Bulgarian Chemical Communications, Volume 46, Special issue B (pp. 20–24) 2014

Physical studies of plant wax from watermelon

I. N .Panchev[1, *], S. D. Pashova[2], R. S. Radev[2], D. N. Petrov[3], D. G. Kovacheva[4]

[1]*University of Food Technologies, Plovdiv,*
[2]*University of Economics, Varna,*
[3]*Plovdiv University "Paisii Hilendarski",*
[4]*Institute of General and Inorganic Chemistry, BAS, Sofia*

Received August 17, 2014; Revised December 12, 2014

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 studied by 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.

Keywords: 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).*

* To whom all correspondence should be sent.
E-mail: exner_ginka@yahoo.com

© 2014 Bulgarian Academy of Sciences, Union of Chemists in Bulgaria

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

- 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 1989). 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] (Dudley 1989).

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

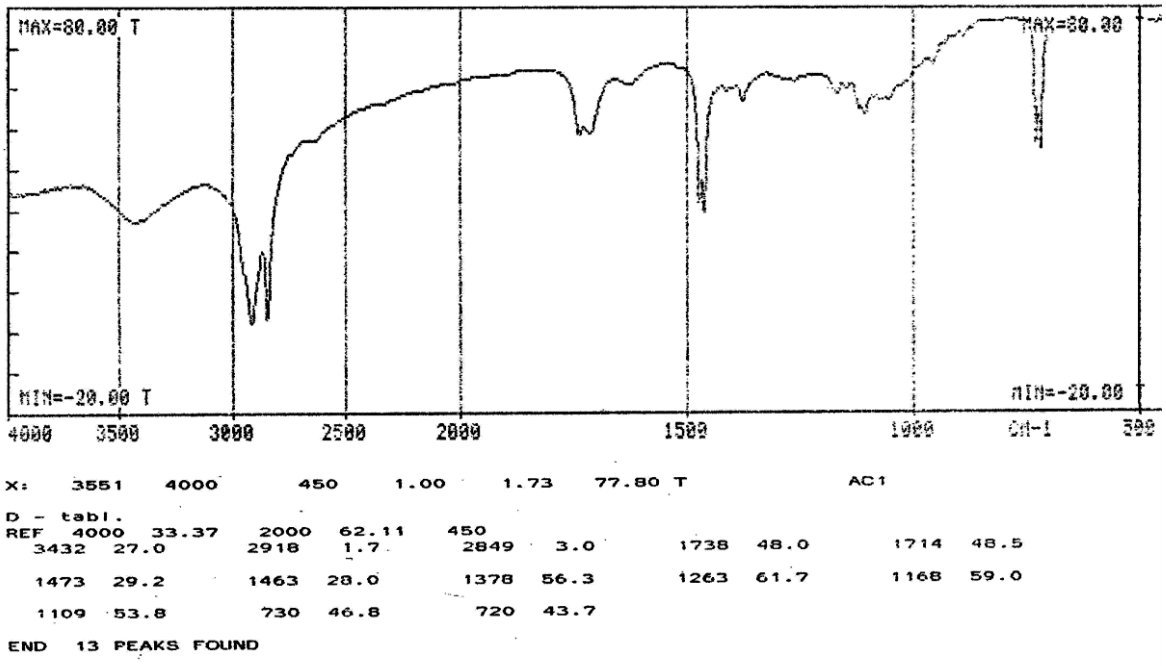

Fig. 1. The IR spectra of the wax from *Citrullus lanatus*

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, the presence of the $– CH_3$ group at about 1463 cm^{-1} also

can be seen. In the spectra there can even be seen peak points at about 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}. The identification if which these 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 sm^{-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 found: 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 sm^{-1} found on the lower side of the strong CH_2 scissors feature at 1473 sm^{-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.

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.

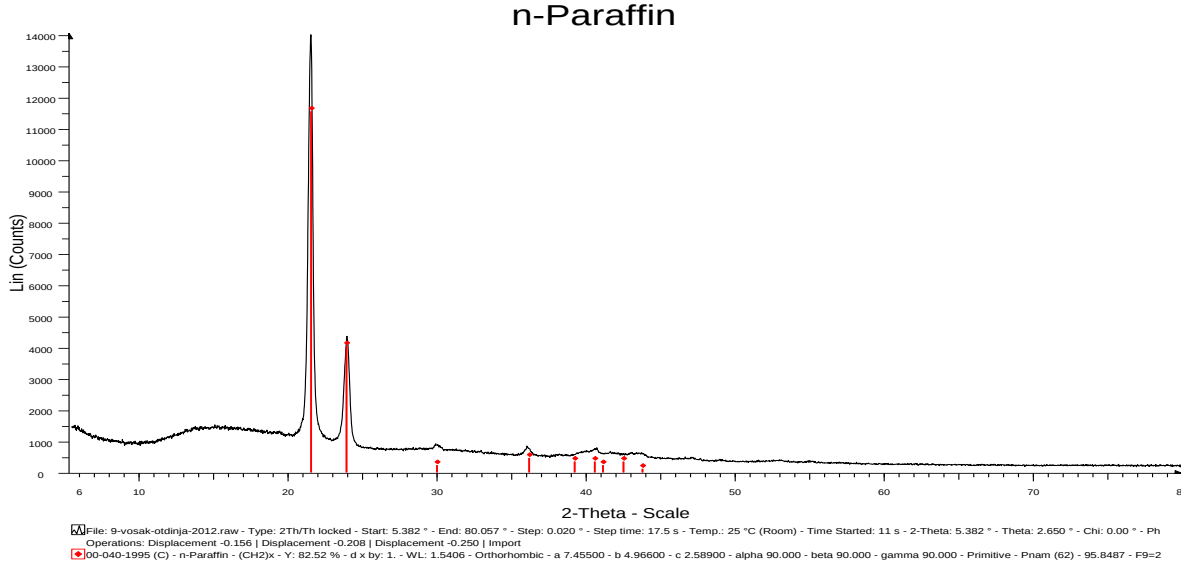

Fig. 2. X-ray structure analysis of the wax from *Citrullus lanatus*

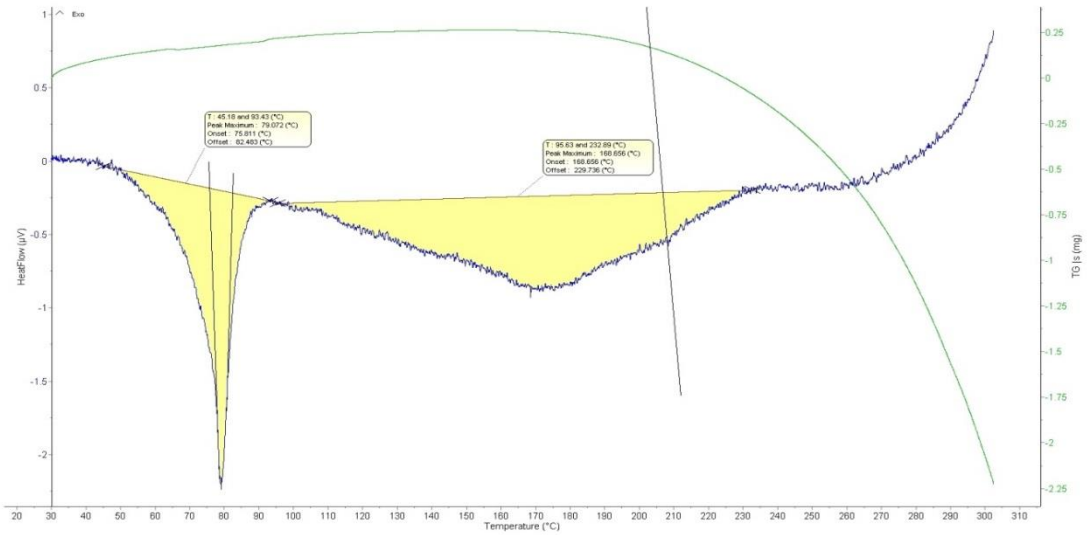

Fig. 3. DTA –TG analysis of the wax from *Citrullus lanatus*

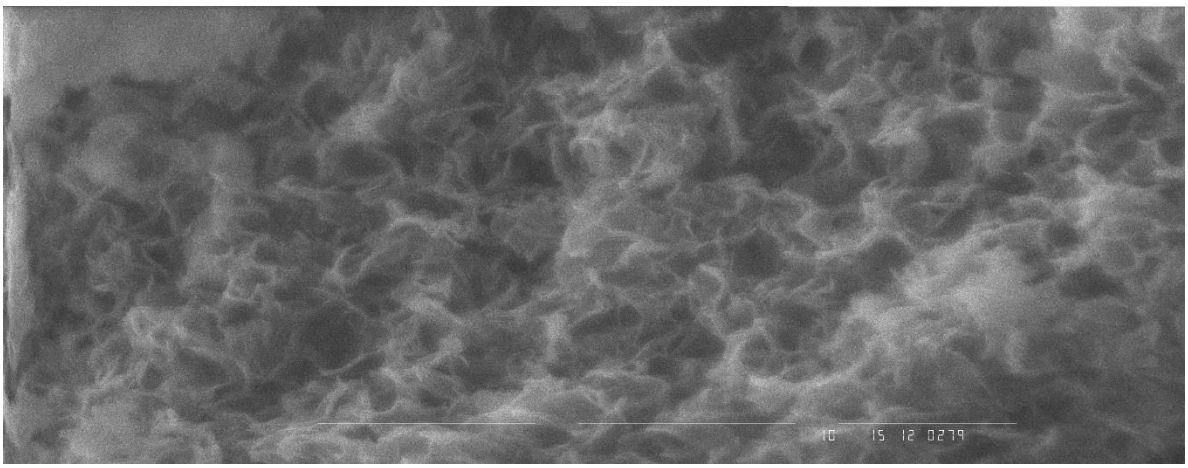

Fig. 4. SEM analysis of the wax from *Citrullus lanatus*

The results of the 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.

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 (mixture of different hydrocarbons) 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-Parafin. 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 by 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, which will spare the thermo sensible biopolymer macromolecules.

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.

REFERENCES

1. J.J. Kester, O. Fennema, *J. Food Science*, **54**, 1383 (2006).
2. J.J. Kester, O. Fennema, *Food Technol*., **12**, 47 (1986).
3. C.J. Weber, Biobased Packaging Materials for the Food Industry. KVL, Frederiksberg, Denmark, (2000).
4. I. Meusel, W. Barthlott, H. Kutzke, B. Barbier, *Powder Diffraction*, **159**, 123 (2000).
5. S.M. Goodwin, M. Jenks, Plant cuticle function as a barrier to water loss. In MA Jenks, PM Hasagawa, eds, Plant Abiotic Stress. Blackwell, Oxford, 14 (2005).
6. Y. Xia, K. Yu, D. Navarre, K. Seebold, A. Kachroo, P. Kachroo, *Plant Physiol*., **154**, 833 (2010).
7. T. Bourtoom, *Int. Food Research J*., **15,** 237 (2008).
8. M.L. Navarro-Tarazaga, A. Massa, M.B. Pérez-Gago, *LWT, Food Sci. Technol.*, **44,** 2328 (2011).
9. E. Velickova, E. Winkelhausen, S. Kuzmanova, V. D. Alves, M. Moldão-Martins, *LWT, Food Sci. Technol.*, **52**, 80 (2013).
10. E. Ochoa, S. Saucedo-Pompa, R. Rojas-Molina, H. de la Garza, A. V. Charles-Rodríguez, C. N. Aguilar, *Am. J. Agr. Biol. Sci*., **6**, 92 (2011).
11. S. Saucedo-Pompa, R. Rojas-Molina, A.F. Aguilera-Carbó, A. Saenz-Galindo, H.D. L Garza, D. Jasso-Cantú, C.N. Aguilar, *Food Research Int.*, 42, 511 (2009).
12. C.L. Weller, A. Gennadios, R.A. Saraiva, *LWT - Food Sci. Technol.* **31,** 279 (1998).
13. R. Hagenmaier, *Florida State Horticulture Society and Citrus Industry*, **111**, 251 (1998).
14. B. Ritter, J. Schulete, E. Sculte, K.P. Their, *Eur. Food Res. Technol*., **212**, 603 (2001).
15. C.M. Galanakis, Trends Food Sci. Technol., 26, 68 (2012).
16. C. Casado, A. Heridia, *J. Exper. Botany*, **50**, 175 (1999).
17. W. Wendlandt, Thermal methods of analysis. John Welly & Sons, Inc. New York, 2 edition, (1974).
18. H. Dudley, I. Fleming, Spectroscopic Methods in Organic Chemistry, Clarendon Press, London, 1989.
19. A.G. Gibbs, *J. Insect. Physiol*., **48**, 391 (2002).

ФИЗИЧНИ ИЗСЛЕДВАНИЯ ВЪРХУ РАСТИТЕЛЕН ВОСЪК ОТ ДИНЯ

И.Н. Панчев[1*], С.Д. Пашова[2], Р.С. Радев[2], Д.Н. Петров[3], Д.Г. Ковачева[4]

[1]Университет по хранителни технологии, Пловдив
[2]Икономически университет, Варна
[3]Пловдивски Университет „Паисий Хилендарски“
[4]Институт по обща и неорганична химия, БАН, София

Постъпила на 17 август, 2014 г. ; приета на 12 декември, 2014 г..

(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.