Scientific report

(Contract number 53/01.10.2015)

Project: Improvement of the biochemical, rheological and technological aspects in bread making by using different composite flours

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The research project aim is to establish the optimal level of pulses and oilseeds flour cultivated in Romania (peas, rape, yellow and brown flaxseed, tomato, pumpkin and mustard) which can substitute the wheat flour in order to obtain baking products of a high quality.

For this purpose we will investigate the physical-chemical composition of wheat flour, pulses and oilseeds that will be used in our research. We will investigate from the nutritional point of view their amino acid and mineral content, we will investigate the pulses and/or oilseed-wheat flour dough behavior during the bread making process and we will evaluate the quality of the obtained bakery products.

For the year 2015 the research objectives proposed in Annex IV of Contract No. 53/01.10.2015 are:

1. *Research concerning the determination of the physical-chemical properties for 2 wheat flours* with the fallowing activities: moisture determination, ash, acidity, wet gluten, gluten deformation index, protein content, mineral elements, amino acids, α-amylase activity;

2. *Research concerning the determination of the physical - chemical properties of pulses and oilseeds* with the fallowing activities: moisture determination, ash, acidity (peas flour, rapeseed flour, yellow and brown flaxseed flour);

3. *Evaluating the validity and precision of the obtained results* with the following activitiesz: setting out the degree to which the results obtained experimentally measured sizes

are consistent with reality, establishing the existence of the causality link, development and substantiation of the scientific conclusions.

I mention that, from activities related to the research objectives proposed for 2015 it wasn't possible to make the amino acid content determination because the reagents and the separation column for the HPLC-DAD device necessary to make these analyzes were acquired at a date which has not allowed us to make the correct and integral experimental determinations. Therefore this activity will be accomplished at a later date then the reporting one during the month of December, 2015 and the data obtained will be reported in the 2016 year. I also mention that a part from the activities that should be realized in 2016 year (moisture determination, ash, acidity for the pumpkin flour, tomato flour and mustard flour) and the mineral elements for the peas flour, rapeseed flour, yellow and brown flaxseed flour, pumpkin flour, tomato flour and mustard flour were in advance made in the 2015 year and will be presented in this scientific report.

In this way, the activities that were not carried out for technical reasons have been replaced with other activities in order to complete the time period necessary to make these experiments.

Results obtained

1. Working methods and the devices used

In order to appreciate the quality of the wheat flours and the peas flour, rapeseed flour, yellow and brown flaxseed seed flour, pumpkin flour, tomato and mustard flour were used for the most part standard analytical methods.

To characterize the quality of the flours, the samples were analyzed by the following methods:

- moisture content determination by oven drying, according to SR EN ISO 712:2010;
- ash content determination according to SR EN ISO 2171:2010;
- protein content determination by Kjeldahl method according to SR EN ISO 20483:2014;
- wet gluten content determination according to SR 90:2007;
- gluten deformation index according to SR 90:2007;
- acidity determination according to SR 90:2007;
- falling number determination according to SR EN ISO 3093:2010;
- mineral elements determination from the flours mentioned above, through an inductively coupled plasma - mass spectrometry (ICP-MS) by a method that will be described

below. The method is based on the measurement by ICP-MS of the mineral elements concentration from an acidic extract obtained from the ash of a vegetal material. The analytical process includes two stages: a dry mineralization and the dosage by ICP-MS of the obtained acid extract.

Description of the method used for the determination of mineral elements by ICP-MS

a. Dry mineralization

For the quantitative determination of mineral elements from the wheat flours, oilseeds and pulses are necessary its separation from the samples. For this purpose we made the dry mineralization in the oven.

In the porcelain crucibles previously cleaned and brought to constant weight by calcinations at 200-250^{o}C we placed about 10 g of the ground material with and accurately weigh of 0,0002g. We put the crucible with the sample on a porcelain triangle at a small gas burner flame. The flour turns without any intervention, and burning should not be too fast. The crucibles with samples are placed in a oven preheated to 200-250^{o}C and were maintained until complete carbonization. Then, we increased the oven temperature up to 550 – 600^{o}C and we incinerated them for 6-8 hours until a white ash was formed. If a carbon-free ash cannot be obtained in this way, we exhausted the charred mass with a few ml of concentrated nitric acid, after this, we dried the samples in a sand bath and incinerated them for 2 hours long at 550 – 600^{o}C. After incineration, the crucible is removed from the oven, placed in an excicator and weighed as soon as it has cooled to ambient temperature.

Further, the ash is dissolved in 5% nitric acid (prepared from concentrated nitric acid, 69%) as follows: the ash sample was dissolved in a porcelain crucible in 5 ml of 5% nitric acid. The solution was filtered under atmospheric pressure of quantitative filter paper, dried and weighed, and then the crucible was washed four times with 5 ml distilled water (deionized) the water wash being then passed still on the filter-paper. The filtrate was brought to a volume of 25 mL.

In parallel a blank sample is prepared for verification.

b. Determination of mineral elements by ICP-MS

The mineral elements from the solution are dosed from the nitric solution obtained through mineralization in the center of the argon plasma connected to the mass spectrometer detector. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer The ions transmitted through the quadrupole are detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system [28]. For the device calibration was used a standard solution of the same concentration of nitric acid, 5%. For each sample the following minerals were determined: Na, Ca, Mg, Fe, Zn, Mn, Cu, Pb, Co, Cr, Ni, Se, V in duplicate.

2. Results and discussions

2.1. The determinations of the selected wheat flours physical-chemical properties

In order to achieve the objectives of this research project, two white wheat flour type 650 were used, obtained from the 2015 processing grain harvest. These are commercial flours from S.C. Mopan S.A. Suceava and S.C. Coza-Rux S.R.L. Suceava County, Romania. Wheat flours were selected as the samples to be of a homogeneous quality. The wheat flours were without any additive or enzymatic corrections.

We chose wheat flour with a lower extraction rate because they are the lowest in mineral elements and in addition they present gluten proteins of a better quality compared to wheat flours with a higher extraction rate and also present a better technological behavior.

According to the first research objective proposed to be achieved in 2015 we determined for the wheat flours the following quality parameters: moisture, ash, acidity, wet gluten, gluten deformation index, protein content, minerals elements and α-amylase activity.

The moisture content for the two wheat flours varies between 14.1 and 14.4%, ash content varied between 0.64 and 0.66%, protein content varied between 12.1 and 12.7%, wet gluten content varied between 27.6 and 34.2%, gluten deformation index varied between 3 and 8 mm, acidity varied between 2.1 and 2.3 acidity grade, falling number varied between 322 and 382 s.

From the results obtained we noticed that the first flour is flour with a very good quality for bread making with gluten with very good visco-elastic properties. The second flour presents a very low gluten deformation index and therefore is strong flour for bread making with resistant gluten, not extensible.

From the point of view of the falling number values both flours analyzed presents low α amylase content because the falling number values are higher than 280÷300 s [24].

2.2. The determination of the physical - chemical properties of pulses and oilseeds (ash, moisture, acidity) for the fallowing flours: peas flour, rapeseed flour, yellow and brown flaxseed flour, tomato flour, pumpkin flour and mustard flour

Moisture is an important characteristic of flours quality and it means its water content expressed as a percentage of their total mass. In moisture content determination the free water content of the sample is eliminated. Flours moisture content value influences its behavior in the technological process and the bread quantitative efficiency. For the pumpkin, yellow flaxseed and mustard flours the moisture content value is between 5.2 and 5.9% and for the rapeseed, brown flaxseed, tomato seed and pea seed flours moisture content value varied between 5.9 and 11.9%.

From the pulses and oilseed flours analyzed the highest moisture value was obtained for the pea flour, a higher value that the one reported by Kohajdová Z. et al. [7] fallowed by the tomato seed flour with a lowest value than the one reported by Persia M.E. et al. [12], brown flaxseed flour and rapeseed flour. For the brown flaxseed flour the moisture content is higher then the one for the yellow flaxseed flour, similar results from this point of view being obtained by the Muller et al. [11], Shim Y.Y. et al. [18]. For the pumpkin flour and for the mustard flour, the moisture content values were similar in results with those reported by Milovanović M.M. et al. [10], respectively Abul-Fadl M.M. et al. [1]. All the values obtained for the moisture content parameter are between the limits established by the set standards for agricultural seeds for consumption.

Ash content from the pulses and oilseeds flours represents the mineral content quantity of its. The results obtained showed that the ash content of them varied between 2.43 and 4.35%. Ash content of the pumpkin flour presented a higher value than the one reported by Milovanović M.M. et al. [10]. For the mustard seed flour and the yellow flaxseed flour were obtained similar results with those reported by Abul-Fadl M.M. et al. [1], respectively Mueller K. et al. [11] and Shim Y.Y. et al. [18]. For the pea flour and the rapeseed flour, lower values were obtained than the ones reported by Kohajdová Z. et al. [7] respectively Vose J.R. [26]. For the tomato seed flour, higher values were obtained for the ash content than the ones reported by Persia M.E. et al. [12].

The ***acidity*** of the oilseeds and pulses flours is the sum of all acids and other chemical substances with the acid reaction present in the flour. It is given by the free acid phosphates, phosphoric acid, the fatty acids resulted by the lipase action, mono amino dicarboxilics acids (glutamic, aspartic acid), organic acids such as lactic, acetic, succinic, citric, malic, e.g. For this parameter the results obtained varied between 2.1 and 3.5 acidity grades for the yellow and brown flaxseeds, pea and tomato seeds flours and between 5.9 and 10.5 acidity degrees for pumpkin, rapeseed and mustard flours.

2.3. Determinations of the mineral elements by ICP-MS for the wheat flours and for the pea flour, rapeseed, yellow flaxseed, brown flaxseed, pumpkin, tomato, mustard flours

The mineral elements content from the materials of vegetable origin is characteristic for each species type and varied function on pedoclimatic factors, culture technologies, e.g. It represents the food constituents absolutely necessary for life contributing to a normal vital activity and a body development. Minerals elements necessary to the human body can be classified in macroelements and microelements [16].

The mineral elements analyzed and their role in the human body

Macroelements

Calcium

It has a role as plastic as well as a dynamic one, participating in the maintenance of the functional integrity of the central and peripheral nervous system, of the cell membrane, of the blood coagulation, in the maintenance of the colloidal protein state. It is also a cofactor of some enzymes [17]. In the human body almost 90% from the calcium quantity is fixed in the in the skeleton and teeth, and the rest it can be found in tissues and fluids.

Sodium

It is a very widespread element in the vegetal reign in various combinations: chlorides, iodinated, citrate, e.g. Sodium ions are involved in the electrochemical impulses transmition, through the cell membrane in order to maintain a normal muscle and nerve sensitivity. Sodium causes water retention and increased resistance to physical and nervous effort. The sodium ions cause water retention in the human body and increase the resistance to the physical and nervous effort [17].

Magnesium

Is an element which participates in the metabolism of carbohydrates, fats; the growth processes and cell permeability [17]. It is a catalytic element and also a plastic one and it is a growth factor, a psychical balancing that helps regulate calcium balance in the human body with an anti-aging role, anti-anaphylaxis, anti-atherosclerosis, e.g.

An insufficient magnesium quantity may increase the incidence of degenerative cardiovascular disease, its concentration in the human blood of the persons suffering from atherosclerosis being inversely proportional correlated with the cholesterol level [16]. Also the magnesium deficiency is involved in digestive diseases, kidney problems, allergies, nerve damage, e.g.

Microelements

Iron

Iron is a component of hemoglobin and of some enzymatic systems from the respiratory chain. It is an oxygen transporter and plays an important role in the cellular respiration. It is the most important from the other microelements, its absence in the human body leading to nutritional anemia characteristic [16].

Zinc

Zinc presents multiple roles in the human body being a part of some metalenzyme and hormones, influencing the metabolism of lipids, proteins and carbohydrates, participating in various cellular activities, such as the synthesis of DNA and RNA, proteins rich in sulfur, e.g. [17]. It is involved in the regulation of the pituitary gland, the functioning of the pancreas, affects the ability of learning and it is involved in sexual development. Zinc deficiency is manifested by a growth delay and sexual maturation, hepato-splenomegaly, ossification of long bones delay, anemia, e.g. [16].

Manganese

It is a constituent of various enzyme systems, is involved in skeletal development, in the reproductive processes, in the carbohydrate and lipid metabolism, it promotes the liver and kidney functions, it accelerates human body combustion, it help in the minerals fixation of iron and vitamins [17].

Manganese deficiency may lead to the development of diabetes and in the reduction of blood coagulation [17]. Also its absence may cause nervous disorders and long bones growth defects.

Cobalt

This element has a very intense activity even if in the human body it amount is the lowest. This element is a part of the vitamin B12, it is an activator of many enzymes and can take place of other ions in the activation of some enzymatic reactions. Deficiency of this element may led to downturn to progressive weakness and anorexia, pernicious anemia, e.g. [16].

Chromium

It is involved in the fat metabolism and in the carbohydrate and protidic ones. Its major effect is the activation of the insulin function. Chromium may protect against atherosclerosis and contributes in maintaining the integrity of the nervous system. Food chromium deficiency leads to an increased in glucose intolerance, which increases the predisposition for diabetes dieses [17].

Nickel

Nickel activates a number of enzymes that are involved in maintaining the integrity of the cell membranes. It is considered that is not possibly to exist nickel deficiency under conditions of rational nutrition [16].

Selenium

It is a cytoprotective agent because it interrupts the metabolic sequences that initiate the neoplasia transformation. It has an important role in increasing young human bodies, in preventing tooth decay in children, in the tissue respiration. A selenium deficiency can lead to heart diseases and to sudden death in babies, e.g. [17].

Vanadium

It is involved in lipid metabolism in the process of reproduction, in the growth process, in the mineralization process and in the erythrocytes formation. It is not very well known the precise vanadium quantity the human body needs [16].

Copper

This element exists in all living tissues and it is indispensable to the cellular life and bone formation. Intervenes in hematopoetic, in iron absorption from the gastrointestinal tract and in it deposits metabolism. It is a dynamic element, anti-infective, antiviral, anti-inflammatory [14].

Lead

It is a heavy metal with a toxic nature, an excess of lead leads to encephalopathy, acute and chronic peripheral neuropathy, appearing on the list of carcinogens. It presents harmful effects on fertility, endocrine system, myocardium and immune mechanisms [14].

Data analysis obtained by ICP-MS

Sodium is the best macroelement represented in all the oilseeds and pulses flours analyzed. The highest amounts are in the tomato and yellow flaxseed flour and the lowest amounts are in the rapeseed flour. Compared to wheat flour, lower sodium content is present in the rapeseed and mustard seed flour. Higher values of sodium were also reported by Hussain S. et al. [6] in flaxseed flour and by Tsatsaronis G.C. [25] in tomato seed flour.

Calcium is a macroelement very important for the human body and it is found in lower amounts in the pumpkin flour. Except this type of flour all the other flours analyzed show much higher values for this macroelement compared with the wheat flours analyzed. The higher amounts of calcium were recorded for the yellow flaxseed flour and for the tomato seed flour. The calcium in the pea flour, was the lowest than to that reported by the Reichert R.D. and MacKenzie S.L. [13].

Magnesium is present in higher amounts than calcium in all the flour samples analyzed, the oilseeds and pulses flours showing higher values than the wheat flours analyzed. The highest magnesium content was obtained for the tomato seed flour. A higher amount of magnesium was also found in tomato seed by Tsatsaronis G.C.[25]. Also, a high amount was obtained in the pumpkin flour a three times higher value than the one obtained by El-Soukkary F.A.H. [3]. For the pea flour the results obtained by us was similar to those obtained by Wang N. and Daun J.K. [27].

Iron is a microelement essential for the human body being presented in high amount in the tomato seed flour. For the rapeseed and brown flaxseeds flour, the values obtained were the lowest for this element compared to the analyzed wheat flours. The presence of iron in mustard seed flour was also found by Lopez-Arquello E. et al. [9].

Zinc, an essential microelement for the human body but who in some amounts may become toxic is present in high amounts in tomato flour seed and in pumpkin flour seed. Higher amount of zinc in pumpkin flour seed was also found by Glew R.H. [5]. The presence of zinc in tomato seed was also found by Tsatsaronis G.C. [25] but in low amounts. In mustard flour seed a high amount of chrome was also reported by Lopez-Arquello E. et al. [9].

Manganese is found in significant amounts in the tomato flour seed. Compared to the wheat flours samples, the rape seed flour and the brown flaxseed flours present inferior values. For the pea flour the magnesium value obtained was similar to those reported by Wang N. and Daun J.K. [27].Higher amount of manganese in mustard flour seed was also found by Lopez-Arquello E. et al. [9].

Cobalt is present in the highest amounts in the pea seed flour. Also, compared to the wheat flours samples, yellow flaxseed flour, tomato flour and mustard flour present higher values.

Chrome is present in low amounts in the rapeseed flour, brown flaxseed flour comparatively with the wheat flours samples analyzed. Significant amounts were found in the tomato seed flour, yellow flaxseed flour and mustard flour.

Nickel was found in low amounts in pumpkin seed flour, brown flaxseed flour and mustard flour. Comparatively with the wheat flours analyzed the rest of the type flours recorded higher values for this element.

Selenium was found in the highest amounts in the mustard seed flour, pea flour, pumpkin flour and yellow flaxseed flour. Compared to the wheat flour samples the rest of the types flours presents lower values. A high amount of selenium was also reported in the pumpkin flour seed by the Glew R.H. et al. [5].

Vanadium was presented in high amount in the tomato seed flour and in the mustard seed flour.

Copper, a microelement essential for the human body but which in some concentrations presents a toxic effect is present in low amounts in the brown flaxseed flour, rapeseed flour and mustard flour. Some amount of copper was also reported by the Lomascolo A. et al. [8] in the rapeseed flour and mustard seed flour [9].

Lead, a heavy metal with a toxic character wasn't present in any flour type except mustard seed flour in low amount.

Conclusions

Following the analysis performed in order to achieve the objectives proposed in Annex IV of Contract No. 53 / 10.01.2015 we can formulate the following conclusions:

1. The wheat flour samples that will constitute the base for the composite flour formation presents a low α amylase activity. From the quality point of view it represents flours with a strong and a very good quality for bread making.

2. From the physical-chemical point of view (ash, moisture, acidity) for all the oilseeds and pulses evaluated, the lowest values for moisture content were obtained for the yellow flaxseed flour and pumpkin seed flour, the lowest values for ash content for pea flour and brown flaxseed flour and the lowest values for acidity for the yellow and brown flaxseed flour.

3. From the point of view of the mineral content analyzed by ICP-MS sodium is the best element represented in all types of flours.

4. The tomato seed flour presents the highest amount of magnesium, chrome, manganese, iron, copper zinc compared to the rest of flours types analyzed.

5. The yellow flaxseed flour presents the highest amount of calcium and sodium compared to the rest of flours types analyzed.

6. Compared to the wheat flours samples analyzed all the pulses and oilseeds samples presented with few exceptions higher values for the elements analyzed.

7. Lead, a metal with a toxic character was present only in low amounts in the mustard seed flour.

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