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ANALYSIS OF THE QUALITY PROPERTIES OF MAIZE EXTRUDATES WITH DIFFERENT ADDITION OF OAT FLAKES

Elżbieta Biller[1], Adam Ekielski[2]

[1]*Food Technology and Gastronomy Institute*
Lomza State University of Applied Sciences, Lomza, Poland
[2]*Faculty of Production Engineering*
Warsaw University of Life Sciences, Warsaw, Poland

E-mail: ebiller@pwsip.edu.pl

Abstract: The aim of this work was the evaluation of quality properties of corn extrudates with the addition of 0, 5, 10 and 15% (w/w) of oat flakes. In the products the following features were analyzed: antioxidant activity (AA), compounds derivatives formed by non-enzymatic browning (NEB) reactions (measure of absorbance at 320 and 420nm) and colour using L*a*b* colour system. AA and NEB were analyzed in methanol extracts of the extrudates. The results shown that, AA values of all extrudates were similar (ANOVA, p>0.05). This indicator was not a significant variable in the classification quality of the products (PCA – principal component analysis). The addition of 5 and 10% of oat to maize caused a significant increase in the absorbance value, and the samples were significantly darker (lower L*). The sample with the addition of 15% of oat to maize was characterized by absorbance values lower than two previous samples and was brighter. The reason for this dependence was probably the increasing share of soluble fiber in the samples, which restricted NEB reactions. On the basis of PCA it was shown that all parameters, except for the AA value, were significant for classification of quality properties of the maize extrudates.

Key words: antioxidant activity; extrudate; non-enzymatic browning; oat flakes

Introduction

An oat is a grain containing many nutrients valuable for the human system. Peterson [1] indicates that oat is a source of active phytochemicals, which have important functions in the body. As a result, oats may be added to many products and may be a source of functional ingredients, which can be extracted, purified and marketed as food supplements. Moreover, it is a source of native antioxidant compounds. It also contains many valuable proteins characterized by relatively good amino acid balance. Oats contain the β-glucan, which belongs to indigestible water soluble gums and are able to reduce the level of plasma cholesterol [2, 3]. Welch et al. [3] studied 35 genotypes of oats. The tested samples contained from about 19 to more than 40% of protein in the groats, from about 4 to more than 10% fat, containing oleic acid (C18:1) from 33% to more than 48%, linoleic acid (C18:2) from about 28 to over 44% and linolenic acid (C18:3) from 0.6 to 1.8%. According to Butt et al. [4] oat groats contain β-glucan (from 2.3 to 8.5 g/100g), 13% of protein, 7.5% of lipids, 10.3% of fiber and of 3.1% ash. Despite the advantages, the application of oats in food processing is limited, and new possibilities of its application should be analyzed. One of the methods is to use grain in the extrusion process, which may improve the nutritional value of raw products, such as maize. Extrusion is the HTST process, which results in favorable as well as less favorable changes in raw materials [5–7]. Sometimes the connection between the benefits and the negative effects is debatable. For example, the negative impact of high temperature on different chemical reactions is known. Extrusion results in forming Maillard compounds, which could be regarded as a marker of accuracy of the undertaken process and quality features of the final products [8–10]. In the case of mixtures of maize and oat, Maillard reaction could be a significant problem because both materials are rich source of protein and carbohydrates. In addition, nowadays there is a tendency to limit the hazard of the Maillard compounds such as acrylamide, furan, glyoxal and methylglyoxal in extruded and other food products [**?**, 12–14]. However, detailed analysis of these compounds is time- and cost-consuming. That is why many authors examine the intensity of the non-enzymatic browning (NEB) reaction, generally analyzing the absorbance of products from extracts [8, 15–17]. During the initial phase of Maillard reaction Amadori compounds are being formed. These products of reaction are unstable and undergo a series of changes, resulting in formation of: UV-absorbing compounds, carbonyl-containing compounds and

coloured pigments [18]. The ability to absorb electromagnetic radiation of different compounds enables, in a simple way, to define extracts derived from food products as an appropriate wavelengths [9, 15]. For the purpose of extraction different solvents are applied [15, 19, 20], but most researchers analyze NEB compounds soluble in water.
Currently alcohol soluble NEB compounds are not used as an extrudate quality indicator, although it is proved that the NEB derivative are not only water-soluble [15, 19–21]. Another feature of derivatives is their antioxidant activity potential (AA) [21–23].

The antioxidant activity potential of derivatives can compensate for the loss of native antioxidants, which may be lost during application of high temperature such as in an extrusion process. The aim of the work was the evaluation of quality properties of maize extrudates with addition of oat flakes. The addition of oat flakes is used to enrich nutritional value of maize products. In the final products the following features were analyzed: antioxidant activity (AA), compounds derivatives formed by non-enzymatic browning (NEB) reactions as well as colour using L*a*b* colour space. The relationship between antioxidant activity of extrudates with the addition of oat flakes, and the degree of non-enzymatic browning measured spectrophotometrically, has been investigated. The analysis of the AA and the NEB was performed for methanol solutions because of the insufficient knowledge on the substances soluble in organic solvents, which are the object of this work.

Materials and Methods

Material for extrusion was made of whole grain maize, which had been ground and a crushed oat flakes. The maize was bought directly from the producer and oat flakes were bought at a local market. Flour mixtures were prepared with: 0%, 5%, 10% and 15% (w/w) of oat flakes added. It was mixed, and water was added to obtain 16% of final moisture content. After 24 hours of conditioning, the material was extruded in a single screw extrusion model Instra-Pro 800 (Instra-Pro International, USA), at a temperature of 120^{o}C, under the pressure of 6MPa. The residence time of material in the device was 25s.

The Preparation of Samples of Extrudate

The extrudates were ground. The powder was used to produce samples weighting about 0.1250g. To each of them 7cm^3 methanol (HPLC grade, Poch SA) was added. Each sample with the solvent was carefully closed, shaken for an hour, and then placed in the dark and cool place (4^{o}C) for 24 hours in order to extract the soluble compounds. After this time, the samples were shaken again, centrifuged at the number of rotations of 5,000 rpm. (Centrifuge type MPW-310 Precision Mechanics, Poland), for 10 minutes and then solute again. A clear filtrate was used for further analysis.

Determination of Dry Matter of Compounds Dissolved in Methanol

In the filtrates the weight of the dissolved compounds was measured. The filtrates of known volume were dried (drier Binder FP, type 115, Germany) to a constant weight.

The Indication of Antioxidant Activities (AA)

The indication of antioxidant properties was performed using the DPPH$^\cdot$ free radical (2,2-diphenyl-1-picryl-hydrazil, Sigma-Aldrich Chemie GmbH, Germany) test, according to the method of Cämmerer and Kroh [22], Zhang and Hamauzu [24] and Molyneux [25] with further modifications. From the obtained filtrate, 0.7, 1, 1.5 and 2cm^3 of solution was collected successively, and each sample was complemented with methanol to a volume of 4 (first series of samples) and 3cm^3 (second series of samples). The first series of samples had been prepared previously, were measured spectrophotometrically at a wavelength of 517nm (control samples). All series were prepared three times.

To the solutions of second series, obtained with different dilution, 1cm^3 of methanolic 0.1mM solution of DPPH$^\cdot$ was added. The reaction was being carried out at the room temperature, in a dark place, for 30 minutes. After that time, the solutions were marked spectrophotometrically (LABOMED, Inc. Spectro UV-VIS SM) and the absorbance at wavelength equal to 517nm, compared to the solvent (methanol) was measured. A blind test was made with 3cm^3 of methanol and 1cm^3 solution DPPH$^\bullet$. The results were calculated using the formula of Zhang and Hamauzu [24], with modification (1):

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

The standard curves were elaborated on the basis of associations between neutralized DPPH$^\bullet$ [%] and the weight of all the compounds dissolved in a sample [mg]. On the basis of equations, compounds dissolved in the extracts were calculated.

Determination of the Calibration Curve DPPH$^\bullet$ Versus Ascorbic Acid

At the same time, calibration curve of reduction 0.1mM DPPH$^\bullet$ methanols solution by 0.05mM ascorbic acid (standard of the vitamin C, Supelco, USA) was elaborated, by examining the absorbance at 517nm for different concentrations of reactants. Finally, AA of the samples was expressed as an adequate amount of ascorbic acid [μm] corresponding to 10mg of all compounds dissolved in the analysed samples.

The Designation of the Degree of Browning

Extrudate browning was carried out in accordance to the methodology used by Fogliano et al [15]. Samples were prepared in the same manner as described above (the first series of the samples), triplicate. A clear set of solutions of settled concentrations was analysed spectrophotometrically at a wavelength of 320 and 420nm. Standard curves were determined depending on total number of compounds dissolved [mg] the absorbance measured at two wavelengths (Abs_λ_{320} and Abs_λ_{420}). On the basis of equations of curves were calculated the values of the absorbance that characterize 10mg of all compounds dissolved in extrudates.

Analysis of Colour of Extrudate

The colour of extrudate samples was measured using instrumental colour system L*a*b*. Colorimeter CR-310 (Minolta, Japan) was used and the measurement was conducted against the standard of white and light type D_{65}. Extrudate samples were gound before measurement and the powder was passed through a sieve before designation. Fragmented material was placed in granular attachment type CR-A50, and then the measurements was done. The action was repeated 10 times. The mean and standard deviation was calculated from 10 measurements. Similarly, there was marked the colour of all samples.

Statistical Analysis

Statistical analysis was conducted using program STATISTICA 10.0 (StatSoft). For the calculations: means and standard deviations, T-test, correlation coefficients, analysis of variance ANOVA and principal component analysis (PCA) were used.

Results: Antioxidant Activity of Methanol Extrudate Extracts

Adding oat flakes to maize generally increased the AA of methanol solutions (Fig. 1), but based on an ANOVA analysis, no statistically significant differences were found between the AA of the samples (p=0.86). No significant differences could occur for two reasons: both types of materials have been a source of natural antioxidants [1, 26–28] or/and mass contribution of oat flakes in relation to the weight of the maize was not high enough(maximum 15%).

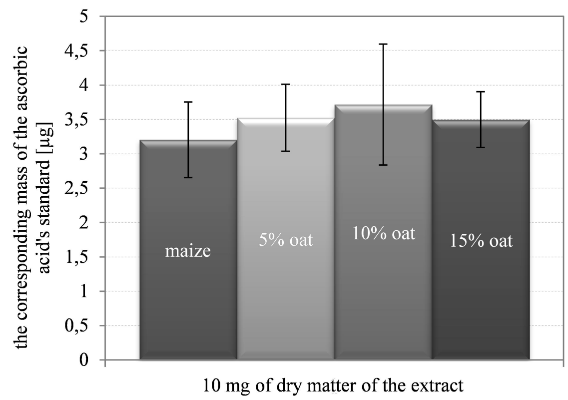

Fig. 1: The antioxidant properties of maize extrudates with various addition of oat flakes; these properties were expressed as the adequate amount of ascorbic acid standard [μg] (y axis), characterized by 10mg dry matter of methanol extracts; in figure mean values and standard deviations is given

Contribution of Derivatives of NEB Reaction in Methanol Extrudate Extracts

Basing on the t-test it can be stated that there were significant differences between the values of absorbance measured at wavelengths of 320nm (Abs_λ_{320}) and 420nm (Abs_λ_{420}) ($p \approx 0.0$). The intensity of absorption at 320nm was significantly higher than at 420nm (Fig. 2 and Fig. 3).

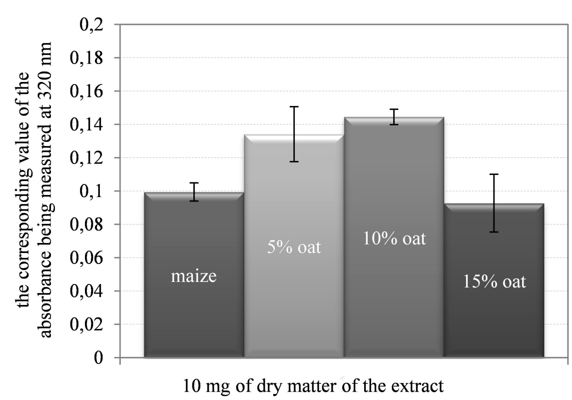

Fig. 2: Absorbance value measured at a wavelength of 320nm (mean values and standard deviations), characterized by equal volume methanol extracts, calculated on dry matter of 10 mg of the extract

The absorbance value at a wavelength of 320nm corresponds to intermediate products of the NEB reactions [15]. It is stated in the literature that these are the reactive derivatives of Amadori compounds' decay which are heterocyclic substances of low molecular weight [18]. Brown pigments (final products of NEB reactions), soluble in methanol, which absorb electromagnetic radiation at wavelength of 420nm [16, 17] were insignificant (maximum absorbance was 0.038).

The trend of changes of intermediate and final products of Maillard reactions in the analysed extrudates was similar,

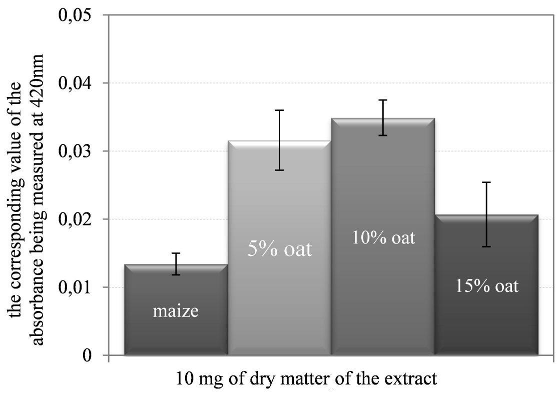

Fig. 3: Absorbance value measured at a wavelength of 420nm (mean values and standard deviations), characterized by equal volume methanol extracts, calculated on dry matter of 10mg of the extract

correlation coefficient R^2=0.92. In the case of samples containing 5 and 10% of oat flakes, increasing the participation of oat in relation to maize, resulted in a significant increase of trade-absorbance both at 320 and at 420nm ($p<0.05$ in both cases). In contrast, 15% share of oats changed the properties of the product – absorbencies were less intensive.

Post-hoc test shown that the values of Abs_λ_{320} of sample 0% of oat and 15% of oat were similar (p=0.30). All values of Abs_λ_{420} were significantly different from each other, but in case of addition of 15% of oat results were significantly lower than for samples 5 and 10% with oat additions ($p<0.05$ in all cases).

Analysis of Extrudate Colour

The colour of extrudates was analysed in the L*a*b* system. The average results of measurements and value of the standard deviation are presented in the Table 1.

Table 1: Values of the indicators of extrudates colour

Oat content	L*[%]	a*[-]	b*[-]
0%	$80.79^a \pm 0.32^b$	-0.07±0.04	23.96±0.21
5%	79.53±0.45	0.41±0.04	23.15±0.33
10%	80.17±0.44	-0.26±0.06	23.62±0.58
15%	80.90±0.39	-0.37±0.07	23.38±0.21

[a] – mean value
[b] – standard deviation

The statistical analysis of the obtained results revealed that the lightness L* depended in most cases on the oat flakes addition to maize (p=0). No difference in lightness between 0% and 15% samples was observed. This was compatible with results obtained from absorbance measurements – sample derived from extrudate with 15% of oat absorb radiation to a lesser extent than respectively the samples with 5 and 10% of oats (results of the ANOVA and post-hoc test NIR). Similar results were obtained during the a* analysis. ANOVA demonstrated significant differences (p=0) of a* depending on the oat flakes addition. All samples were different from each other. The samples with 15% of oat have the smallest value of the a* colour parameter. On the basis of ANOVA it was calculated that oat flake addition to maize, changed significantly the b* parameter of colour ($p<0.05$). The NIR test proved, that there were no significant differences in b* values between: control extrudate of maize (0% oats) and samples containing 10% oats (p=0.62), samples containing 5% and 15% oats (p=0.19) and the extrudate with addition of 10 and 15% oats (p=0.17). However, there was no clear trend for changes of parameter b*.

The Relation Between Oat Flakes Content in the Samples, and the Antioxidant Properties of the Extrudates, as well as Extrudate Colour

All results were obtained as an appropriate value of principal component analysis (PCA). On the basis of PCA results, the variables and their mutual dependence may be classified into categories. The results of PCA analysis are given on Fig. 4, Fig. 5 and Fig. 4.

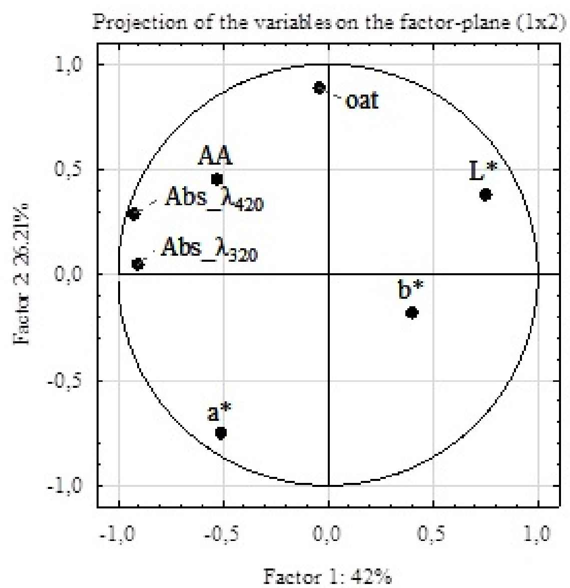

Fig. 4: The classification of variables determining the quality characteristics of extrudates (results of PCA analysis), 4). correlation of all variables with factor 1 and 2, 5). correlation of variables with factors 1 and 2 after the elimination of AA variable

Three variables were associated with factor 1: Abs_λ_{320}, Abs_λ_{420} and L*. The variables Abs_λ_{320} and Abs_λ_{420} were highly negatively correlated and L* highly positively correlated with factor 1. The variables listed above had the largest impact in the explanation of total variance. All three variances can be described as factor „colour change": Abs_λ_{320} and Abs_λ_{420} – reflecting the intensity of the NEB

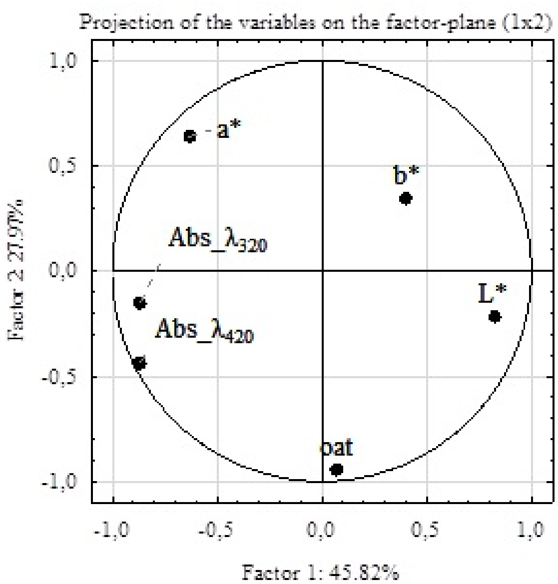

Fig. 5: Classification of variables determining the quality characteristics of extrudates (results of PCA analysis), 4). correlation of all variables with factor 1 and 2, 5). correlation of variables with factors 1 and 2 after the elimination of AA variable

reaction and value L* – the amount of white light reflected from the surface of the samples. A highly positive correlated factor 2 with amount of oats in the extrudate was observed as well as this factor negatively correlated with parameter a*. Factor 2 explained 26.21% of total variance.

Factor 3 was associated only with parameter b*, whereas factor 4 with the AA (data not shown). Factor 3 explained about 15% of variance and factor 4 – 9.34%. Considering the eigenvalues of individual factors, it was found that the first three were characterized by a value >1 and there explained 83.31% of the total variance.

In this analysis the AA factor can be rejected as a low important element (Fig. 5). The AA values were not predictive as variable to characterize the extrudates; however, this factor is important for nutrition. For the purposes of this analysis the AA variable has been removed and the classification of all extrudates display math individual (Fig. 6).

Samples 5 and 10% oats had negative coordinate values for the horizontal axis. An extrudate with 10% oat addition was characterized by the highest values of Abs_λ_{320} and Abs_λ_{420} and were placed in the same quadrant as variables Abs_λ_{320} and Abs_λ_{420} (Fig. 5 and Fig. 6).

Extrudate 5% had significantly highest values of the parameter a*, whereas samples with maize – highest mean b* value. The extrudate samples with a 15% addition of oats were the brightest (maximum L*) and had the highest share oat. The distribution of cases shows the location of relevant variables.

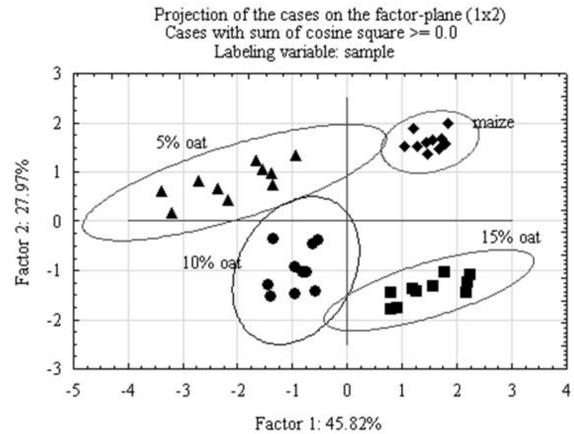

Fig. 6: Grouping cases of extrudates; the ellipses reflect the normal distribution and correlation coefficients R^2=0.95

Discussion

The addition of oat flakes to maize amounting 15% w/w, did not significantly affect the changes of value AA of the methanol extracts of different kinds of extrudates. Methanol is a solvent of many antioxidative substances contained in grain cereals and other plant products which have been proven by many authors. For instance, Stevenson et al. [29] had isolated phenolic compounds and other antioxidative oats substances by methanol, Peterson et al. [1] studied the content of tocopherols and tocotrienols in different varieties of oat insulating them using methanol. Bryngelsson et al. [30] identified in oat avenanthramides, being characterized by strong antioxidant properties. The mentioned compounds were also isolated using methanol.

Antioxidant substances in methanol solutions samples could be derived from both the oat flakes and the maize. Oats are characterized by a content of natural antioxidants, such as tocols, fenolic acids, avenanthramides, flavonoids, sterols and phytic acid. The main tocol is α-tocotrienol, while α-tocopherol is less common. Moreover, in oats there is also a small quantity of β-homologues of vitamine E. Tocols are also detected in maize, containing mainly γ-tocopherol. Individual tocols homologues demonstrate different functional properties, in addition to the antioxidant capacity [27][28]. Phenolic acids in oats are caffeic acid and ferulic acid (soluble mostly in organic solvents). Phytic acid also has the antioxidant properties, but it is nutritionally undesirable. Carotenoids, incorporated in maize, also have antioxidant properties (composition of carotenoids in maize after various processes was presented by Scott and Eldridge [28], similarly as polyphenols and ferulic acid [26]. The study confirmed that maize extrudates with oat flakes addition have antioxidant properties.

There was no correlation between AA value and the absorbance values measured in metanol extracts. Before this study authors expected that AA value would be closely associated with variables reflecting the NEB reaction. This conviction resulted from similar studies published in the literature [22, 31, 32] and from previous experiments conducted by the authors [21, 33]. The experiments mentioned above demonstrated that the AA compounds are type of NEB products. However, studies with the oat addition to maize extrudates have shown that the antioxidant properties were not associated with the intensity of NEB reactions. Negligible impact of non-enzymatic browning compounds on antioxidant properties of analysed extrudate samples was associated with the fact that for the antioxidant capacity were responsible mainly substances other than Maillard reaction products. Basied on the obtained results, it was concluded that the chemical processes of formation of methanol soluble NEB reactions products in the analysed extrudates should be considered regardless of their antioxidant properties.

Absorbance results indicated that in analysed solutions, were present particularly intermediate products of the NEB reactions. Final products of the reactions were much less common. This is a result of only a short time influence of high temperature on the material during the process.

Generally, it was found that there were few compounds soluble in methanol, absorbing electromagnetic radiation at both wavelengths. This result confirmed information published by other authors [15, 19, 20], who studied the derivatives of NEB reactions soluble in solvents other than water. However, examining the broiled pork and pork-beef steak showed that derivatives of Maillard reaction soluble in methanol compared to water-soluble, was from 20 to 77% in the surface layers. In the layers below the surface these proportions were different – in some cases there were more soluble compounds in methanol than in water [21]. The results confirm that the analysis of the intensity of the NEB reaction including studying methanol extracts, may be an important indicator to differentiating quality properties of products. NEB reaction depends on many factors, especially on the heat dose, pH and basic composition of raw materials. Different oat additions to maize extrudate caused changes in this reaction.

The reacting substances may have been protein, carbohydrates and fats derived from maize and oat flakes. Characteristic protein of maize is zein. The zein includes mainly: glutamic acid (21-26%), leucine (20%), proline (10%) and alanine (10%), while there is no lysine and tryptophan [34]. Pedro et al. [35] reported that in the oat the limiting amino acids are lysine and threonine, while there is a lot of the sulfur-containing amino acids and tryptophan. The most reactive amino acid in Maillard reaction is lysine, but the substrates for the reaction are also arginine, tryptophan, cysteine and histidine [36].

In a mixture of oat and maize characterized by a high amount and diversity of protein, fat and carbohydrates, many different products of NEB reactions could be generated. Due to the lack of tryptophan and lysine in zein, oat flakes addition, as the source of mentioned reagents, explains the increase of absorbance in case of extrudate samples containing 5 and 10% of oat flakes addition. The higher addition of oat flakes generated an absorbance reduction at both wavelengths. Oat flakes are a source of amino acids and dietary fiber, especially β-glucans - its soluable fraction [2]. Extrudates before the process were moistened. Fiber can link and retain water in its structure reagents so NEB reactions occurred slowly. At the same time the samples with addition of 15% of oat flakes were significantly brighter than the other two samples containing oat flakes, which seems to confirm this thesis. Lightness (L^*) and a^* parameter were also useful indicators of the quality characteristics of extrudates. The trends of their changes were similar to changes Abs_λ_{320} and Abs_λ_{420}. The samples with addition of 15% oat flakes were characterized by a similar lightness to the control samples of maize extrudate, but has significantly lower value of a^* – was the least żedóf all extrudates. Changes of samples colour are often analyzed as an indicator of the quality of extrudates. Colour parameters reflect changes in composition of raw material, moisture and process conditions [2, 37–41]. Brnčić et al. [38] found a significant correlation between L^* value and the addition of whey protein to maize and the content of moisture in the extrudates.

Depending on the whey protein content parameter b^* also changed, whereas a^* did not. Rodrígez-Miranda et al. [40] studied the extrudates produced from blends of taro flour with nixtamalized and non-nixtamalized maize flour and shown changes in all colour parameters. Stojceska et al. [41] and Head et al. [2] also showed statistically significant variations in all colour parameters such as value L^*, a^* and b^* extrudates, depending on the process conditions. All these results confirm that colour could be a preliminary and fast indicator of the properties of extrudates. In addition, the authors proved that colour of extrudates measured instrumentally was correlated to absorbance values.

Conclusions

Maize extrudates with the addition of oat flakes can enhance the product range of ready-to-eat products containing fiber. Such products are a good source of compounds which have antioxidant properties. The addition of a maximum of 15% of oat flakes to maize extrudates did not cause significant changes of antioxidative activity of the product, while other indicators changed. Depending on the oat participation in extrudates, a different amount of compounds that absorb electromagnetic radiation at wavelengths of 320 and 420nm was created. These were derived from NEB reaction. The addition of 5 and 10% of oat flakes to maize extrudate caused an increase of derived NEB reaction in the methanol extracts, whereas in the extrude containing 15% of oat values of Abs_λ_{320} and Abs_λ_{420} decreased comparing to the previous two. Probably oats were the source of fiber, which could link reactants of NEB so this reaction occurred more slowly. It was shown that the intensity of spectrophotometric determination of NEB reaction in methanol extracts of extrudates can be a useful measurement of assessing the differences among these products. Values of Abs_λ_{320} and Abs_λ_{420} were related to the results of instrumental colour measurement (L*a*b*) and were not related to the AA value.

Finally it was stated that by analyzing Abs_λ_{320} and Abs_λ_{420} value and colour the differences between products can be estimated, while the AA values survey has enabled an overall assessment of their nutritional properties.

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