QUALITY ASSESSMENT FOR NUTRITIVE VALUE OF BISCUITS BASED ON OAT FLOUR FROM *AVENA NUDA* L.

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The purpose of this study was to valorize Avena nuda L. oat species. in order to produce high nutrition value bakery products. The main intention is to bring health benefits to consumers. Biscuits samples with different percentages of oat whole flour were elaborated. These were analyzed by means of classical methods (humidity, protein, ash content, fat total content, sugar, crude fibers and dietary fibers), enzymatic methods (for beta-glucan), chromatographic methods (for acrylamide) and NMR spectroscopic methods (for fatty acids profile). It has been noticed that the use of high percentages of whole oat flour leads to an increase in the content of dietary fiber, beta-glucan, unsaturated fatty acids and minerals, with recognized beneficial effects for human consumption.

Keywords: oat, biscuits, beta glucan, acrylamide, *Avena nuda L.*

1. Introduction

In the last years the amount of oats used for the human consumption gradually increased, due to the benefits of this type of food. The oatmeal is rich in nutrients such as essential amino acids, unsaturated fatty acids, fibers, vitamins, minerals and antioxidants [1]. The main health effect of oats is based on the total dietary fiber and β-glucan content [2]. Food and Drug Administration (FDA) has specified that the consumption of oat β-glucan (BG) can reduce serum cholesterol levels in people with high cholesterol, thus reducing the risk of cardiovascular disease [3]. In developed countries about 75% of the total cultivated oats is used for animal feed and only 25% for human consumption. In less developed countries the percentage of use for feed may be considerably higher [4].

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In fact, the main health effect of oats is that decreases postprandial glycemia, insulin response and blood lipids, especially total cholesterol and "bad" cholesterol (LDL). Apart from BG, oats are rich in other nutrients such as essential amino acids, unsaturated acids, vitamins, minerals and antioxidants. In addition to nutritional properties, some studies have shown that oats can be tolerated by majority of people suffering from celiac disease (gluten intolerance) [5]. Food and Drug Administration (FDA) approved a health claim for β-glucan soluble fibers from oats, for reducing plasma cholesterol levels and risk of heart disease [6]. Oats is being held in category of cereals that contain gluten but was added a footnote specifying: oats can be tolerated by most persons who are intolerant to gluten, but not all [7].

In comparison with wheat, the protein of oats contains considerable amounts of amino acids, in this respect the use of oats in wheat bread improves the biological value of the final product [8]. Some studies have shown that oatmeal is much richer in protein compared to wheat flour, rye, barley, rice, sorghum and maize flour. The average content of oat lipids from grains can be up to 5.9% of triglycerides, with a high proportion of unsaturated fatty acids, which constitute the main fraction [9][10].

Compared with other cereals, oats contain a high amount of lipids. The lipid fraction of oats determines its energy content and has a significant impact on the nutritional quality, through the fatty acid composition. Lipid probably mediates the bonding of starch from oat and therefore influencing functionality. Fats are also involved in the attributes of flavor/non-flavor behavior of oats [11]. Anderson and Bridges, pointed out the importance of water-soluble dietary fiber, flour and oat bran. According to the results obtained from them, introduction of oats products in the human diet decreases blood cholesterol [12] [13].

Acrylamide is a chemical that naturally forms in starchy food products during high-temperature cooking, including frying, baking, roasting and industrial processing, at +120°C and low moisture. The main chemical process that causes this is known as the Maillard reaction; it is the same reaction that ‘browns’ food and affects its taste. Acrylamide is obtained from sugars and amino acids (especially asparagine) that are naturally present in many foods [14]. Creation of acrylamide in bakery products depends on the type of flour used in the recipe, because the asparagine content is different for different cereals: 75-2200 mg/kg wheat; 70-3000 mg/kg corn; 50-1,400 mg/kg oat; 319-880 mg/kg rye; 15-25 mg/kg of rice [15]. According to data reported by the European Food Safety Authority (EFSA) [16], bakery products may contribute to 20%–60% of the total mean acrylamide intakes. This fact involves exposures to acrylamide ranging from 0.13 to 0.31 μg/kg body weight/day in adults and reaching up to 0.55–0.75 μg/kg body weight/day in children and adolescents. In 2013, the European Commission (EC) has adopted new indicative acrylamide values based on the EFSA monitoring data

from 2007 to 2012 for the main sources of acrylamide exposure [17]. These recommendations involve reference values for acrylamide content in the major contributors to the intake of this compound. Risk associated with the acrylamide intake can be regulated and, consequently, controlled.

The purpose of this paper was to highlight oat species Avena Nuda L., in order to produce biscuits with oat whole flour with high nutrition value. These products offerred benefits for human consumption (increased dietary fiber, beta-glucan, unsaturated fatty acids and minerals) and reduce the disease risk for gluten intolerant persons. In order to ensure food safety and consumer protection, the content of acrylamide from biscuits obtained from oatmeal was also evaluated in this study. To guarantee food safety and consumer protection, acrylamide content of these sample-biscuits was also evaluated; it was observed that all samples contained less acrylamide than the maxim recommended level (500 μg/kg).

2. Materials and methods

Naked oat (*Avena nuda* L.) GK Zalan variety was grown in the experimental field at Moara Domnească, Ilfov. Whole grain oat flour used was obtained by grinding the integral grains of naked oat. Experiments were conducted on addition of different percentages of whole grain oat flour in wheat flour while sugar, butter, eggs, baking soda, ammonia, citric acid were added in the same proportion. All samples were baked in an oven (Mondial, Forni, Italy) at 185^0C for 20 minutes. In the table below are presented the types and the percentages of flour used to obtaine the biscuit samples.

Table 1

Types/percentages of flours used to obtaine biscuit samples

Sample	Types/percentages of flours
P1	100% wheat flour
P2	75% wheat flour+25% whole grain oat flour
P3	50% wheat flour t+50% whole grain oat flour
P4	25% wheat flour+75% whole grain oat flour
P5	100% whole grain oat flour

300 g of each biscuit sample were ground, sieved (0.5 mm) and stored in closed containers, protected from light up until analysis, one exception was determination of moisture content. This analyze was performed immediately after grinding.

The characteristics of biscuit samples were determined according to following methods:

- Moisture content according to SR 91:2007 (Bread and pastries. Methods of analysis);

- **Protein content** by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 [18]

- **Fat content** with petroleum ether under reflux conditions in a Soxhlet system (Avanti 2055, Foss). The Avanti 2055 has the capacity to perform extractions on six separate samples simultaneously [19]. Approximately 5 g of ground biscuits were accurately weighed into a filter paper and wrapped. The wrapped sample was transferred to a Soxhlet extractor with a distillation flask containing petroleum ether (40–65 ^{0}C boiling point). The sample was extracted continuously for about 4 h. The excess of solvent was removed by evaporation and the lipid content was determined by weighing [20];

- **Crude fiber** by using the FibreTherm FT 12 system. The method consists in boiling sample with sulfuric acid. The residue is separated by filtration, then washed, dried and weighed. The residue obtained was calcined and the mass loss resulting from calcinations corresponds to the mass of crude fiber from the analyzed sample [21];

- **Acrylamide analysis.** Biscuits acrylamide was quantified by GC-MS/MS. The calibration solutions and the derived extracts sample were analyzed using a gas chromatograph, type TRACE GC Ultra, coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS) from Thermo Fisher Scientific (USA). The analyses were performed in the electron impact ionization operation mode, positive (+EI); acquisition mode: selected reaction monitoring (SRM) and ion scanning mode - Product ("Product").
A capillary column based on polyethylene-glycol, TraceGOLD™ TG-WaxMS (Thermo Fisher Scientific, USA) with a length of 30 m x 0.25 mm inner diameter and film thickness of 0.25 μm for stationary phase. [17] [21] [22];

- **β-glucan** content has been determined by enzymatic methods using the assay kits: K-TDFR “Total dietary fiber” (AOAC Method 991.43) and K-BGLU “Mixed-linkage beta-glucan” (AOAC Method 995.16) obtained from Megazyme International Ltd. (Bray, Ireland). The samples were suspended and hydrated in a pH 6.5 buffer, incubated with purified enzymes from lichenase and then filtered. Part of the final filtrate has been hydrolyzed with β-glucosidase. Beta glucosidase produced has been tested with glucose oxidase/ peroxidase reagent;

- **Minerals content** was measured by F-AAS (Flame atomic absorption spectrometry) using an AAnalyst 400 spectrometer, after dry mineralization at 500^{0}C according to STAS 13013-1:1991. Accurately weighed (1.0 g) samples were transferred into crucibles and kept in a furnace for ash at 550^{0}C and then 5 mL of HCL 1:1 was added, and the crucible was kept on a hot plate and digested to obtain a clean solution (according to STAS 13013/1-91). The final residue was made up to 100 mL. Na, K, Ca, Mg and Zn were analyzed using an atomic absorption spectrometer equipped with air-acetylene flame (1.8 L/min), AAnalyst

400 (Perkin Elmer). Cu, Fe and Mn were analyzed using an atomic absorption spectrometer with graphite furnace, AAnalyst 600 (Perkin Elmer);

- NMR Analysis - ^{1}**H-NMR** spectra were recorded on a Bruker Ascend 400 MHz spectrometer, operating at 9.4 Tesla corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus. Samples were analyzed in 5 mm NMR tubes (Wilmad 507). The NMR samples were prepared by dissolving 0.2 mL fat in 0.8 mL $CDCl_3$.The fat used in the NMR analysis was extracted by Soxhlet standard method.

3. Results and Discussion

Five types of oat biscuit samples were obtained from different mixture of flours, sugar, butter, eggs, baking soda, ammonia, citric acid and water. The results obtained from physical-chemical analysis are presented in Figure 1. An increase content of protein, fat, sugar, dietary fiber, and ash was noticed, directly dependent on the increasing percentage of whole grain oat flour added. The moisture of samples decreases with the increased quantity of whole grain oat flour added.

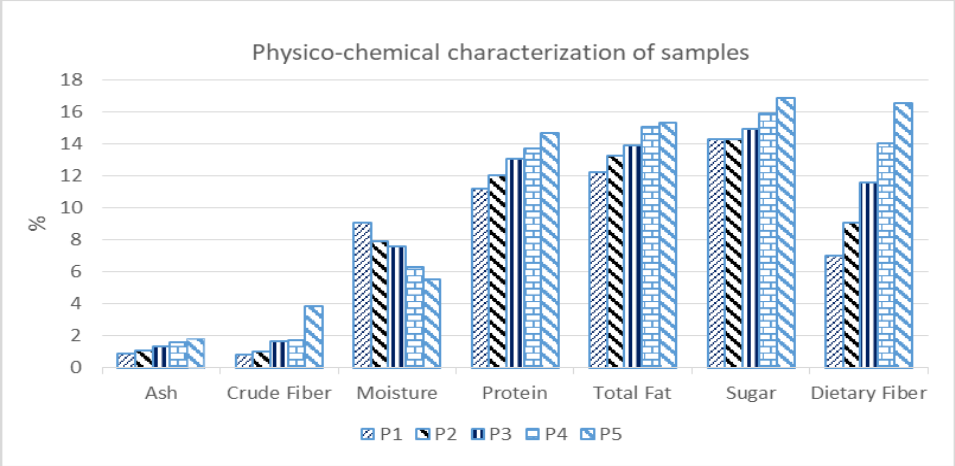

Fig. 1. Physico-chemical characterization (ash, crude fiber, moisture, protein, total fat, sugar, dietary fiber) of studied samples

Regarding the amount of acrylamide that is formed during the cooking process, the obtained results fit in the indicative acrylamide values for 'biscuits' by the EC Commission Recommendation of November 8, 2013 (2013/647/EU)) (500 µg/kg for biscuits) [17]. The content of acrylamide increases with the amount of whole grain oat flour added in the recipe, but the advice indicative acrylamide values are far from being reached.

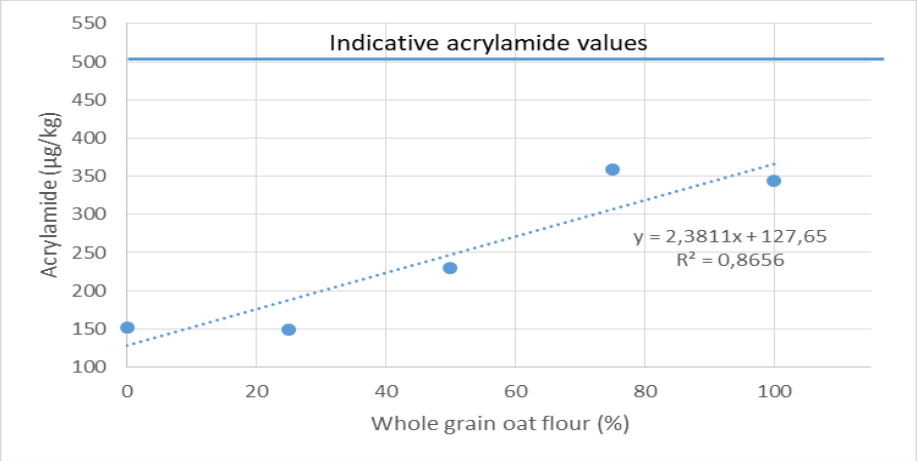

Fig. 2. Acrylamide content in the five types of oat biscuit samples

In terms of β-glucan content, the results for the five types of oat biscuit samples are presented in Figure 3. From the chart below, it can be noticed that β-glucan content increases with the amount of whole grain oat flour added.

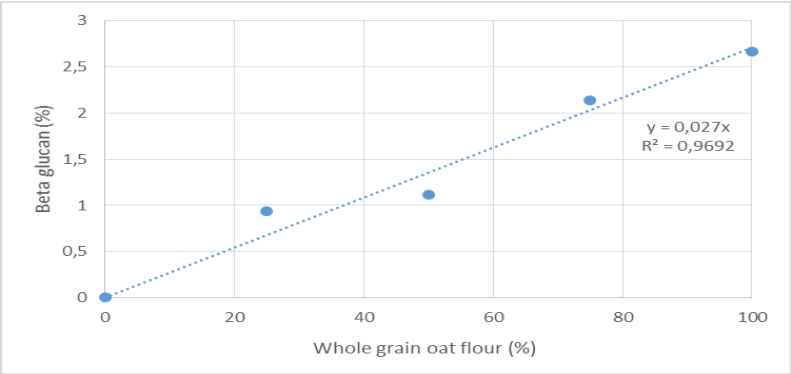

Fig. 3. Beta glucan content in the five types of oat biscuit samples

A higher amount of whole grain oat flour in the recipe could result in an intake of 2.6**%** β-glucan per 100g of biscuits, so the beneficial effect on human health could be obtained by consuming only 115 g of oatmeal biscuits per day. As mentioned in some studies, intake of oat β-glucan at daily doses of at least 3 g may reduce low-density lipoprotein (LDL) cholesterol levels [6].

Correlation equations

Based on the obtained results, there were achieved correlation equations between the patterns of variation on different parameters determined and the amount of added whole grain oat flour. These equations can be used to predict the amount of nutrients or contaminants present in oatmeal biscuits. Ca, Mg, K, Zn

and Mn are directly influenced by the amount of added oatmeal flour, and it has been obtained variation patterns of these parameters.

Table 2

Model equation content for the main minerals contained in biscuits with variation of whole grain oat flour added

Parameters	Equation of the model
Ca	Ca = 54.60+0.25*Oats(%)
Mg	Mg =369.25+6.08*Oats(%)
K	K = 3.07+0.01*Oats(%)
Zn	Zn = 2.66+0.19*Oats(%)
Mn	Mn= 8.75+0.32*Oats(%)

Below are presented patterns of variation obtained from parameters that are directly influenced by the amount of added whole grain oat flour.

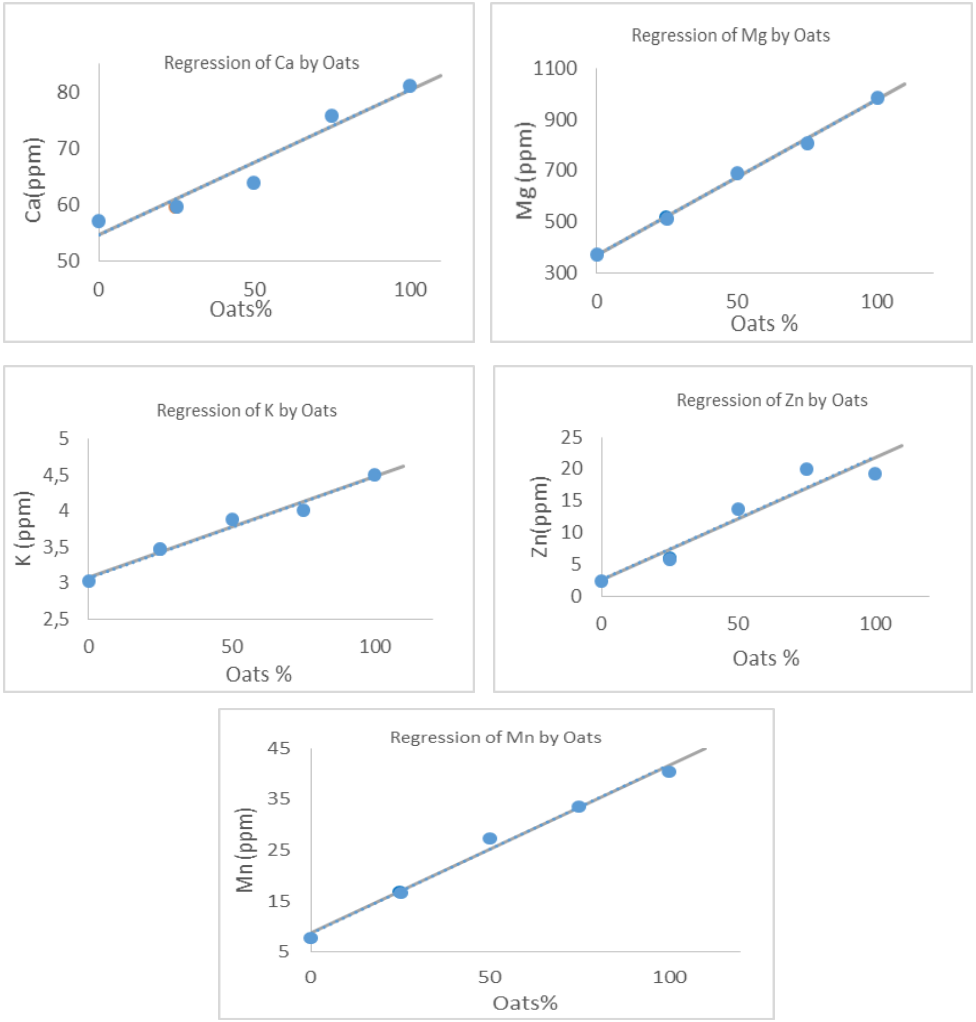

Fig. 4. Regression curves of Ca, Mg, K, Zn and Mn according with whole grain oat flour variation

It has been found that only Ca, Mg, K, Zn and Mn are directly influenced by the increase of added whole grain oat flour, while the content of Fe, Na, Cu and Mn does not increase proportionally with the amount of added oatmeal.

Using the XLSTAT-Premium Academic program, all the results obtained for acrylamide, β-glucan, Ca, Fe, Mg, K, Na, Zn, Cu and Mn, from the five samples of biscuits were used in Principal Component Analyses (PCA).

PCA analysis shows that iron, acrylamide and copper content of biscuits are important for system variability, while sodium content explains less the system variation.

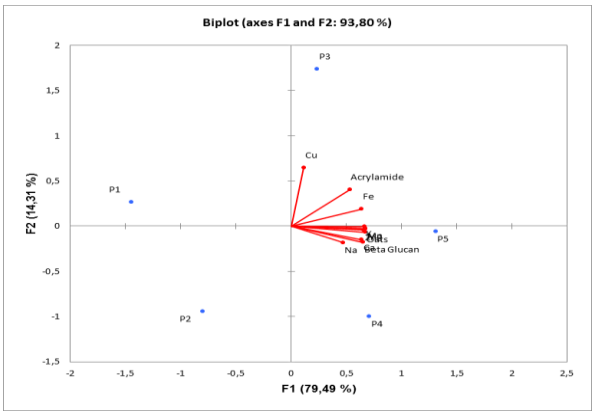

Fig. 5. Principal Component Analysis (PCA) biplot for the five types of oat biscuit samples based on physical and chemical parameters

With the increasing addition of whole grain oat flour the mono-unsaturated and di-unsaturated fatty acids increase. In Fig. 6 the content of mono-unsaturated fatty acids increases from 27.17% to 30.41%. and di-unsaturated fatty acids from 8.68 to 14.75%.

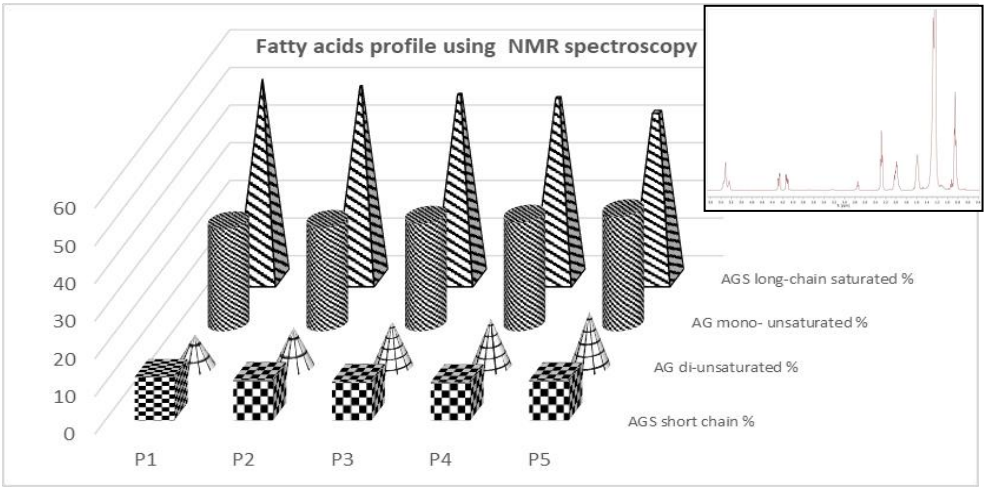

Fig. 6. Fatty acids profile using NMR spectroscopy

4. Conclusions

Different biscuits were prepared with whole oat flour from Naked oat (*Avena nuda*) GK Zalan variety, cultivated at experimental field at Moara Domnească, Ilfov. The results showed that with increasing addition of whole grain oat flour within the biscuits recipe, content of: protein, ash, total fat, sugar, crude fiber and dietary fiber improved. Using whole grain oat flour, biscuits with a higher content of crude fiber and dietary fiber were obtained than biscuits obtained only with wheat flour. Biscuits samples with 2.6% β-glucan per 100g of biscuits were elaborated, therefore by consuming only 115 g of oatmeal biscuits per day, low-density lipoprotein (LDL) cholesterol levels can be reduced. The acrylamide content increases with the quantity of oat whole meal flour added in the recipe, but it is still lower than the 500 μg/kg indicative acrylamide values, recommended by the European Commission for biscuits. The variation model for the content of Ca, Mg, K, Zn and Mg has been established and a prediction of the content based on the amount of oat flour added can be made for the biscuit recipe.

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