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ANTIOXIDANT ACTIVITY OF AQUEOUS SOLUTIONS OF CORN EXTRUDATES WITH THE ADDITION OF OAT FLAKES

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Abstract: The paper presents the results of research on the antioxidant activity of corn extrudates with the addition of oat flakes. The addition of oat flakes to corn increases their nutritional value. However, the process of extrusion decreases the antioxidant content of these products. These losses are compensated by non-enzymatic browning compounds formed during the extrusion process. The objective of this study was therefore to analyze the antioxidant properties of extrudates as a function of the content of Maillard reaction products. The reference point for determining the degree of non-enzymatic browning of the extrudate was the effect on a model reaction of lysine with glucose. The material for the test samples was made from the extruded, ground corn grain with the addition of 0, 50, 100 and 150 g kg^{-1} of crushed oat flakes. The antioxidant properties were investigated using the free radical DPPH$^{\bullet}$ (2.2-diphenyl-1-picryl-hydrazil). RESULTS: Corn extrudate produced without the addition of oat flakes was characterized by higher antioxidant activity, but the differences with other test samples were not statistically significant. CONCLUSIONS: The results of this study show that the antioxidant capacity could be the result of the formation of colored Maillard reaction products and/or pre-melanoidins, determined by absorption at wavelengths of 320 and 420 nm.

Key words: antioxidant activity, extrudate, Maillard reaction, oat flakes

Introduction

Extrusion is a very efficient method of processing food raw materials. The application of a high temperature for a very short time completely changes the original raw materials, giving them entirely new features. The addition of oat flakes to corn extrudates enhances the nutritional value of these products without any substantial fall in their antioxidant capacity. Oats and oat products are good sources of many phytochemicals, including tocopherols, tocotrienols, phenolic acids, flavonoids, phytic acid, avenanthramides, and plant sterols [1]. Oat bran has been demonstrated to decrease the level of serum cholesterol, thereby reducing the risk of developing coronary heart disease, diabetes, blood pressure and obesity [2].

The process of extrusion brings about many favorable and unfavorable physicochemical changes in the final product. The direction of the change is dependent on the type of raw materials and the process conditions. The application of high temperature during food processing causes a decrease in the content of antioxidants [3–5]. Simultaneously, the interaction of reducing sugars with amino acids and proteins leads to the formation of Maillard compounds, which are not present naturally in foods. Evidence of their presence is the browning of the product. Browning, related to the occurrence of the Maillard reaction, makes food more attractive from the sensory point of view [6–8] and the final product has new functional features. The Maillard reaction products include compounds that give extrudates characteristic aromas [9] and antioxidant properties and have the ability to delay the process of oxidation of fats as well as inhibit the action of oxido-reductases, chelate metal ions, and prevent other oxidation processes by free radical scavenging [10].

Consequently, the Maillard reaction products generated during extrusion compensate for the loss of natural compounds, particularly antioxidants present in the raw materials. Therefore, the aim of the present study was to analyze the antioxidant properties of extrudates produced from just corn or from corn with the addition of oat flakes. An attempt has been made to determine whether there are relations between the antioxidant properties of corn extrudate with different levels of added oats and the degree of browning. The existence of such relations would indicate ways for better control of technological processes. It would then be possible to choose the extrusion temperature and time in order to obtain final products with the desired sensory characteristics as well as a high degree of antioxidant properties.

In the experiment the antioxidant activities of aqueous solutions of extrudates were investigated, because natural antioxidants are usually isolated with methanol and/or acetone, hexane and ethyl acetate, instead of with water [11].

Therefore, the analysis of water and non-alcoholic solutions increase the probability that their antioxidant proprieties are the result of the presence of the compounds formed during non-enzymatic browning reactions.

The reference point for determining the degree of non-enzymatic browning effect of the extrudate requires a model reaction of lysine with glucose at their natural pH (pH 9).

Materials and methods

Chemicals: L-lysine (assay ⩾98%, Sigma-Aldrich Chemie GmbH, Germany), α-D-glucose (assay ⩾99.5%, Sigma-Aldrich Chemie GmbH, Germany), ascorbic acid (purity 97.4%, Supelco, USA), 2,2-diphenyl-1-picryl-hydrazyl – DPPH$^{\bullet}$ (Sigma-Aldrich Chemie GmbH, Germany).

Materials: A model Maillard reaction was set by using the interaction of L-lysine and α-D-glucose. A standard curve was determined with the use of ascorbic acid and DPPH$^{\bullet}$, by measurement of the neutralization of free radical DPPH$^{\bullet}$ [%] as a function of the weight of ascorbic acid [μg].

Oat and corn mixtures for extrusion were prepared from whole corn grain, which was subsequently ground, and from crushed oat flakes purchased at a nearby supermarket. The corn meal obtained in this way was used for the preparation of analytical samples, to which 0, 50, 100 and 150g kg^{-1} of crushed oat flakes were added. The mixtures were thoroughly mixed by adding water to them to obtain a final moisture content of 160g kg^{-1}. After 24 h conditioning, the material was subjected to extrusion at a temperature of 120°C and 6 MPa near the extruder outlet.

Methods: Reaction of lysine with glucose – separate solutions of lysine and glucose at concentrations of 0.1 M were prepared in deionized water. 2 ml of each of the solutions was transferred to four 4 ml vials. The vials were closed and inserted into an oven. The reaction was allowed to take place for 24 hours at 80°C. The heating time and temperature were determined experimentally in advance. After the reaction, the mass of the output reaction products was measured using a drying chamber (FP Binder 115). The samples were weighed with a Sartorius OCE-ME5 Micro Balance with 0.001 mg readability. Simultaneously, a reference curve for absorbance of the reaction products was performed at wavelengths of 320 and 420 nm. The relationship between the weight of the products resulting from heating lysine with glucose and absorbance measured at 320 and 420 nm was determined. Lysine and glucose were chosen as the standard for measurement of the non-enzymatic browning reactions occurring in extrudates, because glucose is a component of corn and oats, while lysine undergoes Maillard reactions more rapidly than other amino acids.

Neutralizing DPPH$^{\bullet}$ free radical by ascorbic acid. A 0.1 mM DPPH$^{\bullet}$ solution in methanol (HPLC grade) and a 0.05 mM ascorbic acid solution in deionized water were prepared. The ascorbic acid solution was further diluted to a range of concentrations, which neutralized the DPPH$^{\bullet}$ solution. The reaction was allowed to take place for 30 minutes at room temperature in a dark place. Absorbance was measured at 517 nm (LaboMed, inc. Spectro UV-VIS RS). The amount of DPPH$^{\bullet}$ [%] neutralized by the ascorbic acid [mg] was calculated from the formula (1) described by Zhang & Hamauzu [12].

Preparation of extrudate samples – extrudate samples intended for testing were ground up to prepare analytical samples of about 0.1250 g each. 7 cm^3 of deionized water was added to the analytical samples. Each sample with solvent was shaken for an hour, and then kept in a dark and cool place (4°C) for 24 hours, in order to extract all the compounds soluble in water. After that time, the sample was again shaken, centrifuged at 5,000 rpm (Centrifuge type MPW-310 Precision Mechanics, Poland) for 10 minutes, and then filtered.

The measurement of the mass of compounds dissolved in the extrudate aqueous solutions. 2 ml of each extrudate extract was transferred to 4 ml vials and dried in an oven (Binder FP 115) at 105°C to a constant weight. The samples were weighed with a Sartorius ME5-OCE microbalance. The measurements were made three times. The results obtained were used to calculate the mass of water soluble compounds produced from 1 g of the extrudate sample.

The determination of extrudate antioxidant activity – the determination of antioxidant properties was performed using the free radical DPPH$^{\bullet}$ according to the procedures given by Zhang & Hamauzu [12] Cämmerer & Kroh [13], and Molyneux [14] but with modifications.

From the filtrate obtained, 0.7, 1, 1.5 and 2 ml of solution was collected in succession and diluted with distilled water to a volume of 3 ml. The absorbance of the samples was measured spectrophotometrically at a wavelength of 517 nm (control samples). 1 ml of 0.1 mM DPPH$^{\bullet}$ in methanol solution was added to the solutions obtained using different concentrations of filtrate. The reaction was allowed to take place at room temperature in a dark place for 30 minutes. After that time, the absorbance of the solutions was again measured with respect to the solvent (water). A blank test was made using 3 ml of water and 1ml of distilled DPPH$^{\bullet}$ solution. The results were calculated using the formula (1) [12]:

$$DPPH^{\bullet} decrease[\%] = \\ = 1 - \frac{sample\,absorbance - control\,absorbance}{blank\,absorbance} \times 100 \quad (1)$$

At first, the relationships between DPPH$^\bullet$ neutralization [%] and the mass of the compounds dissolved in water were determined for the different extrudates [mg]. Then the antioxidant activity of the extracts from 1 g of extrudates was calculated in terms of ascorbic acid equivalents [μg] using the standard curve equation (Fig. 1).

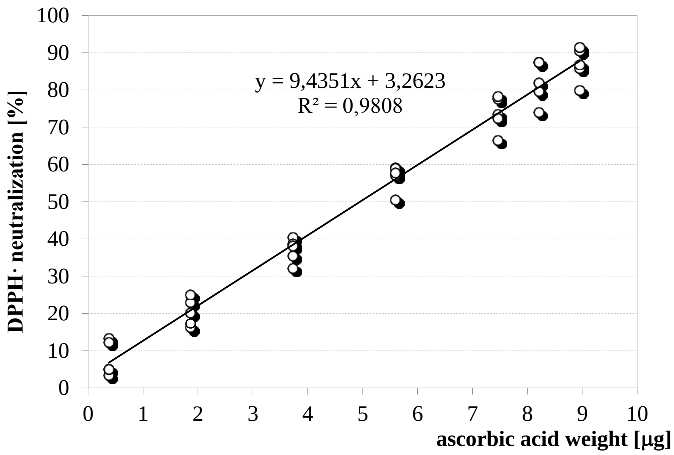

Fig. 1: The standard curve for the reaction of DPPH$^\bullet$ with ascorbic acid

The determination of the degree of browning – extrudate browning was carried out in accordance with the methodology described by Fogliano et al. [15]. Samples were prepared in the same manner as described above. For the analysis, 3 cm^3 of water solutions of the tested extrudates were used with 0.7, 1, 1.5 and 2 cm^3 of filtered extrudate and absorbance measured at wavelengths of 320 and 420 nm.

Statistical Analysis.

Statistical analysis was carried out using the statistical software STATISTICA 6.0 (StatSoft). For the calculations, ANOVA variance analysis and DOE experiment planning were used.

Results and Discussion

The reaction of free radical DPPH$^\bullet$ with ascorbic acid. The characteristics of DPPH$^\bullet$ neutralization by ascorbic acid are shown in Fig. 1. The curve model was used to calculate the antioxidant activity of aqueous solutions of extrudates obtained from 1 g of product as ascorbic acid equivalent [μg]. A model for the proportion of non-enzymatic browning compounds formed with the glucose – lysine reaction.

Fig. 2 presents the relationship between the mass of compounds formed by the reaction of glucose with lysine and the absorbance measured at 320 and 420 nm. After 24 h heating of an aqueous solution of glucose and lysine, a mixture of intermediate (non-brownish; measured at 320 nm) as well as brownish Maillard reaction compounds (absorbance measured at 420 nm) were obtained.

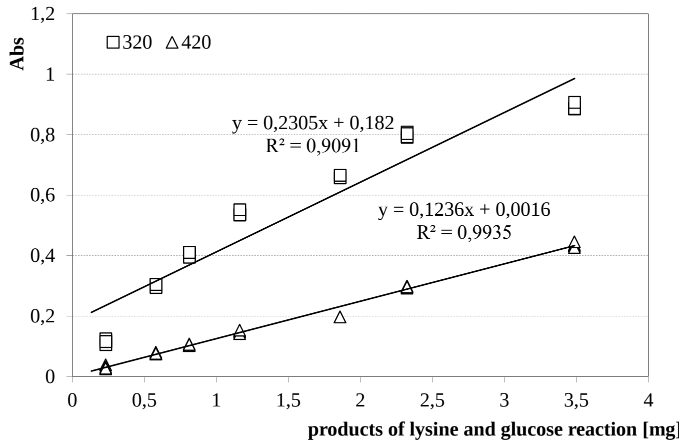

Fig. 2: The relationship between mass [mg] of the products of non-enzymatic browning reaction as determined by the interaction of lysine with glucose and determined by the absorbance [Abs]

The equations of the calibration curves (Fig. 2) were used to calculate the quantities of non-brownish (intermediate) and brownish (final) products derived from the Maillard reaction, present in the water solutions prepared from 1 g of the extrudates. Finally, their amounts were expressed as equivalent [mg] to compounds formed from heating glucose with lysine.

Antioxidant activity of extrudates.

Non-statistically significant differences were observed for the antioxidant properties of extrudates containing various amounts of oat flakes ($p>0.05$). The applied post-hoc test showed a significant difference between the antioxidant activity of extrudate manufactured from corn only and extrudate containing 50 g kg^{-1} oat flakes.

An aqueous solution obtained from 1 g of corn mass without the addition of oat flakes had the ability to reduce the amount of free radical DPPH$^\bullet$ equivalent to approximately 84$\pm$12μg ascorbic acid (Fig. 3).

The addition of 50 g kg^{-1} of oat flakes to the corn flour increased the antioxidant activity of solutions obtained from 1 g of the product to a value equivalent to 170.2$\pm$34 μg ascorbic acid. However, with more than 50 g kg^{-1} of oat flakes (100 and 15g kg^{-1}) such differences no longer appeared (116$\pm$66 and 111.4$\pm$52 μg ascorbic acid, respectively). In spite of the lack of statistically significant differences, the results obtained demonstrate that oat flakes are a source of additional compounds that enhance the antioxidant activity of extrudates obtained from corn flour. The antioxidant properties shown in this study could be the result of the presence of many substances which occur naturally in maize and oat flakes and could be produced during the extrusion process [6]. The antioxidant compo-

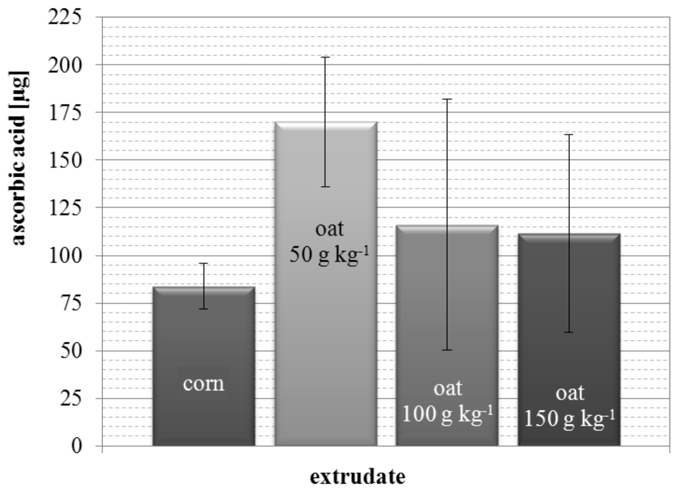

Fig. 3: Antioxidant activity of aqueous solutions produced from 1 g extrudate

unds naturally occurring in oat bran are β-glucans (water soluble) and phenolics. Oat flakes themselves contain significant quantities of tocopherols, tocotrienols, phytic acid, flavonoids and non-flavonoid phenolic compounds, which also exhibit antioxidant properties [2]. Extrusion conditions (mainly temperature) reduce the amount of natural antioxidants 3,5; however, non-enzymatic browning reactions occur simultaneously and new compounds are formed which have antioxidant capacity also [6].

Extrudate browning. There were no statistically significant differences between the concentrations of brownish Maillard reaction products in the samples ($p>0{,}05$), but the post-hoc test showed that the extrudates with 150 g kg^{-1} oat flakes contain statistically significant less brown pigments then the others (Fig. 4).

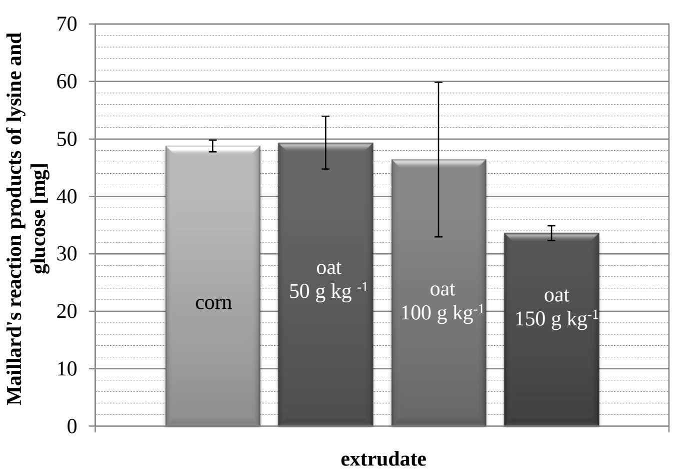

Fig. 4: Absorbance at 420 nm of aqueous solution of 1 g extrudate vs. Maillard reaction products of lysine and glucose

Also, the ANOVA results did not show any statistically significant differences between the concentrations of Maillard reaction compounds from tested extrudates as determined from the 320 nm absorbance ($p>0.05$) (Fig. 5).

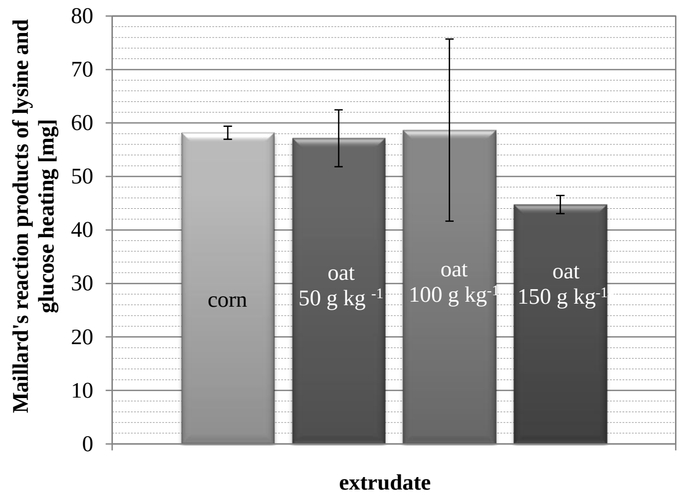

Fig. 5: Absorbance at 320 nm of aqueous solution of 1 g extrudate vs. Maillard reaction products of lysine and glucose

Statistical analysis showed that the amount of compounds identified by their absorption at 320 nm were significantly different from those absorbing at 420 nm. Since the extrusion process was short, the quantity of the final products of the Maillard reaction was less than that of reactive intermediate substances.

In spite of the lack of statistically significant changes in concentrations of intermediates and final non-enzymatic reaction products in the extrudates, it was shown that the increase in the amount oat flakes in relation to the content of corn resulted in the reduction in the formation of brownish Maillard reaction compounds (Fig. 4). The same tendency was observed in the case of the investigation of antioxidant capacity, described above. Oat enriches corn with protein, whose content varies from 139 to 413 g kg^{-1}, depending on the genotype [16], and also with fiber, including β-glucan [17]. The protein present in oats increases the capacity for the browning reaction, but dietary fiber in the form of water soluble β-glucan absorbs water and dissolved substances, reducing their reactivity and extraction to the solution. Therefore, a small amount of added oat flakes resulted in an increase in the amounts of antioxidant compounds soluble in water, while the addition of oat flakes of over 50g kg^{-1} (along with fiber) reduced their presence in the solution. This might have been due to either a lower degree of browning, stronger binding of browning compounds by fiber, and/or stronger binding of natural antioxidants by fiber.

Relation between non-enzymatic browning of extrudates and their antioxidant capacities. Statistical analysis of the results of the present study have enabled the identification of factors that influence the amount of DPPH$^{\bullet}$ reduced by water solutions of the tested extrudates. This was achieved through an analysis of the design of experiments (DOE) using the STATISTICA package. The analysis of variance (DOE method) showed that the antioxidant capacity of extrudate solutions was influenced by the proportion of oat flakes in the mixture (factor name „corn %", Fig. 6) and the concentration of intermediate and final Maillard reaction products (determined from absorbance; factors names respectively: „Abs 320" and „Abs 420"), as well as their interaction. The results of the statistical analysis is depicted as a Pareto chart (Fig. 6), which estimates the ANOVA effects. The Pareto chart shows the estimated effects sorted by their absolute size. A vertical line is shown to indicate the minimum magnitude of statistically significant effects, given the current model and choice of error term, and using the criterion of statistical significance selected in the p=0.05. The Pareto chart is very useful for reviewing a large number of factors, and for presenting the results of an experiment to an audience that is not familiar with standard statistical terminology.

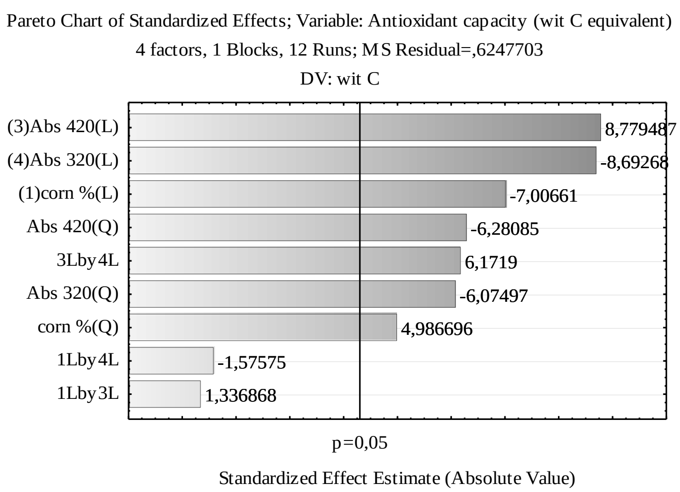

Fig. 6: The Pareto chart of the effects of the dependence between antioxidant capacity of the extrudates and the concentration of intermediate and final non-enzymatic reaction products; Abs 420 (L) – concentration of final reaction products in the first power (x^1), Abs 320 (L) – concentration of intermediates reaction products in the first power (x^1), corn % (L) – oat addition to corn in the first power (x^1), Abs 420 (Q) – concentration of final reaction products in the second power (x^2), Abs 3420 (Q) – concentration of intermediate reaction products in the second power (x^2), corn % (Q) – oat addition to corn in the second power (x^2).

The main factor was the concentration of final non-enzymatic reaction products (in the first power), and the second was the amount of non-brownish products of these reaction, in the first power also. Oat concentration had a lesser effect.

Authors of various publications have rarely discussed the antioxidant properties of non-enzymatic browning compounds that are formed as a result of an extrusion process. The result of the statistical analysis shows that the non-enzymatic browning compounds formed during the extrusion process may be a factors affecting the antioxidant properties of the final product. This has also been confirmed by data from other authors, for example [6]. Such an observation was reported by Camire et al. [18] who studied the antioxidant properties of corn extrudates produced in white corn flour with the addition of sucrose, citric acid and various fruit powders. Antioxidant activity, measured by the ABTS method, did not correlate with anthocyanin or phenolic content. On this basis, the authors found that antioxidant properties were likely to be the result of Maillard browning compounds produced during the extrusion process. Nicoli et al. [4] reported that the formation of Maillard compounds minimizes the loss of natural antioxidants in food.

Antioxidant properties can be affected by both intermediate and final products of non-enzymatic browning reactions. Nicoli et al. [19] studied the oxygen scavenging ability of compounds present in infusions of coffee beans roasted for different periods of time. In that study it was confirmed that the presence of Maillard compounds created in the initial phase of the reaction (intermediate products) rather than the final reaction products (brown pigments) were responsible the for antioxidant capacity. Final Maillard reaction products have high molecular weights. Among these, are melanoidins, which have the capacity to scavenge oxygen radicals and chelate metals [20]. Since color formation is a special feature of melanoidins, some authors have tried to find a correlation between the degree of browning of products measured spectrophotometrically and a constant providing a measure of the course of the antioxidant reactions. The demonstration of such a relationship is not always possible, because residual chromophores attached to melanoidin molecules, are not the main factor responsible for the scavenging properties of melanoidins. It is believed that such a correlation is only possible in those products where the color is mostly based on the Maillard reaction, and where there are few natural thermolabile antioxidants [19].

Conclusion

Extrudates produced from whole corn grain with the addition of oat flakes may prove to be a valuable nutritional product. They are a source of many nutrients, especially proteins, unsaturated fat and dietary fiber. Research has shown that such products have additional antioxidant properties. The addition of oat flakes diminishes the capacity for scavenging the free radical DPPH$^\bullet$, but the differences in antioxidant properties are not statistically significant if the oat flakes were added in an amount not exceeding 150g kg^{-1}. Moreover, the results of this study show that the antioxidant capacity could be the result of the formation of colored Maillard reaction products and/or pre-melanoidins.

Therefore, it could be argued that these compounds are largely responsible for the antioxidant properties of extrudates, as confirmed earlier by other authors.

The optimization of the extrusion process for corn-oat mixtures and control of the degree of product browning may lead to an improvement of the antioxidant properties of the final products. Extrudates made with ground corn grain with the addition of oat flakes may prove to be a valuable nutritional product.

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