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Tunisian acorn (*Quercus Ilex*): An alternative source of flour in cereal and gluten free products

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Abstract.
In the Mediterranean region, especially in the zones where oak trees grow, acorns were widely used as animal feed for thousand years. Nowadays, acorns are traditionally used in bread and cake recipes. Thus, the aim of the present work was to highlight the interest of valorizing Tunisian acorn *(Quercus ilex)* for the preparation of acorn flour as an alternative source of common wheat flour in order to produce cereal and gluten free products. In this regard, the chemical composition of acorn flour was studied. The total content of phenolics (TPC), tannins (TCT), flavonoids (TFC) and the HPLC profiles of the main phenolic acids present in acorn flour were also determined as well as the composition of extracted acorn oil using gaz chromatography. After that, the extracted acorn flour was mixed with semolina, wheat flour and other gluten free flours to determine the effect of its incorporation during the preparation of bread, pasta, muffin, cookies and pizza. Results showed that acorn flour moisture was about 14.2%, whereas protein, ash, fat and carbohydrates contents were 8.5%, 1.02%, 8.5% and 66.78%, respectively. For TPC, TFC and TCT, their contents in acorn flour were respectively, 20.55 mg GAE/g DW, 9.05 mg CE/g DW and 1.21 mg GAE/g DW. In addition, we noted that the main phenolic acids were protocatechuic acid, ferulic acid and gallic acid. Besides, oleic, linoleic, and palmitic were shown to be the most predominant fatty acids in acorn oil. Then, the efficiency of the replacement of wheat flour by acorn flour alone or in combination with other gluten free flours was evaluated by pilot productions of bread, pasta, muffin, cookies and pizza. Results showed that the nutritional and rheological qualities of formulated products were improved with acceptable sensory properties.

Keywords: Acorn flour, gluten free, cereals, nutritional, rheological, phenolics.

Introduction

In some Mediterranean countries, acorns are consumed raw, roasted, or boiled and have been used for the preparation of several products such as bread, cake, coffee and beverages (Polimac & Komlenić, 2015). Nowadays, acorn fruits are being used as an alternative ingredient for flour production and use in food industry. The extracted acorn flour does not contain gluten, therefore it can be used as an alternative to gluten-free flour for the production of cereal products such as bread or pastry products (Korus et al., 2017; Torabi et al., 2020), proposed for people suffering from celiac disease. Several study revealed that the number of people suffering from gluten intolerance-related diseases is increasing (King et al., 2020) in many developing countries including Tunisia. Patient's celiac diseases make up approximately 0.6-1% of the world population (Fasano et al., 2003). To the best of our knowledge, there are a small number of studies about the use of acorn flour as major ingredient in food industry. The aim of this work was to characterize the main components of acorn flour, including its chemical composition, fatty acids and phenolic profiles. The effect of acorn flour addition at different proportion in cereals and gluten free products qualities was also investigated.

Material and Methods

1.1. Acorn flour preparation

Quercus ilex acorns fruits were manually collected from Jendouba region (Ain Drahem site), in the North West of Tunisia, during the month of December 2019. In order to eliminate the anti-nutritive compounds (tannins) from acorn fruits, the hot-water soaking method was used. After tannin extraction, acorn fruits were hand-peeled, dried at 40°C for 3 days and then milled into flour in a blender. The acorn flour was stored in glass flasks at about 4°C for further analysis and utilization.

1.2. Chemical composition

Acorn flour moisture, ash, fat and protein contents were determined according to AOAC Methods (AOAC, 1990). Total carbohydrates were calculated by difference (100 - sum of (% protein, % fat, % ash and % moisture)). Total polyphenols content (TPC) was determined by the Folin–Ciocalteu spectrophotometric method (UV–VIS) as described by Dewanto et al, (2002). For the determination of total flavonoids content (TFC), 250 µL of the methanolic extract was combined with 75 µL $NaNO_2$ (5%) (Dewanto et al., 2002). The protocol followed in the extraction of condensed tannins content (CTC) was that recommended by Sun et al. (1998). The phenolic acids compounds in acorn flour were determined using RP–HPLC equipment (Bourgou et al., 2008). The fatty acid composition of acorn lipid fraction was determined according to the EEC methods (EEC, 1991). The rheological properties of bread dough were measured by Chopin alveograph.

1.3. Cereal products preparation

For the bread making process, straight dough method No.10-09.01 (AACC, 1999) was used. However, the muffin recipe was prepared according to the method of Masmoudi et al. (2020). Pasta and couscous formulations were prepared according to the AACC 66-41 method (AACC, 2000). Pizza

dough was prepared with 63.15% wheat flour, 33.41% water, 1.84% sunflower oil, 1.47 g NaCl and a low amount of fresh yeast (0.13%).

1.4. Data analysis

All analysis was possessed in three replications, and the average values were performed by variance analysis (ANOVA) using STATISTICA software.

Results and discussions

1.1 Acorn flour characterization

Results of the chemical composition of acorn flour are presented in Table 1. The acorn flour presented a moisture content of 14.2% which was within the recommended moisture contents (<15%) for safe storage, minimal microbial growth and chemical deterioration, leading longer shelf life. Protein, ash and carbohydrates contents of acorn flour were 8.5, 1.02 and 66.78 %, respectively. The proteins found in acorn flour do not contain gluten. Silva et al., (2016) reported that the acorn flour is a high carbohydrate product (about 84%) and the dominant carbohydrate is starch (ranging from 31.4 to 49.0%). Fat content in Tunisian acorn flour was lower than that reported by Zarroug et al., (2021), but higher than those found by Masmoudi et al., (2020) for *Quercus suber* L. and Li et al., (2015) for *Quercus lobata*. The observed difference in chemical composition of acorn flour was depending on the location and the used species. In acorn flour the TPC, TFC and CTC were found to be about 20.55 mg GAE/100 g DW, 9.05 mg QE/100 g DW and 1.21 mg QE/100 g DW, respectively. Our results of TPC and TCT were higher than those obtained by Custódio et al. (2013) on acorns collected from Portugal. TPC result was in accordance with that registered by Masmoudi et al. (2020) on *Quercus suber*. L (33.11%). The presence of tannins in acorn flour causes its bitter taste and demonstrates its anti-nutritional properties in higher concentrations. Concerning the phenolic acid contents, acorn flour extract contained 1.4 mg/ml of protocatechuic acid, 1.2 mg/ml of ferulic acid and 1.1 mg/ml of gallic acid. Our finding were in accordance to those found by Santos et al., (2010), who revealed that the most abundant phenol in *Quercus suber* species were gallic and protocatechuic acids. As illustrated in Table 1, the most abundant saturated fatty acid in acorn oil was palmitic acid with value of 11.31%. Acorn oil contained also about 67.5% of oleic acid (C18:1) and 16.5% of linoleic acid (18:2 n6). Since the acorn oil was rich in both oleic and linoleic acids, it might be considered healthier for the human diet. It was worth mentioning that plant oils containing relatively low concentrations of omega-6, and higher levels of monounsaturated fatty acids (mainly oleic acid) may contribute to the lower rate of CHD and a nutritional perspective (Ryan et al., (2007). Based on the above-mentioned findings, it can be concluded that acorn flour could be used as functional ingredient in gluten-free products since it could improve the nutritional value of the final product with acceptable sensory properties.

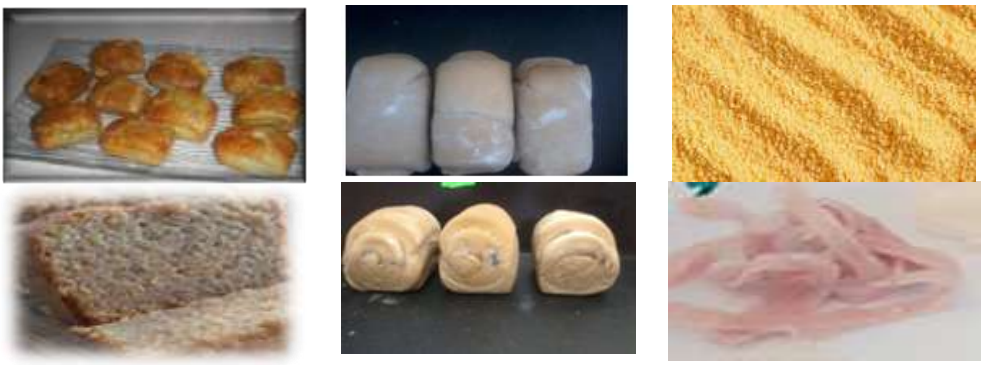
Table 1. Chemical composition of acorn *(Quercus Ilex L.*) flour

Parameters	Values
Moisture (%)	14.2± 0.01
Fat (%)	8.5±0.02
Ash (%)	1.02±0.01
Protein (%)	8.5±0.03
Carbohydrates (%)	66.78± 0.05
TPC (mg GAE /g)	20.55± 0.02
TFC (mg QE /g)	9.05±0.01
CTC (mg QE /g)	1.21±0.04
Fatty acids (% total fatty acid)	
Palmitic acid (C16:0)	11.31±0.12
Oleic acid (C18:1)	67.5±0.13
Linoleic acid (C18:2)	16.5±0.05
Phenolic acids (mg/ml)	
Protocatechuic acid	1.4±0.01
Ferulic acid	1.2±0.02
Gallic acid	1.1±0.21

TPC: Total polyphenols content, TFC: Total flavonoids content, CTC: Condensed tannins content.

1.2 Acorn flour in cereal products

To enhance the nutritional value of the end-products, acorn flour can be used as an alternative raw material in many cereal products (Fig.1). However, the effect of acorn flour addition on the cereal products quality should be made. Because acorn flour is gluten-free, a deterioration of the physical characteristics and rheological properties of the bread and muffin products was observed at high acorn flour levels addition. Similar results were reported by Korus et al. (2015) and Ajo, (2018) in which acorn flour was incorporated at different proportions into traditional bread. Masmoudi et al. (2020) studied also the effect of acorn (*Quercus suber* L.) flour addition on muffin quality. Concerning the incorporation of acorn flour in durum wheat semolina with proportion of 30%, results (data not shown) showed a decrease in protein content and an increase in ash content. In addition, the incorporation of acorn flour in pasta sample showed an increase of the elasticity parameter, but did not affect its viscoelastic properties. However, cooked macaroni has lower cooking loss, medium swelling index and good firmness.



Muffin with 1.5% acorn flour **Bread with 10% of acorn flour** **Couscous and pasta with 30% acorn flour**

Fig. 1. Some enriched cereal products with acorn flour

1.3 Acorn flour in gluten free products

The addition of acorn flour has a positive effect on the nutritional value of cookies and pizza. In gluten-free pizza and cookies enriched with acorn flour, an increased content of fiber and minerals (ash) was observed compared to control pizza sample. In addition, an increase in the antioxidant activity in pizza was also noted with the addition of acorn flour compared to control sample (data not shown).

Gluten free Pizza (30%) **Gluten free cookies (40%)**

Fig. 2. Gluten free pizza enriched with acorn flour

Conclusions

This study revealed that acorn flour had various benefits. This gluten free flour was characterized by an interesting nutritional value with its high contents of starch, essential fatty acids and polyphenols. This could justify its use as functional ingredient for edible purposes, especially for the formulation of cereals and gluten-free products. The characteristics of bread, muffin, pasta and pizza are affected by the incorporation of high proportions of acorn flour. Thus, at optimized concentrations, acorn

flour improved the technological and nutritional properties of the functional final cereal and gluten free products.

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