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Effect of acorn flour on the physico-chemical and sensory properties of biscuits

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ABSTRACT

The aim of the work was to explore the feasibility of using acorn flour as a novel and healthy ingredient in biscuits. The physico-chemical characteristics of acorn flour obtained from three different *Quercus* species were compared. Acorns of *Quercus coccifera* L. were the most antioxidant and were therefore used for preparing biscuits at two levels of addition, 30 and 60 g 100 g^{-1} on wheat flour basis. The physico-chemical and sensory characteristics of the obtained biscuits were then assessed. Acorn-added biscuits showed significantly ($p < 0.05$) higher content of phenolics, antioxidant activity and oxidative stability than control biscuits, prepared without acorn flour. These features improved as the level of acorn flour increased. As for appearance, the acorn-added biscuits were darker, larger, more voluminous and more friable than control biscuits. Higher levels of fermentative alcohols and esters, as well as Maillard reaction volatile compounds (particularly furans), were observed in the acorn-added biscuits.

1. Introduction

Throughout the Mediterranean basin, the acorns, the fruits of the oak, have been used for centuries for edible purposes as a staple food (Hoeche et al., 2014). Collected from wild trees, and milled to flour, acorns were used for the production of traditional flat breads such as *pan 'ispeli* (Pinna, 2013), *talo* and *ogi* (Ayerdi et al., 2016) in Italy (Sardinia region) and Spain (Basque region), respectively. The production of a coffee substitute was another use of acorn in the same area (Pignone and Laghetti, 2010). Being a fundamental high-energy nutritional resource in subsistence economies and during times of shortage, acorns were considered a food for the poor, and were abandoned when the economic conditions improved. Therefore, the culture of using acorns in human nutrition practically disappeared after the Second World War, and these fruits are now barely used for feeding pigs (Pignone and Laghetti, 2010).

Most acorn types, indeed, show a high content of tannins, which are astringent and behave as anti-nutrients (Papoti et al., 2018). In the past, local populations identified and selected the trees bearing sweeter acorns, directly used for edible purposes, whereas the excessively astringent or even bitter acorns, which resulted unpalatable, were subjected to appropriate heat treatments and leaching (Papoti et al., 2018; Pignone and Laghetti, 2010; Rakić et al., 2007) or detoxification with clay (Johns and Duquette, 1991). However, nowadays the knowledge related to the use of acorns as food remains only in the memories of the elderly and is being lost.

In the context of the increased awareness of the nutritional properties of all nuts, acorns have recently become the object of renewed interest being nutritious fruits, with good contents of calcium, iron, magnesium, potassium, phosphorus, as well as A and E vitamins (Li et al., 2015). The lipid fraction is characterized by high levels of unsaturated fatty acids (Al-Rousan et al., 2013; Özcan, 2007). In addition, recent studies highlighted that acorns have a rich phytochemical potential, mostly represented by phenolic compounds (Papoti et al., 2018; Vinha et al., 2016), and display interesting biological activities (Vinha et al., 2016) such as anti-inflammatory and anti-asthma effect (Moon et al., 2013), and a role in the prevention of obesity, dementia and liver diseases (Kang et al., 2004; Lee et al., 2005).

Therefore, a "new age" is going to start for the use of acorns and for

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their reintegration into the human diet (Vinha et al., 2016). The extraction of the lipid fraction has been proposed in consideration of the good level of antioxidants such as tocopherols, carotenoids and phenolics observed in acorn oil (Makhlouf et al., 2018). Furthermore, due to the absence of gluten, acorn flour is being proposed as a new ingredient for producing gluten-free foods, such as bread and biscuits (Korus et al., 2015; Korus et al., 2017).

The use of acorn flour has been proposed also in the production of wheat- and barley-based bread (Švec et al., 2018) and sponge cakes (Molavi et al., 2015). However, the addition of acorn flour worsened the sensory attributes by increasing density, hardness and gumminess of sponge cakes and decreasing the specific volume of bread. These findings were related to a negative effect on the viscoelastic properties of dough, mostly due to the absence of gluten and presence of fiber in the added flour. We hypothesize that biscuits, on the contrary, could tolerate the addition of acorn flour better than bread and sponge cakes, because their characteristic friability requires a weak gluten network. Moreover, biscuits are popular, daily consumed, bakery items and have long shelf-life, all features that make them suitable for the addition of functional ingredients.

In this frame, the aim of the work was to verify our hypothesis, i.e. to explore the feasibility of using acorn flour as a novel and healthy ingredient in wheat-based biscuits. After comparing the physico-chemical characteristics of acorn flour obtained from three different *Quercus* species, the most antioxidant flour was used at two levels of addition (30 and 60 g 100 g^{-1} on wheat flour basis) and its effect on the physico-chemical and sensory characteristics of biscuits was assessed.

2. Materials and methods

2.1. Preparation of acorn flour

Mature acorn fruits were manually harvested from three *Quercus* species, namely *Quercus ilex* L., *Quercus suber* L., and *Quercus coccifera* L. (indicated as QI, QS and QC, respectively) in a forest located in Eastern Algeria (Oum El Bouaghi region) in November 2017. The acorns were shelled and ground using an electric grinder (Moulinex, Groupe SEB, Écully, France) to obtain wholemeal flour whose particle size, determined by a vibratory sieve shaker (Retsch AS200, Haan, Germany), is shown in Table 1. The moisture content of flours accounted for 24.3 ± 0.5, 20.9 ± 0.1 and 22.7 ± 0.7 g 100 g^{-1} for QI, QS and QC, respectively. The obtained flours were slightly sweet, not bitter at all and showed limited levels of astringency (being QC slightly more astringent that QS and QI), as assessed by tasting them. Therefore, the flours did not need any debittering pretreatment and were directly used for preparing biscuits as they were. All flour samples were stored at -20 °C until being used.

2.2. Preparation of biscuits

Ingredients for the preparation of biscuits were wholemeal flour of acorns (*Q. coccifera*) prepared as above described, refined flour of soft wheat (*Triticum aestivum* L.) having protein = 10.8 g 100 g^{-1}, fat = 1.1 g 100 g^{-1}, carbohydrates = 72.7 g 100 g^{-1}, fiber = 2.2 g 100 g^{-1}, ash = 0.5 g 100 g^{-1}, moisture = 12.7 g 100 g^{-1} (Molini Amoruso, Cerignola, Italy), sucrose (Eridania, Bologna, Italy), extra-virgin olive oil (Olearia de Santis, Bitonto, Italy) and sodium bicarbonate (Solvay, Bollate, Italy). Biscuits enriched of acorn flour at the level of 30 and 60 g 100 g^{-1} on wheat flour basis (coded Q30 and Q60, respectively) and control biscuits, made up of soft wheat refined flour, without acorn flour, were prepared. Preliminary trials were made to establish empirically the amount of water to be used for obtaining a similar dough consistency in all three types of biscuits. The amount of water needed was very similar in control and acorn-enriched dough. In fact, wholemeal acorn flour contained more fibers – which absorb a relevant amount of water – than refined wheat flour, therefore potentially requiring more water, however acorn flour was much moister than wheat flour, then reducing the amount of water to be actually added. Therefore the same amount of water was used for all biscuit types. The process consisted of: i) mixing flour (wheat flour 100%, or a blend of wheat flour and 30 or 60 g 100 g^{-1} of acorn flour) (100 g), sugar (35 g), extra virgin olive oil (14 g) and sodium bicarbonate (0.5 g) by a spiral kneader (mod. KM 398 CB, Bomann, Kempen, Germany) for 3 min; ii) adding water (25 mL for all biscuit types), and kneading again for 10 min; iii) sheeting the dough by a rolling pin to the thickness of 6 mm, and shaping as round biscuits by means of a biscuit cutter (4.5 cm diameter); iv) transferring the shaped pieces of dough in a metal tray by mixing all three types of biscuits according to a Latin-square design, to minimize any effect of tray location during subsequent baking; v) baking in a preheated electric oven (Smeg SI 850 RA-5 oven, Smeg S.p.A., Guastalla, Italy) for 12 min at 160 °C. The baking temperature was selected according to other studies (Arshad et al., 2007; Pasqualone et al., 2015; Sharma and Zhou, 2011; Supski, 2006), then cooking time was set up by means of preliminary trials in order to obtain perfectly cooked biscuits without burns. Two independent replicate baking experiments were carried out. Biscuits were finely crushed for all the analyses with the exception of colorimetric, sensory, and textural determinations.

Table 1
Particle size distribution of acorn flour obtained from three different *Quercus* species (*Quercus ilex* L., *Quercus suber* L. and *Quercus coccifera* L., indicated as QI, QS and QC, respectively).

Type of flour	Particle size class (g 100 g^{-1})					
	<106 μm	106–150 μm	150–212 μm	212–300 μm	300–425 μm	>425 μm
QI	3.13	4.35	7.18	15.06	27.63	42.65
QS	2.19	4.81	6.11	17.31	26.37	43.21
QC	1.84	3.76	6.63	17.34	30.90	39.53

2.3. Basic physico-chemical determinations

Moisture content was determined at 105 °C by means of an automatic moisture analyzer (Radwag Wagi Elektroniczne, Radom, Poland). Water activity (a_w) was determined by using the water activity meter Aqua Lab 4TE (Meter Group Inc., Pullman, WA, USA) according to the manufacturers' instructions. Protein content (N × 5.7) was analyzed according to the AACC approved method 46-11.02 (AACC, 2000), whereas fat was extracted and determined by Soxhlet apparatus using diethyl ether as solvent, and total dietary fiber was determined by the enzymatic-gravimetric procedure (AOAC, 1995). Carbohydrates were calculated by difference.

Colorimetric evaluations of red index (a^*), yellow index (b^*) and brown index (BI, defined as 100-L^*) were carried out under D65 illuminant by using a spectro-colorimeter CM-700d (Konica Minolta Sensing, Osaka, Japan) equipped with a pulsed xenon lamp and granular materials attachment CR-A50 (Konica Minolta Sensing, Osaka, Japan).

Chemical determinations were carried out in triplicate, whereas colorimetric evaluations were made with five replications.

2.4. Extraction of phenolic and flavonoid compounds

Five g of sample (acorn flour or powdered biscuits) were mixed with 50 mL of ethanol/water 30:70 (v/v). The mixture was subjected for 35 min to ultrasound treatment (CEIA, Viciomaggio, Italy) at room temperature. The obtained extracts were centrifuged at 6000 × *g* for 10 min at 4 °C (SL 16R Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA, USA), then were filtered with nylon filters having pores of 0.45 μm (Sigma Aldrich).

2.5. Determination of phenolic compounds

The content of total phenolic compounds (TPC) was determined as in

Pasqualone et al. (2016). Each flour extract (100 μL) was mixed with 100 μL of Folin-Ciocalteu reagent. After 4 min, the mixture was added of 800 μL of 5% Na_2CO_3 and incubated for 20 min in a water bath at 40 °C. The absorbance was then measured at 750 nm by an Agilent Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, USA). The total phenolic content was expressed as mg of gallic acid equivalents per 100 g of dry matter based on a gallic acid standard curve made in the same conditions ($R^2 = 0.997$). The analysis was carried out in triplicate.

2.6. Determination of flavonoid compounds

The content of total flavonoid compounds was determined as reported in Makhlouf et al. (2018). Each flour extract (500 μL) was mixed with 2 mL of distilled water and 150 μL of $NaNO_2$ solution (5% w/v). After 6 min, 150 μL of a 10% solution of $AlCl_3$ was added and allowed to stand further 6 min; thereafter, NaOH solution (2 mL, 1M) was added to the mixture. Then, distilled water was added to bring the final volume to 5 mL. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was read at 510 nm. The results were expressed as mg of catechin equivalent per 100 g of dry matter based on a catechin standard curve made in the same conditions ($R^2 = 0.999$). The analysis was carried out in triplicate.

2.7. Determination of antioxidant activity

The antioxidant activity, compared with the standard antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), was evaluated by the assay based on using the radical of the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), as described in Pasqualone et al. (2014a; 2017). Briefly, 50 μL of each extract were added to 950 μL of ABTS reagent previously prepared. After 8 min, the decrease of absorbance was measured at 734 nm. A calibration curve of Trolox was used, and the results were expressed as μmol Trolox equivalents (TE) per g of dry matter.

The antioxidant activity of the extracts was analyzed also by means of the assay based on evaluating the capacity to scavenge the 1,1-diphenyl 2-picrylhydrazyl radical (DPPH). In cuvettes for spectrophotometry, 50 μL of each sample were added to 950 μL of DPPH solution (0.08 mM in ethanol). After 30 min in the dark, the decrease of absorbance was read at 517 nm. The results were expressed as μmol TE per g of dry matter.

The analyses were carried out in triplicate.

2.8. Determination of induction time

The oxidative stability of biscuits – namely, the oxidative stability of their lipid fraction – was evaluated by measuring the induction time (IT) by an automatic testing device (Rapidoxy, Anton Paar, Blankenfelde-Mahlow, Germany). The instrument induces a forced oxidation of the sample (3 g) by subjecting it to conditions of high temperature and high oxygen pressure. According to manufacturer's instructions, IT was defined as the time needed for a 10% drop of the oxygen pressure under the following conditions: T = 140 °C, initial O_2 pressure = 700 kPa. The analysis was carried out in triplicate.

2.9. Determination of volatile compounds

Volatile compounds of biscuits were determined by headspace solid phase micro-extraction (HS-SPME) coupled with gas-chromatography/mass spectrometry (GC/MS). After weighting the samples (500 ± 0.05 mg) in 20 mL vials, the extraction of volatile compounds was carried out by exposing a 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) in the headspace of the sample at 40 °C for 15 min. The fiber was then desorbed in the GC injector (2 min) and the volatile compounds were separated by using an Agilent 6850 gas-chromatograph equipped with an Agilent 5975 mass-spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) and a HP-Innowax (Agilent Technologies Inc., Santa Clara, CA, USA) polar capillary column (60 m length × 0.25 mm i.d. × 0.25 μm film thickness), in the conditions reported in Pasqualone et al. (2014a). Peak identification was performed by computer matching with the reference mass spectra of National Institute of Standards and Technology (NIST) and Wiley libraries. The results were expressed as percentage of the integrated area on the total area. The analysis was carried out in triplicate.

2.10. Determination of dimensional indices of biscuits

Diameter (D) and thickness (T) of biscuits before and after baking were determined by a caliper and used to calculate surface (S) and volume (V). The percentage increases in D, T, S and V due to baking were calculated as follows:

$$\%\ \text{of increase in } D\ (or\ T,\ S,\ V) = \frac{D\ (or\ T,\ S,\ V)\text{after baking} - D\ (or\ T,\ S,\ V)\text{ before baking}}{D\ (or\ T,\ S,\ V)\text{before baking}} \times 100$$

Spread factor was calculated as the ratio between diameter and thickness of baked biscuits, according to the AACC method 10–50.05 (AACC, 2000). Six biscuits were analyzed for each parameter.

2.11. Texture profile analysis of biscuits

The textural properties of biscuits, in terms of fracture force (N mm^{-2}), were determined by a 3-point bending test as in Giarnetti et al. (2015). A Texture Analyzer (Z1.0 TN, Zwick GmbH & Co. KG, Ulm, Germany), equipped with a 1000 N load-cell, was used. The biscuits were placed on supports with their top surface down. The distance between the support bars was 4 cm. The downward movement of the probe, set at a speed of 5 mm min^{-1}, was continued until the biscuit was broken. At least eight biscuits were tested per each type.

2.12. Sensory evaluation of biscuits

Quantitative Descriptive Sensory Analysis of biscuits was performed by a trained panel consisting of 8 members in the conditions described in a previous work (Pasqualone et al., 2011). The list of sensory terms included descriptors of visual-tactile characteristics (color, friability), odor (bran-like, caramel), taste (sweet, bitter) and texture attributes perceived in the mouth (dryness, astringency, graininess). The descriptors were rated on an anchored line scale that provided a 0–9 score range (0 = minimum; 9 = maximum intensity), as reported in Table 2. Three sensory analyses were carried out.

2.13. Statistical analysis

Statistical analysis was carried out using XLSTAT software (Addinsoft SARL, New York, NY, USA). Significant differences were determined at $p \leq 0.05$ by one-way analysis of variance (ANOVA) followed by Tukey's HSD test.

Table 2
Descriptive terms used for sensory profiling of biscuits.

Descriptor	Definition	Scale anchors	
		min (0)	max (9)
Visual-tactile characteristics			
Color	Color of biscuit surface	Beige	Dark brown
Friability	The way the biscuit fractures, when broken by fingers	Very tough, it breaks with difficulty	Very friable and crumbly, it breaks easily
Odor			
Bran-like odor	Smell reminiscent of bran and wholemeal products	Absent	Very intense
Caramel odor	Typical odor associated with caramel	Absent	Very intense
Taste			
Sweet	Basic taste produced by sucrose	Absent	Very intense
Bitter	Basic taste produced by caffeine	Absent	Very intense
Texture attributes perceived during chewing			
Dryness	Dryness perceived at the surface of biscuit	Moist	Very dry
Graininess	Graininess perceived at the end of chewing	Not grainy, leaving finely sized crumbs	Very grainy, leaving differently sized crumbs
Astringency	The shrinking sensation induced by unripe nuts	Absent	Very intense

Table 3
Physico-chemical characteristics of acorn flour obtained from three different *Quercus* species (*Quercus ilex* L., *Quercus suber* L. and *Quercus coccifera* L., indicated as QI, QS and QC, respectively). Values are expressed as mean of three replications ±SD.

Parameter	QI	QS	QC
Protein (g 100 g^{-1} d.m.)	3.06 ± 0.18^{b}	3.28 ± 0.05^{b}	4.45 ± 0.45^{a}
Carbohydrates (g 100 g^{-1} d.m.)	77.91 ± 1.21^{a}	80.45 ± 2.26^{a}	79.57 ± 0.47^{a}
Fat (g 100 g^{-1} d.m.)	7.78 ± 0.07^{c}	8.55 ± 0.07^{b}	8.88 ± 0.01^{a}
Fiber (g 100 g^{-1} d.m.)	11.24 ± 1.09^{a}	7.72 ± 2.27^{b}	7.08 ± 0.57^{b}
TPC (mg GAE 100 g^{-1})	691.1 ± 5.9^{c}	785.9 ± 7.6^{b}	1017.4 ± 22.4^{a}
TFC (mg CAE 100 g^{-1})	102.5 ± 8.3^{b}	63.3 ± 9.7^{c}	119.4 ± 6.1^{a}
AA by DPPH assay (μmol TE g^{-1})	25.53 ± 0.18^{c}	33.06 ± 0.58^{b}	42.56 ± 2.47^{a}
AA by ABTS assay (μmol TE g^{-1})	17.20 ± 1.15^{c}	31.96 ± 0.71^{b}	35.21 ± 0.87^{a}
Colorimetric data			
Red index (a^{*})	5.10 ± 0.03^{a}	4.92 ± 0.01^{c}	5.01 ± 0.01^{b}
Yellow index (b^{*})	21.73 ± 0.09^{b}	22.18 ± 0.03^{a}	20.74 ± 0.06^{c}
Brown index (100 - L^{*})	37.92 ± 0.07^{a}	37.55 ± 0.01^{b}	36.38 ± 0.07^{c}

TPC = total phenolic compounds; TFC = total flavonoid compounds; AA = antioxidant activity; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical; ABTS = 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid); GAE = gallic acid equivalents; CAE = catechin equivalents; TE = Trolox equivalents.
Different letters in row indicate significant difference at $p \leq 0.05$.

3. Results and discussion

3.1. Characteristics of acorn flour

The chemical analyses carried out on the acorn flour obtained from three different *Quercus* species, namely *Q. ilex*, *Q. suber* and *Q. coccifera*, confirmed that acorns are a highly energetic food, rich in carbohydrates, fats, and with relatively low contents of proteins, although the latter two nutrients are more largely affected by environmental and genetic factors (Vinha et al., 2016) (Table 3). Significant differences were observed among species. The highest fiber content was observed in the QI acorn flour, whereas, conversely, the QC flour was the richest in protein and fat. Fat content was abundant in all the flours (7.78–8.88 g 100 g^{-1} d.m.), but it has to be considered that acorn fat has a nutritionally favorable fatty acid profile, as previously reported (Al-Rousan et al., 2013; Özcan, 2007).

The examined flours showed also relevant contents of phenolic and flavonoid compounds, which were significantly more abundant in QC. These findings determined a higher antioxidant activity in the QC flour which, therefore, was selected for enriching the biscuits in bioactive compounds. Overall, the TPC levels of the examined flours were much lower than the range 7.3–11.7 g 100 g^{-1} TPC, reported for extremely astringent acorns (Shimada and Saitoh, 2003). In the present study all the flours were slightly sweet, not bitter at all and showed limited levels of astringency, as assessed by tasting them (*data not shown*), although other authors reported that acorns of QC and QS are very bitter and unpalatable (Ayerdi et al., 2016). Environmental effects and/or genetic causes could not be excluded, being our sampling area (Eastern Algeria) far from that of the study of Ayerdi et al. (2016), i.e. Spain. In addition, differences in the ripening degree of acorns could also occur among different studies, being unripe acorns more bitter and astringent than mature acorns.

As regards their visual appearance, all the acorn flours examined were yellow-brownish. QC flour, however, was lighter in color than the other two flour types.

Table 4
Physico-chemical and sensory characteristics of biscuits enriched of increasing levels of acorn flour (*Quercus coccifera* L.). Values are expressed as mean of three replications ±SD. Control = biscuits without acorn flour; Q30 and Q60 are biscuits prepared with 30 and 60 g 100 g^{-1} acorn flour on wheat flour basis, respectively.

Parameter	Control	Q30	Q60
a_w	0.28 ± 0.01^{a}	0.48 ± 0.01^{c}	0.62 ± 0.02^{b}
Bioactive compounds and antioxidant properties			
TPC (mg GAE 100 g^{-1})	28.19 ± 0.76^{c}	142.97 ± 8.8^{b}	291.91 ± 2.5^{a}
TFC (mg CAE 100 g^{-1})	1.31 ± 0.83^{c}	9.63 ± 0.63^{b}	27.77 ± 1.41^{a}
AA by DPPH assay (μmol TE g^{-1})	0.55 ± 0.42^{c}	3.43 ± 0.02^{b}	8.04 ± 0.26^{a}
AA by ABTS assay (μmol TE g^{-1})	0.23 ± 0.01^{c}	3.17 ± 0.08^{b}	9.15 ± 0.37^{a}
Induction time (min)	242.2 ± 5.6^{c}	291.9 ± 3.9^{b}	392.9 ± 4.8^{a}
Colorimetric data			
Red index (a^{*})	0.17 ± 0.31^{c}	8.32 ± 0.41^{b}	11.04 ± 0.56^{a}
Yellow index (b^{*})	26.23 ± 1.14^{a}	24.87 ± 0.36^{b}	23.66 ± 0.64^{b}
Brown index (100 - L^{*})	20.76 ± 1.96^{c}	46.98 ± 1.26^{b}	55.89 ± 2.18^{a}
Dimensional parameters after baking			
Diameter increase (%)	2.19 ± 0.07^{c}	7.44 ± 1.97^{b}	14.55 ± 0.98^{a}
Thickness increase (%)	84.12 ± 2.24^{a}	84.92 ± 8.41^{a}	73.21 ± 3.78^{a}
Surface increase (%)	4.43 ± 0.15^{c}	19.35 ± 4.32^{b}	31.23 ± 2.60^{a}
Volume increase (%)	92.30 ± 6.10^{b}	113.20 ± 12.70^{a}	127.60 ± 33.90^{a}
Spread factor	3.07 ± 0.35^{b}	4.60 ± 0.11^{a}	5.04 ± 0.29^{a}
Texture			
Fracture force (N/mm^2)	6.00 ± 0.59^{a}	3.67 ± 0.44^{b}	1.42 ± 0.11^{c}
Sensory characteristics			
Color	2.3 ± 0.1^{c}	5.9 ± 0.4^{b}	8.1 ± 0.8^{a}
Friability	2.5 ± 0.2^{b}	4.4 ± 1.2^{a}	5.4 ± 1.1^{a}
Bran-like odor	0.6 ± 0.4^{c}	3.8 ± 0.1^{b}	4.4 ± 0.2^{a}
Caramel odor	0.5 ± 0.3^{c}	2.9 ± 0.8^{b}	5.7 ± 0.8^{a}
Sweet taste	5.1 ± 0.2^{a}	4.2 ± 0.2^{b}	4.6 ± 0.2^{b}
Bitter taste	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Dryness	4.3 ± 0.4^{a}	4.6 ± 0.4^{a}	4.6 ± 0.2^{a}
Astringency	0.0 ± 0.0^{c}	0.3 ± 0.2^{b}	0.7 ± 0.1^{a}
Graniness	1.1 ± 0.2^{c}	3.1 ± 0.1^{b}	4.3 ± 0.2^{a}

TPC = total phenolic compounds; TFC = total flavonoid compounds; GAE = gallic acid equivalents; CAE = catechin equivalents; AA = antioxidant activity; TE = Trolox equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical; ABTS = 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid); IT = induction time.
Different letters in row indicate significant difference at $p \leq 0.05$.

3.2. Characteristics of biscuits

The QC flour was used for preparing biscuits at 30 and 60 g 100 g^{-1} on wheat flour basis, inducing a significant increase of the phenolic content and antioxidant activity of the end products (Table 4). The Q30 biscuits were significantly richer of phenolics and flavonoids than control without acorn flour, and showed higher antioxidant activity. These positive features further increased in Q60 biscuits. Accordingly, the oxidative stability of biscuits, evaluated by measuring the time needed to oxidize biscuits under forced conditions (induction time), was significantly longer as the level of acorn flour increased.

The addition of acorn flour resulted also in a relevant increase in red and brown indices, whereas the yellow index decreased compared to control (Table 4). This browning effect was imputable to the phenolic fraction of the composite flour, which is known to easily undergo oxidation by polyphenoloxidase developing brown quinones and related compounds (Pasqualone et al., 2014b; Taranto et al., 2012). Other authors similarly reported a significant browning effect of the addition of acorn flour in gluten-free bread (Korus et al., 2015). Color was evaluated because of its great influence on consumer acceptance. Therefore, the observed color alterations, with respect to control biscuits, should be properly communicated to consumers together with the positive antioxidant features, to avoid rejection.

As in other bakery products, several dimensional indices can be measured in biscuits to evaluate the extent of thermal expansion occurred during baking, which is due to the thermal increase in volume of the carbon dioxide developed by leavening agents, dough moisture, and air entrapped in the dough during kneading. Subsequent baking to dryness renders biscuits having a greater degree of expansion more friable than those less expanded and more compact. The expansion is influenced by dough extensibility and strength, therefore its measurement highlights any variation of dough induced by newly added ingredients.

The increase of diameter induced by baking varied significantly with the addition of acorn flour (Table 4). Biscuits appeared progressively larger as the level of acorn enrichment increased, probably due to the effect of gluten dilution with a gluten-free flour. Other authors found that gluten-free biscuits increased in diameter more than conventional biscuits (Hossain et al., 2017). The addition of acorn flour, however, did not significantly influence the increase in thickness due to baking.

As a consequence of large increase in diameter, the acorn-enriched biscuits showed a greater increase of surface compared to control biscuits. The expansion in volume, although significantly higher in the acorn-enriched biscuits than in the control, was less influenced by the amount of added acorn flour (30 or 60 g 100 g^{-1}), because of the reduced effect on thickness.

The spread factor is another physical indicator of biscuit quality; higher values of this index are appreciated owing to a positive influence on the acceptability of biscuits (Klunklin and Savage, 2018). The spread factor of acorn flour-added biscuits was higher than in the control.

If gluten is abundant and elastic (which is undesired in biscuits), it can inhibit biscuit expansion. High values of spread factor were observed in gluten-free biscuits, compared to conventional wheat biscuits (Sharma et al., 2016). Further, the addition of non gluten containing ingredients, such as rice flour or minor millet flour, increased the spread factor of wheat-based biscuits (Chung et al., 2014; Sharma et al., 2016). However, opposite results were observed by adding flours of bean and sesame (Hoojjat and Zabik, 1984), or lentil flour (Zucco et al., 2011). Especially if complex ingredients are added to the dough, a number of interactions occur among proteins, starches, fiber, lipids and minor compounds, thus the results obtained could not be exhaustively explained only by the dilution of gluten. In our case, being acorn flour a relatively fatty ingredient, the additional fat component contributed by this flour could have had a positive effect on spread ratio (Sudha et al., 2007).

The measure of the friability of biscuits was carried out through a three-point bending test ("snap test") until the break, simulating the breaking force exerted during chewing. The fracture force, lower for more friable biscuits, was significantly greater in control biscuits and progressively decreased as the level of acorn flour increased (Table 4). Also these findings were mainly imputable to gluten dilution, although a contribution by the lipid fraction of the acorn flour cannot be excluded. Lipids are known to interfere with gluten formation and play an essential role in determining the friability of biscuits (Caponio et al., 2006).

The sensory properties showed significant differences among the examined biscuits for the majority of descriptors (Table 4). The sensory evaluation of color surface paralleled the colorimetric measurement, with acorn-enriched biscuits darker than the control. A significantly more pronounced friability of biscuits enriched of acorn flour was also observed, compared to control, in agreement with the instrumental data

Table 5
Volatile compounds of biscuits enriched of increasing levels of acorn flour (*Quercus coccifera* L.). Values are expressed as percentage of the integrated area on the total area (mean of three replications ±SD). Control = biscuits without acorn flour; Q30 and Q60 are biscuits prepared with 30 and 60 g 100 g^{-1} acorn flour on wheat flour basis, respectively.

Compounds	Control	Q30	Q60
Alcohols			
Ethanol	n.d.	16.28 ± 0.16^{b}	24.54 ± 3.43^{a}
2-Butanol	n.d.	1.79 ± 0.73^{b}	11.73 ± 0.49^{a}
3-Methyl-1-butanol *or* isoamyl alcohol	n.d.	1.18 ± 0.11^{a}	n.d.
1-Pentanol *or* amyl alcohol	n.d.	1.13 ± 0.02^{a}	n.d.
2-Pentanol	n.d.	n.d.	0.96 ± 0.46
2-Heptanol	3.18 ± 0.34^{c}	13.96 ± 0.38^{a}	6.23 ± 0.16^{b}
Aldehydes			
2-Methylpropanal	2.00 ± 0.52^{a}	1.93 ± 0.12^{a}	2.08 ± 0.68^{a}
2-Methylbutanal	2.49 ± 0.29^{b}	3.43 ± 0.44^{a}	4.32 ± 0.62^{a}
3-Methylbutanal	4.19 ± 0.69^{b}	5.66 ± 0.47^{a}	7.13 ± 1.06^{a}
Hexanal	6.41 ± 0.54^{a}	6.20 ± 0.53^{a}	4.39 ± 0.32^{b}
Ketones			
2-Butanone	9.20 ± 0.18^{a}	9.50 ± 0.16^{a}	7.45 ± 1.79^{a}
2-Pentanone	n.d.	5.37 ± 0.4^{a}	5.26 ± 1.04^{a}
Carboxylic acids			
2-Oxopropanoic acid *or* pyruvic acid	n.d.	2.92 ± 0.59^{a}	1.00 ± 0.19^{b}
2-Methylpropanoic acid or isobutyric acid	n.d.	n.d.	1.44 ± 0.35^{a}
Esters			
Ethyl acetate	1.69 ± 0.03^{b}	5.90 ± 1.00^{a}	4.72 ± 1.44^{a}
Ethyl butanoate	1.84 ± 0.25	n.d.	n.d.
2-Methylpropyl propanoate	n.d.	2.16 ± 0.08^{a}	1.08 ± 0.29^{b}
Furan compounds			
2-Furancarboxaldehyde *or* 2-furaldehyde *or* furfural	4.56 ± 0.21^{b}	6.37 ± 0.38^{a}	7.48 ± 0.68^{a}
2-Furanmethanol *or* furfuryl alcohol	0.97 ± 0.08^{b}	1.76 ± 0.34^{a}	1.83 ± 0.15^{a}
Dihydro-2(3H)-furanone *or* γ-butyrolactone	n.d.	0.88 ± 0.26	n.d.
Pyrazines			
2-Methylpyrazine	n.d.	1.23 ± 0.15^{a}	1.71 ± 0.42^{a}
Pyrazine	2.03 ± 0.06	n.d.	n.d.

Different letters in row indicate significant difference at $p \leq 0.05$; n.d. = not detected.

of breaking force.

The odor sensations prevailing in biscuits were bran-like and caramel notes, both much more intense when acorn flour was added. The bran-like note was associated to the typical odor of bran and wholemeal products, therefore more perceivable in biscuits from acorn-wheat composite flours, being acorns milled to wholemeal flour whereas refined wheat flour was used in control biscuits. The caramel note, particularly intense in Q60 biscuits, was imputable to Maillard reaction during baking.

The sweet taste of biscuits was perceived significantly more intense in control, whereas bitterness was absent in all biscuit types.

Dryness was not different among biscuits. The biscuits added of acorn flour showed higher graininess than control, i.e. they were more friable and crumbly due to fat contributed by acorns, but the crumbs were granular and slightly heterogeneous in size having this flour also a high fiber content. The addition of acorn flour caused the appearance of an astringent sensation, although the latter was scored very low.

The volatile compounds were significantly different among biscuits (Table 5). The acorn-enriched biscuits showed higher levels of fermentative alcohols and their esters, particularly ethanol and ethyl acetate, probably due to some fermentative activity in the raw material. 2-Methylbutanal and 3-methylbutanal, Strecker aldehydes deriving from Maillard reaction, were more present in the volatile pattern of biscuits added of acorns. On the contrary, the amount of hexanal was lower in Q60 than in the other types of biscuits, indicating a lower level of lipid oxidation, which agreed with higher contents of phenolic compounds. 2-Butanone, with no significant differences among biscuits, was also abundant. This compound was already detected in biscuits in previous researches (Pasqualone et al., 2015) and its origin was attributed to β-chetoacids, arose from thermal treatment of tryglicerides, via decarboxylation (Chung et al., 1993). The furan compounds were mostly represented by furfural, a caramel-like odorant deriving by Maillard reaction, significantly more abundant in acorn-added biscuits than in control. Furfural is typically present in biscuits, being reported in several studies (Giarnetti et al., 2015; Mohsen et al., 2009; Pasqualone et al., 2014a, 2015). Little amounts of pyrazines, produced by Maillard reaction, were also detected.

4. Conclusions

Our study aimed at rediscovering endangered knowledge on the use of acorns for edible purposes, while at the same time taking into account the expectations of the modern consumer, used to foods with good sensory quality. The characteristics of biscuits are known to be less affected than those of bread from gluten dilution. In particular, the most important quality feature for biscuits is friability. After baking, the QC acorn-enriched biscuits were darker, larger, more voluminous and more friable than control biscuits. Only a very slight astringency was perceived. Therefore, according to our starting hypothesis, the enrichment of biscuits with QC acorn flour proved to be a very effective strategy for conferring antioxidant properties to biscuits, without excessively altering the sensory features.

Nowadays, the keyword is sustainability. Bringing light on the use of acorns for edible purposes could limit the waste of a nutritious and healthy food source. Acorns are an edible wild food, largely underutilized, which could supplement cultivated plants and help facing the increasing demand of food for a rapidly growing global population.

Declarations

Author contribution statement

Antonella Pasqualone: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Fatima Makhlouf: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Malika Barkat, Carmine Summo, Francesco Caponio: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Graziana Difonzo, Giacomo Squeo: Performed the experiments; Analyzed and interpreted the data.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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