World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:9, No:4, 2015

Determination of Some Chemical Properties of Uncommon Flours

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***Abstract*—**Flours of wheat, chestnut, acorn and lupin were evaluated in relation to phenolic compounds, antioxidant activity, and oxalate content. At the chemical level the results show some variability between samples by type of flour, and the sample of chestnut flour presented the higher value of oxalate (0.00348 mg/100g) when compared to the other samples in the study. Considering the content of phenolic compounds, the sample that stood out was the acorn flour, having a high value of 0.812 g AGE/100 g. All the samples presented intermediate content of antioxidant activity and the sample that showed a slightly higher value was the wheat flour with a value of 0.746 mM TRE/g sample.

***Keywords*—**Wheat, Acorn, Lupine, Chestnut, Flour, Antioxidant properties, Oxalate.

I. Introduction

THE antinutritional factors in flours include phenol, tannin, oxalate, phytate and alkaloids [1]. Oxalic acid is a dicarboxylic acid commonly found in microorganisms, plants and animals. The oxalate can be obtained by eating some foods, as well as through metabolism of ascorbic acid and glyoxylate synthesized by the body.

The excessive intake of oxalate can lead to death in humans due to the formation of oxalosis or calcium oxalate deposits in vital tissues or organs of the body. Individuals with kidney stone problems should moderate the intake of foods that may contain oxalates (40-50 mg/day recommended by [2]. Therefore, the determination of oxalate content in foods is very important for individuals with kidney stone problems [3].

Reactive oxygen species (ROS) are produced when an imbalance occurs between the oxidant and antioxidant defense system of the human body, called "oxidative stress". Free radicals damage different biological molecules, such as DNA, proteins, lipids, and carbohydrates, causing molecular and physiological damage to cells giving rise to disease conditions. Antioxidants have the overall objective of reducing oxidative free radicals and may thus reduce oxidative stress and keep the balance between oxidants and antioxidants in the human body [4]. Antioxidants are molecules that inhibit the oxidation of other molecules, and they are widely used in dietary supplements. Antioxidant activity is the total capacity of antioxidants for eliminating free radicals in the cell and in food [5].

Phenolic compounds are important secondary metabolites that are common in many fresh and processed food products. Phenolic compounds are also largely known to be very effective in combating free radicals, thus their regular consumption has been associated with benefits for human health, such as anti-inflammatory, antimutagenic, and anticancer effects [6].

Wheat is one of the most important food for the world population, in which about 35% of the total world population regularly consume wheat-based foods [7]. Nowadays, there has been growing interest in using chestnut flour for use in bakery because its chemical composition is close to many cereals [8]. The acorn is a fruit which is available on the market with a great variety of industrial products made from its flour or starch, including breads, cakes, soups, jelly, among others [9]. Also, the lupin flour is of enormous importance, being widely used in the food industry to improve the nutritional value of other foods [10]. The nutrition value of these flours could be an opportunity to create new flours to the bakery industry.

The objective of this study was the evaluation of the antioxidant and the antinutritional properties, more precisely the phenolic compounds, antioxidant activity, and oxalate content in wheat, chestnut, acorn and lupin flours.

II. Experimental Procedure

A. Product Preparation and Formulation

Chestnuts (*C. sativa*) were collected from Soutos da Lapa a Protected Designation of Origin region, Portugal. Acorns from *Q. rotundifolia* were collected in Idanha-a-Nova region, Portugal. Three sets of 1 kg each of chestnuts and acorns fruits were randomly harvested at the maturity stage. Samples were stored in dark conditions at 4◦C until *the* experiments began. Before milling, the fruits were prepared as follow: (1) pre-dehydration at 40◦C during 24 h in a FD 115 Binder ventilated drying chamber (with an air flow of 300 m^3/h), (2) peeling and chopping into little pieces, (3) drying in the same equipment at 60◦C, (4) milling in a SK 100 Cross Beater Retsch knife mill to pass a 1 mm sieve. Lupin flour was purchased from local market, Viseu, Portugal. Two wheat flours were also purchased from two different milling factories, with the designation of Ceres and Cerealis. These wheat flours are both used commercially for bakery proposes.

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B. Chemical Analyses

The chemical analysis involved the determination of phenolic compounds, antioxidant activity and oxalates.

To make the extraction for the determination of phenolic compounds and antioxidant activity, three different solvents (ethanol, acetone and methanol) were used in triplicate. The extracts were performed as follows: 5 g flour was added to 50 mL of 50% ethanol in water, 50% acetone in water and 50% methanol in water. Subsequently, the samples with the solvents were subjected to a constant stirring at 100 rpm at a temperature of 50°C for 75 minutes. Finally vacuum filtrations with a paper filter Whatman Nº.1 was made to filter the extracts. At the same time, similar experiences were performed in order to determine the dry extract of all samples. Thus, the extracted material was recovered by vacuum filtration, through a filter Whatman Nº 1, which was previously weighed. The filters with the residues from the extractions were taken to the oven at 105 ° C for 5 hours. Finally, the dry filters with the extract were weighed. Dry extract was calculated as the weight loss percentage of the initial material (1):

$$\%\ \text{Dry extract} = \frac{\text{Dry extract}}{\text{Weight of Sample}}\ \text{x}\ 100 \qquad (1)$$

The determination of total phenolic compounds was estimated according to Folin–Ciocalteu method. The results were based on the gallic acid equivalents (GAE). To a tube were added 125 µL of sample, 750 µL of water, 125 µL Folin–Ciocalteu reagent which was left standing for 6 minutes. Then were added 2 mL of sodium carbonate 5%, and it was placed in the dark at room temperature for 60 minutes. After that the absorbance of the samples was read in a spectrophotometer at a wavelength of 760 nm.

The antioxidant capacity was determined by the method DPPH (2,2-diphenyl-1-picrylhydrazyl). The results were based on the percentage of inhibition, compared with a standard antioxidant (Trolox) in a dose–response curve being expressed in Trolox equivalents. To a tube were added 100 µL of sample and 2 mL of DPPH, previously prepared, and it was placed in the dark at room temperature for 30 minutes. After that the absorbance of the samples was read in a spectrophotometer at a wavelength of 515 nm. The antioxidant activity was expressed as mM Trolox (TE) per gram.

The DPPH radical-scavenging activity of phenolic extract was calculated according to (2):

$$\text{DPPH}(\%)=\left[\frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control}\right] x100 \qquad (2)$$

The oxalate content was determined using the volumetric analysis method. The titration method described by [11] was used to determine the oxalate content. One gram of the sample was weighed into a 100 ml conical flask where 75 ml of 3 mol equi/L H_2SO_4 were added and stirred intermittently with a magnetic stirrer for 1 h. It was then filtered using Whatman No.1 filter paper. From the filtrate, 25 ml was taken and titrated while hot (80-90 ºC) against 0.1 mol equi/L $KMnO_4$ solution until a faint pink color persisted for at least 30 s. The concentration of oxalate in each sample was got from the calculation: 1 ml 0.1 N permanganate = 0.006303 mg oxalate. All procedures were carried out in triplicates.

III. Results and Discussion

A. Dry Extract

The extraction yield is presented in Fig. 1. The results showed that the extraction yield was similar for the three solvents used for each type of flour, regardless of the extraction method. Furthermore, it was found that chestnut flour was the one that gave a lower extraction yield compared to the other flours under study (Lupin, Chestnut, Acorn, Ceres Wheat and Cerealis Wheat), lower than 60%.

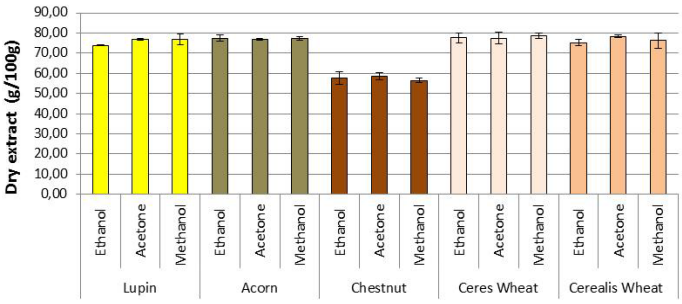

Fig. 1 Dry extract for extractions performed with different solvents in lupin, acorn, chestnut and wheat (Ceres and Cerealis) flours

B. Phenolic Compounds

The total phenolic compounds are shown in Fig. 2. The data make it possible to classify these flours as low polyphenol content. It was also observed that the acorn flour stood out with a higher content in this type of compounds, for all methods of extraction. Furthermore, in the extract acetone/water it was observed the highest value (0.812 ± 0.173 g GAE/100 g).

Generally, the extract that showed the lower content in phenolic compounds was the methanol/water, and the wheat flour type Ceres presented the lowest value (0.088 ± 0.008 g GAE/100 g).

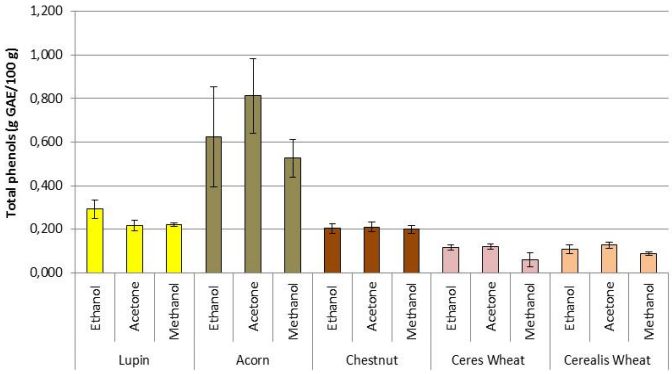

Fig. 2 Phenolic compounds in lupin, acorn, chestnut and wheat (Ceres and Cerealis) flours

The encountered values are similar to the results found by other authors for lupin flour [10] and chestnut flours [12], but for acorn flours the results are higher than those found by [13]. Both wheat flours presented lower content of phenolic compounds when compared with the results found by [4]. These differences could be due to the different species and varieties under study and also due to the extraction solvent used, as well as to their concentration and extraction time.

C. Antioxidant Activity

The results for antioxidant activity revealed that only three types of flour (acorn, chestnut and wheat Ceres) from the five samples studied showed antioxidant activity (Fig. 3).

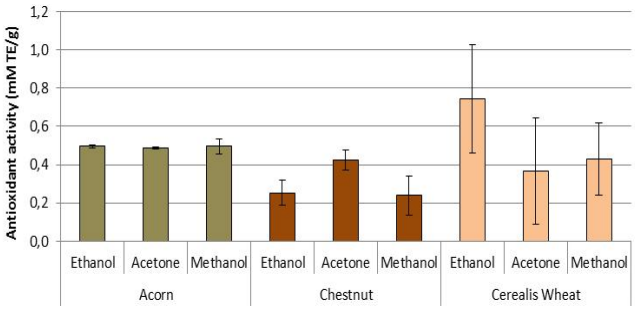

Fig. 3 Antioxidant activity in acorn, chestnut and Cerealis flours

The sample wheat Ceres with extraction ethanol/water presented the highest value (0.746 ± 0.283 mM TRE/g), whereas the type of flour that showed the lowest antioxidant activity was the chestnut flour in the ethanol/water and methanol/water extractions with values of 0.252 and 0.239 ± 0.102 mM TRE/ g, respectively.

The acorn flour presented a more or less constant antioxidant activity independently of the solvent used for extraction. Moreover, in the study performed by [13] with methanol extraction in acorn flour, it was reported a value of 2.44 ± 0.03 g TRE/100g in the antioxidant activity, clearly higher than the values found in our study (0.496 ± 0.039 mM TRE / 100g). Higher values were also found by [10]. But, for chestnut flours and wheat flour; [11] and [4] found lower values, respectively. However, in a study to determine the chestnut flour antioxidant activity; [14] found that these values were 3.24 mmol TRE /g and 2.95 mmol TRE/g methanol and ethanol extracts, respectively. These differences could be due to the extraction method used, as well as the varieties of acorn, chestnut and wheat flours studied.

D. Oxalate Contents

The oxalate contents are shown in Fig. 4. The greatest value was found in chestnut flour, with a value of 0.00348 ± 0.01 g/100g. On the other hand, lupin flour presented the lowest value (0.0012± 0.49 g/100g), showing the other flours similar values.

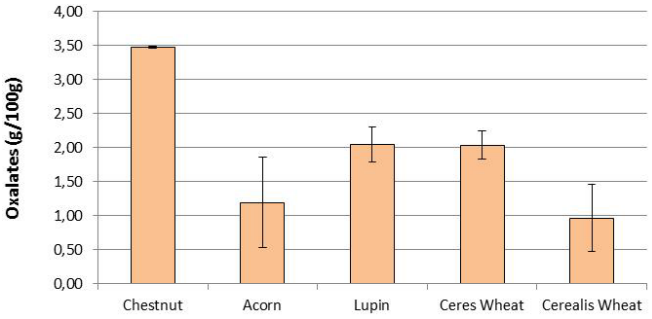

Fig. 4 Oxalate content in lupin, acorn, chestnut and wheat (Ceres and Cerealis) flours

The values reported in this study were low when compared to other flours [15], other cereals, legumes and nuts [16]. The differences found may be because of different analytical methods used and/or sampling sources and regions.

IV. Conclusion

There were no significant differences in the estimation of dry extract using different types of extraction methods, and this tendency was observed for all samples.

Samples of acorn flour showed the highest values of total phenols, and particularly for the extraction with aqueous acetone 50%.

The antioxidant activity was greater in the sample of wheat Cerealis, when the extraction was done with ethanol 50%.

The content of oxalates was higher for the chestnut flour, but they are quite low when compared with other values reported by other authors.

Generally, the lupin, chestnut, acorn, and wheat flours studied presented good functional properties, considering total phenols content and antioxidant activity. Furthermore, the results of the study revealed that these flours have a great potential to be used in food industry either for the purpose of formulating new products or for the replacement in food products made from various conventional flour sources.

Acknowledgment

The authors thank CI&DETS Research Centre and Polytechnic Institute of Viseu for financial support.

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