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EPR study of free radicals in pasta products

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The features of the EPR spectra of different kinds of pasta products purchased from the local market and other home-made products are reported. All commercially available samples exhibited one singlet EPR line with g=2.0049±0.0003 and line width ΔH=0.95±0.05 mT. The only difference between the EPR signal intensities at equal weights of different pasta products is that those prepared from wheat flour and water only exhibited a three to fifteen times lower signal in comparison with these containing additives as fats, milk, and egg powder.

The effect of drying parameters on the relative free radical concentration in freshly home-made spaghetti was also studied. The used wheat flour itself showed a very weak EPR singlet line with g=2.0040±0.0003 and ΔH=0.83±0.02 mT. The spaghetti prepared from it and water were dried in open air at 28, 65, 75 and 93±1°C. After drying all samples exhibited an EPR signal with g=2.0047±0.0003. At equal weights the relative number of paramagnetic species depends on the temperature and time of drying. At low drying temperature this effect may be attributed to the oxidation processes whereas at higher temperatures (65, 75, 93 °C) additionally Maillard reaction takes place. Upon storing the pasta products in inert atmosphere the number of free radicals remains constant in comparison with those exposed to open air where the significant increase in the number of free radicals is attributed to the development of an additional oxidation process.

Keywords: Pasta, spaghetti, drying, free radicals, EPR.

INTRODUCTION

The pasta is a widely consumed food. It is assumed that the best quality pasta is that produced in accordance with French (9 July 1999, law № 99-574), Italian (Presidential decree №187, 9 February 2001) or Greek (Ministries of Finance and Commerce. Approval of the Decision No 359/93 of the Supreme Chemical Council Food Code – Article 115 "Pasta") laws from durum wheat semolina flour and water. The traditional pasta is dried at 40-50°C for a long time (up to 40 h). However, such drying cycles, called "low-temperature" (LT), can cause hygienic and qualitative problems because the combined effects of low temperature, high relative humidity and long drying time open the possibility of promoting microbiological growth. On the other hand, there is a big variety of pasta containing many ingredients added to their dough as fats, eggs, milk, spices, cheeses, etc. These ingredients, as well as the drying process in production of the pasta products influence their color [1-3], structural [4], textural [4,5] and technological [6] properties. Commercially available pasta products are typically dried at high (HT) (60-75°C) or very high temperature (VHT) (75-100°C) for a short time (2-15 h depending on the temperature). The resulting product is characterized with less sticky, firmer consistence, lower cooking loss and improved color properties. On the other hand, in contrast to LT cycles, it is established that at HT and VHT drying cycles the Maillard reaction takes place [1-3] causing browning of the product. However, in previous studies from this laboratory it was shown that in the early stages of the Maillard reaction free radicals are recorded [7-9]. Other authors assigned them to radical cations of the type of 1,4-bis(5-amino-5-carboxy-1-pentyl)pyrazinium (CROSSPY) assuming that the latter are transient products in the process of protein oligomerization and melanoidins formation [10-13].

In the first part of the present study EPR spectra of different kinds of pasta products available on the local market were recorded, in order to find more thorough information on the semiquantitative concentration of free radicals present in them. The second part is devoted to the influence of the drying and storing conditions on the free radical generation in home-made pasta.

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Table 1. Pastas studied in the present paper

Type of pasta products	Origin	Content
Pasta ”Pagani” (alfabeta)	Italy	Durum wheat flour
Macaroni “Stella” (helical)	Greece	100 % durum wheat semolina
Macaroni “Stella” (tubes)	Greece	100 % durum wheat semolina
Spaghetti “Melissa-Primo Gusto”	Greece	100 % durum wheat semolina
Vermicelli “Melissa-Primo Gusto”	Greece	100 % durum wheat semolina
Spaghetti “Gold ear”	Bulgaria	Wheat flour and water
Pasta “Cala” (helical)	Bulgaria	Common wheat semolina flour, water, natural color flours
Macaroni (over done) “Etar”	Bulgaria	Wheat semolina and water
Home made noodles “Etar”	Bulgaria	Flour type 500, semolina, salt, vegetable fat, egg powder
Noodles “Inko”	Bulgaria	Wheat flour type 500, wheat semolina, salt, milk, egg powder
Spaghetti “Choice” with vegetables (semi-cooked)	Korea	Wheat flour, palm fat, salt, soya sauce, onion, garlic, red and black pepper
Vermicelli “Zagaria”	Bulgaria	Durum wheat semolina, common wheat semolina and water

EXPERIMENTAL

Materials and method

Wheat flour and different types of pasta products (spaghetti, macaroni, noodles and vermicelli) produced by different producers were purchased from the local market. The type of pasta products, their origin and content are shown in Table 1.

Sample preparation, storage and kinetic measurements

The commercially available samples were crushed and accommodated in quartz EPR sample tubes (ϕ = 5mm) up to 40 mm length.

In order to study the effect of drying conditions on the free radical generation, fresh home-made spaghetti were prepared and dried at different temperatures and atmospheres for different periods of time. The spaghetti dough obtained by kneading of wheat flour produced from common wheat semolina and distilled water were extruded in form of cylinders (d = 1.5±0.2 mm, length 60 mm). The samples were dried in a laboratory oven at 28, 65, 75, 93 ± 1°C for different times. The process of drying was monitored by weighing the samples. The samples were regularly taken out from the oven and weighed. The moment when the samples were dry was when the weight of two consecutive measurements was constant. After finishing the drying process, the home-made spaghetti were crushed and one part of the samples was accommodated into quartz EPR sample tubes in open air. Another part was accommodated in sample tubes closed with special rubber caps. Through this cap the sample was flushed with argon and heated in a drying oven at 65±1°C for a given time. The EPR spectra were recorded regularly with time. A third part was lyophilized in order to study the effect of oxygen on the relative number of free radicals during the drying process. In all cases the sample tubes were filled up to a 40 mm height and were with equal weight.

EPR measurements

EPR spectra were recorded at room temperature on a Bruker ER 200D SRC spectrometer operating in X-band. The g-values of all samples were estimated using “EPR marker” available in the “FF Lock” module (ER 033) of the ER 200D SRC spectrometer, calibrated in advance with DPPH for which g = 2.0036 [14]. The filled part of the sample tube was positioned exactly in the centre of the cavity. Every point in the kinetic studies was obtained as an average of at least three independent measurements.

The changes in the relative number of free radicals (N) in the studied samples per gram, was calculated using the formula $N \sim Ipp(\Delta Hpp)^2$, where Ipp is the peak-to-peak intensity and ΔHpp is the peak-to-peak line width of the first derivative of the EPR signal.

RESULTS AND DISCUSSION

EPR spectra of commercially available pasta samples

The pasta prepared only from wheat flour and water exhibited a weak singlet EPR line with g=2.0049±0.0003 and ΔH=0.95±0.05 mT. The g-factors of the recorded spectra of the pasta suggest that the detection of O-centered radicals [15] is probably due to starch free radicals in the wheat flour. If this pasta is additionally backed, it exhibits about three times more intensive spectrum with the same EPR parameters. Most probably, the thermo-generated free radicals may be attributed to the development of a Maillard reaction [10-13].

The pasta containing additives such as fats, milk, egg powder, etc., showed about three to fifteen (in noodles) times more intensive EPR line with the same g-factor. The intensive spectrum may be due to oxidation processes of fats from the additives to pasta in addition to the Maillard reaction.

EPR spectra of home-made pasta

The wheat flour sample exhibited a weak singlet EPR signal with g=2.0040±0.0003 and ΔH=0.83±0.02 mT, suggesting C-centered free radicals [15]. This EPR signal is probably due to mechanically induced free radicals in the process of milling the wheat grains. This assumption is in concert with the data available in the literature reporting that the intensity of the EPR signal increases with the extent of grinding [16].

After kneading a dough from flour and distilled water, the EPR parameters of the samples dried at 28, 65, 75 and 93 ± 1°C changed to g=2.0047±0.0002 and ΔH=0.93±0.03 mT. This result may be connected with the fact that in starch and proteins, which are the major components of wheat semolina, structural transformation during pasta processing takes place [17-19] and forms free radicals [7].

The experimental results showed that the relative number of paramagnetic species depends on the drying temperature and time (Fig. 1).

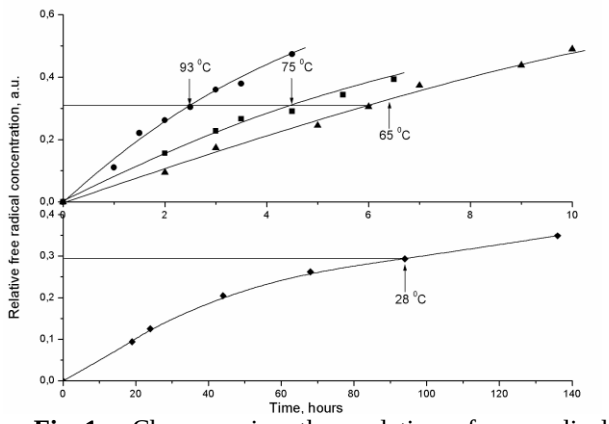

Fig. 1. Changes in the relative free radical concentration of home-made spaghetti during the process of drying in a laboratory oven at 28, 65, 75 and 93 ± 1 °C. The arrows show the points at which the weight of the samples becomes constant.

The home-made spaghetti were dried at different temperatures, as follows:
at 28 °C for 94 h; at 65 °C for 6.5 h; at 75 °C for 4.5 h; at 93 °C for 2.5 h.

The relative number of free radicals in these samples was approximately equal at these drying conditions. Samples dried at 28 °C needed longer time and probably oxidation processes took place at the high drying temperatures (65, 75, 93 °C). In addition, development of a Maillard reaction was expected. Realization of oxidation processes was confirmed by the fact that samples dried at room temperature for equal times in air and under vacuum, exhibited different free radicals number. The sample dried in absence of oxygen atmosphere was EPR silent.

In order to investigate the influence of storage conditions on the relative free radical concentration two home-made samples dried at room temperature in open air were taken – the one was kept in open air whereas the other - in argon atmosphere. The two samples were heated at 65±1 °C for 50 h. The results presented in Figure 2 clearly show that the relative free radical concentration in the sample stored in argon atmosphere remains constant during the whole period of study whereas the number of free radicals in the sample kept in air was almost constant up to the 30th hour, then sharply increased.

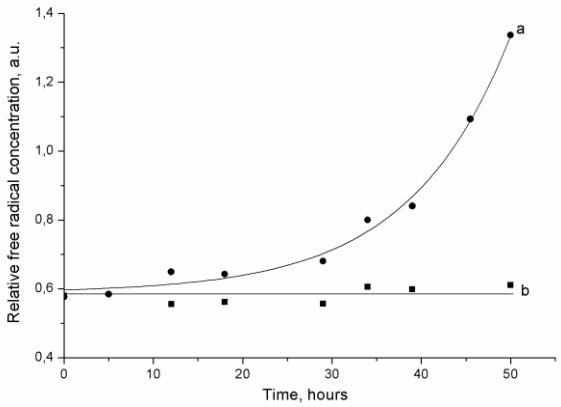

Fig. 2. Changes in the relative free radical concentration of dry home-made spaghetti samples heated at 65 ± 1 °C in a laboratory oven for a time interval of 0-50 h and stored in: a) open air and b) in argon atmosphere.

It may be assumed that at the beginning (up to the 30th hour), some antioxidants naturally present in the pasta protected it from oxidation [20]. After their consumption, the sharp increase in the number of free radicals suggests that a typical chain oxidation reaction [21] takes place. From these results it could be concluded that during the storage period, oxidation processes take place in spaghetti samples if kept in air access.

CONCLUSIONS

The present EPR studies of pasta show that:

Under kneading of wheat flour and water a chemical reaction takes place and free radicals with g=2.0047±0.0003 are generated;

The increase in relative free radical concentration in the drying samples on increasing the heating temperature may be most probably attributed to free radicals generation by oxidation processes at 28 °C whereas at higher temperatures (65, 75, 93 °C) the increase may be attributed to the development of an additional Maillard reaction. In conclusion, the processes of free radicals generation taking place in pasta products are lipid oxidation and Maillard reaction in presence of heat;

Drying under vacuum prevents the pasta samples from free radicals generation;

Storage of pasta in an inert atmosphere prevents it from developing of oxidation processes.

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EPR ИЗСЛЕДВАНЕ НА СВОБОДНИТЕ РАДИКАЛИ В МАКАРОНЕНИ ИЗДЕЛИЯ

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(Резюме)

Характеристиките на ЕПР спектрите на различни видове тестени изделия, закупени от местния пазар и на други домашно приготвени продукти са докладвани. Всички налични в търговската мрежа проби, показаха една синглетна ЕПР линия с g=2.0049±0.0003 и ширина на линията ΔH=0.95±0.05 mT. Единствената разлика, в интензитета на ЕПР сигналите, при равни тегла на различните тестени изделия е, че тези, приготвени само от пшенично брашно и вода показват от три до петнадесет пъти по-нисък сигнал в сравнение с тези, съдържащи добавки, като мазнини, мляко и яйца на прах.

Ефектът от параметрите на сушене върху относителната концентрация на свободните радикали в прясно приготвени домашни спагети, също е изследван. Използваното пшенично брашно показва много слабо интензивна синглетна ЕПР линия с g=2.0040±0.0003 и ΔH=0.83±0.02 mT. Спагетите, приготвени от него и вода бяха сушени на открито при 28, 65, 75 и 93 ± 1 °C. След изсушаване, всички проби показват ЕПР сигнал с g= 2,0047 ± 0,0003. Относителния брой на парамагнитни частици зависи от температурата и времето на сушене, при еднакви тегла на пробите. При ниска температура на сушене, този ефект може да се дължи на окислителните процеси, докато при по-високи температури (65, 75, 93 °C), в допълнение протича и Майлардова реакция. При съхранение на паста продуктите в инертна атмосфера, броя на свободните радикали остава постоянен в сравнение с тези, които са изложени на открит въздух, където значителното нарастване на броя на свободните радикали се дължи на развитието на допълнителен процес на окисление.