properties of fruits juices.

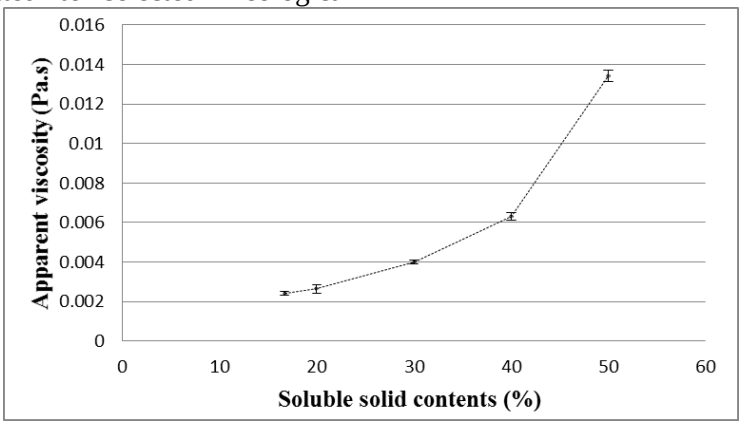

Fig. 1. The change of apparent viscosity as a function of soluble solids content of pomegranate juice during concentration process.

Table 1. The statistical evaluation of rheological models applied to fit the experimental shear stress-shear rate data.

% Soluble Solid Content	Statistical Criteria	Models			
		Bingham model	**Power Law model**	**Hershel-Bulkley model**	**Newton model**
Raw (15.73)	R^2	0.991	0.996	NC*	0.984
	RMSE	0.0038	0.00014	NC*	0.0017
	χ^2	0.01863	0.011	NC*	0.0397
20	R^2	0.996	0.998	0.998	0.987
	RMSE	0.0015	0.0002	0.0006	0.0046
	χ^2	0.0352	0.0157	0.022	0.0660
30	R^2	0.996	0.997	0.998	0.989
	RMSE	0.0047	0.0004	0.0013	0.0038
	χ^2	0.0653	0.02	0.0334	0.0599
40	R^2	1.0	1.0	NC*	0.999
	RMSE	0.001	0.0008	NC*	0.0019
	χ^2	0.032	0.0260	NC*	0.0423
50	R^2	NC*	1.0	1.0	1.0
	RMSE	NC*	0.0070	0.0070	0.0024
	χ^2	NC*	0.0800	0.0754	0.0478

NC*: statistically non compatible.

For different soluble solids contents of pomegranate juices, the statistical evaluation of rheological models fitted to experimental data were given in the Table 1. For raw, 20 – 40 % soluble solids contents, the best model fitted was chosen as the Power Law due to its highest R^2, lowest RMSE and χ^2. On the other hand, the 50 % concentrated juice showed Newtonian character. Similarly, Yildiz et al. [6] reported that non-concentrated pomegranate juice has non-Newtonian and dilatant properties depending on temperature (20 - 90 °C) and pressing method used. On the contrary, Kaya and Sözer [12] found that pomegranate juice samples at higher concentrations (45.7 – 71 % soluble solid contents) at different temperatures (5 – 60 °C) showed Newton model. Altan and Maskan[5] reported that the pomegranate juice samples having the soluble solids contents of 17.5 – 65 % at different temperatures (10 – 55 °C) showed Newtonian fluid behavior. They found that R^2 values of Newtonian model are greater than 0.966 at all concentration processes. However, the change of consistency coefficient and flow behavior indexes during concentration process of pomegranate juice was not investigated in these studies. The contrary between rheological characters of pomegranate juices given in different studies may be due to differences on the type of pomegranate used, the processing pressure, the temperature range applied, the concentration method, method of the rheological measurement, etc.

The change in the consistency coefficient and flow behavior index of pomegranate juice during vacuum concentration process were predicted by using Power Law model (for raw juice and 20 – 40 % soluble solids), which was obtained as the best model

Table 2. The consistency coefficient and flow behavior indexes of pomegranate concentrates.

% Soluble Solid Content	Consistency coefficient K, Pa.s^n	Flow behavior index, n
Raw (15.73)	0.005 ± 0.001	0.836 ± 0.04
20	0.005 ± 0.001	0.854 ± 0.053
30	0.007 ± 0.001	0.872 ± 0.019
40	0.007 ± 0.001	0.974 ± 0.031
50	0.013 ± 0.00	1.0 ± 0.00

describing the rheological changes. To make the comparison of the change in rheological properties of pomegranate concentrates in the range of 15.73 – 50 %, the viscosity value for the concentrated juice having 50 % soluble solids was used as its consistency coefficient with n = 1. It was obtained that consistency increased as the soluble solids increased ($p < 0.05$). Similar to the result obtained regarding the change of the apparent viscosity, the consistency coefficient increased more than two-folds as the soluble solids content increased from 20 % to 50 %. On the other hand, the flow behavior index increased from 0.836 to 1.0 as the soluble solids content increased from 15.73 % to 50 % (Table 2). Although pomegranate juice samples having the soluble solids content in the range of 15.73 – 40 % showed non-Newtonian and pseudoplastic behavior, the juice having soluble solids content of 50 % has Newtonian fluid character. Results showed that the consistency coefficient and flow behavior index could be used to discuss the change of rheological properties of pomegranate juice during concentration process in detail.

The apparent viscosity of pomegranate juices increased as their soluble solids increased. As the soluble solids content increased from 20 % to 50 %, the apparent viscosity increased as 4 folds. Although the pomegranate juice having soluble solids content up to 40 % showed pseudoplastic non-Newtonian fluid character, the juice having soluble solids content of 50 % has Newtonian character. During the concentration of pomegranate juice, the consistency coefficient K values increased, and the flow behavior index approaches to unity (1). These results could give valuable data for designing and setting up of pumping and mixing systems for pomegranate concentrates. The effects of novel concentration methods on quality and rheology of fruit juices should be studied in detail. Further studies on the determination of the rheological properties of different fruit juices during concentration process are recommended.

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ПРОМЕНИ В РЕОЛОГИЧНИТЕ СВОЙСТВА НА СОК ОТ НАР (Punicagranatum L.Hicaznar) ПРИ ПРОЦЕСА НА КОНЦЕНТРИРАНЕ

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(Резюме)

Изследването на промените в реологичните свойства по време на процеса изпарение би могло да намали неблагоприятните ефекти на термична обработка върху разтворими твърди вещества. В настоящата работа са определени реологичните свойства на сок от нар при различно съдържание на разтворими твърди вещества (20, 30, 40 и 50 %) по време на процеса на концентриране, извършващ се от ротационен изпарител. Реологичните измервания бяха осъществени чрез ротационен вискозиметър с датчик тип „цилиндър в цилиндър“ при градиент на скоростта в интервала 0–264 s^{-1}.

Беше установено, че както може да се очаква, вискозитетът нараства при увеличаване съдържанието на разтворимите твърди вещества. За необработен сок от нар (концентрация на твърдите вещества 15.73 ± 0.30 %) вискозитетът беше 0.0024 ± 0.0001 Pa.s, докато той се увеличи до 0.01342 ± 0.0003 Pa.s за концентриран сок, съдържащ 50% разтворими твърди вещества. С цел установяване на подходящ модел, най-добре описващ експерименталните данни, бяха приложени четири различни реологични модели: Нютонов модел, степенен модел, модел на Бингам и модел на Хершел-Балкли. При подбора на най-подходящ модел беше избран статистически критерии за най-висок регресионен коефициент, най-малка средно-квадратична грешка и най-малък χ-квадрат. Беше установено, че за сок от нар с различни концентрации, експерименталната зависимост на тангенциалното напрежение от градиента на скоростта се описва най-точно от степенния закон. Беше прогнозирано, че вискозитетният коефициент на сок от нар се увеличава от 0.005 $Pa.s^n$ до 0.013 $Pa.s^n$ при увеличаване съдържанието на твърди вещества от 20 % на 50 %. Получените при това изследване реологични параметри могат да послужат като ценни данни при изчисляването на промените в поведението на течене в помпени системи на сок от нар в зависимост от неговата концентрация.

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The effects of ultrasound application durations on the rheological properties of tomato (*Lycopersicon Esculentum*) juice

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In this study, effects of ultrasound application durations on the rheological properties of tomato juice were investigated. Tomato juice having 6.18 ± 0.04 % soluble solid contents was ultrasonicated using a 1500 W ultrasonic processor at a constant frequency of 20 kHz for different application durations (0, 5 and 10 min). The empirical data for tomato juice samples obtained from the viscometer (Brookfield LVDV II Pro, USA) were converted into viscosity function. Rheological properties of juice at 20 °C were determined by fitting shear stress-shear rate data to some rheological models (Newtonian, Bingham, Power Law, Herschel Bulkley). It was found that samples showed Non-Newtonian fluid (pseudoplastic) behavior. As the ultrasonic application duration increased the apparent viscosity of the tomato juice increased. Herschel Bulkley model was the best model for all application durations investigated (For raw juice and chi-square (χ^2) 0.203, root mean square error (RMSE) 0.045, Regression coefficient (R^2) 0.995; for 5 min: χ^2 = 0.219, RMSE = 0.053, R^2 = 0.994 and for 10 min: χ^2 = 0.225, RMSE = 0.056, R^2 = 0.994). Rheological data obtained in this study showed the importance of ultrasonic application duration. Application time should be optimized for ultrasonic processing of tomato juices by considering pumping requirements and changes on quality attributes.

***Key words**: Ultrasound, rheology, tomato juice, models*

INTRODUCTION

Tomato (Lycopersicon Esculentum) has important place in daily consumption in the form of fresh or manufactured products (tomato paste, ketchup, tomato juice), and hence provides important economic contributions in the food industry. Tomato juice is obtained by squeezing the whole tomatoes. After the squeezing, skin and seeds are removed with a fine sieve. Tomato juice is composed of serum and colloidal particles [1] larger than diameter of 150 μm.

Tomato juice is produced generally by using conventional production methods [2,3]. In addition, the studies about using of new technologies for increasing of yield, enzyme inactivation, microbial inactivation, reduction of color changing, improvement of rheological properties etc. have been increased in recent years [4-8]. Ohmic heating, microwave treatment, high pressure applications, pulsed electric field, ultrasound are common technologies [2,3,9-11]. Further studies are needed to investigate the effects of process conditions for these technologies on the quality of variety of fluid foods.

High power ultrasound technology, which is one of the non-thermal techniques, provides desired molecular physical and chemical changes in the food. The ultrasound technology is mainly used for enhancing the emulsion applications, cell fractionations, chemical reactions, cutting the sensitive foods, and inactivation of enzymes and microorganisms [12-14]. The application of ultrasound affects as the viscosity and water binding properties of biopolymers [15]. It is applied as an alternative technique for different purposes such as microbial and enzyme inactivation, extraction, drying, filtration, crystallization, degas, cutting etc.[14]. Especially, the combination of ultrasound with heat and pressure improves the rheological properties of tomato juice [2,3].

Rheology is a physical property and is important for product development, process control, design and feasibility [16, 17]. The considerable amount of studies on the changes of rheological properties of tomato juice during processing have been made [2, 3, 18-20]. In the light of this information, it can be said that the tomato juice has a pseudoplastic character. On the other hand, any study regarding the determination of the effects of ultrasound application on the changes of rheological properties

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of tomato juice was not found, within the knowledge of the authors.

The aim of this study was to investigate the effects of ultrasound durations on the changes of rheological properties of tomato juice, and to obtain the rheological model best fitting the experimental rheological data. This data will be useful in the adaptation of this novel technology in tomato juice processing lines and the optimization of ultrasonic processing conditions for tomato juices by considering pumping requirements and changes on quality attributes.

EXPERIMENTAL

In this study, tomato juice having 6.18 ± 0.04 % soluble solid contents was supplied from a local commercial firm, and transported at cold conditions (4°C) after production immediately. Tomato juice was ultrasonicated using a 1500 W ultrasonic processor (Selecta Ultrasons H-D Model, Spain) at a constant frequency of 20 kHz for different application durations (0, 5 and 10 min). Ultrasonic bath was filled with 500 ml water, and 25 ml tomato juice in the glass beaker was submerged into the ultrasonic bath. Rheological measurements were done at constant temperature of 20 °C, immediately.

Rheological measurements

Brookfield viscometer (Model LVDV-II Pro, Brookfield Engineering Laboratories, USA) was used for rheological measurements. Shear stress, shear rate, viscosity and % torque values were recorded. Percent changes in the apparent viscosity were obtained from the rheological measurements of the ultrasound-treated samples and control group (0 min) samples as given in Equation (1).

$$\text{Percent change in the apparent viscosity} = \frac{\mu_{app,ultrason} - \mu_{app,control}}{\mu_{app,control}} \times 100 \quad (1)$$

The experimental shear stress-shear rate measurements were fitted to selected the rheological models to obtain the rheological properties of tomato juice. Four different rheological models were applied; Newton model, Power Law Model, Bingham model and Herschel Bulkley model [16].

Statistical analysis

Compatibility of the model with experimental data were determined by using a non-linear regression analysis of statistical software package (SPSS. ver. 20). Regression coefficient (R^2), root mean square error (RMSE) and chi-square (χ^2) values were calculated [21]. Duncan test was applied to compare the differences between any rheological property depending on ultrasound durations. The statistical criteria of having highest R^2, lowest RMSE and lowest χ^2 were chosen for selection of the best model fitted.

RESULTS AND DISCUSSION

According to rheological measurement results of the tomato juice, the apparent viscosity decreased as the shear rate increased (Figure 1). Therefore rheological characteristics of tomato juice show Non-Newtonian pseudoplastic behavior. Similarly, this nature of tomato juice has been previously found [22,23]. However, there was no enough information on the change of rheological behavior during ultrasound treatment in the literature, according to the knowledge of authors.

While the time extent of ultrasound treatment was increased, the apparent viscosity of tomato juice is increased (Figure 1). The difference between apparent viscosity of untreated (0 min) and ultrasonicated tomato juice for 10 min samples was statistically significant for shear rate range of 0 – 36.4 s^{-1} ($p < 0.05$). On the other hand, the viscosity of the ultrasonicated samples for 5 min was similar to that of the untreated samples ($p > 0.05$). The percent change in the apparent viscosity of tomato juice ultrasonicated for 10 min was between 1.83 % and 11.97 % for shear rates applied in the range of 0 – 36.4 s^{-1}. At higher shear rates applied, there was no significant effect of ultrasonication duration on the changes of viscosities ($p > 0.05$). The rheological behavior of tomato juice is influenced with water soluble and non-soluble pectin amount and cellulose, hemicelluloses compounds which are present in structure. This pectin amount could influence the viscosity with possible interesterification occurred by ultrasound [7,20]. Similarly, there is limited information that the combination of ultrasound with other thermal/nonthermal methods could enhance the rheological properties of tomato juice [3].

Different rheological models (Newton, Bingham, Power Law and Herschel Bulkley models) were used to determine the changes on the consistency coefficients and flow behavior indexes of ultrasound treated tomato juices. The rheological models were fitted to the experimental shear stress- shear rate data. The statistical evaluation of model agreement was given in Table 1. Statistical criteria were the biggest regression coefficient (R^2) for the model and the lowest errors (RMSE and χ^2) between experimental and predicted shear stresses for each shear rate value.

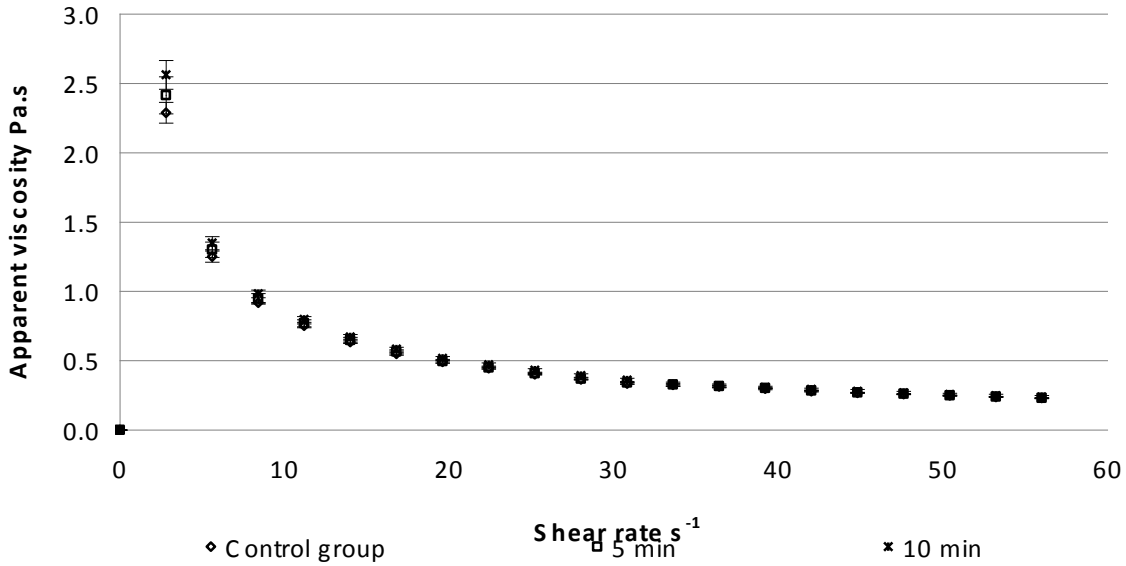

Fig. 1. Change of apparent viscosity of untreated and ultrasonicated tomato juices depending on shear rate.

Table 1. Statistical evaluation for the agreement of rheological models with experimental data.t.

Treatment	Statistical Criteria	Newton model	Bingham model	Power Law model	Hershel-Bulkley model
Untreated (0 min)	R^2	NC	0.769	0.993	0.995
	RMSE	11.38	1.95	0.06	0.04
	χ^2	3.29	1.33	0.23	0.20
Ultrasound treatment (5 min)	R^2	NC	0.742	0.992	0.996
	RMSE	12.27	2.14	0.06	0.05
	χ^2	3.41	1.39	0.24	0.21
Ultrasound treatment (10 min)	R^2	NC	0.743	0.991	0.993
	RMSE	13.49	2.38	0.06	0.05
	χ^2	3.58	1.47	0.24	0.22

NC*: statistically non compatible.

Table 2. Rheological properties of untreated and ultrasonicated tomato juice.

Treatment	Consistency coefficient (K, Pa.s^n)	Flow behavior index, n (-)	Yield Stress (τ_0, Pa.s^n)
Untreated (0 min)	4.067 ± 0.28	0.273 + 0.01	0.052 ± 0.02
Ultrasound treatment (5 min)	4.365 ± 0.32	0.261 ± 0.01	0.059 ± 0.01
Ultrasound treatment (10 min)	4.674 ± 0.65	0.251 ± 0.02	0.098 ± 0.01

It was determined that Herchel Bulkley model was described best the rheological behavior of untreated and ultrasonicated tomato juices since its regression coefficient was highest and its statistical errors were lowest (Table 2). The good agreement of this model to the experimental data has been shown in Figure 2 for each of the ultrasound durations. Similarly, Sharma et al.[18] established the most proper model is Herchel Bulkley model for untreated tomato juice samples having different compositions.

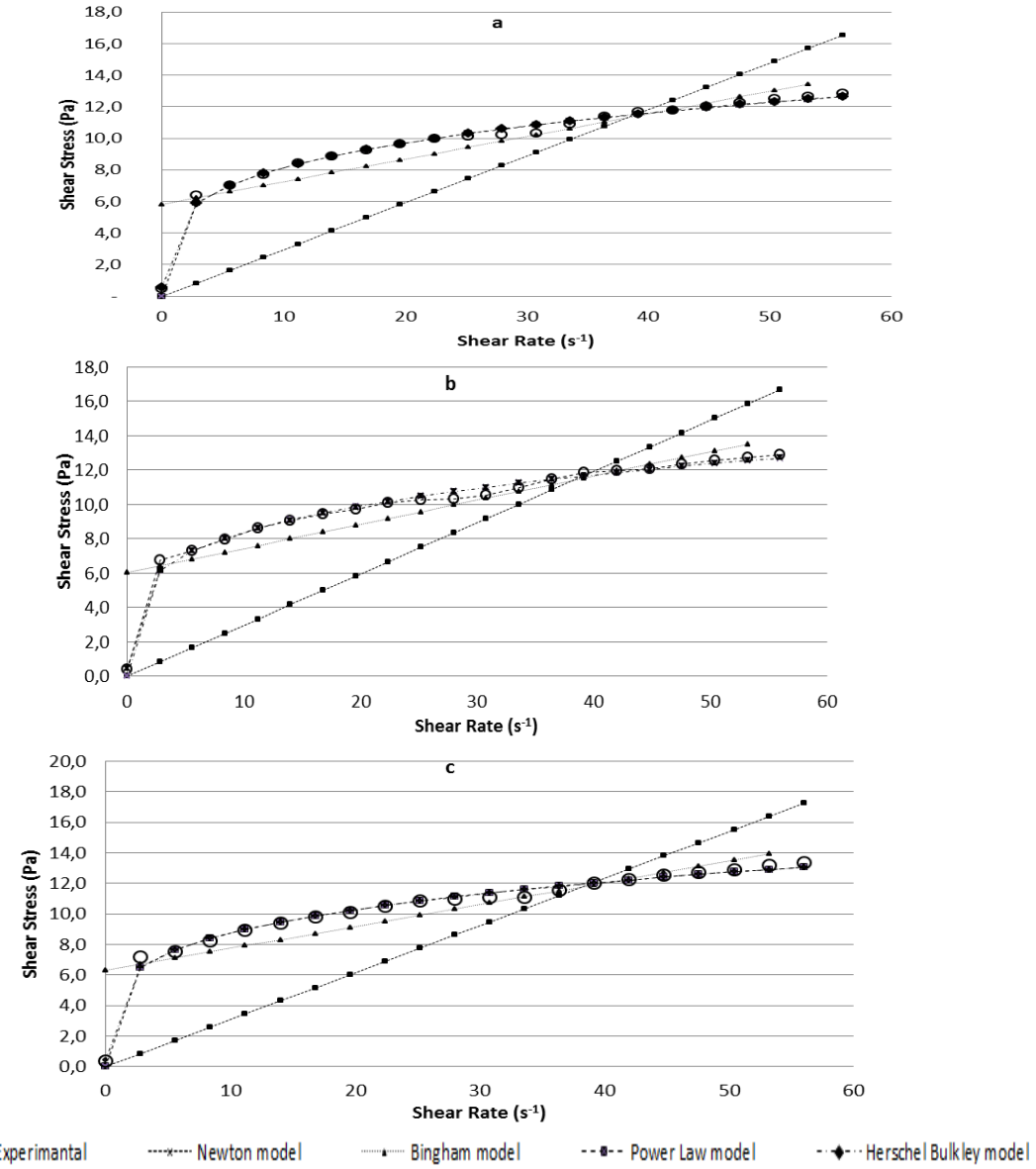

Fig. 2. Comparison between the model predictions and experimental rheological data; a) untreated sample, b) ultrasonicated sample for 5 min, c) ultrasonicated sample for 10 min.

The consistency coefficient, flow behavior index, yield stress values of tomato juices were determined by using Herschel-Bulkley model since it was found as the most proper model for describing the rheological behavior best (Table 2). It was found that the consistency coefficient of tomato juice statistically changed after ultrasound application ($p < 0.05$). As the ultrasound treatment time increased consistency coefficient increased and the flow behavior index decreased. However, the rheological behavior of tomato juice remained unchanged as non-Newtonian fluid having yield stress. Similarly, the change of viscosity has showed similar trend demonstrating the general evaluation of change in its rheological behavior. It could be said that ultrasound treatment caused the increasing of the apparent viscosity value of tomato juice by increasing its consistency coefficient and decreasing the flow behavior index.

It is recommended that the ultrasound treatment time for 10 min and above at low shear rates (< 36.4 s^{-1}) could be used if the main purpose is to increase the consistency of tomato juice higher than 12 %. The optimization of ultrasound conditions regarding the treatment time and shear rate is necessary for design and setting up of piping and ultrasound processing equipment for each individual fluid foods.

The effects of ultrasound applications on the rheopectic and thixotropic characteristics of tomato juice will be determined in flowing projects. In addition, the effects of frequency and power density of ultrasound applications on both rheological properties and other quality attributes of different

fruit and vegetables juices should be investigated further in future studies.

The considerable change on viscosity of tomato juice has been obtained for ultrasonication for 10 min. On the other hand, the consistency coefficient increased and the flow behavior index decreased as the ultrasonication duration increased. Herchel Bulkley model was determined as the most proper model describing the rheological changes of untreated and ultrasonicated tomato juices. It was characterized that tomato juice had Non-Newtonian fluid having yield stress. Since it was determined that the ultrasound could increase the overall consistency of tomato juice, the application conditions for ultrasonication applied to tomato juices could be optimized for the individual purposes such as being used in sauce making and high consistency past production, etc.

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ВЛИЯНИЕ НА ПРОДЪЛЖИТЕЛНОСТТА НА УЛТРАЗВУКОВОТО ВЪЗДЕЙСТВИЕ ВЪРХУ РЕОЛОГИЧНИТЕ СВОЙСТВА НА ДОМАТЕН (Lycopersicon Esculentum) СОК

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(Резюме)

В настоящата работа е изследван ефектът на продължителността на ултразвуковото въздействие върху реологичните свойства на доматен сок. Доматен сок със съдържание на разтворими твърди вещества 6.18 ± 0.04 % беше подложен на ултразвук, използвайки 1500 W ултразвуков генератор при постоянна честота 20 kHz за различно време (0, 5 and 10 min). Емпиричните данни за образците доматен сок получени чрез вискозиметър Brookfield LVDV II Pro, USA бяха конвертирани като функция на вискозитета. Реологичните свойства на сока при температура 20 °C бяха получени чрез интерполация на зависимостта на тангенциалното напрежение от вискозитета с някои реологични модели: Нютонов модел, степенен модел, модел на Бингам и модел на Хершел-Балкли. Беше установено, че образците проявяват ненютоновско (псевдопластично) поведение на течене. При увеличаване на продължителността на ултразвуковото въздействие, вискозитетът на доматения сок нараства. Моделът, който най-добре описва експерименталните данни при всички продължителности на ултразвука, е този на Хершел-Балкли (за необработен сок и χ^2=0.203, средно-квадратична грешка RMSE=0.045 регресионният коефициент е R^2=0.995; за 5 min ултразвуково въздействие χ^2 =0.219, RMSE = 0.053, R^2=0.994 и за 10 min ултразвуково въздействие: χ^2 =0.225, RMSE=0.056, R^2 =0.994). Реологичните данни, получени при това изследване, показват важността на продължителността на ултразвуковото въздействие. Тази продължителност следва да бъде оптимизирана при ултразвуково обработване на доматен сок съобразно с изискванията за помпене и промените в качествените показатели.

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Industrial applications and potential use of ohmic heating for fluid foods

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Ohmic heating technology is generally used for processing liquids and solid-liquid mixtures (or pumpable foods) in the food industry. In ohmic processing, the product is heated volumetrically by dissipation of electrical current through it. The main advantages of ohmic heating are the rapid processing and relatively uniform heating achieved. In this review, the application and potential of ohmic heating in food industry were examined. Nowadays, the availability of novel ohmic heating systems more advanced than their predecessors makes this technology even more attractive for food processors. Ohmic technology is currently being used commercially throughout the world (USA, Japan, UK, several European countries, etc.) for the pasteurization or sterilization of pumpable foods such as fruit and vegetable products (juices, purees, pulps, etc.), milk, ice-cream mix, egg, whey, soups, stews, heat sensitive liquids, soymilk, etc. and aseptic packaging. Much research is still being carried out to improve the current ohmic systems. In recent years, industrial ohmic heating systems have been developed in different countries by companies. The potential applications of ohmic heating technique in food industry are very wide such as cooking, thawing, blanching, peeling, evaporation, extraction, dehydration and fermentation. Researchers should more investigate the potential applications and its effects on food quality and safety before its industrialization.

***Key words**: Food industry, ohmic heating, pumpable foods, current application, potential application*

INTRODUCTION

Consumers are increasingly demanding foods being safety and having improved taste and nutrition [1]. The processing of the particulate foods by conventional thermal methods could damage the food product due to slow conductive and convective heat transfer [2]. To overcome this problem, food manufacturer have begun to apply the electrical energy for food processing in the food industry in recent years [3]. The novel food technologies utilizing the electrical energy in thermal processing have been increasingly attracting the attention of food processors because of its capability of improving the quality and reducing processing costs [4]. Nowadays, ohmic heating systems are more sophisticated and cheaper than previous systems. Hence ohmic heating is more attractive for food industry [5].

Ohmic heating refers to resistive dissipation of electrical energy in the conductive food product which is in contact with electrodes (Figure 1) [6,7]. Also it is known as joule heating, electroheating, electroconductive heating, electrical resistance heating, and direct electrical resistance heating in the literature [2,4]. The uniform heat is generated within the food which allows the transition of electric current when food materials include sufficient water and electrolytes [8]. Ohmic heating

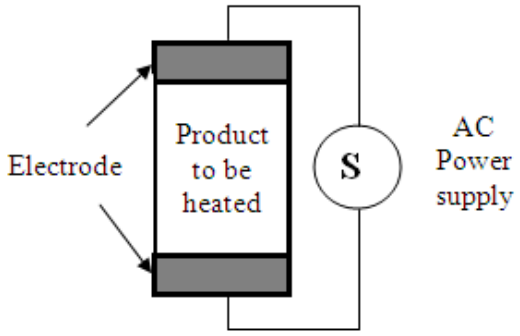

Fig. 1. Operating principle of an ohmic heating device [6].

technology is generally used for processing of liquids and solid-liquid mixtures or pumpable food in the food industry, however its use in the solid foods are still under the research [9-11]. The aim of this review is to provide a general perspective of the ohmic heating technology for currently available industrial applications and future trends in the food industry.

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INDUSTRIAL APPLICATION OF OHMIC HEATING

Brief history. Ohmic heating is investigated in several studies in the beginning of the 19th century. It was firstly used for milk pasteurization in food industry in the last century. But, it did not succeed at this time due to loss of insulation material, higher electricity prices, inadequate process regulations, and other technical restrictions. Later researches [3] declined during 1930s to 1960s. In 1980s, the Electrical Council Research (UK) was secured by a patent for continuous ohmic heating equipment. After this development, the first industrial unit is produced in 1989 in the UK [12]. Today, there are many commercial plants which are operated in food industry [13].

Ohmic heating systems. Ohmic heating systems for food processing have basically a container, a pair of electrodes, and an alternating power supply [14]. Power supply units in ohmic heating systems give electrical energy to system at low frequencies [3]. The electrodes should be made from most conductive materials and also these should have low cost and corrosion resistance [15]. Ohmic heating systems are relatively small equipments. Instant start/stop can also be made, and process temperature can be controlled accurately [3,16].

Ohmic systems can be operated to batch or continuous for processing of food [14]. They have high potential which can be designed to wide variety depending on the application [3] The typical batch ohmic systems have a horizontal cylinder with two electrodes placed at both ends [17]. Continuous ohmic heating systems can vary greatly for industrial applications [3]. They may have several designs; a simple tube with pairs of opposing electrodes mounted on the tube walls opposite to each other, coaxial tubes acting as electrodes with the food flowing between or a vertical tube with the electrodes embodied at regular intervals, etc. [14]. These systems have main parts of flow system and cooling parts. They have several columns for electrical heating. The columns include insulating materials [3].

Selection of the system depends on processing methods of foods and aims of the process. Batch and continuous systems are used for liquid (pumpable) and solid foods [11,14,17].

Current commercial applications. Ohmic heating technology is currently being used commercially throughout the world (USA, Mexico, Japan, UK, and other several European countries) for the pasteurization or sterilization of pumpable foods (viscous or liquid foods) such as fruit and vegetable products (juices, purees, pulps, etc.), milk, ice-cream mix, egg, whey, soups, stews, heat sensitive liquids, soymilk, etc. and aseptic packaging [3,12,14]. It is reported that at least 18 commercial plants were operated in Europe, USA, and Japan. Application of this technology has particularly succeeded to processing of fruits and vegetables [6].

Manufacturer companies. A great number of researches were completed for optimization of process parameters, improving of equipment materials, and design [19]. Ohmic heating systems must have effective control of heating and flow rates, as well as investment and operating costs should be low for successful commercially in food industry [17].

Nowadays, industrial ohmic heating systems are produced commercially in different countries by companies such as APV Baker Ltd. (UK)[12], C-Tech Innovation (UK) [16], Agro process (IAI Group, Canada) [20], Yanagiya Machinery Co. Ltd. (Japan) [21], Kasag (Switzerland) [22], Alfa Laval (Sweden) [23], Raztek (USA) [24], Emmepiemme SRL (Italy) [4,25]. A list of some industrial ohmic heating systems and features for processing of foods is shown in Table 1.

Leadley reported [6] that APV Baker Ltd. (UK) produced two industrial ohmic heating systems. One of these had power output of 75 kW and product capacities of 750 kg/h and the other had power output of 300 kW and product capacities of 3,000 kg/h. Approximate prices for these systems including aseptic line were £ 1,300,000 to £ 2,000,000. On the other hand, Emmepiemme SRL (Italy) produced systems in the range of 60 kW to 480 kW for production throughputs of 1,000 kg/h to 6,500 kg/h. Approximate prices estimated in 2004 were € 60,000 to € 220,000 due to the power output of systems. Tucker informed [27] that Raztek (USA) manufactured one industrial ohmic heating system which was used for pasteurizing the liquid egg at a flow rate over 11,300 kg/h.

Anderson reported [25] that the costs of industrial ohmic heating systems, including installation, can be in excess of $ 9,000,000. Although these systems have an enormous investment for a manufacturing plant, their processing costs are comparable to commercial conventional systems [3]. Costs of ohmic heating were found to be comparable to processing of low acid products [17].

Table 1. Some industrial ohmic heating systems and their features [16,21,23,26,27].

Company	Type of Technology	Type of Electrodes	Products	Heating Power, kW	Frequency, Hz	Capacity, kg/h
C-Tech Innovation	Continuous	-	Beverages, fruits and vegetables through to meat based products, ready meals*	20-240	-	300-7,200
Alfa Laval	Continuous	-	Fruits and vegetables products, prepared foods, liquid egg, ready meals, sauces*	60-300	50	-
Emmepiemme SRL	Continuous	Stainless steel Rising	Fruits and vegetables products*	60-480	25,000	1,000-6,500
APV Baker Ltd.	Continuous	Platinum coated Intrusive	Ready meals and particulate fruit product*	75-300	50	750-3,000
Raztek	Continuous	Pure carbon	Liquid egg*	-	50	11,300
Yanagiya Machinery	Continuous	-	Tofu production	-	-	-

*Pasteurization or sterilization of pumpable food.

POTENTIAL APPLICATIONS OF OHMIC HEATING

Ohmic heating is investigated on various areas in food engineering. It has a lot of potential for commercial use in food processing [18]. It has a large number of potential future applications such as cooking, thawing, blanching, peeling, evaporation, extraction, dehydration, fermentation, and online detection of starch gelatinization for food industry [2,3,8,28].

Cooking. Solid foods of ohmic heating are restricted due to the difficulty in providing good contact between the electrodes and food surface [29]. In recent years, several studies have been completed about ohmic cooking [11,14,30-32]. Studies have been conducted especially in meat processing. Although ohmic cooking has offer some advantages such as rapid cooking, energy efficiency, and food safety, it has not yet used industrially due to several limitations [14,29,30]. Icier et al. [11] reported a new method for cooking of meatball in continuous type ohmic cooking system resulting the highest product quality such as lowest hardness, maximum chewiness, and resilience and providing safety foods.

Thawing. Ohmic heating is an alternative method for thawing of frozen foods [12]. Frozen food samples must be good contact with the electrodes for efficiency of this method. The size, shape, and electrical conductivity of the frozen food should also be carefully optimized to provide higher efficiency [33]. Ohmic thawing have some advantages such as less treatment times, the less microbial growth and the better quality of the thawed product compared to the conventional methods [12,34]. Although ohmic thawing is not applied commercially in food industry due to several limitations, it has potential to use in the future [33,35].

Blanching and peeling. The ohmic heating appears to be an alternative method for blanching and peeling method for vegetables and fruits. Cell membranes of vegetables and fruits are damaged by combination of electrical and thermal effects in this method [36]. Mizrahi [37] reported that ohmic blanching considerably reduced the hot water requirement and blanching time compared to conventional method. Vegetables and fruits may be peeled efficiently without using any chemicals [13].

Evaporation. Ohmic heating is a new method for vacuum evaporation of orange juice. It can evaporate faster and gives higher quality of final product than conventional processes at the same time [12].

Extraction. Ohmic heating can be used for extraction. It affects the structure of biological tissue so that increasing of extraction yield [36].

Lakkakula et al. [38] informed that ohmic heating increased the extraction yields for sucrose from sugar beets, beet dye from beet root, soymilk from soybeans, and total percent of lipids extracted from rice bran. Wang and Sastry [39] showed the improved apple juice extraction yields with ohmic heating.

Dehydration. Ohmic heating can be used for dehydration of vegetables and fruits. It may be causes electroporation of cell membranes. Hence, it increases the permeabilization of the cell and so facilitates dehydration [8,12]. Lima and Sastry [40] showed the faster hot-air drying rate of sweet potato with ohmic heating.

Fermentation. Ohmic heating applications may be useful for fermentation in food industry. It was reported that lag period decreased when ohmic heating was used in fermentation of some foods. This technique may decrease the lag period of fermentative bacteria and so decrease of fermantation time in processing of yogurt, cheese, beer, or wine [25,41].

CONCLUSION

Ohmic heating have been used for several purposes including blanching, heating, cooking, etc.. Several manufacturers developed ohmic systems especially for processing of liquid foods. However, further studies are recommended to development of new industrial ohmic systems for the potential applications into industrialization. New industrial ohmic systems should also be convenient for processing solid food products. Nowadays, although industrial ohmic systems have high investment cost, their cost will be decreased by the time with technological developments. In future studies, toxicological and mutagenic effects should also be investigated for electrically processed foods.

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ИНДУСТРИАЛНИ ПРИЛОЖЕНИЯ И ВЪЗМОЖНОСТИ НА ОМОВОТО НАГРЯВАНЕ ЗА ТЕЧНИ ХРАНИ

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(Резюме)

В хранителната индустрия технологията на омовото отопление се използва главно при преработката на течности или смеси на твърди вещества и течности (или храни, които могат да се изпомпват). При омовата преработка обемът на продукта се нагрява чрез пропускане на електрически ток през него. Сред основните предимства на метода са бързата преработка и постигането на относително равномерно нагряване. В настоящия обзор са разгледани приложенията и възможностите на омовото отопление в хранителната индустрия. В днешно време, наличието на нови омови отоплителни системи, по-напреднали от своите предшественици, прави тази технология още по-привлекателна за производителите на хранителни продукти. В момента в световен мащаб (САЩ, Япония, Англия, някои европейски страни и др.) омовата технология е комерсиализирана и се използва за пастьоризиране или стерилизиране на храни, които се изпомпват, като например плодови и зеленчукови продукти (сокове, пюрета, каши и др.), мляко, миксове за сладолед, яйца, суроватка, супи, яхнии, чувствителни към топлина течности, соево мляко и др., а така също и при асептично опаковане. Все още се извършват много изследвания за подобряване на съществуващите омови системи. През последните години, в различни страни са разработени промишлени омови отоплителни системи. Потенциалните приложения на техниката на омово отопление в хранително-вкусовата промишленост са много широки, като включват готвене, размразяване, бланширане, пилинг, изпаряване, екстракция, дехидратация и ферментация. Учените трябва повече да изследват потенциалните приложения, като обърнат внимание на въздействие на омовото отопление върху качеството и безопасността на храните преди индустриализацията на метода.

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Rapid monitoring of volatile organic compounds: selected ion flow tube mass spectrometry (SIFT-MS)

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Selected ion flow tube mass spectrometry (SIFT-MS) is an analytical mass spectrometry technique that offers real time and rapid identification and quantification of gases in air and human breath, even it is trace amount. This technology is used rapid measurements of volatile organic compounds (VOC) and some inorganic gases.

SIFT-MS can be applied in food science, environment, medicine, and health and safety practice. In food science, several studies of volatile compounds in fruit and vegetables, nuts, cocoa and cocoa liquors have been reported. The applications of this technology is allowed to air analysis in following areas; hazardous toxic chemicals in shipping containers, diagnosis of non-invasive diseases through breath analysis and screening urine of workers for levels of toxic compounds.

Detection of ppt level of VOCs, real-time ambient air monitoring without any required sample preparation, and the direct analysis of moist samples are considered as the advantages of this technique. However, conflicts in compound identification and quantification between ionized fragments with the same molecular weight are the challenges of SIFT-MS.

The aim of this review is point out a new technology for measuring VOCs and outlines some results from several studies to show the application area of SIFT-MS in food science and other areas.

Keywords: Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), volatile compound, fruits and vegetables, rapid monitoring.

INTRODUCTION

Selected ion flow tube mass spectrometry (SIFT-MS) was intended in the mid 1990's as an analytical method for the direct, accurate analysis of humid exhaled breath while avoiding redundant sample preparation into bottle or onto traps [1]. SIFT-MS is reversed the process which had been in use for the previous 30 years for determining rate coefficients. Main objective is if a rate coefficient and product ion(s) were known for an ion-molecule reaction, the concentration of neutral analyses can be found. The researchers assumed that this technology could be useful for breath research and the non-invasive diagnosis of illnesses [2]. Nowadays, SIFT-MS is used effectively an analytical mass spectrometry technique that provides rapid identification and quantification of trace amount of gases in air and human breath. This technology is offered for real-time analysis of trace concentrations of VOCs, which normally present in the vapour phase at room temperature, and some inorganic gases [3].

SIFT-MS has been applied in many areas, including food science, environment, medicine, and health and safety practice. The applications of this technology is not limited with fruit and vegetable flavors, also this technique can be used in detecting levels of hazardous toxic chemicals in shipping containers, in rumen gas, research on diagnosis of volatile markers of infection and tumors in urinary headspace, and screening urine [4]. Ability to simultaneous measurement of the concentrations of many compounds in a single sample without chromatographic separation make SIFT-MS preferred for VOCs measurements [5].

OVERVIEW OF SIFT-MS

The operation of a SIFT-MS instrument is to determine the kinetic parameters of an ion-molecule reaction, which helps to determine a reaction rate coefficient and the product ion branching ratios. Francis [2] clearly explained the reactions during SIFT-MS operation. The basic principle of SIFT-MS is soft chemical ionization. An external ion source produces selected ions as H_3O^+, NO^+ and O_2^+, and forwards them to the ion injection orifice separately. Reagent ions do not react with air, N_2, O_2, Ar, CO_2 and water vapour [4], despite those ions react with most other gases and vapours [6]. These ions move through a flow

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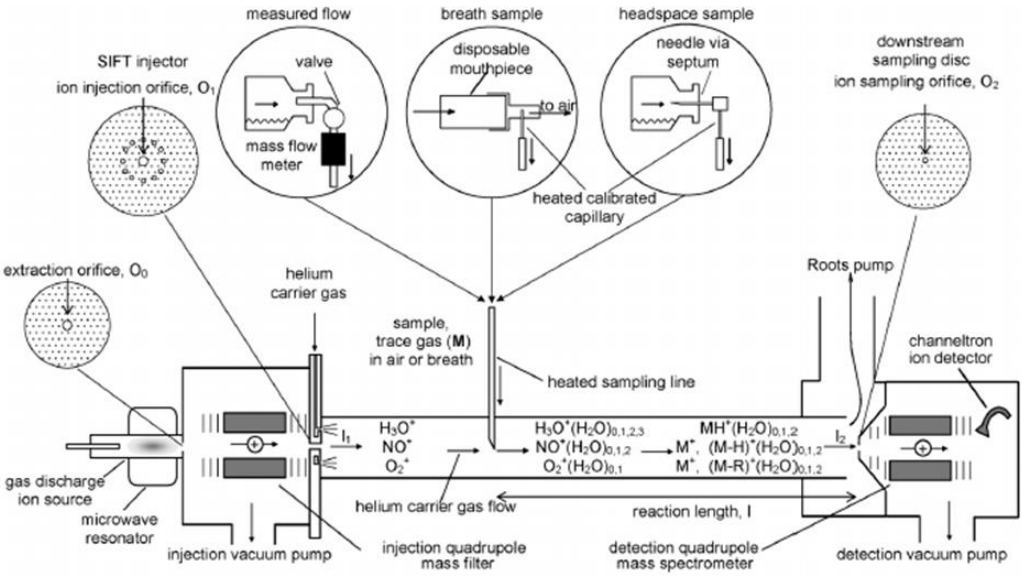

Fig. 1. Scheme of SIFT-MS [10].

tube carried by helium as a carrier gas [7]. Sampled air or reactant gas is injected into the flow tube via the inlet port and travels in the flow tube with the inert carrier gas at a known flow rate [8]. Trace gases in the sample react with precursor ion and generate product ions [7]. Product ions are detected by the quadrupole mass spectrometer and the target gas concentration can be calculated by using the reaction rate constant (k value) and product ion to precursor ion rate of the specific volatile compounds [8, 9]. The concentration [M] of selected volatiles is calculated using the product count rate (Ip), reaction rate constant (k), precursor ions count rate (I) and reaction time (t) as follows: [M]=Ip/Ikt [9].

Schematic of SIFT-MS is shown in Figure 1 [10].

OTHER ANALYTICAL TECHNIQUES FOR VOCS

There are different types of instruments exist for measuring VOCs in the air, headspace and breath. The traditional analytical methods to measure VOCs in the air and the headspace mostly require an isolation step before identifying the compounds. Some of the isolation methods used in previous studies is extraction, distillation, dynamic headspace, solid phase micro extraction (SPME), and liquid-phase micro extraction (LPME) [11-16]. To determine the VOCs in the breath requires real time measurements; therefore extraction step should be eliminated. In this respect, instrumentation that will be chosen need to be suitable for real time measurements.

Gas chromatography (GC) was used for separation and identification of compounds [14,16] equipped with Mass Spectrometry (MS), Flame Ionization Detector (FID) [12,17], Electron Capture Detector (ECD), Photoionization Detector (PID) [18], Flame Photometric Detector (FPD) [5,20], Nitrogen Chemiluminescence Detector (NCD) and Sulfur Chemiluminescence Detector (SCD) [21,22].

Proton transfer reaction mass spectrometry (PTR-MS) is a newly developed technique, which allows rapid detection of aroma compounds in alcoholic beverages [23], breath analysis of banana aroma during eating [24], breath profile of smoker and non-smokers [25]. This technique was developed for on-line gas monitoring [26]. PTR-MS also used in chemical ionization (CI) techniques for detection of ionizing molecules with minimum fragmentation [27]. CI offers high sensitivity when the primary ions have enough time to react with the neutral compounds. PTR-MS instruments have recently incorporated linear and quadrupole ion traps and time-of-flight mass spectrometers (TOF-MS) for greater compound specificity. TOF-MS is well designed to give accurate mass data; however, a TOF tube does not give data associated with structural isomers [28,29].

Since there was interconnection between quantities of reacting species and reaction time, atmospheric pressure chemical ionization (APCI) systems have been developed [30]. Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) is another technology which was developed to monitor aroma compounds in the breath during eating[31]. These two techniques use soft chemical ionization with the H_3O^+ as a reagent ion to monitor volatile organic compounds. APCI-MS was used for quantification of acetic acid, formic acid and furfural, which were biomass degradation products, within pretreated wheat straw hydrolyzates and bioethanol formation during fermentation[32]. While APCI-MS requires minimal sample preparation, results were found similar with high-performance liquid chromatography (HPLC) analysis.

All of these instruments have advantages and disadvantages. For example, instruments based on gas chromatography have the potential to analyze and quantify a large variety of molecules concurrently, with high sensitivity and specificity. However, some chromatographic techniques are allowed to sample loss during pre-concentration of the air.

Chemical ionization techniques can achieve high time resolution but some molecular species are difficult to identify and separate [33].

SIFT-MS APPLICATIONS IN FOOD SCIENCE

In food science, several fruit and vegetable studies of VOCs focuses on formation of Lipoxygenase (LOX) derived compounds. Monitoring the real time changes is essential to show quick formation of these compounds during ripening, storage or after tissue disruption. Studies showed that the concentration of (*Z*)-3-hexenal which is produced during the decomposition of 13-hydroperoxides of linolenic acid by LOX and hydroperoxide lyase in the LOX pathway [34] increased to a peak level in 3 min after tissue disruption by blending in tomatoes and tomatillos [35,36]. Also the concentration of (*E*)-2-hexenal, which is formed from the isomerization of (*Z*)-3-hexenal by cis/trans-isomerase in the LOX pathway [37,38], increased and reached peak level slightly later than (*Z*)-3-hexenal in tomatillos, tomatoes [39], jalapeno peppers [40], and strawberries [41].

The concentration change of volatile compounds during blanching, refrigerated storage, frozen storage and thawing was studied in jalapeno peppers [40], and strawberries [41]. The effect of ripening stage on volatile formation was studied in strawberries [41]. In early ripening stage, the concentration of LOX-derived volatiles was high and fruity esters were low, oppositely in last stage of ripening, reverse results were observed.

While this technology offers real time monitoring, some studies focused on volatile change in mouthspace during chewing and after swallowing. While concentrations of tomatoes and strawberries' volatile compounds increase in the mouthspace and nosespace during chewing, consumption of some foods, such as water, milk, and sodium caseinate solutions, can reduce volatile levels in the mouth, including malodorous garlic breath [42]. The Maillard reaction is also responsible for the formation of many volatiles. Real time formation of alkylpyrazines, aldehydes, acids, alcohols, esters, and ketones were easily measured during roasting with SIFT-MS [43]. Formation of many volatiles were peaked after 15 min. of cocoa roasting then decreased. Also volatile formation increased with increasing temperature and peak time was shortened with decreasing pH during roasting. Both roasting and drying increase the formation of furan compounds in cocoa and carrots [43,44]. Langford et al.[45] compared the headspace VOCs concentrations of New Zealand cheeses marketed as parmesan, Italian Parmigiano Reggiano and Grana Padano cheeses in real time without any sample pre-concentration.

APPLICATIONS IN OTHER AREAS

SIFT-MS has been applied in many areas, including environment, medicine, and health. Some VOCs in exhaled breath are an indicator of non-invasive disease [5,8,46,47]. SIFT-MS techniques can be used in biological researches, which help to determine several metabolites in breath such as isoprene, ethanol, acetone and ammonia [48,49]. Monitoring these compounds by SIFT-MS, give an insight about the health condition of patient. Isoprene is a non-invasive marker of endogenous cholesterol in human breath and may help diagnosis of coronary artery disease as a result of increased cholesterol levels [50]. Also isoprene and ammonia are countable as markers of end-stage renal failure [51]. Measuring compounds concentration can be helpful to identify specific bacteria in the headspace of blood or urine sample [52], which can be helpful to identify antibiotics for target microorganism. Boshier et al. [53] used SIFT-MS to analyze trace gases within the breath of anaesthetized patients. This study showed the utility of on-line breath analysis during surgery for the monitoring of endogenous metabolites, anaesthetic gases and potential biomarkers of metabolic and oxidative stress during operation. Smith et al. [54] investigated the production of acetaldehyde from cancer cell lines (specifically the human non-small cell lung cancer cell lines SK-MES and Calu-1) *in vitro* and found that concentration of acetaldehyde in the headspace was proportional with the number of cancer cell lines in the environment. Another study focused on presence of formaldehyde in the headspace of the urine from the cancer patients. Spanel et al. [55] resulted that patients with bladder and prostate cancer had higher formaldehyde compared with urine from the healthy controls. Spanel and Smith [56] quantified trace levels of formaldehyde, acetaldehyde and propanol, potential cancer biomarkers, in breath by SIFT-MS. Smith et al. [57] determined ammonia, nitric oxide, acetone, ethanol and methanol which are the major volatiles in the urine. Abnormal levels of these compounds cause suspicions about alkaline urine, acidic urine or bacterial infection.

SIFT-MS is used for identifying dominant gases as hydrogen sulfide, methyl sulfide, and dimethyl

sulfide, in the rumen headspace [58]. Exhaust gas mixture was analyzed with SIFT-MS and found that aliphatic and aromatic hydrocarbons, aldehydes, ketones and alcohols were present with their fractions in both petrol and diesel engines [59].

CONCLUSION

Distinction of SIFT-MS from the other mass spectrometry analytical techniques is analyzing of VOCs in real-time without the requirement for calibration curves to calculate or determine analyze concentrations. It quantifies VOCs based on the ratio of product ion count to reagent ion count [5].

The advantages of SIFT-MS over other analytical techniques are detection of ppt level volatile by volume level, in real-time ambient air monitoring without any required sample preparation, and the direct analysis of moist samples. This technique allows to determine breath volatiles in real-time during eating and swallowing. Also determining of trace gases as a marker of cancer cells is beneficial to track metabolic activity both *in vitro* and *in vivo* [54], in addition to this, SIFT-MS technique may be used to predict early-stage tumors in the body as a non-invasive indicator in the near future [55].

However, the challenges of this technique are conflicts in compound identification and quantification between ionized fragments with the same molecular weight. SIFT-MS is in the early stage of development and further applications in the above-mentioned areas of researches and applications will be come out. With the continued progress to the SIFT-MS instrumentation and methodology, the new features of the technique become more widely applicable in other areas of researches.

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БЪРЗ МОНИТОРИНГ НА ЛЕТЛИВИ ОРГАНИЧНИ КОМПОНЕНТИ: SIFT-МАССПЕКТРОМЕТРИЯ

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(Резюме)

SIFT-мас-спектрометрията (SIFT-MS) е аналитична мас-спектроскопска техника, която предлага бърза идентификация и количествено определяне в реално време на газове във въздуха и човешки дъх, дори и в случаите на незначително количество на газа. Тази технология се използва за бързо измерване на летливи органични компоненти и някои неорганични газове. SIFT-MS може да се прилага в науката за храненето, околната среда, медицината, здравеопазването и техниката за безопасност. В науката за храненето са представени редица изследвания на летливи компоненти в плодове и зеленчуци, ядки, какао и какаови ликьори. Приложенията на тази технология позволява анализ на въздуха в следните области: опасни токсични химикали в контейнери за транспортиране, неинвазивна диагностика на заболяванията чрез дъха, анализ и скрининг на урина за нива на токсични съединения на работниците. За предимствата на тази техника се считат откриване на ppt нива на летливи органични компоненти, наблюдение в реално време на атмосферния въздух без никаква подготовка на пробите и на пряк анализ на влажни проби. Въпреки това, предизвикателства на приложение на метода са трудности в идентификацията и количественото характеризиране на химични съединения и йонизирани фрагменти със същата молекулна маса.

Целта на настоящия обзор е представянето на една нова технология за измерване на летливи органични съединения и запознаване с резултатите от някои изследвания, които дават информация за областта на приложения на SIFT-MS в науката за храната и в други области за анализ на следи от газ във въздуха, издишвания от човека въздух и парите в горната част на колони с течности.

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Sensory and instrumental evaluation of the whey cheeses

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Whey as a relevant dairy by-product is still not used in completely effective way, not only in the Czech Republic. There is the possibility of typical whey cheese production as mainly in the Scandinavian countries. Experimental whey cheeses were made according to the experimental recipes. Samples were evaluated by a sensory evaluation and texture analysis. The most sensory acceptable ($P < 0.05$) was the sample with the addition of caramel. Changes of consistency of cheese samples during storage were detected. The result was the increase in hardness as confirmed by sensory analysis and instrumental measurements. Hardness of all produced whey cheeses was significantly affected by raw material and production technology. Following this model experiment will continue further research, particularly stabilization ratio of ingredients and verification of selected recipes for sensory panel.

Key words: dairy product, sensory evaluation, texture, hardness

INTRODUCTION

In the Czech Republic like everywhere in the world, whey remains in large volumes from the cheese production. It is still not completely solved this problem with this dairy by-product. One of possible utilization is the whey cheese production. Moreover to the better known Ricotta, whey can be processed into the form of a brown caramel cheese typical and known especially in the Scandinavian countries. Their different names depend on the region or country of origin. In Norway, e.g. Mysost is made from the whey cows and Gjetost from goat whey, while Mesost is typical for Sweden [1].

The lactose caramelisation and whey protein coagulation are typical for the production of these brown whey cheeses. Steam is the sole resulting by-product. The highly concentrated whey arises and other ingredients, such as cream or milk, can be possibly added. Evaporation, stirring and cooling are the main technological processes [2,3]. Brown whey cheeses are mostly of rectangular shape, rind-less, characterized by a distinctive sweet and typically caramel flavor, aroma reminding fine caramels, and by rougher texture [2,4-6].

The texture is regarded as one of the main cheese quality attributes and plays an important role as a determinant of the final use of the cheese, as well as for the consumer acceptance. Various cheeses are characterized by different textures. Therefore, different predominant attributes of texture are expected at each type of cheese. E.g. Mozzarella cheese is "fibrous" or "elastic" and Parmesan is "friable", etc. Among the most commonly used terms to describe the cheese structure or texture include hardness, adhesion, fragility, creaminess, friability, chewiness, cohesiveness, staleness, firmness, gumminess and elasticity [7,8].

EXPERIMENTAL

All samples of cheeses were manufactured according to the designed recipes in the laboratory of Department of Food Technology (Mendel University in Brno). They were used for the texture and sensory analysis. The sample of goat whey was used directly from the farm and was not subjected to any heat treatment. Whey was vaporized in the amount specified in the recipe up to the desired pasty consistency. The resulting paste was cooled and then evaporated again. After achieving of optimal consistency, resulting paste was put into plastic containers and stored in a refrigerator at a temperature of 7 °C. The samples were divided according to the designed recipe into three groups – the samples with addition of cream (No. 3, 4, 7, 8), samples without addition of cream (No. 1, 2, 5, 6) and samples flavored with various ingredients (No. 9 – 25). To produce the samples without the addition of cream, 2.6 l of goat whey was used (samples No. 1, 2, 5, 6). To produce samples with addition of cream, 0.17 l of cream was added to whey (samples No. 3, 4, 7, 8). According to the same recipe, to produce samples variously flavored, these ingredients were chosen to be added into goat

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whey: vanilla sugar (No. 9); apples with cinnamon sugar (No. 10); dried prunes (No. 11); chocolate with cream (No. 12); extract of dried sweet marjoram (No. 13); plum butter and poppy seeds (No. 14); extract of wild garlic (No. 15); bananas with cinnamon sugar and cream (No. 16); prunes and cream (No. 17); dried cranberries with cream (No. 18); light brown caramel (No. 19); chocolate (No. 20); medium brown caramel (No. 21); sweetened condensed cream (No. 22); chocolate and dried prunes (No. 23); dark brown caramel (No. 24); and caramel with chocolate (No. 25).

Overall, it was therefore analyzed 25 samples of whey cheese – 4 samples with the addition of cream (No. 3, 4, 7, 8), 4 samples without the addition of cream (No. 1, 2, 5, 6) and 17 samples flavored with various ingredients (No. 9 – 25) in order to find the most organoleptic acceptable one. For the sensory evaluation of the appearance of the entire sample, whole loaf of cheese was used. 10 g of the samples were evaluated by eight assessors (with ISO 8586-1) in the sensory laboratory (under conditions ISO 8589). Sample of cheese was given to the evaluators on a white porcelain plate 15 minutes after removing from the refrigerator. To ensure the anonymity of the samples, four-digit codes were used. Milk was used as a neutralizer. Following descriptors were monitored: overall pleasantness of appearance, color, uniformity in coloring, overall pleasantness of smell, intensity of caramel smell, typical cheese smell, foreign smell, goat smell, hardness, spreadability, sandiness, friability, the overall pleasantness of taste, flavor intensity of sweet, salty, caramel, cooked, foreign taste and goat taste. Evaluation was based on unstructured graphical scale with a length of 100 mm with a verbal description of the extreme points. When the higher value of the descriptor was detected, the more positive evaluation was estimated. Texture analysis of produced samples was performed in the 7th day after the production. The samples were left for 30 min at room temperature before the measurement. Determination of selected textural properties was performed by the universal testing machine. The penetration test was used for the determination of the hardness of whey cheeses. After the sensory evaluation, the most sensory acceptable whey cheese was sample No. 21. Therefore, this sample was analyzed (texture and sensory analysis) for longer time period – every seventh day for 6 weeks (7th, 14th, 21st, 28th, 35th and 42nd day). All data were analyzed using the software program Unistat 5.1 by the analysis of variance (ANOVA) with multiple comparisons followed by Tukey's test.

RESULTS AND DISCUSSION

Sensory evaluation of whey cheese has detected no statistically significant difference ($P > 0.05$) within all descriptors characterizing flavor and aroma between samples with or without added cream. Thus, the addition of cream recipe has no effect on the taste and smell of the products. In both groups of samples, there was identified high intensity of salty taste which was not accepted as positive characteristic by the evaluators. Such excessive salty taste may be caused by whey because most of the minerals of milk in cheese production are transferred to whey. Statistically significant difference ($P < 0.05$) was not observed at descriptor of coloration uniformity. It was the most frequently detected by evaluators as creamy yellow color within the both groups of samples (with and without the addition of cream).

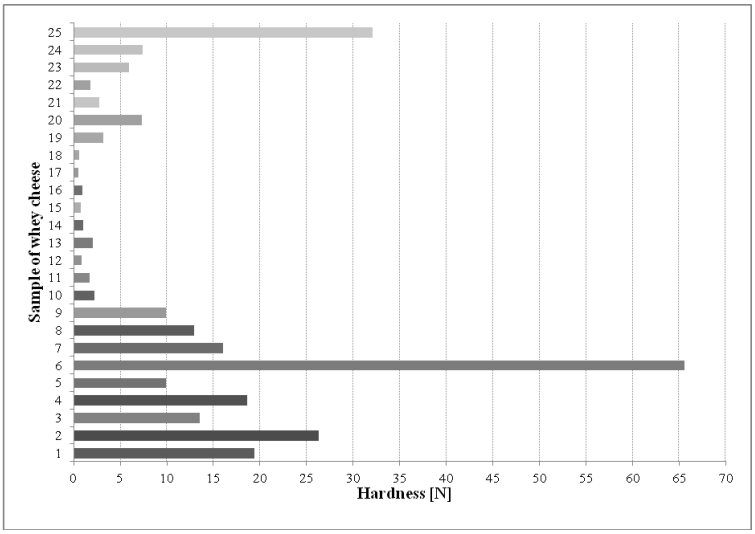

Fig. 1. Instrumental determination of hardness of whey cheeses.

Table 1 shows results of sensory evaluation of the data obtained from sensory questionnaires. Due to the high intensity of the salty taste of produced whey cheese, which was perceived negatively, it was necessary to adjust production technology and upgrade existing recipes using different ingredients that are listed in materials and methods. Whereas the each sample was made according to a specific recipe, it was not possible to statistically compare obtained results of flavored whey cheeses, because of its distinctive taste. The values of hardness determined by penetration test are shown for individual samples of whey cheeses in the Figure 1.

Sample with medium brown caramel (No. 21) was the most acceptable according the evaluators. For this reason, it was analyzed during storage in detail with sensory and texture analysis. The results in Table 2 and Figure 2 show changes in consistency of the cheese samples (No. 21) during storage. These data are in increasing the hardness of the sample. The highest difference in hardness was noticeable between the 7th and the 14th day of storage when the hardness value increased approximately 3 times. Significant increasing hardness was slowed after three weeks. This was compared with the results of sensory analysis. There is the significant positive correlation between these methods. Sample (No. 21) was the most acceptable in the fourth week of measurement.

Table 1. The sensory evaluation of whey cheeses produced according to recipe with or without the addition of cream (mean +/- SE).

Descriptor	Without cream	With cream	P
Overall acceptance of appearance	61.72±3.93	63.25±3.41	SN
Coloring uniformity	81.99±2.82	84.48±2.73	SN
Overall pleasantness of smell	69.18±3.13	75.63±3.87	SN
Intensity of caramel smell	41.68±5.36	50.92±4.79	SN
Intensity of typical cheese smell	38.54±4.89	39.20±4.42	SN
Intensity of goat smell	17.08±3.57	16.33±3.34	SN
Intensity of foreign smell	93.77±2.64	96.38±1.61	SN
Hardness	48.37±3.61	52.00±4.48	*
Spreadability	57.81±3.32	54.70±5.13	SN
Sandiness	56.09±5.84	67.65±4.87	*
Friability	66.64±4.72	74.48±3.90	*
Overall pleasantness of taste	47.72±4.87	55.50±4.45	SN
Intensity of sweet taste	49.28±4.46	53.33±4.79	SN
Intensity salty taste	32.78±4.35	33.79±4.55	SN
Intensity of caramel taste	53.23±4.29	55.24±4.50	SN
Intensity of goat taste	74.85±4.72	75.03±5.28	SN
Intensity of foreign taste	93.75±2.34	92.97±2.45	SN

SN – statistically non-significant (P>0.05);
* – statistically significant (P<0.05)

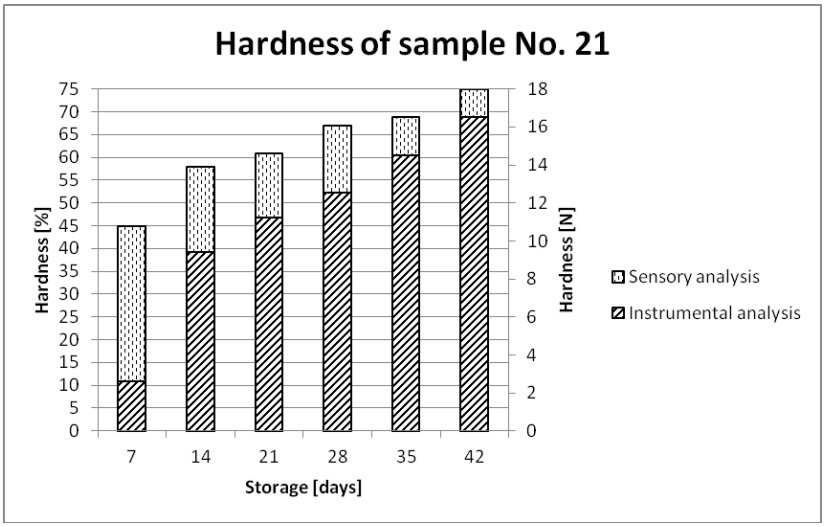

Fig. 2. Comparison of hardness of sample No. 21 determined by instrumental and sensory methods during storage.

Table 2. Comparison of hardness of sample No. 21 determined by instrumental and sensory methods during storage (mean +/- SE).

Duration of storage [days]	Hardness of sample No. 21	
	Instrumental analysis [N]	Sensory analysis [%]
7	2.65±0.15^{a}	45.3±8.3^{a}
14	9.44±1.01^{b}	58.2±8.0^{ab}
21	11.23±1.22^{bc}	60.9±9.0^{ab}
28	12.58±1.01^{c}	67.1±8.9^{b}
35	14.52±0.91^{d}	69.2±7.7^{b}
42	16.53±1.15^{d}	75.4±9.8^{b}

$^{a, b, c, d}$ – means between rows with different superscripts indicates statistically differences (P< 0.05)

CONLUSION

The assessors did not notice the difference in taste and smell of the samples produced by designed recipe with or without the cream addition. The intensity of the salty taste of these two groups was not perceived positively by evaluators. Regarding mentioned facts, it was necessary to modify and upgrade the technology of existing recipes using various additives in order to reduce the salty taste of the produced whey cheeses. From these samples, the most acceptable one from a sensory point of view was the sample with the addition of medium brown caramel (No. 21). For this reason, this sample was analyzed by instrumental and sensory method during longer period of time and the results show significant changes in consistency of the product during storage, which resulted in an increase in hardness with the ideal values around the fourth week of storage. Hardness of produced whey cheese was significantly affected by raw materials and production technology. On this model experiment will be followed by further research. In this research will be selected a few recipes which will be tested by consumers.

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СЕНЗОРНА И ИНСТРУМЕНТАЛНА ОЦЕНКА НА СИРЕНЕ ОТ СУРОВАТКА

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(Резюме)

Суроватката е млечен вторичен продукт, който не само в Чешката република все още не се използва достатъчно ефективно. Главно в Скандинавските страни съществува възможност за производството на характерно сирене от суроватка. Направени са експериментални сирена от суроватка според рецептата, създадена от МЕНДЕЛУ. Образците са оценени посредством сензорен и текстурен анализ. С най-висока сензорна приемливост (P < 0.05) беше образецът с добавка на карамел. Бяха забелязани промени в консистенцията на сиренатa по време на съхранението. Резултатът беше увеличаване на твърдостта, което беше потвърдено от сензорния анализ и инструменталното измерване. Твърдостта на всички произведени сирена от суроватка беше значително повлияна от суровината и технологията на производство. Следвайки описания моделен експеримент, бъдещият експеримент ще включва главно оптимизиране на състава и верификация на избрани рецепти за сензорен панел.

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Application of Black Sea sapropels for increasing of grain beans yield cv. "Smiljan", cultivated on cinnamonic pseudopodzolic soil (Planosol)

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In the period 2012 – 2013 was studied the effect of application of deep water Black Sea sediments (sapropels) for recultivation of acidic cinnamonic pseudopodzolic soil (Planosol) [CPS (Planosol)], used for growing of beans for grain, cv. "Smiljan". The results obtained showed that in an amount 20 g/kg, the sapropels (Variant 1) neutralize the acidity of CPS (Planosol) from 4.53 to pH 6.87 after incubation period of a month and to pH 7.05, 7.12 and 7.27 respectively after 2, 3 and 4 months incubation. The sapropels increase the content of humus in the used soil from 6.7 to 11.2 g/kg and the content of $CaCO_3$ from 0.22 to 1.46 g/kg. At Variant 1 the grain yield was 33.2 % more higher, compared to Control. The content of crude protein increases with 12.8 % and the content of phosphorus in the grain – with 1.1 % more, in comparison to Control.

Keywords: *Organic-mineral sediments, soil acidity, crude protein, dry matter*

INTRODUCTION

The grain legumes collections are the richest group in plant world, with their economic value and dissemination they take second place after cereals. They supply about 20 % of total fund of plant protein. The seeds are 2 to 5 richest of proteins compared to cereals, and most of them consists between 20 – 35 % crude protein. Beans (Phaseolus vulgaris) according to Terziev et al. [1] as a cultural plant is well known since ancient times. Mature seeds of beans are valuable food for humans because of its high content of protein, amino acids, carbohydrates, minerals, vitamins B, C and others. Beans is a strategic food because its seeds can be stored for years without losing their nutritional value. The legumes are for a big importance for agriculture as trench culture, because they enrich the soil with nitrogen. According to Crepon [2] bean protein is easily assimilated by the human body, unlike those of meat foods for which the degradation toxic products cause pathological changes in human organism. Bean seeds are a source of valuable amino acids for the human body. Beans is included in the diet of people suffering from various diseases - cardiovascular, diabetes, etc.

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In our country areas and production of beans are concentrated mainly in northeastern Bulgaria, but is also is grown in other areas, including on acid soils that are not favorably for its cultivation. The optimum soil pH lies in neutral and weakly alkaline medium. So that is needed the acidic soils to be neutralized with appropriate ameliorants. As such, currently are used various lime ashes, lime cream, calcium carbonate and others. A disadvantage of these ameliorants according to Dimitrov et al. [3] may indicate that they do not provide long-term stabilization of pH, because of an absence of buffering capacity.

According to Koteva et al [4] from about 46 millions da cultivated agriculture lands in Bulgaria, 3.5 millions are classified as strong acidic (pH 4.1 - 4.6), and 4.6 - 5.0 millions – as acidic soils (pH 4.6 - 6.0) as a consequence of long standing using of nitrogen fertilizers, acidic rains, waterlogging related to reduction processes etc., leading to destruction of the soil structure and low yields. In fact more than 8 millions decares lands need a chemical recultivation.

The Black Sea organic-mineral sediments (sapropels) according to Dimitrov et al. [5] in agreement with Bmins [6] represent an unique natural phenomena, having no analogue between the sediments found in other watersheds on earth. During last years they are a subject of special research, because of the opportunity for application in several aspects of agriculture. The soil fertility according to Petrova [7] depends on the content of

nutrients, pH of the soil solution, the soil structure, irrigation regime etc. According to Nikolov et al. [8] the introducing of sapropels in acidic soils leads to increasing of soil buffering capacity and enriching of exhausted soils with useful for the plants nutrients and organic matter. The sapropels are widespread on the Sea bottom at a depths 1200 – 2000 m and in fact are practically inexhaustible. An important their advantage according to Shnukov et al [9] in agreement with Dimitrov [10] is that they are completely sterile, because of their forming in non-living hydrogen sulphide zone on the sea bottom.

The aim of present work was to study the opportunity to use Black Sea sapropels for increasing the grain yield of beans cv. "Smiljan", cultivated on the acidic soil CPS (Planosol).

EXPERIMENTAL

Elemental analysis

Sample sapropels, taken from a depth 1200 m was analysed for a content of Si, K, P, Ti, Al, Ca, Na, as well as some micronutrients, as Fe, Mn, Mg, Cr, Mo, Cu, and heavy metals Zn, Ni, Pb. They were determined in the form of oxides. An inductively coupled emission spectrometry (Jobni Yvon Emission - JY38S, France) was used. The quantitative measuring was carried out with apparatus ICP.

Neutralization of Planosol.

For preparing of the trial parcels, acidic CPS (Planosol) from the area of Zlatosel village (Plovdiv district), taken from deep horizon 0 - 40 cm was mixed with sapropels at amounts 20 g/kg. After pouring on with water, samples were left for incubation. The samples were periodically filled up with water and mixed. Parallel was prepared two control parcels with CPS. During a month of incubation period, the beads were closed to avoid an access of atmospheric air. After incubation period of 1, 2, 3 and 4 months at a temperature 303 K were determined pH of the detached varieties in water medium by pH-meter, model OP-211/1 (ISO 10390). Parallely were established the pH value of sample sapropels.

Determination of exchange ions, humus content and calcium carbonate.

The content of exchange ions - Ca^{2+}, Mg^{2+}, Al^{3+} and H^{+} was determined by standard method[11] in extract of 1n KCI. The humus content of CPS (Planosol) in the soil horizon 0 - 40 cm, as well as in the soil-sapropels mixtures and in sample pure sapropels was determined by the method of Turin. The content of active calcium carbonate was established by the method of Druino and Gale [11].

Field experiment.

Bean seeds were sown on the 15th of April in four replications of 12 plants in two trial parcels, every of them with surface 1 m^2. Parralely were sown bean seeds in four replications on the two control parcels with the same surface. There were made all necessary agro technical activities. Feeding with NH_4NO_3 in the both variants was made twice at a dose 4 g for a plant, as the first was at the 30th of April in a phase 3th - 4th leave and the second - at the 15th of June in phase flowering. The beans plants at Control and Variant 1 were grown by the same irrigation regime. For the normal development of bean plants was used wire structure with a height 3 m. Picking of grain harvest for the two investigated years was made at the 15th of November.

Analysis of beans grain

After the end of vegetation were determined the following indicators of beans grain: absolute dry matter by the weight method, weight of 100 bean grains, total yield of grain, kg/da. The content of crude protein was calculated on the base of nitrogen content, multiplied by coefficient 6.25. The content of nitrogen was determined by Kjeldhal method with apparatus "VELP UDK 132" (Jones 1991).

Statistical data analysis

The statistical processing of the obtained experimental data from the two investigated years was made with the program "BIOSTAT".

RESULTS AND DISCUSSION

The data from the elemental analysis of sapropels are shown at Tables 1 and 2. The content of macro- and micronutrients was established, calculated as oxides. The data show that content of calcium – 154.6 g/kg, calculated as CaO is more than its content in the most soil types. The content of some other basic nutrients as Mg, Mn, Fe exceeds their content in soils too. The rich content of macro- and micronutrients as well as of organic matter determines sapropels as a complex organic-mineral fertilizer.

The experimental data for the content of exchange ions in the samples are shown in Table 3. It was established that the neutralizing ability of sapropels used was most significant after the first month of incubation – from 4.53 to pH 6.87 and after that changes weakly reaching pH 7.27 in the.

Table 1. Chemical composition of sapropels. Content of N, P and micronutrients.

Content of nutrients in sample Black Sea sapropels								
Cr_2O_3	MoO_3	ZnO	MnO	PbO_2	CuO	NiO	P	N
g/t	g/t	g/t	g/t	g/t	g/t	g/t	g/kg	g/kg
50	36.4	65.8	383.4	28.2	36.6	49.8	9.6	25.4

Table 2. Chemical composition of sapropels. Content of humus, micro- and macronutrients.

Content of humus and nutrients.in sample Black sea sapropels, g/kg										Humus /g/kg/
1	SiO_2	TiO_2	Al_2O_3	FeO	MnO	MgO	CaO	Na_2O	K_2O	
2	397.6	7.0	117	45.6	0.4	26.8	154.6	21.3	18.3	68.6

Table 3. Exchange ions content and pH of CPS (Planosol) and soil-sapropels mixture.

Variants	Exchange ions after 1 month, mgeq/l				pH after months of incubation			
	Ca^{2+}	Mg^{2+}	$Ca^{2+}Mg^{2+}$	Al^{3+}, H^+	1	2	3	4
Variant I	41.7	227.3	65.4	-	6.87	7.05	7.12	7.27
Control	193.7	158.3	352.0	1012.3	4.53			

end of four months incubation period. Sapropel used has almost a neutral reaction – 7.28 pH units, but as the main reason for pH change should be noted the activation of exchange bases, in the marine sapropels. pH change depends on the content of humus and exchange ions. The presence of organic carbon in the form of humic acids salts immobilized in gel structures and exchange ions (Ca^{2+}, Mg^{2+}) in the sapropels composition, improve the buffer ability of the investigated CPS (Planosol).

The content of some nutrient nutrients and compositions changes after incubation of sapropels in the used CPS soil. The humus content increases from 6.70 to 11.20 g/kg and $CaCO_3$ – from 0.22 to 1.46 g/kg. The content of P and N changes insignificantly (Table 4).

Table 4. Content of humus, $CaCO_3$, N and P in CPS (Planosol). (Planosol), sapropels and soil-sapropels mixture.

№	Variants	Humus, /g/kg/	$CaCO_3$, /g/kg/	N, /g/kg/	P, /g/kg/
1	Variant 1	11.00	1.46	7.28	3.84
2	CPS	6.70	0.22	7.30	3.80
3	Sapropels	68.6	62.2	4.50	4.00

The yield in the four trial replications and the median value is represented on a Figure 1.

The most expressed difference was established in the grain yield of beans – with 33.2 % higher in Variant 1, compared to Control. At the second place increases the content of crude protein in the grain with 12.9 %, compared to Control, most probably due to the imported with the sapropels nutrients in the soil – macro-, micronutrients, humic substances etc. Another reason is the acidic soil medium which is not favorably for growing of legumes. There is not remarkable difference in weight of 100 grains and dry matter between variant 1 and Control – 1.02 % and 1.1 % (Table 5).

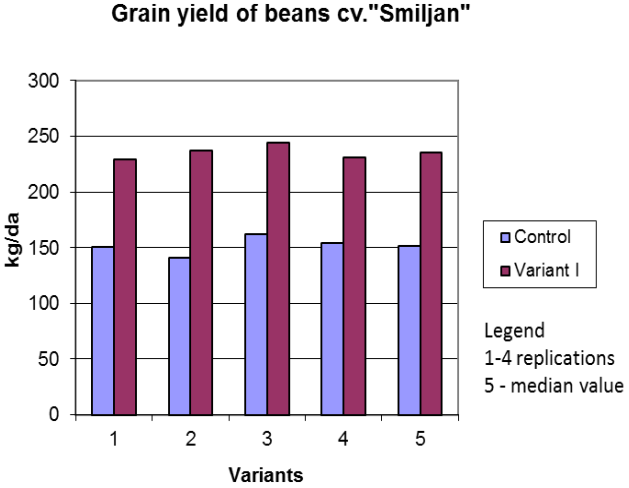

Fig. 1. Grain yield (kg/da) in replications and median value of beans, cv. "Smiljan".

The differences between the Control and Variant 1 in the total yield are well warranted (Table 6).

The marine sapropels neutralize the soil acidity of cinnamonic pseudopodzolic soil (Planosol) from 4.53 to neutral medium – pH 6.87 - 7.27 after 1 – 4 months incubation period by insignificant amount of sapropels - 20 g/kg. It influents positively on the final grain yield and the content of crude protein in the grain.

The study show that marine sapropels could be used as ameliorant and complex organic-mineral fertilizer for acidic soils in order to optimize the

yield and biochemical indicators of legumes, which are sensitive to acidic soil reaction.

Table 5. Biochemical indicators and weight of 100 beans grains.

№	Variants	Dry matter, g/kg	Crude protein, g/kg	Weight of 100 grains, g	Total yield, kg/da	P, g/kg
1	Variant I	879.6	253.8	109	215.2	10.53
2	Control	864.3	198.8	99	161.8	9.6
3	Difference	1.02	12.8	1.1	33.2	1.1

Table 6. Total grain yield of beans, cv. "Smiljan".

Variants	Total yield, kg/da	± D	Rank
Control	152.05	0	IV
Variant 1	235.4	+ 83.35	I
GD 5 %	9,82		
GD 1 %	14.23		
GD 0.1 %	21.34		

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ИЗПОЛЗВАНЕ НА ЧЕРНОМОРСКИ УТАЙКИ ЗА УВЕЛИЧАВАНЕ ДОБИВА НА ФАСУЛ ЗА ЗЪРНО, СОРТ „СМИЛЯН", КУЛТИВИРАН ВЪРХУ КАНЕЛЕНА ПСЕВДОПОДЗОЛИСТА ПОЧВА (Planosol)

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(Резюме)

В периода 2012-2013 г. е изследван ефектът от прилагането на дълбоководни черноморски утайки (сапропели) за рекултивация на кисела канелена псевдоподзолиста почва (Planosol), използвана за отглеждане на фасул за зърно, сорт Смилянски. Получените резултати показват, че в количество 20 g/kg, сапропелите (вариант 1) неутрализират киселинността на канелената псевдоподзолиста почва (Planosol) от 4.53 до pH 6.87 след инкубационен период от един месец и до pH 7.05, 7.12 и 7.27 съответно след 2, 3 и 4 месеца инкубация. Сапропелите увеличават съдържанието на хумус в използваната почва от 6.7 g/kg до 11.2 g/kg и съдържанието на $CaCO_3$ от 0.22 g/kg до 1.46 g/kg. При Вариант 1 добивът на зърно е с 33.2 % по-висок, в сравнение с контролата . Съдържанието на суров протеин се увеличава с 12.8%, а съдържанието на фосфор в зърното - с 1.1% повече, в сравнение с контролата.

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Reducing the rate of nitrogen fertilization for growing of early tomatoes, cv. "Dar", using modified fertilizing granules

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Growing early tomatoes, cv. "Dar" on alluvial meadow soil (Calcaric Fluvisol) in non-heated greenhouses in the period 2008 – 2009, the nitrogen fertilization was optimized, thank to introduction of modified granules, containing ammonium nitrate, deep water Black Sea sediments (sapropels) and water swelling polymer. In variant I, using granules coated with a polymeric layer of partially acetylated (20 %) polyvinyl alcohol (PVA-M), the early yield increased with 12.4 %, and the total standard yield increased with 11.9 % in comparison to control. Due to reduced fertilization rate and once introduced modified granules, was realized economy of 12.5 % ammonium fertilizer. Ecological effect is expected, due to decreased pollution of groundwater and tomato fruits with nitrates.

Keywords*: Black Sea sediments, yield, modified granules, polymeric membrane, modified polyvinyl alcohol.*
Abbreviations: **SAS** – surface active substances (surfactants)
PVA - polyvinyl alcohol,
PVA-M - modified PVA/ ,
$\mathbf{M_m}$ – median molecular mass

INTRODUCTION

The traditional methods of nitrogen nutrition in many cases lead to number of unfavourable on the environment phenomena. According to Koteva [1] the long standing mineral fertilizing leads to increasing of soil acidity, destruction of soil structure and lower humus content, especially by using of ammonium nitrate. A part of ammonium nitrogen can not be assimilated, due to evaporation by decomposition of salt in the soil. According to European Environment Agency the presence of nitrates is a common problem for many European regions. In many cities groundwater are contaminated with nitrates. In regions with intensive agricultural activities, nitrate content is higher than the allowable 50 mg/l [2]. According to Revich [3] the intensive feeding with nitrogen deteriorates the quality of plant production, related to changes in population health.

In recent decades, the Black Sea sapropels are subject of special research, because of possibility for their application in various aspects of agriculture, related to increase of soil fertility. Their origin according Dimitrov at al. [4], have started after an ecological cataclysm.

As a consequence the more from the available flora and fauna perished and formed 1-2 m sediments on the sea bottom. An important motive for studying of sapropels according to Bmins [5] in agreement with Dimitrov at all, [6] and Shnukov et all [7] is the favorable macro- and micro- componential composition of sapropels. Georgiev [8] has established that they improve the agrotechnical properties of soils and stimulate the growth of plants. Acording to Cholakov et all [9], tomatoes do not tolerate high nitrogen fertilization rates. Overdosing especially those fertilizers with acidic character as ammonium nitrate, ammonium sulphate, calcium nitrate etc. have a negative environmental impact on soil and plant production. It is recommended feeding to be realized by parts - before planting the seedlings and twice during the vegetation, According to Terziev et all [10] the use of chemical fertilizers with acidic character leads to progressive acidification of soils with poor buffering capacity. Parallel to this is getting worse their structure, leading to reduction in yields and nitrate accumulation in plant production over allowable concentrations.

As SAS many water-soluble and amphiphilic polymers in the form of solutions are widely used in the practice. The limited solubility of some amphiphilic polymers in water make them suitable for certain specific applications, such as adhesives, coatings with membrane properties etc

According to Nikolov et al. [11] the Black sea sapropels represent complex organic-mineral fertilizer and ameliorant for various type of soils. Thank to their buffering ability they can be used to control acidity by soils with pH less 5.5. Using fertilizing agents with acidic reaction the

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introduction of sapropels regulate the soil medium, which is favorable, especially by sensitive to acidic reaction crops.

The aim of present work was to reduce the rate of nitrogen fertilization by growing of early tomatoes, cv.Dar, using modified fertilizer granules, containing polymers and Black sea sediments (sapropels).

MATERALS AND METHODS

1. Elemental analysis

The content of some micro- and macro nutrients was determined in sample sapropels, taken from a depth 1200 m. K, P, Si , Ti , A1, Ca, Na., as well as some micronutrients as Fe, Mn, Mg, Cr, Mo, Zn, Mn, Cu, Ni and the heavy metal Pb was determined, (calculated in the form of oxides) The content of the main nutrient nutrients N, P, K, Ca and Mg (ppm) in the alluvial meadow soil was determined. The inductively coupled emission spectrometry (Jobni Yvon Emission - JY 38 S. France) was used. The quantitative measures were carried out with apparatus ICP. The humus content of sample pure sapropels was determined by the method of Turin (Trendafilov et al., 2007)

2. Determination of pH and EC (mS/cm)

pH values (H_2O) of the tested alluvial meadow soil (Calcaric Fluvisol) was determined using pH meter, model OP-211/1, (ISO 10390). Additionally was determined electrical conductivity (EC) of the tested soil (mS/cm) by EC meter, model SOPs.

3. Preparation of modified granules

For the purpose of the field experiment modified granules tomatoes cv."Dar" with the following composition were prepared:

- 440 g/kg ammonium nitrate in the form of clasical granules,
- 300 g/kg Black sea sapropels and 1,3 g/kg polymer PVA-M
- 258.7 g/kg kaolin, (IUPAC Standard InChl, with formula $Al_2O_7Si_2$ and molecular weight 222.13).

The filler and sapropels were pre-screened through a sieve of 1.0 mm.

The granulation was carried out by mixing and injection (pressing), by using aqueous solution of PVA-M with concentration 5 g/kg as an adhesive. PVA-M represents partially acetylated PVA with a rate of acetilating 20%. The starting PVA was with M_m = 73 000. After 24 h drying at room temperature granules obtained were additionally coated with a polymeric layer by immersion in aqueous solution of the same polymer with concentration 20 g/kg. The size of granules was in borders 9-10 mm (Fig.1). After drying they were used for fertilizing of tomato plants at variant 1.

4. Greenhouse experiment

The two years experiment was carried out in unheated greenhouse. Tomato plants cv."Dar" were planted at the 6th of April in two variants, every of them in four replications as follows:

1. Variant 1 - fertilizing with modified granules containing sapropels
2. Control - traditional fertilizing with ammonium nitrate

The total number of tomato plants in the both variants was 80, as every replication contained 10 plants. The four trial replications were separated from both sides with 2 guardian plants, or for the both variants - 16 plants. Planting of tomato seedlings was conducted in two-roll band in scheme 90 +60 x30 cm. The plants were grown by technology for early production. For irrigation was used drop irrigation system.

The soil used was alluvial meadow (Calcaric Fluvisol)**.** Fertilization of the test plot with superphosphate and potassium sulphate was done with the last treatment at doses backing respectively 18 and 12 kg/da active substance.

Tomato plants were formed to one stem in phase "Five wrist". Fertilization at variant 1 was made once during the planting of seedlings by introducing of modified granules. There was applied a reduced fertilization rate - 12 g ammonium nitrate for plant, corresponding to 48 kg/da, calculated for 4 000 plants/da. In the control feeding was performed three times with 15 g total amount ammonium nitrate for plant, conforming to the 60 kg/da, as follows:

1. First feeding - a week after planting of seedlings
2 .Second feeding - at the 15th of May in phase flowering.
3 . hird feeding - at the 5th of June, by an appearance of the first red tomatoes.

Two weeks after transplanting as a preventive measure against attack of plant pathogens was made treatment at variant 1 and control plants with fungicide preparation "Ridomil Gold". Both variants have grown under the same irrigation regime by using drop irrigation system, with placed nozzle at each tomato plant, including the guardian plants.

5. Statistical analysis.

The statistical processing of the obtained experimental data from the two investigated years was made with the program "BIOSTAT".

Table.1. Agrochemical analysis of Alluvial meadow soil, (*Calcaric Fluvisol*) , water extract and recommended fertilization rates

pH deep horizon 0-40cm	EC mS/cm	Nutrient elements, ppm					Recommended fertilizers, kg/da		
		N	P	K	Ca	Mg	NH_4NO_3	$Ca(H_2PO_4)_2.H_2O$ triple superphoshate	K_2SO_4
6,72	0,14	15	4,1	32	28	18	20	20	30

Table 2. Chemical composition of sapropels. Content of N, P and micronutrients

№	Chemical composition of sample Black sea sapropels								
1	Cr_2O_3 g/t	MoO_3 g/t	ZnO g/t	MnO g/t	PbO_2 g/t	CuO g/t	NiO g/t	P g/kg	N g/kg
2	50.0	36.4	65.8	383.4	28.2	36.6	49.8	9.6	25.4

Table 3. Chemical composition of sapropels. Content of humus, micro- and macronutrients

№	Chemical composition of sample Black sea sapropels, g/kg									Humus
1	SiO_2	TiO_2	Al_2O_3	FeO	MnO	MgO	CaO	Na_2O	K_2O	/g/kg/
2	397.6	7.0	117	45.6	0.40	26.8	154.6	21.3	18.3	68.6

RESULTS AND DISCUSSIONS

The data for the agrochemical analysis of water extract of Alluvial meadow soil used show that pH value - 6.72 (neutral medium) is favorable for growing of tomatoes. The insignificant content of mobile forms of N (15 ppm), P (4.1 ppm) and K (32 ppm) requires the soil used to be enriched additionally with ammonium nitrate, triple superphosphate and potassium sulphate (Table 1).

The data analysis for macro- and micronutrients (Tables 2, 3) showed that some of contents in the sapropels composition are more, than in soils and substrates used for seedlings production of vegetable crops. The content of macro- and micronutrients was established, calculated as oxides. The data show that for some important for the crops vegetation micronutrients, such as Cr, Mo, Fe, Mn, their content in sapropelles exceeds many times the same in soils. The presence of CaO is 154,6 g/kg, which is over the limits in comparison to most soil types. The content of K_2O - 1,83 g/kg, MgO – 26,8 g/kg and some other nutrients determine the deep water marine sapropels as a natural micro- and macro fertilizer. The lost by heating at 1273 K, (table 2) is 199,7 g/kg, because of organic matter and carbonates. Humus content according to Koteva et all (1983) is an important factor for the soil fertilitybecause it improves the nitrogen assimilation from theplants. Sample used sapropels contains 68,6 g/kg humus The content of heavy metals Zn, Ni, Pb is in admissible borders (Table 2).

Fig.1 represents modified granules, containing NH_4NO_3, kaolin, sapropels and PVA-M as adhesive and film-forming substance on the granule surface. The result obtained showed that the use of granules coated with polymer membrane has a

Fig.1. Modified nitrogen fertilizer granules with PVA-M membrane

significant impact on economic productivity of tomato plants. This effect was better pronounced in the early yield of tomato fruit (Table 4). The increase compared to control was 24.4% as reported differences has good statistical reliability. Data from Table 4 show significant change in the proportion of early yield compared to the total standard yield. Quantity obtained in the first three harvest standard production was 40.5% of the total amount harvested to the end of the harvest period. Reliable method to include scheduled nitrogen fertilizer in granules coated with PVA-M significantly increase the total amount of the resulting standard yield – with 12.0% compared to control, although the application rate of fertilizer was reduced to 48 kg/da NH_4NO_3, compared to control - 60 kg/da. The results obtained for the early and total standard yields were well warranted. The study show that marine sapropels could be used as ameliorant and complex organic-mineral fertilizer for acidic soils in order to optimize the yield and biochemical indices of legumes, which are sensitive to acidic soil reaction.

Table.4. Early and Total Yield of Standard Tomato Fruits, cv."Dar"

Variants	Early yield of standard fruits 2008-2009				Total yield of standard fruits 2008-2009			
	kg /da	% to control	+-D	rate	kg /da	% to control	+-D	rate
1.Control	2704	100.0	0	4	7417	100.0	0	4
2,Variant 1	3365	124.4	+661	1	8308	112.0	+891	1
GD 5%	108.1				404.1			
GD 1%	156.5				584.3			
GD 0.1 %	234.8				876.5			

Analysis of results and the fact that despite the smaller quantity of fertilizer inputs, were obtained higher yields, give rise to claim that modified granules provide more effective assimilation of ammonium and nitrate nitrogen, which is an important factor for growth in all plant organs, including fruit. Formation of polymer membrane after swelling of the polymer coating, due to soil moisture, results in more even and prolonged releasing and assimilation of nitrogen in the soil nutrient solution. It reduces losses, due to decomposition of ammonium nitrate, associated with separation and departure of ammonia, as well as washing and removal of nitrate nitrogen in groundwater. The main reason for the losses is the dynamics of nitrates in the soil. They are highly mobile and pass faster the plow horizon to groundwater, thereby contaminate them. Second should be reported loss of ammonium nitrogen, due to hydrolysis of ammonium nitrate in the soil solution. Part of the nitrogen in the form of ammonia flies in the atmosphere during the decomposition of the ammonium base, which is perishable. Improved mineral nutrition of plants determines more higher yield of fruits. PVA-M as amphiphilic polymer formes stable polymer membrane, which is a prerequisite for more longer diffusion of nitrogen, associated with its more effective assimilation by plants. Should take into account the presence of marine sapropels. It is reasonable to assume that partly the established biological effect was due to additional introduction of macro-, microelements and organic matter in the soil medium.

CONCLUSIONS

1. Fertilization by modified granulates, containing ammonium nitrate, sapropels and amphiphilic water swelling polymer offers qualified new opportunities to improve nitrogen nutrition in tomato plants, cultivated in greenhouse conditions. The granules covered with polymeric layer are stable in the soil medium and assure more evenly and prolonged action of nitrogen in soils.

2. The application of modified granules has significant economical effect, manifested in increased early and total standard yield of tomato fruit with 12.4% and 11.9% respectively by reduced nitrogen fertilizer dose with 20%.

3. The tested method of nutrition is recommended to sensitive of nitrates vegetable crops, thanks to regulating entry of nitrogen.

4. Expected is realizing of ecological effect, due to decreased pollution of plant production and groundwater with nitrates under the exposure limit 50 mg/l.

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НАМАЛЯВАНЕТО НОРМАТА НА АЗОТНОТО ТОРЕНЕ ПРИ ОТГЛЕЖДАНЕ НА РАННИ ДОМАТИ, СОРТ "ДАР", С ИЗПОЛЗВАНЕ НА МОДИФИЦИРАНИ ТОРОВИ ГРАНУЛИ

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(Резюме)

При отглеждане на ранни домати сорт " Дар " върху алувиално ливадна почва (Calcaric Fluvisol) в неотопляеми оранжерии в периода 2008-2009 е оптимизирано азотното торене, благодарение въвеждането на модифицирани гранули съдържащи амониев нитрат, дълбоководни Черноморски утайки (сапропели) и водонабъбващ полимер. При вариант I, използващ гранули покрити с полимерен слой от частично ацеталиран (20%), поливинил алкохол (PVA-M), ранният стандартен добив се увеличава с 12,4% , а общият стандартен добив с 11.9% в сравнение с контролата. Благодарение на намалената торова норма и еднократно внесени модифицирани гранули се реализира 12.5% икономия на амониев тор. Очаква се реализиране на екологичен ефект, поради намалено замърсяване на подземните води и доматените плодове с нитрати.

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Ohmic thawing of frozen ground meat

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In this study, the applicability of ohmic heating on tempering of the frozen ground meat was investigated. Ground beef meat has been shaped as a block of 1 cm × 7 cm × 10 cm in a specially designed container, and then frozen at air blast freezer (-30 °C). It was aimed to reach the center temperature of the frozen block meat to +20 °C from –18 °C. The ohmic tempering was performed by the application of different voltage gradients (10, 15 and 20 V cm^{-1}) whereas conventional tempering was performed at controlled conditions in the refrigerator (4 °C). The effects of the thawing method and the voltage gradient on tempering time, temperature distribution, color and pH were investigated. As the voltage gradient increased, the tempering time decreased in the range of 92-95 % comparing to conventional tempering. Average initial thawing temperature was measured as -1.1 °C for the samples. At the end of the tempering , the cold point of the ground meat was 0 °C while the minimum temperature value of surface were -4.7 °C, -3.3 °C and -3.4 °C for 10, 15 and 20 V cm^{-1} voltage gradients, respectively. Similarly, the thawing method and the voltage gradient affected the color properties (L*, a*, b*, Hue angle, chroma and total colour differences), significantly ($p<0.05$). The value of a*, which is important for meat samples, was similar for conventional thawing and ohmic thawing applied at 10 V cm^{-1}. It is thought that the results of this study will provide data for the scaling up of the industrial ohmic tempering or thawing systems and give useful insights to further studies on these subjects.

Keywords: Ohmic heating, Tempering, Ground meat

INTRODUCTION

Thawing of frozen foods by using conventional methods require long time, provide non-homogeneous thawing. They are generally applied at uncontrolled conditions under high temperatures (except for refrigerators) affecting the microbiological and nutrient quality of foods adversely. In recent years, the applicability of alternative thawing methods such as microwave, radio frequency and ohmic thawing has been studied [1-4]. Ohmic thawing could provide homogeneous and fast heating with minimum loss on the food quality and the nutrient value while obtaining microbiologically safe food. Optimum thawing procedures should be of concern to the food industry, and it is commonly accepted that it should be the rapid thawing at low temperatures and avoid a notable rise in temperature, and prevent the excessive dehydration of. However, it is difficult to accomplish them by using conventional thawing processes because the use of low temperatures reduces the temperature difference between the frozen sample and the environment, which is the principal driving force for the thawing process [5].

Ohmic treatment is one of the electroheating methods, and is based on the passage of electrical current through a food product having electrical resistance [6-8]. Heat is generated instantly inside the food [9,10]. The ohmic treatment is an alternative method for thawing of meat products. The application of ohmic thawing required less treatment times than conventional methods at the same temperature range [11]. Ohmic thawing technology showed high potential to supply thawed foodstuff on high quality [12]. However, very little research about the application of ohmic thawing has been carried out in the literature, and the primary food studied for the application of ohmic thawing on meat processing has been shrimp blocks [13-20].

This study focused on the applicability of ohmic thawing procedures for semi-solid type food at different voltage gradients. The main objectives of this work were to investigate the effect of voltage gradients on thawing time, temperature distribution, color properties, total dry matter content and pH of ground meat during ohmic thawing; and to compare the effects of ohmic and conventional thawing on these selected attributes. The result of this study would be beneficial for the setting up of the industrial or pilot-scale ohmic thawing units for meat products.

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EXPERIMENTAL

Meat was ground twice by meat grinder (Arçelik, Turkey) and ground meat was frozen in air blast freezer (Electrolux, Sweden) quickly by replacing it into an ohmic cell with dimensions of 1 cm x 7 cm x 10 cm. Frozen samples were tempered ohmically (10, 15 and 20 V cm^{-1}) and conventionally (+4 °C). Ohmic thawing experiments were performed using a custom-designed laboratory scale ohmic thawing system which consisted of a power supplying system (an isolating transformer and a variable transformer), a microprocessor board, the computer connection and the thawing cell (Fig. 1). The thawing cell used was made up of Teflon®. To compare the effect of ohmic thawing process, the conventional thawing method was performed in a refrigerator (Arçelik, Turkey) having controlled temperature (+4 °C).

During freezing and thawing, temperature values and time were recorded to find out temperature distribution, tempering time and initial freezing point. Temperature measurement was performed with insulated thermocouples and microprocessor (Omega, UK). Thermocouples located to the cold point of ground meat before freezing. Colour measurements of the samples were carried out by using a HunterLab Colorflex (CFLX 45–2 Model Colorimeter, HunterLab, Reston, VA). pH was determined by using a membrane pH meter (Hanna Instrument, USA). The instrument was standardized each time with a black glass and a white tile (*X*: 79.09, *Y*: 83.98, *Z*: 88.69 and *L*: 93.44, *a*: -1.12, and *b*: 1.02). Color values were expressed as *L*, a, and *b* at any time. Lightness value, *L*, indicated how dark/light the sample was (varying from 0-black to 100-white), *a* was a measure of greenness/ redness (varying from -60 to +60) and *b* was the grade of blueness/yellowness (also varying from -60 to +60). Four readings were performed for each replicate. The combination parameters (Hue angle) were calculated by using tristimulus values measured (Eqs. 1-6).

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad \text{Eq.1}$$

$$Hue\ angle = tan^{-1}\frac{b^*}{a^*} \quad \text{Eq.2}$$

$$\Delta E = \sqrt{(L^* - L_0{}^*)^2 + (a^* - a_0{}^*)^2 + (b^* - b_0{}^*)^2} \quad \text{Eq.3}$$

$$\Delta C = \sqrt{(a^* - a_0{}^*)^2 + (b^* - b_0{}^*)^2} \quad \text{Eq.4}$$

$$\text{Chroma ratio} = \frac{\text{Chroma value of thawing sample}}{\text{Chroma value of raw material}} \quad \text{Eq.5}$$

$$\text{Hue angle ratio} = \frac{\text{Hue angle ratio thawed sample}}{\text{Hue angle ratio raw material}} \quad \text{Eq.6}$$

The total dry content and pH of each sample were measured using AOAC procedures[24]. Statistical evaluations were performed by using SPSS Version 13.0.1 statistical package (SPSS, 2004). The comparison was made to analyze the effects of voltage gradient and thawing methods on thawing time, color, pH, total dry matter content properties by using Post Hoc (Duncan) test. The confidence level used to determine statistical significance was 95%.

RESULTS AND DISCUSSIONS

All samples were thawed successfully from -14 to 0 °C by the application of ohmic thawing at different voltage gradients (10, 15 and 20 V cm^{-1}). Thawing times were 2040 s, 1455 s, 1185 s for 10, 15 and 20 V cm^{-1} voltage gradients, respectively. On the other hand, conventional thawing time was 27520 s. Generally, thawing times were similar up to the initial thawing temperature for different voltage gradients during ohmic thawing, however after this point; it decreased as the voltage gradient increased ($p<0.05$) (Figure 2). Ohmic application decreased the thawing time in the range of 92-95 % comparing to conventional thawing (Figures 2 and 3). Average initial thawing temperature was measured as -1.8 °C for the sample having the moisture content of 77%. The amount of heat generation during ohmic treatment was directly related to the current induced by the voltage gradient in the field [21,22]. Similarly, Icier *et al.*[3,19] have already concluded that the increase in the voltage gradient provided the decrease in the ohmic thawing time. The change in color values has been reported as the most important criteria to predict the behavior of ground meat during thawing, and it could provide reliable information about organoleptic characteristics. It is known that the myoglobin protein is the primary heme pigment responsible for meat color, but there are other species contributing to color changes during thawing of meat samples (deoxymyoglabin, oxymyoglabin, sulfmyoglabin, metmyoglabin). The spectral features in the visible region allow us to explain these changes. Color changes were evaluated in terms of *L, a, b*, Hue angle, ΔE, ΔC, chroma and hue angle ratio values (Table 1). Color values of thawed samples were significantly different from raw material for both thawing methods ($p < 0.05$). The value of a*, b*, total color differences and chroma differences was highest at the high voltage gradient.

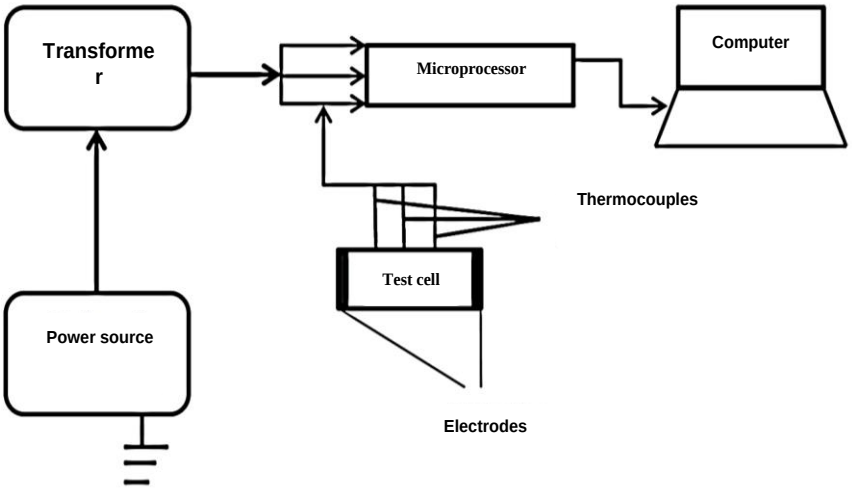

Fig. 1. Schematic diagram of laboratory scale ohmic thawing unit [8]

Table 1. Changes on color attributes of ground meat thawed by different methods

	L*	a*	b*	ΔE	ΔC	HUE	HUE ANGLE RATIO	CHROMA RATIO
Raw material	46.90+0.01	15.88±0.06	16.64±0.08	-	-	46.33±0.03	-	-
20 V cm^{-1}	47.78±0.42	19.82±1.04	17.87±1.08	4.35±1.15	4.25±1.11	42.01±1.48	0.91±0,03	1.16±0.06
15 V cm^{-1}	44.38±0.89	16.73±0.68	17.14±0.35	6.70±0.67	6.55±0.76	40.63±0.52	0.70±0.06	0.60±0.08
10 V cm^{-1}	47.38±0.66	19.54±1.67	19.26+0.4	3.58±1.75	3.34±1.69	44.68±1.94	1.06±0.05	0.90±0.05
Conventional	48.59+0.17	21.71±1.06	19.91±0.44	2.85±0.44	1.13±1.10	42.55±0.8	1.00+0,01	0.97+0.03

Table 2. Changes on total dry matter contents and pH of ground meat thawed by different methods

	THAWING METHODS				
Analysis	Raw material	10 V cm^{-1}	15 V cm^{-1}	20 V cm^{-1}	Conventional
Total dry matter	76.97 ± 0.04	77.18 ± 0.03	77.41 ± 0.01	76.53± 0.05	76.79± 0.08
pH	5.78 ± 0.04	5.81 ± 0.04	5.86 ± 0.05	5.88± 0.01	5.81 ± 0.01

There was a decrease in Hue angle values of thawed samples comparing to raw materials. When 10 V cm^{-1} voltage gradient was used, the color of ground meat was maintained successfully with comparably fast thawing then conventional thawing. Hence, 10 V cm^{-1} was recommended as the best voltage gradient for ohmic thawing of the ground meat. The effects of thawing method and voltage gradient on pH and total dry content values were not statistically significant (Table 2) ($p>0.05$).

The pH of meat that has been frozen and thawed tends to be lower than prior to freezing[23]. As pH is a measure of the amount of free hydrogen ions (H^+) in a solution, it is possible that freezing with subsequent exudate production could cause denaturation of buffer proteins, the release of hydrogen ions and a subsequent decrease in pH. Alternatively, the loss of fluid from the meat tissue may cause an increase in the concentration of the solutes, which results in a decrease in the pH. In this study, the effect of thawing method and the voltage gradient was not statistically effective on pH

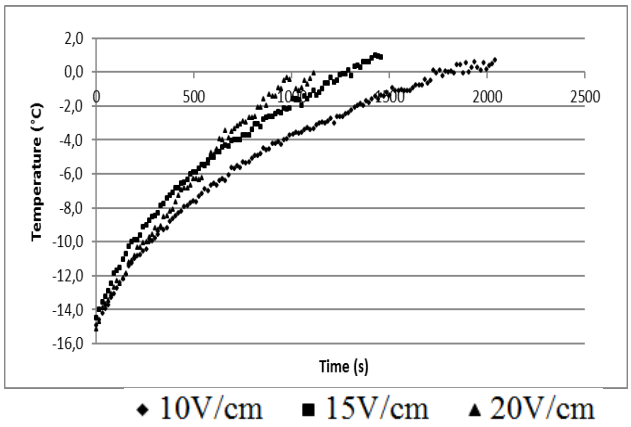

Fig. 2. Changes in the temperature of ground meat during ohmic thawing processes

value of ground meat ($p>0.05$). However, there is an increasing trend in pH value as the voltage gradient increased to 20 V cm^{-1}. It could be concluded that ohmic thawing at low voltage gradients could not result to the denaturation of buffer proteins, the release of hydrogen ions, etc. However, further studies should be conducted on the determination of the effects of ohmic thawing conditions on protein content and the release of hydrogen ions.

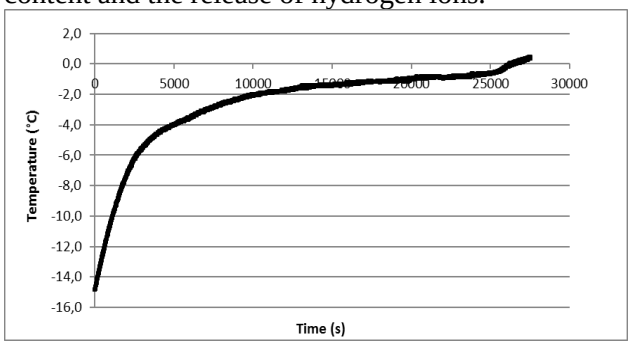

Fig. 3. The increase in the temperature of ground meat during conventional thawing processes

CONCLUSION

In the present study, the application of ohmic treatment as a thawing process was investigated by making the comparison with the conventional thawing method in terms of selected quality attributes. The applied voltage gradient did not have any significant effect on the pH, total dry matter content whereas it had significant effect on the thawing time, and color attributes of ground meat. In this study, it was aimed to obtain basic information on the effects of ohmic thawing on selected attributes of ground meat before the scaling up of industrial and pilot-scale thawing systems. In addition, further studies should be performed to determine whether the undesirable components in ohmic thawing have been occurred.

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ОМОВО РАЗМРАЗЯВАНЕ НА ЗАМРАЗЕНА КАЙМА

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(Резюме)

В настоящата работа е изследвана възможността за приложение на омовото отопление за темперирање на замразена кайма. Кайма от говеждо месо беше формувана в специално проектиран контейнер като блокче с размери 1 cm × 7 cm × 10 cm и след това замразена във въздуходувен фризер (-30 °C). Целта беше в центъра на замразеното блокче да се достигне температура от +20 °C, като се стартира от температура –18 °C. Омичното темперирање се извърши чрез прилагане на различни градиенти на напрежението (10, 15 and 20 V cm^{-1}), докато конвенционалното темперирање се извърши при контролирани условия в хладилник (4 °C). Беше изследван ефекта на метода на размразяване и на градиента на напрежението върху времето за темперирање, разпределението на температурата, цвета и pH. При увеличаване на градиента на напрежението, времето за темперирање намалява 92-95% в сравнение с конвенционалното темперирање. За образците беше измерена средна начална температура на размразяване -1.1 °C. В края на темперирането студената точка в каймата беше 0 °C, докато минималните стойности на температурата на повърхността бях -4.7 °C, -3.3 °C и -3.4 °C за градиенти на напрежението съответно 10, 15 and 20 V cm^{-1}. По подобен начин вида на размразяването и градиента на напрежението оказват съществено влияние ($p<0.05$) върху цветните характеристики (L*, a*, b*, ъгъл на Hue, хрома и цялостна цветна разлика). Стойността на a*, която е съществена за месни продукти, беше подобна за конвенционално размразяване и омово отопление, приложено при градиент на напрежението10 V cm^{-1}. Смята се, че резултатите от това проучване ще осигурят данни за постепенно повишаване на промишленото приложение на омовото темперираци или размразяващи системи и дават полезна информация за по-нататъшни изследвания по тези въпроси.

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Use of supercritical CO_2 in food industry

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Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids (SCF) as the extracting solvent. Carbon dioxide is the most commonly used supercritical fluid in food industry, especially being used for decaffeination. In this study, supercritical fluid extraction technique and its uses (especially carbon dioxide) in food industry (as essential oil production, fractional separation of oils, removing of cholesterol, debittering, inactivation of pectinmethylesterase, sterilization, extraction of aromas in juices and antioxidant compounds from vegetables, dealcoholisation of alcoholic beverages etc.) will be detailed.

***Key words**: supercritical fluid, extraction, carbon dioxide, food*

INTRODUCTION

Extraction can be defined as the removal of soluble material from an insoluble residue, either liquid or solid, by treatment with a liquid solvent. It is therefore, a solution process and depends on the mass transfer phenomena [1].

A fluid is termed supercritical when the temperature and pressure are higher than the corresponding critical values (Figure 1). Thus, the physicochemical properties of a given fluid, such as density, diffusivity, dielectric constant and viscosity can be easily controlled by changing the pressure or the temperature without ever crossing phase boundaries [2]. Supercritical fluids (SCF) are suitable as a substitute for organic solvents in a range of industrial and laboratory processes because of regulatory and environmental pressures on hydrocarbon and ozone-depleting emissions. SCF-based processes has helped to eliminate the use of organic solvents such as hexane and methylene chloride [1,3]. The close relationship between the fluid density and its dissolving power and its favorable mass transfer properties makes supercritical fluids a useful processing medium for extraction and separation techniques [2,4].

Supercritical fluid extraction (SFE), supercritical gas extraction, and dense gas extraction are alternative terms to name the operation with a fluid at temperatures and pressures near the critical point [5]. It is defined as separation of chemicals, flavors from the products such as coffee, tea, hops, herbs, and spices which are mixed with supercritical fluid to form a mobile phase [2]. It can be used as sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils) [1,6-9]. Supercritical fluids can offer a good catalytic activity and produce a product with no solvent residues [1,3].

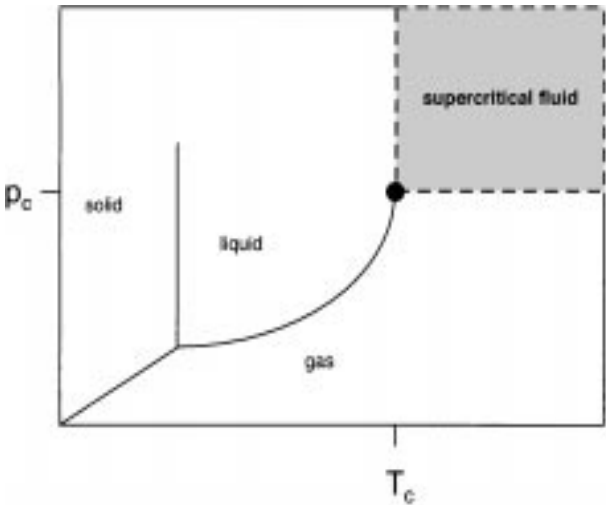

Fig. 1. Phase diagram for a single substance. P_c – critical pressure; T_c – critical temperature.

The first commercial supercritical fluid extraction was performed in Germany in 1978 by Hag AG for the decaffeination of green coffee beans. Two years later Carlton and United Breweries in Australia developed a process for the extraction of hop flavors using liquid carbon dioxide [10]. Both applications were commercially successful and have given rise to numerous variations and improvements which have also been developed on an industrial scale [11].

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A simplified process-scale SFE system is shown in Figure 2.

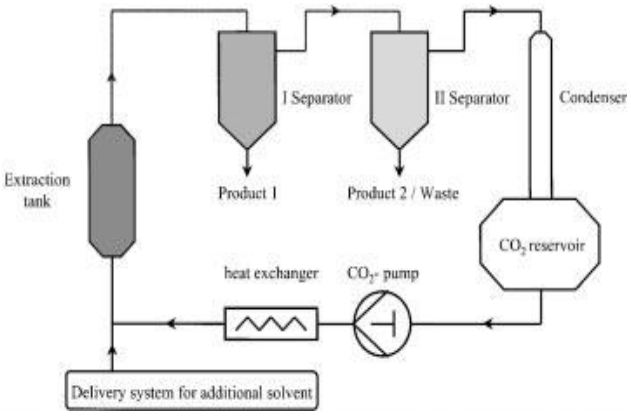

Fig. 2. A simplified drawing of a process-scale supercritical fluid extractor.

Raw material is charged in the extraction tank which is equipped with temperature controllers and pressure valves at both ends to keep desired extraction conditions. The extraction tank is pressurized with the fluid by means of pumps, which are also needed for the circulation of the fluid in the system. From the tank the fluid and the solubilized components are transferred to the separator(s), where the solvation power of the fluid is decreased by increasing the temperature, or more likely, decreasing the pressure of the system. The product is then collected via a valve located in the lower part of the separator(s) [2].

Advantages of SFE

In summary, the advantages of SFE are as follows [3]:

1. SCFs have solvating powers similar to liquid organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension.
2. Since the solvating power can be adjusted by changing the pressure or temperature, separation of analytes from solvent is fast and easy.
3. By adding modifiers to a SCF (like methanol to CO_2), its polarity can be changed for having more selective separation power.
4. Involving food or pharmaceuticals, one does not have to worry about solvent residuals if a "typical" organic solvent were used in industrial processes.
5. Candidate SCFs are generally cheap, simple and many are safe. Disposal costs are much less and in industrial processes, the fluids can be simple to recycle.
6. SCF technology requires sensitive process control, which is a challenge. In addition, the phase transitions of the mixture of solutes and solvents has to be measured or predicted quite accurately. Generally the phase transitions in the critical region is rather complex and difficult to measure and predict.

Table 1. The critical properties for some components commonly used as supercritical fluids.

Critical properties of various solvents				
Solvent	Molecular weight, g/mol	Critical temperature, K	Critical pressure MPa, atm	Critical density, g/cm^3
Carbon dioxide (CO_2)	44.01	304.1	7.38 (72.8)	0.469
Water (H_2O) (acc. IAPWS)	18.015	647.096	22.064 (217.755)	0.322
Methane (CH_4)	16.04	190.4	4.60 (45.4)	0.162
Ethane (C_2H_6)	30.07	305.3	4.87 (48.1)	0.203
Propane (C_3H_8)	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C_2H_4)	28.05	282.4	5.04 (49.7)	0.215
Propylene (C_3H_6)	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH_3OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C_2H_5OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C_3H_6O)	58.08	508.1	4.70 (46.4)	0.278

In Table 1, the critical properties for some components commonly used as supercritical fluids are shown [1]. All these fluids have a low critical temperature and pressure.

General applications of supercritical fluid include recovery of organics from oil shale, separations of biological fluids, bio separation, petroleum recovery, crude de-asphalting and dewaxing, coal processing (reactive extraction and liquefaction), selective extraction of fragrances, oils and impurities from agricultural and food products, pollution control, combustion and many other applications [3,12]. The natural antioxidants produced by SFE are also of interest to the food industry because they do not alter the aroma, flavour or colour of foodstuffs. They are very easily dispersed since they are highly soluble, and they do not evaporate during frying or baking, unlike other synthetic antioxidants [4,5]. In dealcoholisation using SFE the separation efficiency is far greater than in distillation. Furthermore, extraction temperatures are moderate (between 15 and 40 °C) which means that the thermolabile components, which are largely responsible for the aroma and flavour, do not break down [13]. The supercritical extrusion fluid has the potential to produce a range of puffed food products, such as ready-to-eat cereals, pasta and confectionery with improved texture, colour and taste [4]. Additionally, SFE products from plants are complex mixtures of essential oils, esters, terpenes, fatty acids, waxes, resins, and pigments (cited in order of decreasing solubility) [14-16]. Figure 3 shows supercritical fluid technology applied to everyday's food [16].

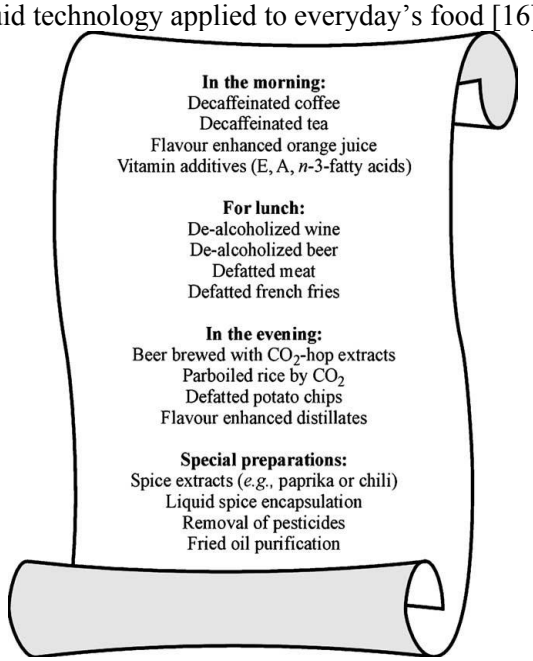

Fig. 3. Supercritical fluid technology applied to everyday's food

Supercritical CO_2

Carbon dioxide is the most commonly used supercritical fluid in food industry, especially being used for decaffeination [4,17]. It is sometimes modified by co-solvents such as ethanol or methanol [1]. It usually behaves as a gas in air at standard temperature and pressure (STP), or as a solid called dry ice when frozen. If the temperature and pressure are both increased from STP to be at or above the critical point for carbon dioxide, it can adopt properties midway between a gas and a liquid. Supercritical carbon dioxide is a fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure [3]. The density of the supercritical CO_2 at around 200 bar pressure is close to that of hexane, and the solvation characteristics are also similar to hexane; thus, it acts as a non-polar solvent. Around the supercritical region, CO_2 can dissolve triglycerides at concentrations up to 1 % mass [1].

Carbon dioxide is non-toxic, nonflammable, odorless, tasteless, inert, and inexpensive. These properties make it suitable for extracting, for example, thermally labile and non-polar bioactive compounds but, because of its non-polar nature, it cannot be used for dissolving polar molecules. On one hand, it decreases the processing times, increases yields and makes it possible to use milder processing conditions, but on the other, it complicates [2,18]. Extraction conditions for supercritical CO_2 are above the critical temperature of 31 °C and critical pressure of 74 bar (Figure 4) [17,19]. As detailed, carbon dioxide (CO_2) has become the ideal supercritical fluid in the food industry due to its characteristics: the critical temperature is 31.06°C, the critical pressure is 73.83 bar and the critical density is 0.460 g/cm^3 [2,4]. Addition of modifiers may slightly alter this. Due to its low critical temperature, carbon dioxide is known to be perfectly adapted in food, aromas, essential oils and nutraceutical industries [1,20,21]. In addition, the solubility of many extracted compounds in CO_2 vary with pressure, permitting selective extractions. So, supercritical extraction mostly uses carbon dioxide at high pressure to extract the high value products from natural materials.

Supercritical CO_2 is particularly suitable for applications in which (i) processing costs are a limiting factor, (ii) conventional solvent extraction is restricted by environmental regulation, consumer demands, or health considerations, (iii) products have improved quality and/or marketability (for example, when the "natural" character of the

product increases the market price), or (iv) traditional processing is not applicable because the product is thermally labile or morphologically unique [14].

Supercritical CO_2 tends to be selective towards lower molecular weight compounds (< 250 g/mol) or weakly polar groups such as lipids, cholesterol, aldehydes, ethers, esters and ketones, while high molecular weight (> 400 g/mol) or polar groups such as hydroxyl, carboxyl, and sugars, polysaccharides, amino acids, proteins, phosphatides, glycosides, inorganic salts, are relatively insoluble in dense carbon dioxide [4].

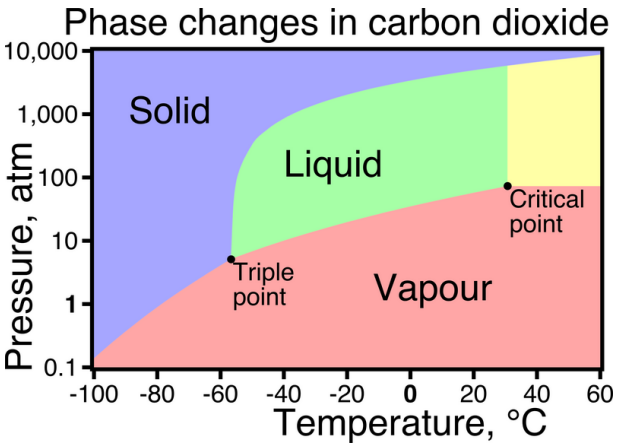

Fig. 4. Phase diagram of carbon dioxide as a function of temperature and pressure.

Carbon dioxide is forced through the green coffee beans which are then sprayed with water at high pressure to remove the caffeine. The caffeine can then be isolated for resale (e.g. to the pharmaceutical industry or to beverage manufacturers) by passing the water through activated charcoal filters or by distillation, crystallization or reverse osmosis. Supercritical carbon dioxide is also used to remove organichloride pesticides and metals from agricultural crops without adulterating the desired constituents from the plant matter in the herbal supplement industry[3]. Supercritical CO_2 extraction of various oil seeds, such as soybean, flaxseed, safflower, and cottonseed, has been reported [22-24]. Other researchers have studied the solubilities of various vegetable oils including canola, millet bran, and rice bran in supercritical CO_2 [25-27].

How does supercritical CO_2 extraction exist?

It involves heating the CO_2 to above 465 °C and pumping it above 75.84 bar. Usually, this is between 413.69 – 689.48 bar. Supercritical fluid CO_2 can best be described as a dense fog when CO_2 is used in a dense liquid state. Low-pressure CO_2 is often the best method for producing high quality botanical extracts. CO_2 loading rate in this state means that you have to pump many volumes of CO_2 through botanical. The loading rate is typically 10 – 40 volumes of product. For this reason, it is important to have pumped CO_2, which has a much faster loading rate 2 – 10 volumes and a wide range of uses [1]. The CO_2 storage tanks and extractors must be properly isolated and equipped with relief systems [18].

CONCLUSION

The successful commercial developments involves the processing of a high-value product, relatively simple extraction processes, that has finally brought about the level of growth that was initially forecast for SFE. The many advantages of the SFE can be summarized as follows: high quality and purity of the obtained product; quick extraction and separation phases; extract free of residues; selective extraction by a specific compound; reduction in separation cost. But the use of SFE in the food industry also has some disadvantages. The lack of continuous systems for extracting solid substrates imposes serious capacity restrictions on installed apparatus.

It is clear from this review that the application of supercritical fluid technology in the food industry is a field in which there is much research and development at present. In some cases, such as the extraction of caffeine from tea and coffee or aromas from spices and hops, SFE is already being used in industry. In other cases its practical applications could be seen, particularly in the area of food colourings and the refining of seed oils due to its economic potential.

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ПРИЛОЖЕНИЕ НА СУПЕР КРИТИЧЕН CO_2 В ХРАНИТЕЛНАТА ИНДУСТРИЯ

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(Резюме)

Екстракцията със суперкритичен флуид е процес на разделяне на една компонента (екстракта) от другата (матрицата), при което като екстрахиращ разтворител се използва суперкритичен флуид. Въглеродният диоксид е най-често използвания в хранителната индустрия суперкритичен флуид, особено при процеса декофеинизиране. В настоящото изследване ще бъде детайлизирана техниката на екстракция чрез суперкритичен флуид и нейните приложения (най-вече на при използването на въглероден диоксид) в хранителната индустрия (при производството на етерични масла, фракционното разделяне на масла, премахване на холестерол, отстраняване на горчивия вкус, деактивация на пектинметилестераза, стерилизация, екстракция на аромати в сокове и на антиоксидантни компоненти от зеленчуци, намаляване на алкохолното съдържание на алкохолни напитки и др.)

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Impact of innovative technologies on fruit and vegetable quality

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Food processing operations have a major impact on the stability of the nutrients and generally damage antioxidants in fruit and vegetables, and their products. Domestic, conventional, industrial and even non-thermal processing is reported to degrade the level of phytochemicals in processed food products. Recent concerns in non-thermal technologies are not only to obtain high quality food with "fresh-like" properties, but also to provide food with better functionalities. However, some researchers reported that results obtained from non-thermal processes might not be different from the thermal treatments. The main focus of this review is to clarify the dependence of non-thermal technologies such as high hydrostatic pressure (HHP), pulsed electric field (PEF) and ultrasound (US) processing on key nutrients of fruits and vegetables.

Key words: *Non-thermal food processing, high hydrostatic pressure processing, pulsed electric field, ultrasound, fruit and vegetables, phytochemicals*.

INTRODUCTION

The optimization of food processing and storage conditions is an essential step to reduce the degradation of phytochemicals for potential health benefits [1]. The effect of several non-thermal techniques such as high pressure processing (HPP), pulsed electric field (PEF), and ultrasound/sonication (US) techniques on the fruit and vegetables, and their products have been investigated [2].

High hydrostatic pressure (HHP processing could preserve nutrients and the organoleptic properties of fruits and vegetables due to its restricted effect on the covalent bonds of low molecular mass compounds and vitamins. HHP treatment may enhance the antioxidant activity of juices comparing to the untreated one [3]. While most researchers have reported that HPP helps retaining the antioxidant activity of the individual fruits, Keenan et al.[4] found out that the retention of ascorbic acid (AA), antioxidant and polyphenols contents in HHP processed smoothies was not better than that of thermally processed samples.

PEF has proved to be a validated technology for the production of safe beverage products as shown by the positive influence on the texture of solid plant foods, leading to increased yields of extraction of metabolites, as well as enhanced juice yields [5]. However, Morales-de la Peña et al. [6] determined PEF treatment to cause a reduction in vitamin C content and antioxidant capacity which might decline over time compared to conventional thermal treatment.

US treatment of fruit juices is reported to have a minimal effect on the AA content during processing and results in improved stability during storage when compared to thermal treatment [7]. Moreover, Rostagno et al.[8] used the US-assisted extraction for isoflavone extraction from soy beverages blended with fruit juices and determined that total and individual isoflavone concentration obtained with the optimized method were not significantly different from that of the conventional methods.

In order to retain the nutraceutical and pharmacological properties of phytochemicals in processed fruit and vegetable products, the food processor must optimize relevant processing steps in order to restrict the loss of phytochemicals [9]. Furthermore, positive effects of these new technologies arise from all process parameters consistency. Generally, new technologies are presenting great solutions about keeping nutrients but in some situations, parameters may affect the nutritional values of the processed product in a negative way. For example, longer PEF treatment times may induce reduction in the product retention of vitamin C due to product heating. Besides, some researchers reported that results obtained from non-thermal processes might not be different from the thermal treatments [10]. Thus for obtaining processed products in good qualities and sufficient nutritional values and having positive results after the treatments, these new technologies had to be studied thoroughly.

This review considers the impact of processing on both key nutrients and antioxidants, taking an example of fruit and vegetables as a case study to

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demonstrate how the nutritional quality of fruits and vegetables may be affected during processing.

High hydrostatic pressure processing

HHP processing is an established non-thermal food processing and preservation technique with reduced effects on nutritional and quality parameters compared to conventional thermal processing. In HHP processing, the products are treated under pressure above 100 MPa. The great advantage of HHP treatment is that pressure at a given position and time is the same in all directions, transmitted uniformly, immediately through the pressure transferring medium and independent of geometry. Literature data indicate that HPP preserves the nutritional value of HPP processed food and food products. HPP treatment at ambient temperature is reported to have minimal effect on the bioactive content of various fruits and vegetables [11].

Yen and Lin [12] investigated the effects of HHP and thermal pasteurization on AA content of guava puree during storage at 4 °C. After treatment under pressure of 600 MPa, at temperature 25 °C and 15 min treatment time, the product exhibited no change in AA content as compared to the fresh samples. The authors concluded that samples retained good quality similar to the freshly extracted puree after storage at 4 °C for 40 days. It is suggested that further AA degradation after HHP processing could take place during storage and it could be eliminated by lowering storage temperature. The AA content of untreated and pressurized (400 MPa/room temperature/15 min) guava puree started to decline after 10 and 20 days, whereas it remained constant in thermal (88–90 °C/24 s) and in higher pressure (600 MPa/ room temperature/15 min) treated guava puree during 30 and 40 days, respectively. The latter could be caused by inactivation of endogenous pro-oxidative enzyme during treatment at high pressure level[12]. Isaacs [13] - reported that at elevated temperatures, pressure treatment could degrade vitamin C to a large extent for long treatment time, e.g., pressurization up to 600 MPa/75 °C/40 min resulting in 70 % and 50 % losses of vitamin C, respectively in pineapple and grapefruit juices. In addition, at constant pressure (600 MPa/40 min), increasing temperature enhanced the vitamin C degradation of pineapple juice as a decrease of 20–25 % at 40 °C; 45–50 % at 60 °C, and 60–70 % at 75 °C respectively was observed.

Ferrari et al. [14] investigated the effects of HHP (400–600 MPa) at 25, 45 and 50 °C for 5 or 10 min on anthocyanin and polyphenol contents of pomegranate juice. Their experimental results indicated that anthocyanin content was mainly influenced by pressure and temperature levels. At room temperature, the concentration of these molecules decreased with the intensity of the treatment in terms of pressure level and processing time. The authors also indicated that high pressure treatments modified the mechanism of anthocyanin degradation by affecting the molecules involved in the kinetics of reaction, such as enzymes. The residual activity of the enzymes along with a small concentration of dissolved oxygen could cause the degradation of the anthocyanins during the storage of the processed juice, as widely supported [15].

Distinct from the application of HPP for preservation purposes, high pressure treatments have been extensively used in the extraction process of secondary plant metabolites from fruits and vegetables. For example, De Ancos et al.[16] successfully employed HHP processing (50–400 MPa/25 °C/15 min) for extraction of carotene from persimmon fruit purees. As a result, different pressure levels at constant temperature gave different release of various carotenes depending on their chemical properties and chromoplast location.
The extraction of bioactive compounds can be described as a mass transport phenomenon where solids contained in plant structures migrate into the solvent, up to their equilibrium concentration. Additionally, mass transport can be increased by some factors like heating, changes in concentration gradients, and the influence of new technologies such as ultrasound, high pressure, and pulsed electric field [17]. HHP enhances the mass transfer rates, which increase cell permeability as well as secondary metabolite diffusion [18,19]. Prasad et al.[20] extracted long an fruit pericarp in 50 % ethanol applying both HHP (500 MPa) and conventional methods. Their results demonstrated that HHP extraction showed excellent antioxidant and anticancer activities and were found to be higher than conventional extraction. Three phenolic compounds, gallic acid, corilagin and ellagic acid were identified and quantified. Compared with conventional extraction, HHP extraction exhibited higher extraction effectiveness in terms of higher extraction yield, phenolic content and antioxidant and anticancer activity with shorter extraction time. Increased extraction yields caused by high pressure were probably related to its aptitude to deprotonate charged groups and to disrupt salt bridges and hydrophobic bonds in cellular membranes, which may lead to higher permeability [19].

Pulsed electric field

PEF is a technology that has been extensively investigated in recent years for its applications in food processing. By the mechanism of electropermeabilization, PEF has been proven as a valid technology for the production of safe beverage products showing positive influence on the texture of solid plant foods, leading to enhanced yields of extraction of metabolites, as well as increased juice yields. One of the principal differences in the use of PEF consists of the intensity of field which were classified as high intensity field (15–40 kV/cm/5–100 pulses/40-700 μs/1.1-100 Hz) that are more effective towards microbial inactivation and low and medium intensity field (0.6–2.6 V/cm/5–100 pulses/ short treatment time within 10^{-4}–10^{-2} s; 1 Hz) which have been successfully used for enhancing mass transfer in solid foods [17,21]. With the increasing interest in availability of bioactive compounds from fresh to processed foods, the effects of this technology have been reviewed comprehensively [22]. Morales-de la Peña et al.[6,10] investigated the effect of PEF on vitamin C in exotic fruit based drinks immediately after the treatment and concluded that levels were not different from the thermally processed juices such as orange/kiwi/pineapple and soymilk based drinks. However, the beneficial effect of the PEF was noticeable over 31 days storage period, (800 μs/35 kV/cm) showed significantly greater vitamin C retention than both a 1400 μs and a thermal treatment. Generally, longer treatment times may induce reduction in the retention of vitamin C of the juice related with product heating. Longer exposure time may also generate free radicals which may speed up vitamin C degradation. Watermelon juice, product with a low initial vitamin C concentration, was PEF treated (25 kV/cm/50 μs/50 Hz), and it exhibited the highest vitamin C retention (96.4–99.9 %). On the other hand, vitamin C loss was higher than 50 % when HIPEF (high-intensity PEF) treatment parameters were risen (35 kV/cm/2050 μs/250 Hz). However, this treatment found to be appropriate for product safety [22].

In cases where PEF has caused a loss of anthocyanins, this probably might come from the direct impact of the treatment on these compounds and the partial inactivation of enzymes (β-glucosidase, peroxidase and polyphenoloxidase) which were induced. Aguiló-Aguayo et al.[23] reported an increase of β-glucosidase activity in strawberry juice, which could explain the corresponding degradation of anthocyanin following PEF treatment (35 kV/cm, 1000 μs, 50 Hz). In another research, the total phenolic content of a blend of orange/kiwi/pineapple juice and soymilk was not affected by PEF treatments (35 kV/cm, 4 μs bipolar pulses, 200 Hz) for a total treatment time of 800 μs and 1400 μs [10].

The effect of PEF processing on the bioactive compounds in watermelon juice was extensively studied by Oms-Oliu et al.[22]. While severe PEF strength proved to increase the rate of vitamin C loss in the juice, the lycopene retention in HIPEF processed changed it from 87.6 % to 121.2 % over the range of processing parameters (25–35 kV/cm, 1–7 μs or 50–2050 μs, 50–250 Hz). Enhancement of lycopene content might be related to PEF-induced cell permeabilization and the release of intracellular pigments (lycopene) from watermelon. Consequently, it was determined that such an increase at these electric field intensities could have been a stress induction for cells and subsequent production of lycopene as secondary metabolite stimulating metabolic activity [24]. These results were similar to those observed by Odriozola-Serrano et al.[25] in strawberry juice processed by PEF. Keeping constant 35 kV/cm electric field strength, and the treatment time of 1000 μs, the treatments were set at frequencies from 50 to 250 Hz, pulse width from 1 to 7 μs. The authors determined that the presence of health-related compounds (vitamin C, anthocyanins and antioxidant capacity) were maximal at a treatment frequency of 232 Hz and a pulse width of 1 μs. Under all experimental conditions, the relative retention of anthocyanins ranged between 87 % and 102 %.

Main causes of degradation in antioxidants during thermal processing could be attributed to oxidation and isomerization [26]. According to Morales-de la Peña et al.[6,10], the antioxidant capacity of a mix kiwi/orange/pineapple juice and soy milk was not affected by the PEF. Moreover, antioxidant capacity of this product - decreased to a greater degree in thermally treated (90ºC, 60 s) sample than in PEF-treated one after storage period of 60 days [6,10]. Conversely, Aguiló-Aguayo et al.[23] reported that total antioxidant capacity of watermelon juice was affected by the treatment conditions. In the process with 35 kV/cm, 2050 μs, 250 Hz did not seem to affect the antioxidant capacity of the juice when treated with a 7 μs pulse width, though it was significantly reduced when the pulse width was applied as 1 μs and the frequency was reduced to 50 Hz.

The application of this technology as a treatment for enhancing yield extraction has been reported for

several plant foods (apple, sugar beet, grapes, carrot) [17,27-29]. Ade-Omowaye et al.[30] applied successfully the PEF technology, as a pre-processing step in coconut milk processing, with an increase in milk yield but the bioactive compounds contents were not reported. However, to date there are limited reports focusing on the application of similar methods for extraction of juice and/or on the effect of such processing technology on bioactive metabolites from exotic fruit sources. Although the existing data, other plant foods may provide a solid base for studies on bioactive compounds and further investigations are required.

Ultrasound processing

Ultrasound with frequencies in the range 20-100 kHz has been a subject of research and development for many years in the food industry. Such processing requires the presence of a liquid medium for power transmission. It causes chemical and physical changes in the biological structures due to intracellular cavitation. Ultrasound processing on its own or in combination with heat and/or pressure is an effective processing tool for microbial inactivation and phytochemical retention. Advantages of ultrasound include reduced processing time, higher throughput at lower energy consumption [31].

Salleh-Mack and Roberts [32] investigated the effects of temperature, sugar concentration (8, 12, and 16 g/100 ml), organic acids (citric and malic acids) and pH (2.5 and 4.0) on ultrasound pasteurization in fruit juices. For this aim, *Escherichia coli* ATCC 25922 was used as a model organism and US treatment times were chosen to achieve a 5 log (base 10) reduction. Temperature was set at 30 °C and below in order to eliminate the thermal inactivation effects. Consequently, US increased the sensitivity of *E. coli* to thermal inactivation. The presence of soluble solids had a protective effect where the sonication time requirement increased. Similar to heat sensitivity, the lower pH environment resulted in *E. coli* having less resistance to sonication and the type of organic acid had the least significant effect on US inactivation. Additionally, it was reported that US could negatively modify some food properties including flavor, color or nutritional value.

US treatment of fruit juices is reported to have a minimal effect on the AA content during processing and results in improved stability during storage when compared to thermal treatment. This positive effect of US is attributed to the effective removal of occluded oxygen from the juice as this is a critical parameter influencing the retention of AA [33]. With regard to exotic fruits, Cheng et al.[34] reported a significant increase in the AA content of guava juice during sonication from 110 ± 0.5 mg/100mL (fresh) to 119 ± 0.8 mg/100 mL (sonication) and to 125 ± 1.1 mg/100 mL (combined sonication and carbonation). The authors also observed that during carbonation, sample temperature decreased substantially which could have disfavoured AA degradation.

Rawson et al. [35] determined that sonication temperature played a significant role in preservation of bioactive compounds. Freshly squeezed watermelon juice was subjected to thermosonication treatments with processing variables of temperature (25-45°C), amplitude level (24.1-60 μm) and processing time (2-10 min) at a constant frequency of 20 kHz and pulse durations of 5 s and pulse repetition time and 5 s. The authors observed a higher retention of AA and lycopene at low amplitude level and temperature. They determined a decrease in the phenolic content of sonicated watermelon juice with temperature rise from 25 to 45 °C. Temperature effect was more pronounced at higher processing times (10 min) [35]. Additionally, in another study US processing was reported to enhance extraction yield of bioactive compounds like polyphenols and carotenoids in both aqueous and solvent extraction systems about 6 % and 35 % depending on the processing conditions [36].

Ultrasonic extraction is a well-known commercial method for increasing mass transfer rate by cavitation forces. Bubbles in the liquid-solid extraction using ultrasonic extraction can explosively collapse and produce localized pressure while improving the interaction between the intracellular substances and the solvent to facilitate the extraction of the phytochemicals. The extraction of lycopene from tomato using ultrasonic assisted extraction (UAE) and ultrasound/microwave assisted extraction (UMAE) was reported in Lianfu and Zelong's [37] research. They showed that the optimal conditions for UMAE were 98 W microwave power together with 40 KHz ultrasonic processing, the ratio of solvents to tomato paste was 10.6:1 (V/W) and the extracting time should be 367 s; whereas for UAE, the extracting temperature was 86.4°C, the ratio of the solvents to tomato paste was 8.0:1 (V/W) and the extracting time should be 29.1 min. Additionally, the percentage of lycopene yield was determined 97.4 % and 89.4 % for UMAE and UAE, respectively. The comparison of these two methods showed that UMAE overcomes theshortcomings of UAE and would be a more attractive extract method in the future.

CONCLUSION

Ensuring food safety and at the same time meeting the demands of conscious consumers for good quality and nutritious foods has resulted in increased interest in non-thermal preservation technologies. In parallel with the processing conditions, innovative technologies such as HPP, PEF and ultrasound might have a positive or negative effect on nutrients and phytochemicals of fruits and vegetables. However, some researchers determined that nutritional values were not significantly affected by the non-thermal processing parameters. Therefore, to properly evaluate the impact of these technologies further researches on this topic is still required.

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ВЛИЯНИЕ НА ИНОВАТИВНИТЕ ТЕХНОЛОГИИ ВЪРХУ КАЧЕСТВОТО НА ПЛОДОВЕТЕ И ЗЕЛЕНЧУЦИТЕ

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(Резюме)

Процесите, свързани с преработката на храните, оказват значително въздействие върху стабилността на хранителните вещества и като цяло увреждат антиоксидантите в плодовете, зеленчуците техните продукти. Домашните, конвенционалните, индустриалните и дори нетермичните обработки намаляват нивото на фитохимикали в преработените хранителни продукти. Най-новите тенденции в нетермичните технологии са не само да се получи високо качествени продукти със запазени свойства, но също така да се осигури по-добра функционалност на храната. Въпреки това, някои изследователи съобщават, че резултатите от не-термични процеси, не могат да бъдат различени от тези, получени при термични обработки. Главният фокус на настоящото ревю е да изясни влиянието на не-термични технологии, като високо хидростатично налягане (ВХН), импулсно електрично поле (ИЕП) и ултразвук (УЗ) върху ключови хранителни вещества в плодовете и зеленчуците.

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Nano-spray drying applications in food industry

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Transforming a raw material into a suitable product for industrial use is a complex operation including drying processes. One of the most common drying processes is spray drying which produces powder with a defined particle size out of solutions, dispersions and emulsions. It is generally used for pharmaceuticals, food, biotechnology and other industrial materials synthesis. Spray drying has several advantages such as operational flexibility, applicability for heat-sensitive materials, as well as an affordable cost, but it has limitations in particle size, volume and the yield. Nowadays, nanotechnology, which refers to objects that are one-billionth of a meter in diameter, gained importance as a newest application trend in science. Accordingly, nano-spray drying technology was improved to create particles in nanometer range for efficient spray process for small quantities, narrow particle size distribution and highest yields of fine particles. In this review, new technological developments about nano-spray dryers and some of its applications in food industry will be discussed.

Key words: *Nano- spray dryer, microencapsulation, nanoencapsulation, food*

INTRODUCTION

Developing new technologies for obtaining standardized herb and food extracts is an important research topic nowadays and dried extract products have several advantages over conventional liquid forms. These advantages could be listed as low storage costs, providing more concentrated products and stable form of the active ingredients [1]. Several techniques can be applied for this purpose such as spray drying, freeze drying, spouted bed drying and fluidized bed drying. Spray-drying is a common technique used in the food industry since decades old. The first time idea was patented in 1872 by Samuel Percy where in 1920s, the industrial use had been launched with the production of milk powder and detergent [2].

Spray drying is commonly used in food, pharmaceutical, chemical and aroma industry [3-8]. Whole milk, skimmed milk and whey powders are the most popular products produced by spray drying processes. The other food products can be indicated as: instant coffee and tea, baby food, egg powder, cheese powder, ice cream mixes, fruit juices, flavoring agents, anthocyanins, enzymes, microorganisms, yeasts and pesticides [9-15].

Substantially, spray drying is a process where the liquid solution or suspension is dried to particles rapidly with atomization in a heated chamber (Figure 1). The powder obtained after this process has a flowing structure and a certain size distribution of spherical particles. Its quality depends on many factors interacted with each other like: physical and chemical properties of the raw material, feed rate and its concentration, atomizer rate and the temperature parameters of the drying medium. Among other drying processes, spray drying has relatively short drying time and it allows to dry heat-sensitive products [16]. Spray drying occurs in process steps like:

- pre-concentration of the liquid (generally for decreasing costs it is applied to low concentrated liquids),
- atomization (composing of liquid drops),
- drying in hot air/gas flow, formation of powder particles,
- separation of product from humid air/gas (cyclone stage) [17].

Removing water from the solution by spray drying is a common engineering application. In spray drying, liquid feed is atomized into hot gas flow, usually air and inert gases like nitrogen, and instantly dry. Feed liquid can be a solution, emulsion or a mixture suspension [3]. Powders obtained after drying process properties changes due to the physical and chemical properties of feeding fluid, design of the spray dryer and drying conditions as very thin powder (10-50 μm) and granule or agglomerate [18] (2-3 mm).

Advantages of spray drying could be listed as:

- if drying parameters are fixed, powders specifications could be stabilized,
- drying system can provide continuous and can be controlled automatically,

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- different dryers can be designed according to heat-sensitive, heat-resistant, corrosive or abrasive solutions.

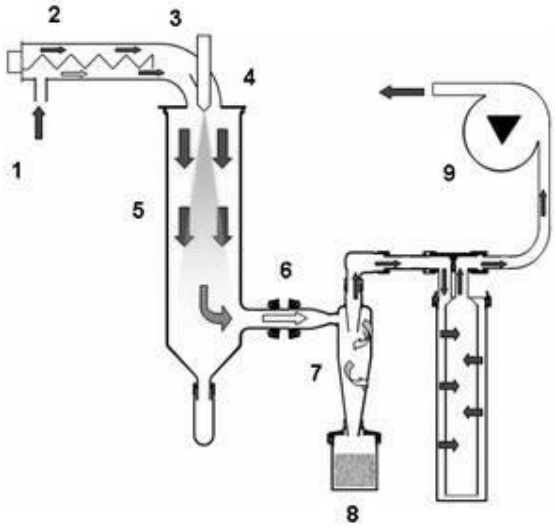

Fig. 1. General units of a spray drying system (1. air intake, 2. heating, 3. temperature sensor at air entry point, 4. atomizer, 5. drying chamber, 6. temperature sensor at air exit point, 7. cyclone: separation of the product from the air stream, 8. collection vessel for finished product, 9. aspirator)[14].

However, high installation costs, low thermal efficiency, energy consumption and workability of dried powders in humid conditions are the main disadvantages of spray drying process [2]. In addition to this, spray dried foods encounter with aroma losses and particle stickiness seen in high temperatures during the process. In order to solve these problems and enabling drying process thus producing the last powder in acceptable quality parameters; different applications can be combined such as processing spray dried powder to granules in fluid bed dryer. By this way, solubility of powders and physical resistance could be raised and flavorings could be added to the product. Another solution is microencapsulation of the raw material directly by spray drying with using different carrier agents. Microencapsulation protects the powder for oxidation reactions, prevents volatile flavor compounds and raises its storage stability. In microencapsulation, several gums such as arabic gum, carob gam, carboxymethyl cellulose (CMC), carrageenan, starch products like maltodextrins (MD19, MD12) and modified starches are generally used in order to obtain good product recovery [19,20].

Atomization constitutes the most important process step and atomization of the liquid as small droplets is carried out with pressure or centrifugal energy. For this purpose, different atomizer types such as pneumatic atomizer, pressure nozzle, two-fluid nozzle and sonic nozzle are used. The purpose of atomization is to create the maximum heat transfer surface which allows to optimum heat and mass transfer between dry air and the liquid. Proper atomization configurations depend on the viscosity, the nature of the feeding fluid and the desired properties on the final product. As the supplied energy increases, droplets obtained by atomization decreases [21].

Spray drying process can be designed according to atomizers settlement position corresponding to hot air distributors, as co-current or counter-current. In co-current flow, feeding fluid is sprayed in the same flow direction with hot air and inlet temperature changes between 150 – 220^{o}C, as it is suddenly evaporated. In these systems, dry powder product is subjected to moderate temperatures like 50 – 80^{o}C, and products thermal degradations are restricted. Thus, drying of some bacterial suspensions without harming any living organisms might be a possibility. Correspondingly, in counter-current systems, feeding fluid is sprayed in opposite directions to hot air flow. Dry powder product exposes to high temperature in this way. Therefore, drying of heat sensitive products is limited in this system. However, the most important advantage of this system is its economical properties with low energy consumption [3,22].

Nowadays, nanotechnology is a rapidly growing field which has impact on every area in science and technology. Especially some areas such as physics, engineering, chemistry, biology, agriculture, and food sciences are essential for the development of nanotechnology [23].

Nanotechnology has good potential to improve agriculture and food systems. Safety, efficiency, bioavailability and keeping nutritional values of foods and the molecular synthesis of new products or ingredients can be affected by nanoscale level. These principal links of nanotechnology are refining flavor and nutrition, food security and processing, functionality of foods, protection of the environment and the cost-effectiveness of storage and distribution [24]. Additionally, at the Second International Food Nanoscience Conference, the immense opportunities of nanoscience are asserted possible in previously mentioned areas [25].

In the food industry, significant advancements by nanotechnology are new functional materials, micro and nanoscale processing, product development, the design of methods and instrumentation for food safety and biosecurity [26]. In this context, nano-spray dryers which use nanotechnology in the design of the classical spray dryers, create particles in the nanometer range. Besides some challenges like high sensitivity of

proteins during processing and storage and product recovery in pharmaceuticals brought a necessity of a new spray dryer design. To overcome these kinds of technical challenges, Bürki et al. [14], designed to generate very fine droplets resulting in particle sizes between 300 nm and 5µm. Therefore novel technologies at the spray head, heating system and particle collector made 'nano' spray drying a possibility. Differently from conventional dryers, nano-spray drying utilizes a vibrating mesh technology. With using a piezoelectric driven vibrating membrane in the spray head, millions of tiny droplets are generated every second. Dried particles are separated by the use of an electrostatic particle collector with high particle recovery rates even for nanoparticles of milligram sample amounts. However, substantial limitations of nano-spray drying were the particle size (minimum 2 µm), the yield (maximum around 70 %), and the sample volume (minimum 50 mL for devices in lab scale).

A typical spray-drying process consists of four steps that include; atomization of the liquid stream, vaporization/drying of the liquid stream through the drying gas, particle formation and separation and collection.

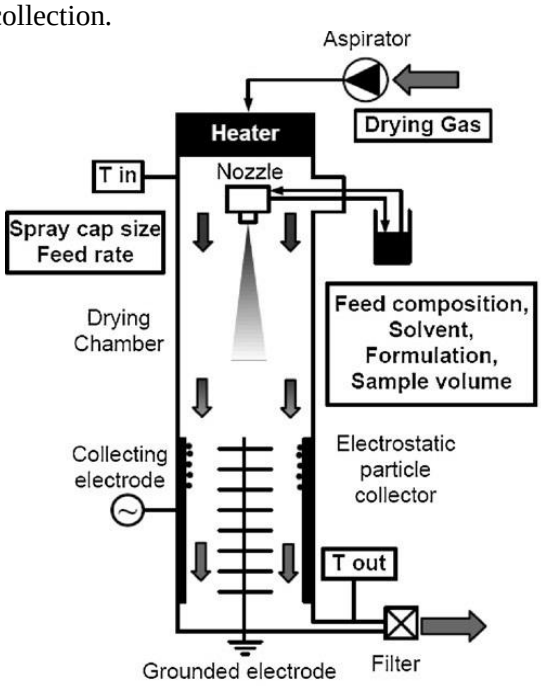

Fig. 2. Nano-spray dryer [14].

In nano-spray dryer technology, the unique feature is, the droplet generation through a piezoelectric driven actuator (with a thin stainless steel membrane and the membrane has an array of micron-sized holes; spray meshes of 4.0, 5.5 or 7.0 µm hole size) that operates at a specific ultrasonic frequency (60 kHz) and thus creating a mist of droplets with extremely ultra-fine particle size. The dried particles are electrostatically charged (solid particles are accumulated at the wall of the cylindrical particle collecting electrode by a strong electrical field, the electrical field is generated via high voltage) and gathered at the collecting electrode surface with minimal wastages and high formulation yields. Besides, a new heating system is used to provide the drying gas to produce the particles. The gas flow is laminar as in common spray drying. The advantage of laminar flow is that the particles fall straight down from the spray head and do not stick to the glass wall. Nano-spray dryer design is shown in Figure 2.

Later, these new design is used to evaluate the early stages of product development for a variety of applications including preparation of sub-micron particles of polymeric wall materials[27] producing protein nanoparticles [14,28], the encapsulation of nano-emulsions [29], as well as the drying of pharmaceutical excipients and model drugs [30].

In a study, Tewa-Tagne et al.[31] used a spray-drying method for the production of nanocapsules which partly reduced the moisture content of the product while the carrier concentration increased. Researchers also stated that moisture content of spray-dried powders depends on the nature of the carrier and its interaction with water molecules. Outlet temperature is another factor that effects the final products moisture content. Goula and Adamopoulos[32] reported that high outlet temperatures often reduce moisture content of product.

Liu et al.[27] spray dried micro and nano-scale particles in hybrid composites of alginate and silica nanoparticles in their study and observed uniform micro particles formed in a single step micro-fluidic jet spray drying. Here, evaporation induced self-assembly during spray drying then formed uniform micro particles with shells enriched with silica nanoparticles. Then the final structures of particles were determined by the drying temperature and the ratio of alginate to silica indicating that this could be used for manufacturing diverse structural motifs. As a result, combination of polymer-mediated self-assembly with a moderate temperature spray drying could be a versatile process to synthesize thermal sensitive biomaterials for food and pharmaceutical related applications.

Lately, there has been an interest in the development of protein nanotherapeutics for diseases such as cancer, diabetes and asthma. To meet that demand, Lee et al. [28] investigated spray drying technology for obtaining these kinds of powders. However, the separation and collection of protein nanoparticles with conventional spray dryer

setups has been known to be extremely challenging due to its typical low collection efficiency for fine particles less than 2 μm. Accordingly, there has not been any approach to fabricate protein nanoparticles in a single step and with high yield (> 70 %). In their study, they explored the feasibility of the novel Nano Spray Dryer B-90, for the production of bovine serum albumin (BSA) nanoparticles. They reported that particle size and morphology were predominantly influenced by the spray mesh size and surfactant concentration while the drying air flow rate and inlet temperature had minimal impact.

Process parameters were determined respectively as, 4 μm spray mesh at BSA concentration of 0.1 % (w/v), surfactant concentration of 0.05 % (w/v), drying flow rate of 150 L/min, inlet temperature of 120 °C and the yield 72 ± 4 %. In conclusion, nano spray drying offered an alternative approach for the production of protein nanoparticles.

Li et al.[29], used nano spray drying technology in their research to provide submicron particles with high yields (70 % to 90 %) and small sample amounts (50 mg to 500 mg). For this aim, some polymeric wall materials (arabic gum, whey protein, polyvinyl alcohol, modified starch and maltodextrin) were spray dried and the resulting size distributions were shown to be below the 1 μm scale, attaining sizes as low as ~ 350 nm with a standard deviation of ~ 100 nm for arabic gum (0.1 wt.% solid concentration), which was a very noteworthy result. They also expressed, size and standard deviation depend on the nature and the type of the wall material and predominantly on the concentration of the spray dried solution. Consequently, they reported that, preliminary results of encapsulated nano-emulsions and formulated nano-crystals using this novel technology offer promising perspectives for new pharmaceutical applications using spray drying.

Nano and microparticle engineering of water soluble drugs was conducted using a novel piezoelectric spray-drying method in another research. Cyclosporin A (CyA) and dexamethasone (DEX) were encapsulated in biodegradable poly (D, L-lactide-co-glycolide) (PLGA) grades of different molecular weights. Spray drying process parameters such as spray mesh diameter, sample flow rate, spray rate and sample concentration were found to play a key role on the particle engineering and the obtained product yield. CyA was found to be molecularly dispersed within the PLGA nanoparticles while DEX's crystallinity varied according to the lactide/glycolide ratio. In concluding, this novel process proved to be efficient for nano and microparticle engineering of water insoluble active substances [30].

CONCLUSION

Nanotechnology offers new possibilities in food science especially in the field of functionality like enriched and fortified food products. One of these topics is obtaining nanoscale powders by spray drying which presents a platform for many applications in food technology. It can be applied to foods with nanoencapsulation of active compounds such as flavors, vitamins, minerals, antimicrobials, drugs, colorants, antioxidants, probiotic microorganisms, and micronutrients. The aim of nanoencapsulation is to maintain these active compounds at suitable levels for long periods of time. As a result, nanoparticles have better properties for encapsulation and release efficiency than traditional encapsulation systems. By this way optimization of powders inhalation properties would be useful to improve stable products and to keep aroma active subtances.

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ПРИЛОЖЕНИЯ НА СУШЕНЕТО ЧРЕЗ НАНОРАЗПРАШАВАНЕ В ХРАНИТЕЛНАТА ИНДУСТРИЯ

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(Резюме)

Трансформирането на суровия материал в подходящ продукт за индустриално приложение е комплексен процес, включващ сушене. Един от най-често срещаните процеси на сушене е сушене чрез разпрашаване, при което от разтвори, суспенсии и емулсии се получава прах и дефиниран размер на частиците. Обикновено той се използва във фармацията, хранителната промишленост, биотехнологиите ипри синтеза на други индустриални материали. Сушенето чрез разпрашаване притежава редица предимства, като операционна гъвкавост, приложимост на термо-чувствителни материали, достъпна цена, но то има ограничения по отношение на размера на частиците, обема и добива. В днешно време, нанотехнологиите, които се отнасят до обекти, имащи диаметри една милиардна част от метъра, придобиха значение като най-новите тенденции на приложения в областта на науката. В съответствие с това, сушенето чрез наноразпрашаване беше подобрено да създаде частици в нанометровия диапазон при ефективен процес на разпрашаване на малки количества, тясно разпределение на размера на частиците и най-високи добиви на фини прахови частици. В настоящото ревю ще бъдат дискутирани нови технологични развития на изсушителите чрез наноразпрашаване и някои от техните приложения в хранителната индустрия.

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Effect of active packaging material on milk quality

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Effect of active electret package on milk quality was studied. Electric charge of electret package was shown to reduce milk acidity growth by 2 °T a day in average and to elongate milk shelf life by half. Electric field of the package affects milk composition: lactose content in milk decreases, density grows, while protein content remains unchanged. Milk particle micellization model in electric field of the package was suggested.

Keywords. Milk, active package, electret.

INTRODUCTION

Role of packaging in food quality preservation is known worldwide. Basic function of packaging is protection from climatic factors (oxygen, moisture, light and temperature), transportation and storage damages and biological factors (microorganisms, insects, etc) [1]. However, many food products contain microorganisms that cause food spoilage when stored. Milk and dairy products are generally very rich in nutrients which provide an ideal growth environment for many microorganisms. Milk, for example, contains *Lactobacillus, Pediococcus, Lactococcus, Streptococcus,* etc.

Dairy products are exposed to different treatment at milk processing factory. Bactofugation, pasteurization, sterilization and ionization are the most common techniques used to reduce microorganism content in milk products [2]. Their combination eliminates up to 99 % bacteria [2]. Pasteurization process involves heating milk for 15 – 20 s at 90 – 92°C; during sterilization milk is heated above 100 °C under pressure. Such thermal treatment efficiently kills many forms of microbial life initially contained in milk, but it leads to destruction of vitamins and nutrients. Ionization radiation treatment is advanced technique used to elongate shelf life of products sensitive to high temperatures.

However, the objective is not only to delete undesired microorganisms in raw milk but to exclude new bacteria and prevent resurgence of the remained ones. Preserving agents are not always used as they can have negative health affect since a lot of nutrient additives may cause digestive disorder and skin problems being allergen or carcinogen. For this reason manufacturers are looking for advanced packaging that enables to preserve nutrients as long as possible. The challenge to extend shelf life of a product due to packaging specific physical properties is up to date in both economic and environment protection aspects.

Materials with targeted active action on packed products are classified as active packaging. They replace gradually traditional materials that perform mechanical and barrier properties. Production technologies of these materials are manufacturer's know-how [1]. These technologies include application of electric field produced by the packaging material as sterilant. Such kind of materials that have constant electric field is known as electrets. They are used in radio electronics, mechanical engineering and medicine [3-5]. It would be interesting to study their effect on packed products.

The corona poling of polyethylene packaging films results in a decrease in the total migration of different compounds from the film into food products[6]. The decrease in the amount of the migrant transferred from the poled polyethylene films into different solvents is due to the effect of their electric field on the wetting, dissolution, and diffusion processes.

In this paper we have studied application of electrets material as active packaging for milk. The objective of the paper is to determine effect of packaging material electric field on biochemical and biological processes that occur in milk during storage.

EXPERIMENTAL

At the first stage active packaging material was manufactured by LDPE film corona poling [3]. Samples were charged in the field of negative

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corona discharge after being heated at 90 °C for 10 min. Corona treatment unit is shown in Figure 1. Corona electrode consists of 196 needles equally spaced on 49 cm^2 area. The gap between corona electrode and the sample was 20 mm, polarization time was 30 s, polarization voltage – 30 kV.

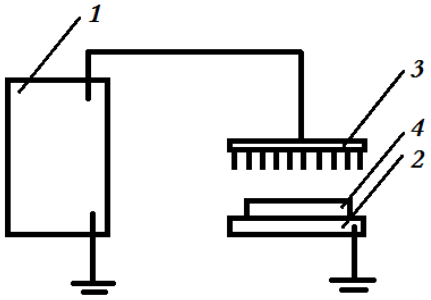

Fig. 1. Corona discharge system: 1 – high voltage source, 2 – grounded electrode, 3 – corona electrode, 4 – sample.

Corona electret characteristics were following: surface potential (~ 1500 V), efficient surface charge density (~ 0.85 μC/m^2), electric field strength (~ 60 kV/m).

Packages for milk were made of electret material and industrial PE film using thermal impulse sealer IS - 600. Quality changes of milk packed according to GOST 13277-79 (Russian Standard) was studied. It was examined for bacterial number (total bacteria number per volume unit), number of Escherichia coli (coli-titer), organoleptic characteristics and acidity (GOST 13264-88).

RESULTS AND DISCUSSION

Over time, off-flavor appears in milk that is not specific to fresh product (greasiness, rust and etc.). This disadvantage can appear due to accumulation of free fatty acids resulting from life activity of microorganisms and lactose fermentation to form lactic acid. Electric field was shown [8] to reduce respiratory rate, heat production and other energy parameters of bacteria. It indicates retarded metabolic process i.e. electric field inhibits bacterial activity.

Research performed showed that milk packed in electret film preserved initial organoleptic characteristics for a longer time. Electret package elongated milk shelf life by half. Figure 2 shows organoleptic evaluation of milk stored in active (curve 1) and traditional (curve 2) packages.

Electric charge of electret package reduced acidity growth by 2 °T a day in average i.e. it inhibited microorganism life activity. Acidity level decay (Turner degree) is given in Figure 3.

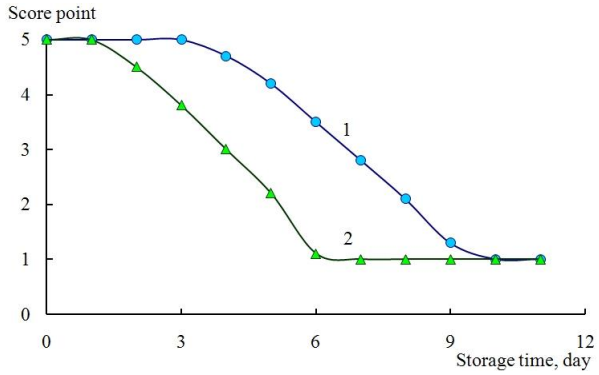

Fig. 2. Organoleptic evaluation of milk in electret (1) and industrial (2) package.

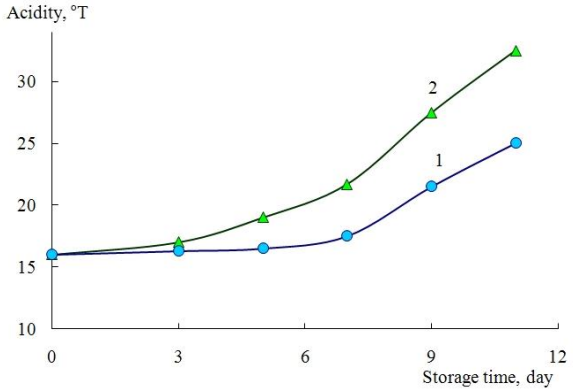

Fig. 3. Milk acidity when stored in electret (1) and industrial (2) package.

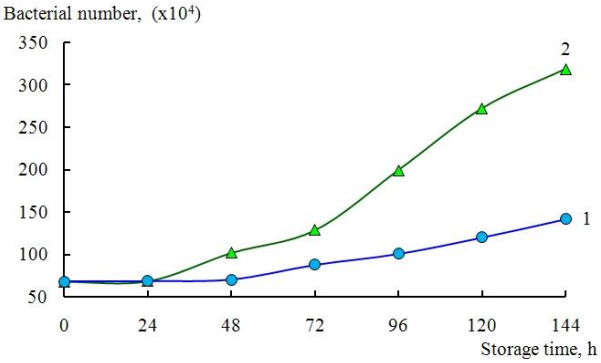

Fig. 4. Total bacterial content in milk packed in electret (1) and industrial (2) film.

Microbiological analysis data also point to the fact of bacterial activity inhibition. When industrial polyethylene film was used as packaging, number of mesophilic microorganisms in milk in two hours was higher compared to active package. Figure 4 illustrates that total bacterial content in milk packed in industrial film (curve 2) almost two and a half times exceeded total bacterial content in milk packed in active package (curve 1) by the sixth day. There were no Escherichia coli found in both raw and packed milk.

Besides, electric field of the package was revealed to affect dairy products composition (Table 1).

Table 1. Milk composition changes during storage.

Milk parameter	industrial PE film			electret PE film		
	0	**24 h**	**48 h**	**0**	**24 h**	**48 h**
Milk density, kg/dm^3	1026	1035	1066	1026	1031	1047
Protein weight fraction, %	3.427					
Lactose content, %	3.01	2.9	2.4	3.01	2.95	2.8

Lactose content in milk decreased, while density grew that represents microorganism life activity processes. At the same time protein content remains unchanged. These data correlate with changes of organoleptic, physical and chemical values.

Milk nutritional content associated with bioactive substances slightly decreased. However, when electret package was used, this process was slowed down significantly. It occurs due to the presence of electric-field sensitive substances in bacteria cells (e.g. coenzyme A) that determine, in particular, cell respiration system activity [7].

Milk is complex disperse system that includes components in different aggregate state: molecules and ions (some salts, lactose, water-soluble vitamins, etc.) and charged (mainly negative) colloid particles (casein, whey proteins, calcium phosphate and butterfat). There is a strong interaction between specific disperse phases i.e. single equilibrium system is formed. Any change in content and state of milk dispersion components under different factors (temperature, pH and etc.) may result in disequilibrium and system stability loss [2].

Colloid particle stability in milk is determined by electric charge. For example, casein micelles have negative surface charge. There are attractive and repulsive forces between colloid particles. If interparticle repulsive forces prevail, then the whole system is stable. As repulsive forces decline and attractive forces increase, system stability gets broken with coagulation and agglomeration of colloid particles. Visually it corresponds to cream layer formation on a milk surface. This process is accompanied by casein negative charge decay.

Electric field of active package effects colloid system stability. In this case more uniform emulsion particle distribution is observed.

In this regard, premature coagulation of casein and other milk components can be prevented due to package negative charge distribution in milk.

Milk is bad electric conductor. Its conductivity is associated with Cl^-, Na^+, K^+, H^+, Ca^{2+}, Mg^{2+} and other ions. Although casein, whey proteins and fat globules have electric charge on surface, they move slowly owing to large size and increase viscosity of the solution and in practice reduce electric conductivity. Calcium phosphates are of the most interest. One part of them is molecular solution, another one is colloid and equilibrium is established between them. Equilibrium stability is affected by milk acidity. Milk acidity is increased due to growth of microorganisms that ferment lactose to form milk acid. It decreases negative charge of protein particles and disrupt the balance between calcium salts i.e. some colloid calcium salts are transformed into ionic-molecular state.

This process can be presented by the following model (Figure 5).

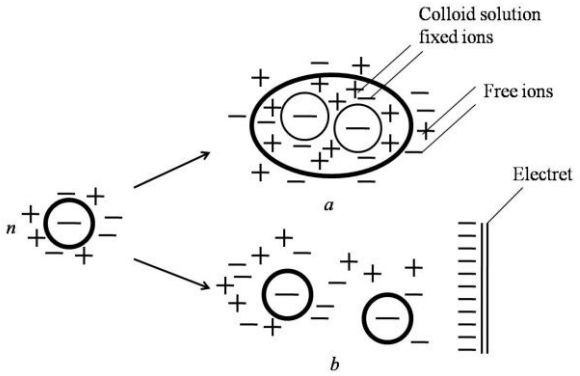

Fig. 5. Milk particle micellization model: a – micelle coagulation when attraction forces prevail during storage; b – micelles in solution exposed to negative electric field of the corona electret.

Typically, equilibrium is shifted to excess of calcium and magnesium ions with the phosphorous and citric acid distribution breaking [8-10]. As milk acidity grows, electric conductivity and number of coagulated particles (Figure 5a) increase as well. Milk in active package shows another pattern (Figure 5b). Electric field is seen to affect colloid system equilibrium in dairy products –more uniform distribution of emulsion particles is observed.

Hence, application of electret material as active package has following peculiarities. On the one hand cumulated electric charge affects microorganisms; on the other hand it stimulates initial equilibrium state of milk dispersive system. Moreover, electric field - that has negative charge – improves milk keeping qualities. More uniform emulsion particle distribution was observed for milk packed in electret films.

Based on obtained results one may state that active package with negative electric field enables to extend shelf life, preserve milk organoleptic and physical characteristics.

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ВЛИЯНИЕ НА АКТИВНИТЕ ОПАКОВЪЧНИ МАТЕРИАЛИ ВЪРХУ КАЧЕСТВОТО НА МЛЯКОТО

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(Резюме)

Изследвано е влиянието на активни електретни опаковки върху качеството на млякото. Показано е, че електричният заряд на електретните опаковки намалява нарастването на млечната киселинност средно с 2°T на ден и увеличава срокът на годност на млякото с 50 %. Установено е, че електричното поле на опаковката влияе на състава на млякото: съдържанието на лактоза в млякото намалява, плътността расте, докато съдържанието на протеини остава непроменено. Предложен е модел на мицелизиране на млечни частици в електричното поле на опаковката.

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A study on the environmental situation in the area of the Kardzhali lead-zinc plant using the moss technique, neutron activation analysis, atomic absorption spectrometry, and GIS technology

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The results of a study on atmospheric deposition of trace elements using the moss biomonitoring technique in the area of the lead-zinc plant in Kardzhali are reported. This plant is the main source of lead, cadmium, zinc and sulphur oxide contamination. Various moss types were used to study atmospheric deposition of trace elements in 54 sampling sites. The concentrations of the following elements Na, Mg, Al, Cl, K, Sc, Ca, Cr, Ti, V, Mn, Ni, Fe, Co, Zn, Se, As, Br, Rb, Sr, Mo, In, Sb, I, Cs, Ba, La, Ce, Sm, Tb, Dy, Hf, Ta, W, Au, U, Th were determined by means of instrumental epithermal neutron activation analysis (ENAA) at the IBR-2 reactor of FLNP JINR, Dubna. Multivariate statistics was applied to characterize the pollution sources. GIS technology allowed apportioning the deposition patterns of element pollutants in the study area. The present investigation is a continuation of the environmental surveys in Bulgaria used to regulate the Bulgarian industries.

Keywords*: atmospheric deposition of trace elements, moss biomonitoring, lead-zinc plant, neutron activation analysis*

INTRODUCTION

The use of terrestrial mosses as biomonitors in large-scale multielement studies of heavy metal deposition from the atmosphere is a well-established technique in Europe. The present study is part of the UN European Cooperative Programme „Atmospheric Heavy Metal Deposition in Europe" aimed at investigating the long-range transboundary air pollution in Europe at 5-year intervals [1].

This paper presents results from the moss survey in 2011 around the Kardzhali lead-zinc plant (LZP) known for its non-ferrous metal deposits. According to the Ministry of Environment and Water, Kardzhali is one of the ecological "hot spots" in Bulgaria.

The manufacturing process in Kardzhali is a major source of contamination with significant amounts of heavy metals and other toxic elements (*e.g.* Pb, Zn, Cd, As, Sn) in the soil, water and air. These inflict environmental damage to the region of research interest. Due to the constant circulation of chemical elements in nature, contaminants such as trace metals and their compounds are effectively deposited onto mosses after being released into the air.

Since mosses have no root system, the soil has insignificant influence on the concentration of trace metals found in them.

EXPERIMENTAL

Study Area

Figure 1 shows allocation of the sampling area in the map of Bulgaria. The study area of about 110 km^2 is located around LZP Kardzhali. It spans 6 km to the North and South and 5 km to the West and East from the plant's chimney.

Fig. 1. Study area.

About 6% of the world zinc deposits and about 3% of the lead deposits are located in Bulgaria. 75% of the lead-zinc ore is located in the Rhodope Mountains and over 70% of the total available ore

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is mined. Annually, LZP Kardzhali produces 27,200 tons of zinc, 30,000 tons of lead and 45,000 tons of sulfuric acid. After a project to increase lead production in the plant was accepted, the annual production will rise up to 60,000 tons by 2014 [2].

Surveys in the area of Kardzhali, particularly in the last few years, show increased trace metals content in the soil and the plants, including mosses, as a consequence of mining polymetallic ores, flotation and heavy metal production. The approximate size of the land affected by aerogenic lead contamination with higher than the admissible concentrations is about 40,000 da of virgin soil and about 18,000 da of arable lands [3].

Sampling

A total of 77 moss samples, of which *Hypnum cupressiforme* was the dominant type, were collected according to the sampling strategy of the UNECE ICP Vegetation Programme on atmospheric deposition studies in Europe [1]. For each site 5-10 subsamples were taken within a 50 X 50 m area and were combined in the field.

Sample Preparation

The unwashed samples were air-dried to constant weight at 40 °C for 48 h, and extraneous plant material was removed. The whole living part corresponding approximately to three years growth of the moss was subjected to analysis. Therefore, the results from the survey represent the average deposition situation over the period 2008-2010 for the elements retained in the moss. Moss samples of about 0.3 g in weight were packed in polyethylene foil bags for short-term irradiation to determine short-lived isotopes, and in aluminum cups for long-term irradiation for determination of long-lived isotopes.

Analysis

Neutron activation analysis (NAA) was performed in the radioanalytical laboratory at the pulsed fast reactor IBR-2 of the Frank Laboratory of Neutron Physics, JINR, Dubna, Russia [4].

Long-lived isotopes were determined using epithermal neutrons in cadmium-screened irradiation channel with neutron flux density Φ_{epi}.= 3.6×10^{11} n/(cm$^2\times$s). Samples were irradiated for 5 d, re-packed and then measured twice after 4–6 and 20 d of decay, respectively. Measuring time varied from 1 to 5 h. To determine the short lived isotopes (Cl, V, I, Mg, Al, and Mn) conventional irradiation channel was used. Samples were irradiated for 3 min and measured twice after 2–3 min and the second one for 20 min after 9–10 min of decay.

The concentration of the environmentally meaningful element Pb cannot be determined by INAA, and Cu and Cd are difficult for determination at low concentration levels. These elements will therefore be determined by atomic absorption spectrometry (AAS) at the Sts. Cyril and Methodius University, Skopje, Macedonia in the nearest future.

RESULTS AND DISCUSSION

At the time of writing, 54 samples were analyzed. The results of the descriptive statistical analysis of the elemental concentrations determined in the moss samples (min, max, mean and median) are given in Table 1 along with data for the neighboring Macedonia[5].

For comparison, the corresponding values for the Norwegian moss data representing territories with minor influence from air pollution[6] are given in the same table.

Multivariate statistics (factor analysis) was used to identify and characterize different pollution sources. The results of factor analysis are presented in Table 2.

Values of the four factors are given in Table 2.

Factor 1 has particularly high values of K, Rb, Sr, Mo, Cs, Ba, typically found in plants and of rare earth elements (REEs), Hf, Ta, U, and Th, which are soil indicators. Most of these elements are typical of heavy crustal material, and they partly reflect the contamination of moss samples with soil particles.

Factor 2 has high factor loadings particularly for Na, Mg, Al, Sc, Ti, which are typical light crustal material (silicic rocks), as well as for V, Fe, and Co, which could be attributed to a basaltic component.

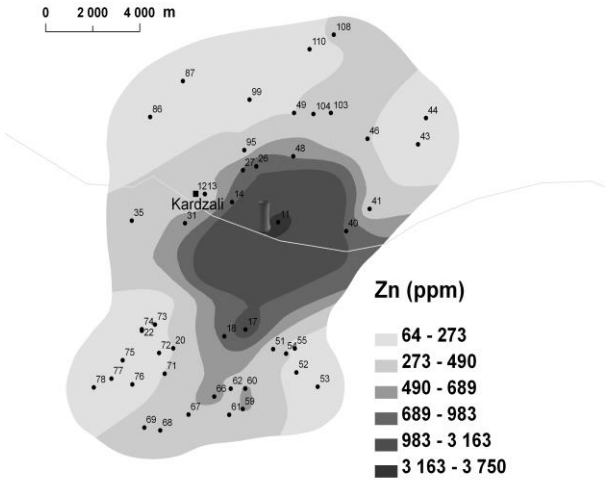

Fig. 2. The geographical distribution map of Zn relevant to factor 3.

Factor 3 has high loadings for Zn, Se, As, In, Sb, Au associated with the Kardzhali LZP. This group of elements correlates very well with metal contamination of surface soils around the lead and zinc smelter in the Republic of Macedonia [7] .

Factor 3 is illustrated by the distribution map for Zn (Figure 2) as the main polluting elements of ZLP in Kardzhali with the maximal concentrations of Zn exceeding the normal (unpolluted values, taken from the Norwegian data), by the order of 10-100. The highest concentrations of Zn are observed in sampling points 11, 14, 18, and 17 and 40. The town of Kardzhali is experiencing tremendous impact of elements, associated with lead-zinc ores. To construct this map the program ArcGIS 9.3 with geostatistical analysis was used.

Factor 4 seems to be a mixture of two factors: high values of Cr, Ni, Co, and As indicate the presence of industrial pollution. High values for Br and I usually considered as "marine" elements, given the lack of elements such as Na and Cl in this factor, suggest heavy fuel oils used in the smelting process at the LZP plant as a source of pollution.

Table 1. Comparison of the results obtained with Macedonia and Norway, mg/kg

Elements	Bulgaria, LZP		Macedonia [5]		Norway [6]	
	median	range	median	range	median	range
Na	1525	179-8190	419	118-8673	–	–
Mg	1006.5	366-3730	2377	674-7421	1730	940-2370
Al	17400	4120-53800	3736	825-17600	200	67-820
Cl	162	78.6-601	149	43-693	–	–
K	7585	3650- 30900	8615	2861-18190	–	–
Ca	11450	5520- 21700	5593	1207-23640	2820	1680-5490
Sc	2.415	0.12- 13-	0.81	0.12-6.79	0.052	0.009-0.220
Ti	716	170- 3990	163	12-1365	23.5	12.4-66.4
V	20	6.3- 124	6.9	1.79-43	0.92	0.39-5.1
Cr	14.4	2.71- 260	7.47	2.33-122	0.55	0.10-4.2
Mn	424	56- 1700	186	37-1475	256	22-750
Fe	6440	1250- 32400	2458	424-17380	209	77-1370
Co	2.64	0.43- 23.5-	1.09	0.24-13.6	0.202	0.065-0.654
Ni	10.7	1-213	2.4	0.09-24	1.14	0.12-6.6
Zn	269	28.8-3750	39	14-203	26.5	7.9-173
As	3.18	0.479-22.4	0.80	0.12-8.0	0.093	0.020-0.505
Se	0.54	0.1-2.54	0.18	0.013-0.61	0.33	0.05-1.30
Br	6.65	1.66-19.5	2.16	0.06-7.7	4.5	1.4-20.3
Rb	31.5	6.93-229	10.9	5-47	7.7	1.3-51.5
Sr	81.2	19.7-527	31	11.8-136	15.8	3.6-43.3
Mo	0.54	0.121-1.78	0.19	0.03-1.12	0.135	0.065-0.70
In	0.074	0.014-0.424	–	–	–	–
Sb	3.93	0.162-46.5	0.2	0.039-1.4	0.033	0.004-0.240
I	2.26	0.90-4.87	1.18	0.36-2.8	2.5	0.6-41.7
Cs	1.46	0.19-8.81	0.39	0.097-1.7	0.072	0.016-0.88
Ba	165.5	35.2-847	54	14-256	17.1	5.6-50.5
La	6.93	0.924-227.2	2.32	0.50-22	0.189	0.045-2.56
Ce	17.35	1.67-52	5.60	0.83-42	0.342	0.095-4.61
Sm	1.27	0.199-3.82	0.46	0.07-3.4	0.33	0.05-1.34
Tb	0.174	0.026-0.476	0.06	0.01-0.56	0.003	<0.002-0.030
Dy	1.09	0.218-3.55				
Hf	1.04	0.25-7.62	0.26	0.05-3.8	–	–
Ta	0.22	0.04-1.48	0.09	0.013-0.79	0.01–	<0.01-0.07
W	0.55	0.107-2.15	1.21	0.25-3.9	0.127	0.009-1.23
Au	0.004	0.00107-0.0235	0.0061	0.001-0.034	–	–
Th	3.215	0.244-19	0.67	0.12-7.6	0.033	0.004-0.240
U	0.856	0.102-5.22	0.21	0.03-1.45	0.015	0.001-0.138

Table 2. Factor analysis of NAA data on moss samples collected in the vicinity of Kardzhali LZP (Varimax normalized)

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Na	0.48	**0.65**	0.25	-0.06
Mg	0.37	**0.73**	-0.03	0.29
Al	**0.55**	**0.71**	-0.03	0.05
Cl	0.34	-0.38	0.23	0.03
K	**0.86**	0.24	0.23	-0.06
Sc	0.30	**0.64**	-0.11	0.18
Ca	0.46	-0.09	-0.04	0.14
Cr	-0.02	0.34	-0.02	**0.84**
Ti	0.14	**0.91**	-0.05	0.06
V	-0.01	**0.91**	0.05	0.07
Mn	0.33	0.45	-0.06	0.11
Ni	-0.04	0.34	-0.03	**0.84**
Fe	0.45	**0.69**	0.01	0.28
Co	0.11	**0.57**	0.01	**0.72**
Zn	0.07	-0.03	**0.96**	-0.04
Se	-0.02	-0.12	**0.82**	0.08
As	0.15	0.21	**0.65**	**0.57**
Br	0.10	-0.14	0.36	**0.59**
Rb	**0.89**	0.12	0.12	0.06
Sr	**0.72**	0.23	0.01	-0.23
Mo	**0.62**	0.24	0.49	0.20
In	0.15	0.03	**0.89**	0.07
Sb	0.07	-0.08	**0.95**	-0.10
I	0.29	-0.09	0.16	**0.73**
Cs	**0.92**	0.055	-0.01	0.15
Ba	**0.62**	0.53	0.18	-0.05
La	**0.67**	0.49	0.18	0.19
Ce	**0.75**	0.48	0.17	0.20
Sm	**0.74**	0.52	0.09	0.15
Tb	**0.64**	**0.61**	0.07	0.16
Dy	0.47	**0.69**	-0.05	0.08
Hf	**0.81**	0.41	0.08	0.037
Ta	**0.92**	0.24	-0.02	0.08
W	**0.82**	0.23	0.08	0.23
Au	0.13	0.05	**0.64**	0.18
U	**0.81**	0.29	0.28	0.01
Th	**0.91**	0.26	0.14	0.05
Expl. Var	*11.1*	*7.5*	*5.0*	*3,7*

CONCLUSIONS

As evident from the median values in Table 1, it is clear that the study area around the Kardzhali LZP is considerably polluted compared to previously obtained data for the other areas of Bulgaria [8].

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ИЗСЛЕДВАНЕ НА СЪСТОЯНИЕТО НА ОКОЛНАТА СРЕДА В РАЙОНА НА ОЛОВНО-ЦИНКОВ ЗАВОД КРАЙ КЪРДЖАЛИ ЧРЕЗ ИЗПОЛЗВАНЕ НА МЪХОВА ТЕХНИКА, НЕУТРОНЕН АКТИВАЦИОНЕН АНАЛИЗ, АТОМНО-АБСОРБЦИОННА СПЕКТРОМЕТРИЯ И ГИС ТЕХНОЛОГИИ

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(Резюме)

Представени са резултатите от изследванията на атмосферното замърсяване с тежки метали с помощта на биомониторинг на мъхове в района на оловно – цинковия комбинат в Кърджали. Този комбинат е основен източник на замърсяване с олово, кадмий, цинк и серен диоксид. За изучаване на атмосферното замърсяване бяха събрани проби от мъхове от 54 точки. Определени са концентрациите на следните елементи Na, Mg, Al, Cl, K, Sc, Ca, Cr, Ti, V, Mn, Ni. Fe, Co, Zn, Se, As, Br, Rb, Sr, Mo, In, Sb, I, Cs, Ba, La, Ce, Sm, Tb, Dy, Hf, Ta, W, Au, U, Th с помощта на инструментален епитермичен неутронен активационен анализ (ЕНАА) при реактор ИБР – 2 в лабораторията по неутронна физика на ОИЯИ, Дубна. За характеризиране на различните източници на замърсяване е използван многофакторен анализ. С помощта на GIS технологии са построени карти на разпределението на замърсяванията с различни елементи в изучавания район. Представените резултати са продължение на изследванията на околната среда в България, които се използват за регулиране на българската индустрия.

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EPR study of gamma – irradiated cereal foods

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The results from the EPR studies on wheat and oat bran, rolled oats, buckwheat and different kind of rice – white, brown and parboiled rice before and after gamma-irradiation are reported. Before irradiation all samples exhibit one weak singlet EPR line characterized with common g-factor of 2.0048±0.0005 and six lines due to Mn^{2+} naturally available in the plants. Only parboiled rice did not show any EPR spectrum before irradiation. After irradiation, in addition of the Mn^{2+} signal a typical "cellulose-like" triplet EPR spectrum appears, attributed to cellulose free radicals, generated by gamma-irradiation. This EPR spectrum is superimposed by a partly resolved "carbohydrate" spectrum, which however is the main spectrum for parboiled rice samples. The fading kinetics of radiation-induced EPR signals were studied for a period of 90 days after irradiation. The reported results unambiguously show that the presence of characteristic EPR spectra of cereal samples can be used for identification of previous radiation processing.

Keywords. Cereals, irradiation, EPR

INTRODUCTION

Irradiation of various food products with high energy radiation have been used as effective tool for their disinfestations and microbial decontamination, suitable also for their long-term preservation [1]. Gamma-irradiation has established itself as a safe, secure and clean procedure for sterilization of foodstuffs including in the final packing. The results show that the quantity of irradiated foods in the world in 2005 was 405,000 ton [2]. However, this manipulation should be under control, which is the reason for many recent studies creating suitable analytical methods [3]. As a result, ten protocols were adopted by the European Committee of Normalization, from which six primary and the remaining for screening purpose. Three of the main protocols use EPR (also known as ESR) since high energy radiation yields free radicals in foods, which are stable for a given period of time and therefore easily detected by EPR. One of them (EN 1787) treats cellulose containing foodstuffs, in which "cellulose-like" EPR spectrum appears after irradiation [4].

Cereals, especially wheat is an important nutritive for mankind because of its unique quality characteristics and the fact that large quantities can be produced, harvested, stored and transported in an efficient way. EPR spectroscopy was applied to the studies on laser induced free radicals in wheat grains [5] and in oat grains [6]. Free radicals in irradiated wheat flour were investigated by EPR [7]. The possible use of oat, wheat and corn kernels as dosimeters for high-energy radiation were discussed [8]. Basmati rice were studied by EPR and Thermoluminescence methods. EPR investigation of 0.5-2.0 kGy irradiated basmati rice samples showed short lived free radicals. In view of this the possibility to identify irradiated rice by the relaxation characteristics and thermal behaviour of the free radicals were examined [9] in accordance with our previous research [10]. Rice noodles could be confirmed for irradiation treatment using ESR spectroscopy [11].

In the present communication we report the EPR spectra obtained before and after gamma-irradiation of some widely spread non investigated cereal foodstuffs in order to prove radiation treatment.

EXPERIMENTAL

Samples

Wheat and oat bran, rolled oats, buckwheat and different kind of rice – white, brown and parboiled rice were purchased from local market, and were divided in two portions. The first batch were passed for irradiation, the second was separated as a control samples.

Irradiation

All samples were simultaneously gamma-irradiated by "Gamma 1300" irradiator with a single dose of 10 kGy. The irradiation was performed at room temperature and in the air. All further manipulations of the irradiated cereals were performed at least 72 h after irradiation in order to avoid any interference by the radiation induced short living paramagnetic species.

Instrumentation

EPR measurements were performed at room temperature on Bruker ER 200 D SRC spectrometer operated in X-band. Standard rectangular cavity

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(ER4102ST) operating in TE102 mode and 100 kHz magnetic field modulation were used. All samples were accommodated in quartz EPR sample tubes (i.d./o.d. 4/5 mm). The g-values of all samples were estimated using "EPR marker" available in the F-F Lock module (ER 033) – in our spectrometer g- mark is 2.0050.

RESULTS AND DISCUSSION

EPR spectra before irradiation

Before irradiation EPR spectra of all samples exhibit one weak singlet line (Figure 1a) characterized with common g = 2.0048±0005, which is equal to that observed in all plant origin foodstuffs. It is attributed to free radicals of semi-quinones [12] or to oxidation products of fatty acids present in some fruits and vegetables [13]. In addition to the weak singlet another spectrum can be detected (Figure 1b). It consists of six lines (marked with asterisks in Figure 1b) due to Mn^{2+} naturally available in the plants (nuclear spin of ^{55}Mn, which is 100% in the natural abundance, is 5/2). The spectrum of Mn^{2+} is characterized with g = 2.0014±0.0005 and A_o = 75±1G. Only parboiled rice did not show EPR spectrum before irradiation.

EPR spectra after irradiation

As for non-irradiated samples a spectrum due to Mn^{2+} ions (Figure 2a) can be detected for the samples after radiation treatment. Manganese spectrum is radiation independent. More precise analysis of the EPR spectra after irradiation of wheat and oat bran, rolled oats, buckwheat, white and brown rice (Figure 2b) show that they are similar, consisting of central intense line with g=2.0048±0.0005 and two weak satellite peaks separated *ca*. 3 mT left and right to it. The central line of the triplet is buried by the natural singlet. The presence of two satellite lines is considered in the Protocol EN 1787 as unambiguous evidence for previous radiation treatment of plant origin foodstuffs. Radiation induced triplet, called "cellulose-like", is attributed to cellulose free radicals [14]. On the other hand, additional doublet of lines (marked with asterisks in Figure 2b) are recorded in the EPR spectra of studied samples overlapped with the "cellulose-like" (marked with arrows in Figure 2b) spectrum. This doublet may be attributed to free radicals of starch [15] (Chabane et al., 2001) known as "carbohydrate" spectrum [16].

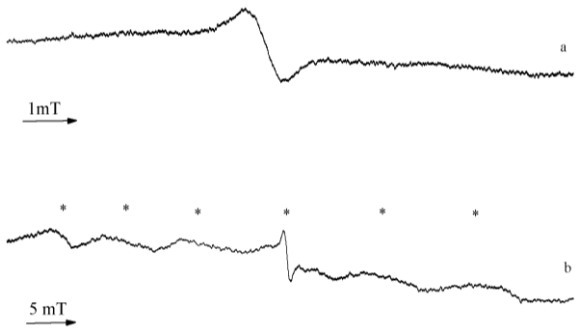

Fig. 1. EPR spectra of non - irradiated samples, recorded at magnetic field sweep 10 mT (a) and 60 mT (b).

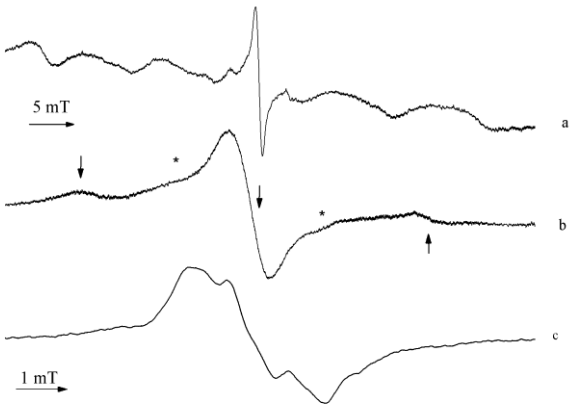

Fig. 2. EPR spectra of irradiated wheat and oat bran, rolled oats, buckwheat, white and brown rice, recorded at magnetic field sweep 60 mT (a) and 10 mT (b); parboiled rice 10 mT (c).

There is no difference between EPR spectra of wheat and oat bran, rolled oats, buckwheat, as well as white and brown rice except intensity. EPR spectrum of irradiated parboiled rice (Figure 2c) differs from the spectra of all other samples. For this sample "carbohydrate" spectrum is better pronounced and "cellulose-like" spectrum is not registered.

Study of the EPR fading kinetics

In order to find the time stability of radiation-induced EPR signals of cereal food samples, their decay kinetics was studied for a period of 90 days after irradiation. It follows from Figure 3 that the intensity of the radiation induced signals of the food samples gradually decrease in identical way with the storage time. Nevertheless the following order of stability is recorded: parboiled rice > white, brown rice > buckwheat > rolled oats > wheat and oat bran.

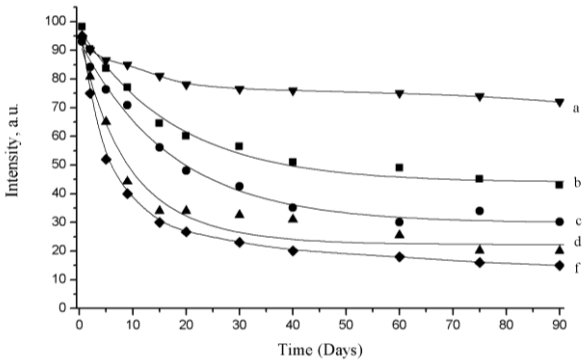

Fig. 3. Kinetics of fading of the radiation induced signals in parboiled rice (a); white, brown rice (b); buckwheat (c) rolled oats (d); wheat and oat bran (f).

CONCLUSION

The obtained EPR spectra of some gamma-irradiated cereal foodstuffs are complex because of the overlapping signals of different free radicals due to carbohydrates. Nevertheless, the individual components of each spectrum lead to the specific EPR spectra. Identification of radiation treatment can be proved by these characteristic EPR spectra, thus enriching European protocols concerning irradiated foodstuffs.

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ЕПР ИЗСЛЕДВАНЕ НА ГАМА-ОБЛЪЧЕНИ ЗЪРНЕНИ ХРАНИ

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(Резюме)

Представени са резултати от ЕПР изследване на пшеничени и овесени трици, овесени ядки, елда и различни видове ориз - бял, кафяв и бланширан ориз преди и след гама-облъчване. Преди облъчване при всички проби се регистрира слаба синглетна ЕПР линия, характеризираща се с g-фактор 2.0048±0,0005 и шест линии, които се дължат на Mn^{2+} йони природно съдържащи се в растенията. Единствено бланшираният ориз не показва ЕПР спектър преди облъчване. След облъчване заедно със сигнала от Mn^{2+} йони, се детектира типичен „целулозоподобен“ триплетен ЕПР спектър, който се приписва на свободни радикали от целулозата, генерирани в следствие на гама-облъчване. В допълнение на този ЕПР спектър е насложен и частично разрешен „въглехидратен“ спектър, който е основен спектър за пробите от бланширан ориз. Кинетиката на затихване на радиационно-индуцираните ЕПР сигнали е проследена за период от 90 дни след облъчване. Докладваните резултати показват недвусмислено, че присъствието на характерни ЕПР спектри на проби от житни растения може да се използва за идентифициране на предходна радиационна обработка.

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Effect of gamma radiation on some saccharides: an EPR study

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In the present work an Electron Paramagnetic Resonance (EPR) spectroscopic study of γ irradiated mannitol and stevia is reported. It is found that the EPR spectra of irradiated samples strongly depend on the applied microwave power and modulation amplitude. On the basis of microwave saturation is found that both mannitol and stevia EPR spectra are complex, containing at least two individual and overlapping signals. The produced free radicals are stable at room temperature at least six months. This is the fundament to distinguish irradiated products containing mannitol or stevia from non-irradiated ones even six months after irradiation.

Keywords: *EPR spectroscopy, γ radiation, mannitol, stevia*.

INTRODUCTION

In the last three decades EPR or ESR (Electron Spin Resonance) spectroscopy has expanded significantly in the field of practical applications. This is mainly due to its selectivity (only paramagnetic substances), high sensitivity (10^{-11} – 10^{-12} M) and non-destructive analysis. One direction in this field is detection of radiation induced defect in the matter. On the other hand gamma radiation is considered as a clean, not too expensive and effective method for sterilization of foodstuffs [1], medical and pharmaceutical products [2], etc. The irradiation procedure must be controlled because generated paramagnetic defects or free radicals in the mater and especially their recombination products in the food form new, unknown substances with unknown effects in regard to human health. This fact justifies the necessity of control of the radiation processing. A lot of works described the application of EPR method for identification of irradiated foodstuffs [3-7] as well as for dosimetric control [8,9] were published. In the literature there is only one publication about EPR investigations of gamma irradiated stevia [10] who study it as a material for accidental dosimetry. This study demonstrated the potential use of sweeteners for retrospective dosimetry. No data about EPR investigation of gamma-irradiated mannitol are reported up to now.

In the present work for the first time EPR spectra of gamma irradiated mannitol and stevia in respect to their structure and time stability in order to their potential application for identification of irradiated foodstuffs containing them, as well as for dosimetric purposes are reported.

EXPERIMENTAL

Materials. Mannitol ($C_6H_8(OH)_6$) was purchased from Aldrich whereas stevia (made from the leaves of the plant species Stevia rebaudiana with sweet taste coming from steviol glycoside ($C_{20}H_{30}O_3$)) was purchased from local market. The substances were used as obtained.

Irradiation. The samples were irradiated with single dose of 10 kGy γ-rays on "Gamma-1300" irradiator (^{137}Cs) in air and at room temperature.

Instrumentation. The EPR spectra were recorded at room temperature on a JEOL JES-FA 100 EPR spectrometer operating in the X–band, equipped with a standard TE_{011} cylindrical resonator.

RESULTS AND DISCUSSION

Features of the EPR spectrum of mannitol and stevia

In non-irradiated samples of mannitol and stevia no EPR spectra were recorded. After irradiation the samples exhibit an unresolved and a complex signal containing several overlapped and not well resolved EPR patterns. The nature of these radicals is not clear up to now. Figure 1a shows the EPR spectrum of gamma irradiated mannitol which is characterized with g factor 2.0170 ± 0.0002 of the most intense peak. The first step after irradiation was to study the influence of the instrumental settings microwave power and modulation amplitude on the EPR signal in order to find more deep information about their possible structure. It is seen from Figure 1a that the radiation induced EPR spectrum of mannitol is consists of four peaks (noted as P1, P2, P3 and P4) all sensitive to the magnitude of the applied microwave power (Figure 1b).

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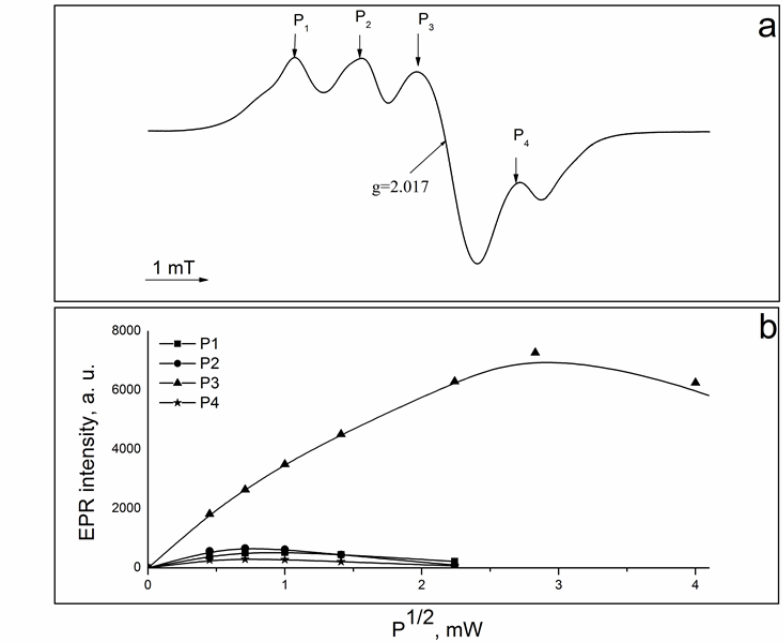

Fig. 1. (a) EPR spectrum of irradiated mannitol; (b) EPR signal intensity of irradiated mannitol as a function of square root of microwave power.

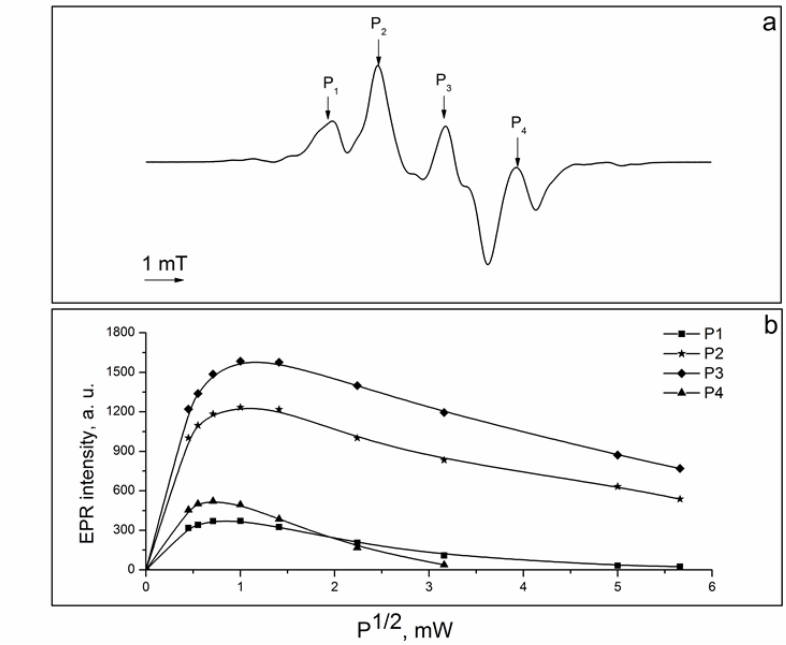

Fig. 2. (a) EPR spectrum of irradiated stevia; (b) EPR signal intensity of irradiated stevia as a function of square root of microwave power.

It was found (Figure 1b) that the peaks P1, P2 and P4 have equal behavior of saturation - they are saturated at microwave power less than 0.2 mW and above 8 mW they disappear. Because of the same behavior of saturation from the applied microwave power it was concluded that the peaks P1, P2 and P4 probably are belonging to one and the same free radical. The peak P3 is slightly broadened at microwave power higher than 1mW, which unambiguously indicate saturation effect but it is well observed at 8 mW and higher power. In respect of the applied modulation amplitude the EPR spectrum of irradiated mannitol linearity depends of it up to 0.16 mT and after that over-modulation of the separate EPR lines appears. In view of this it may be concluded, that in gamma irradiated mannitol at least two free radicals are present.

Figure 2a shows the EPR spectrum of irradiated stevia which is composed of four peaks marked in the figure as P1, P2, P3 and P4. These peaks are characterized with g-values 2.0379, 2.0280, 2.0155

and 2.0045 (± 0.0002) respectively. The behavior of to microwave power saturation and modulation amplitude shows increasing of the EPR signal intensity to about 1 mW but linear dependence is obtained to and down 0.3 mW (Figure 2b). On the other hand the peak P4 disappear over 10 mW. In view of the dependence of the EPR signal intensity from the applied modulation amplitude as a whole there are not changes in the EPR spectrum of stevia up to 0.6 mT modulation amplitude, after that the spectrum become slightly broadening.

Time stability of radiation-induced EPR signals

Previous works [11,12]show that immediately after irradiation, the shape and the intensity of the EPR spectra of saccharides undergo changes during a certain period of time, characteristic for each material. Having this in mind, the EPR spectra of γ -irradiated mannitol and stevia were monitored for six months after the irradiation. It was found that the EPR spectra of both samples undergo small transformations in the first days after irradiation. After that the shape of the spectra of irradiated mannitol and stevia remains unchanged only decreasing of the intensity of the EPR spectra with 250 $g.kg^{-1}$ was recorded. This means that six months after irradiation these substances are suitable for identification of radiation processing. Moreover, mannitol and stevia can be considered as appropriate substances for dosimetric purposes.

The obtained results describe for the first time some features of EPR spectra of manitol and stevia. It is shown that each spectrum consist at least two independent paramagnetic species. Also it may be suggested that using EPR technique is possible to investigate irradiated foodstuffs containing them as well as for their potential as dosimetric materials. The present initial studies about EPR investigation of mannitol and stevia suggest that some additional work must be done though, before it can be taken in regular use.

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ЕФЕКТ НА ГАМА РАДИАЦИЯ ВЪРХУ ЗАХАРИДИ: ЕПР ИЗСЛЕДВАНЕ

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(Резюме)

В настоящата работа са представени резултати от изследване на гама облъчени манитол и стевия с Електрон Парамагнитен Резонанс (ЕПР) спектроскопия. Установено е, че ЕПР спектъра на облъчените проби зависи силно от приложената микровълнова мощност и амплитуда на модулация. Въз основа на насищането на ЕПР сигнала при увеличаване на микровълновата мощност е установено, че ЕПР спектрите на манитол и стевия са сложни и съдържат най-малко две отделни и припокриващи се ЕПР линии. Образуваните свободни радикали са стабилни при стайна температура най-малко шест месеца. Това дава възможност да се разграничат облъчени манитол и стевия съдържащи продукти от необлъчени, дори шест месеца след облъчване.

BULGARIAN CHEMICAL COMMUNICATIONS

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General remarks: Manuscripts are submitted in English by e-mail or by mail (in duplicate). The text must be typed double-spaced, on A4 format paper using Times New Roman font size 12, normal character spacing. The manuscript should not exceed 15 pages (about 3500 words), including photographs, tables, drawings, formulae, etc. Authors are requested to use margins of 3 cm on all sides. For mail submission hard copies, made by a clearly legible duplication process, are requested. Manuscripts should be subdivided into labelled sections, e.g. **Introduction, Experimental, Results and Discussion**, *etc*.

The title page comprises headline, author's names and affiliations, abstract and key words.

Attention is drawn to the following:

a) **The title** of the manuscript should reflect concisely the purpose and findings of the work. Abbreviations, symbols, chemical formulas, references and footnotes should be avoided. If indispensable, abbreviations and formulas should be given in parentheses immediately after the respective full form.

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The abstract should be self-explanatory and intelligible without any references to the text and containing not more than 250 words. It should be followed by key words (not more than six).

References should be numbered sequentially in the order, in which they are cited in the text. The numbers in the text should be enclosed in brackets [2], [5, 6], [9–12], etc., set on the text line. References, typed with double spacing, are to be listed in numerical order on a separate sheet. All references are to be given in Latin letters. The names of the authors are given without inversion. Titles of journals must be abbreviated according to Chemical Abstracts and given in italics, the volume is typed in bold, the initial page is given and the year in parentheses. Attention is drawn to the following conventions:

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b) Only the initials of the first and middle names should be given.

In the manuscripts, the reference to author(s) of cited works should be made without giving initials, e.g. "Bush and Smith [7] pioneered...". If the reference carries the names of three or more authors it should be quoted as "Bush *et al*. [7]", if Bush is the first author, or as "Bush and co-workers [7]", if Bush is the senior author.

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Tables are numbered with Arabic numerals on the left-hand top. Each table should be referred to in the text. Column headings should be as short as possible but they must define units unambiguously. The units are to be separated from the preceding symbols by a comma or brackets.

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The author(s) name(s), the title of the manuscript, the number of drawings, photographs, diagrams, etc., should be written in black pencil on the back of the illustrative material (hard copies) in accordance with the list enclosed. Avoid using more than 6 (12 for reviews, respectively) figures in the manuscript. Since most of the illustrative materials are to be presented as 8-cm wide pictures, attention should be paid that all axis titles, numerals, legend(s) and texts are legible.

The authors are asked to submit **the final text** (after the manuscript has been accepted for publication) in electronic form either by e-mail or mail on a 3.5'' diskette (CD) using a PC Word-processor. The main text, list of references, tables and figure captions should be saved in separate files (as *.rtf or *.doc) with clearly identifiable file names. It is essential that the name and version of

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EXAMPLES FOR PRESENTATION OF REFERENCES

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