Melanoidin Formation in Cooked Meat Products†

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The processes of melanoidin formation in fried meatballs and schnitzel have been studied through a preparative quantitative isolation of the brown pigments. The influence of the duration of using the frying fat on the quantity of the latter has been established. The melanoidins have been characterized by spectra and fractionated using gel chromatography. It has been shown that their content is in direct relationship with the organoleptic properties of the end products.

A great number of cooked products and semiprocessed foods are offered in the sphere of public catering. Meat is the major component of most. The technological modes of heat processing applied (i.e., baking, frying, boiling, stewing, etc.) as well as the content of carbonyl compounds and nitrogen bases create the prerequisites for processes of nonenzymatic browning to take place with the formation of melanoidins as their end products. Investigations of the latter are rather limited. Most often they deal with the change in color depending on different factors (Hornstein and Grome, 1960; Horvat et al., 1962; Linko and Johnson, 1963; Lobanov and Wolfson, 1958; Macy et al., 1964; Pinto and Chichester, 1966; Sharp, 1957; Wasserman and Spinelli, 1970; Wolfson, 1964; Wood, 1961). Wolfson and Lobanov (1958) observed the browning of meat broth, and after the complete evaporation of water, they obtained soluble and insoluble dark brown products. Analogical interest has been shown in the browning of fish products (Adrian, 1972, 1973), melanoidins having been isolated from cuttlefish (Safronova, 1980). The Maillard reaction in meat has been the object of investigation of Dworschak (1970) using the derivatographic method. In most of these investigations conclusions about the flow of the nonezymatic processes of browning are drawn from the decrease in the content of amino acids and reducing carbohydrates or from the measurement of water extract absorption in the UV and visible spectrum ranges.

In previous works (Kuntscheva et al., 1983) we have described a method for preparative quantitative isolation of melanoidins from heat-processed meat (Obretenov et al., 1982) and the opportunities for using it in laboratory experiments and public catering. Then we studied the processes of melanoidin formation in meat under model conditions using a round of beef (Obretenov et al., 1983), a large portion of semiprocessed meat products with a high degree of processing (Somov et al., 1983), and fried meatballs (Ivanova et al., 1990). The results have shown that the amount of melanoidins is influenced by the technological mode of processing. Spectral and gel chromatographic characterizations have been made.

In the present work we offer a discussion of melanoidin formation in two culinary products and their characterization.

MATERIALS AND METHODS

The meat was prepared in accordance with Bulgarian State Standard (BSS) 7220-81 and was a homogeneous mass with a uniform distribution of meat and fat (lean meat and fatty tissue). It contained 55–62% of fats against dry matter, 48–62% water, and 1–2% sodium chloride against total mass. No pathogenic microorganisms were present.

With its organoleptic and physicochemical indices, sunflower oil met the requirements for freshly extracted vegetable oil in accordance with BSS 1-77. Its relative humidity at 20 °C was 0.9165–0.9235, n_D^{20} 1.4735–1.5755, acidity up to 0.2%, peroxide number 5 mequiv/kg.

The meatballs were prepared from beef and pork mince in a ratio of 1.5:1 with the addition of onions, bread, eggs, and spices. The mixture was homogenized and left for 20–30 min at 4–6 °C. The meatballs were round in shape with diameter of 60–70 mm and weight of 63 g. They were rolled in flour before frying. After frying, a meatball weighed 50 g (*Unified collection of recipes*, 1981).

The schnitzels were prepared from beef and pork mince in a ratio of 4:1 with the addition of salt and black pepper. They were oval in shape with diameter of 120–140 mm, thickness of 20–30 mm, and weight of 106.5 g. They were rolled consecutively in flour, milk and egg mixture, bread crumbs, and then again in the milk and egg mixture. Their weight after frying was 100 g (*Unified collection of recipes*, 1981).

Both the meatballs and the schnitzels were fried in a frying pot (EF-24M-1) with automatic temperature regulation (±5 °C) in 1200 mL of oil. The meatballs were fried at 200 °C and the schnitzels at 160 °C. The product-to-frying-fat ratio was 1:4. The frying fat was used for 5 days. Each day 200 meatballs and 50 schnitzels were fried for 1 h, which was the duration of one "frying cycle". Therefore, the technological process comprised five cycles for 5 days, the total duration of using the fat before 5 h. Fresh oil was added to maintain a constant frying fat volume. The frying fat was filtered and stored at 0–4 °C (*Technological instruction*, 1988) after each cycle.

Melanoidins were isolated from two meatballs (with a total weight of 100 g) and one schnitzel (100 g) and were extracted from the above products after the fat was removed, purified using column chromatography and dialysis, dried, and stored in a dark place (Kuntscheva et al., 1983; Obretenov et al., 1982).

The gel chromatographic separation was done on the Sephadex G-50 device with a column of 25/800 mm, distilled water as the eluent, elution velocity of 100 mL/h, V_0 = 80 mL against blue dextran. Fractions of 10 mL were added together. Consecutive fractions with equal UV characteristics were united and marked A, B, C, ...

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Table I. Data for the Melanoidins in Fried Meatballs

frying cycle, day	amount of isolated melanoidins, g	5-point assessment
first	0.360	good, 4.00
third	0.873	very good, 5.00
fifth	1.373	very good, 4.60

The organoleptic assessment of the culinary products was made by expert tasters according to a 5-point scale (*Methodological instructions*, 1986). Five points were given for excellent quality, four for good, three for satisfactory, two for unsatisfactory, and one for poor quality. The results were statistically processed.

The IR spectra of the melanoidins were done on a UR-20 apparatus, Karl Zeiss, Jena, and on an infrared Fourier transform spectrometer 1750, Perkin-Elmer, in tablets of potassium bromide, at similar weight concentrations.

The UV and visible spectra were done on a UV–vis spectrometer, Perkin–Elmer Lambda 15, in distilled water.

RESULTS AND DISCUSSION

The investigation was carried out under production conditions where it was natural to expect that the processes of melanoidin formation would take place in a more complicated way compared to model investigations (Obretenov et al., 1983; Somov et al., 1983). Large amounts of samples were used in industrial equipment. The investigated products—meatballs and schnitzel—had different components with their respective specific behaviors at high temperatures and impacts on melanoidin formation. The products were fried in preheated deep fat, and it could be expected that melanoidins would be formed both in the samples and in the fat.

Melanoidin Formation in Fried Meatballs. The amount of melanoidins formed in the fried meatballs depended greatly on the duration of using the frying fat (Table I). The melanoidins from the fifth frying cycle (1.373 g) were 3.9 times greater compared to the melanoidins from the first cycle (0.360 g) and 1.6 times greater compared to the third cycle (0.873 g). The frying time being the same (1 h), obviously the influence of the duration of using the fat on the processes of melanoidin formation was essential. The well-known oxidation destruction of the higher fatty acids took place while the products were fried in vegetable fat, as a result of which organic compounds different in structure were accumulated. Among them were the carbonyl compounds with different chain lengths which are important for nonenzymatic browning (Fors et al., 1983; Nawar, 1985). During the first cycle melanoidin formation was probably caused above all by the presence of carbonyl and amine compounds in the raw meatballs, and therefore the amount of melanoidins was smallest. With the advance of the destruction processes in the fat, the amount of melanoidin precursors increased. After diffusing in the sample, they interacted with the nitrogen bases and the amount of melanoidins was increased.

The organoleptic assessment of the fried meatballs was highest (very good, 5.00) for the third frying cycle. It was lower (very good, 4.60) in the fifth frying cycle despite the increase in the amount of melanoidins. The fat, destroyed to a high degree, favored the synthesis of melanoidins but deteriorated the product quality.

The gel chromatographic separation indicated that the melanoidins from the fist frying cycle did not contain a measurable amount of fractions eluting close to V_0. The highest molecular fractions were eluted from 100 to 150 mL, and their content increased with increasing duration of frying in the fat (Figure 1). The major amount was eluted from 150 to 250 mL with a maximum of around 200

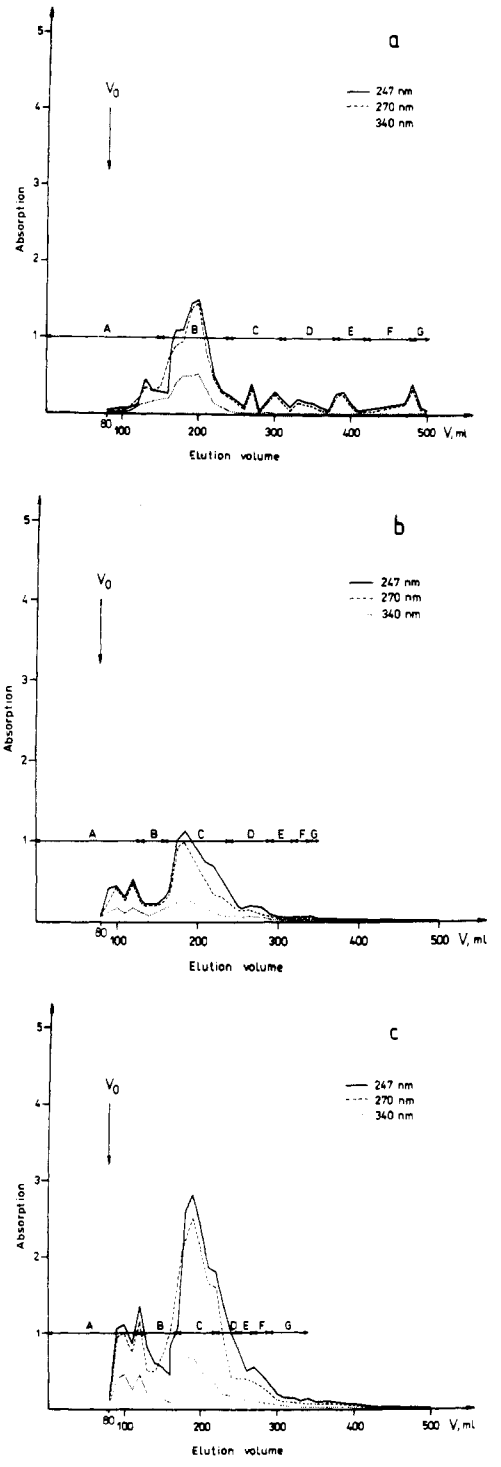

Figure 1. Gel chromatograms of melanoidins from fried meatballs, according to Table I: (a) first cycle; (b) third cycle; (c) fifth cycle.

mL for the first cycle, 190 mL for the third cycle, and 180 mL for the fifth cycle. The presence of low molecular fractions was best expressed in the melanoidins from the first cycle. They were eluted up to 500 mL and were absent from the other two samples.

The UV spectra of the nonfractionated melanoidins showed an intensive plateau-like absorption within the 250–280-nm range. The separate melanoidin fractions did not possess characteristic absorption maxima but the plateau-like absorption specific for high molecular compounds. The melanoidins obtained in the last period of frying contained fractions with a more intense absorption at around 300 nm, which gradually deteriorated. The

Table II. Data for the Melanoidins in Fried Schnitzel

frying cycle, day	amount of isolated melanoidins, g	5-point assessment
first	0.227	good, 4.00
second	0.285	good, 4.12
third	0.559	very good, 4.50
fourth	0.579	good, 4.00
fifth	1.213	good, 3.85

melanoidins of the fifth cycle had strong absorption with a maximum at 270 nm.

The IR spectra of the nonfractionated melanoidins were very close in character with those of the isolated melanoidins from other food products (meat, bread, coffee, etc.) (Obretenov et al., 1984, 1991) with intensive absorption within the ranges 1030–1120, 1350–1450, 1600–1650, and 3300–3500 cm^{-1} without a maximum for carbonyl group.

Melanoidin Formation in Fried Schnitzel. These processes were measurable from the first frying cycle (0.227 g) when the frying fat was slightly changed (Table II). With increasing time of using the fat, the amount of melanoidins increased (1.213 g) and was 5.3 times greater compared to the first cycle. Having in mind that the same types of raw schnitzels were used, here, as was the case with the meatballs, the important role of the destruction products from the frying fat on the synthesis of melanoidins became clearly apparent. Their lower amount in comparison with the meatballs was probably due to the lower temperature of frying (200 °C for the meatballs and 160 °C for the schnitzels) and the difference in their composition.

Here, as with the fried meatballs, the tasters' assessment was rather low for the first frying cycle (good, 4.00), highest for the third cycle (very good, 4.50), and dropped (good, 3.85) in the fifth cycle despite the highest content of melanoidins in the fried schnitzel.

The gel chromatograms of the isolated melanoidins (Figure 2) indicated essential changes in the fraction composition depending on the frying cycle. The melanoidin fractions from the first frying cycle began to elute immediately after V_0; their amount gradually increased and reached its maximum at around 240 mL. The elution of the column ended at about 380 mL. The major amount of melanoidins from the third frying cycle was eluted within a comparatively narrow interval: 150–240 mL with a clearly expressed maximum. This elution maximum for the melanoidins from the fifth frying cycle was around 170 mL. After about 330 mL, the elution was complete. Therefore, with increasing time of using the frying fat, conditions were created for obtaining higher molecular melanoidins and for narrowing the range of their molecular masses.

The total melanoidins and their fractions from schnitzels did not differ essentially in their absorption in the UV range from those of meatballs.

The IR spectra of the total melanoidins from schnitzels had absorption bands at 600–900, 1020–1080, 1262, 1350–1415, 1600–1660, 2300–2360, and 2900–3500 cm^{-1} due to >CH, >COC<, >C=C<, >C=N, >NH, $>N^+H$, COO^- and other functional groups. There was no clearly expressed absorption maximum for a carbonyl group.

This investigation showed convincingly that the processes of melanoidin formation in fried culinary products related intensively and directly to the organoleptic properties of the end product. It also revealed the major role of frying fat in these complex transformations. From the publications in the literature it is well-known that lipids are active participants in the reaction of nonenzymatic

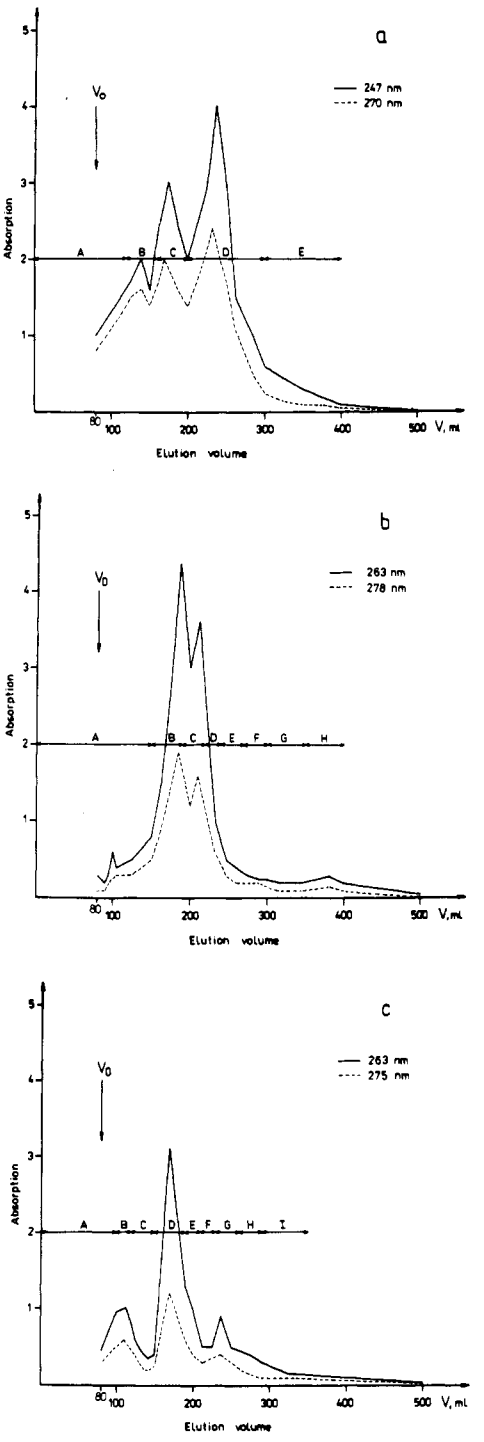

Figure 2. Gel chromatograms of melanoidins from fried schnitzel, according to Table II: (a) first cycle; (b) third cycle; (c) fifth cycle.

browning. The problem here is more concrete and sufficiently interesting. Here are some of its aspects. The increase in the amount of melanoidins in the fried product with increasing duration of using the frying fat undoubtedly presupposes a diffusion of melanoidin precursors (mainly carbonyls) from the fat into the product, where they react with the nitrogen bases to form brown polymers. A diffusion of nitrogen bases from the product into the frying fat was possible. At the end of the period for which the fat was used, it was strongly colored. Judging from our previous experiments, the melanoidins accumulating in it were slightly polar and strongly lipophilic. They could be the product of aldol condensation of the destruction products of lipids, or (if they contain nitrogen) the α-amino

acids in the frying fat have undergone to a high degree a Strecker degradation whose products result in nonpolar polymers. We should not exclude the diffusion of the already synthesized melanoidins: the polar ones to be adsorbed on the product and the nonpolar ones to leave it because of their better solubility in the frying fat. Along with all of this, it is also possible to have heat destruction of melanoidins to low molecular flavor components. These will be some of the trends for our forthcoming investigations into the processes of melanoidin formation in food products.

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