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ORIGINAL ARTICLE

Physical and textural properties of biscuits containing jet milled rye and barley flour

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Abstract The biscuit-making performance of flour depends on both its botanical source and particle size. Several quality parameters of biscuits produced by partial replacement of wheat flour by barley and rye flours at 0, 10, 20, 30 and 40% were measured. Moreover, in order to investigate the effect of particle size, a commercial and two jet milled finer samples of both rye and barley flours were used. For most of the composite flours, the level of substitution was not statistically significant for the weight and the spread ratio of the biscuits. Biscuits with composite flours were softer and darker than the control biscuit (100% wheat flour). In addition, their total phenolics content and antioxidant activity were greater. Among composite flour biscuits, the finer barley flour biscuits were harder than those with the commercial flour. Moreover, as rye flour is darker than wheat and barley flours, rye biscuits were the darker of all. Porosity, bulk and true densities were affected by the particle size of the substitute flours.

Keywords Biscuits · Barley flour · Rye flour · Jet mill · Physicochemical properties · Mechanical properties

Introduction

Nowadays ready-to-eat processed food products, like biscuits, are widely consumed. These products are characterized by long shelf life, satisfying taste, good nutritional quality, ease of portability and low cost (Chavan and Kadam 1993). Flour, sucrose and fat along with other minor constituents (e.g. milk, flavour agents etc.) are mixed to produce a palatable dough that transforms into biscuit by baking in the oven (Mamat et al. 2010).

Wheat flour is the flour mostly used for biscuit preparation. However, its protein content is inferior to that of most cereals (Chavan and Kadam 1993). Furthermore, wheat has to be imported by countries that they can not grow it (Okpala and Egwu 2015). Thus, the use of composite flours seems a good solution. Composite flours are mixtures of flours of roots, tubers, cereals, legumes etc. with or without the addition of wheat flour. They are of interest due to the consumers' demand for variety in their diet as well as for a superior nutrition (Fellers and Bean 1988).

Barley, due to its content of health-related bioactive compounds, is used for the formulation of new food products for human diet (Charalampopoulos et al. 2002). Barley is rich in soluble fiber, especially β-glucan, and has a high phenolic content (Alu'datt et al. 2012). Thus, it has greater antioxidant activity than rice and wheat (Madhujith et al. 2006). Moreover, barley is used as protein diet fortification as its proteins are a rich source of limiting essential aminoacids (Sarac and Henry 1998).

Rye is a cereal used in bread and other products of human consumption or as animal feed. It has a high content of dietary fibers, mainly arabinoxylans. In addition, its β-glucan content is lower than that of barley (Rakha et al. 2010). Compared to wheat flour, rye contains higher levels of arabinoxylans and has lower gluten content.

The botanical source of flour and its particle size are significant factors for its physicochemical and mechanical properties (Drakos et al. 2017). In the present study, finer flours were produced by jet milling commercial flours. Jet

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milling is a fluid energy impact-milling technique producing ultrafine powders (Drakos et al. 2017). Apart from the material itself, the final particle size depends on various factors; the feed rate of the mill is one of them (Teng et al. 2009). Feed rate is affected by the vibration rate of the feeder. Lower vibration rate of feeder results in lower feed rate and thus, smaller particle size.

The present work was focused on the biscuit-making performance of combinations of wheat flour with rye and barley flours. The effect of barley and rye flours' particle size was also investigated. These flours were jet milled and thus, finer flours were produced. For all flour combinations, the physical and textural characteristics of the produced biscuits were studied and compared to those of biscuits with 100% wheat flour.

Materials and methods

Materials

Flours

Commercial soft wheat (W), barley (B) and rye (R) flours were kindly donated by Loulis Mills S.A. (Keratsini, Greece). Barley (B) and rye (R) flours were further pulverized using an air jet mill (Model 0101S Jet-O-Mizer Milling, Fluid Energy Processing and Equipment, Telford, PA, USA) with an air pressure of 8 bar operated at two vibration rates of feeder (90 and 70%). The characteristics and the particle size of the studied flours are shown in Table 1. Particle size data are reported as weighted mean diameters, d_{43}.

Reagents

Vegetable fat (Nea Fytini, Elais-Uniliver, Greece), white sugar (Hellenic Sugar Industry S.A.), salt and baking soda were bought from a local supermarket. Folin–Ciocalteau reagent was from Merck (Darmstadt, Germany) whereas all the remaining reagents were purchased from Sigma-Aldrich (Steinheim, Germany). Distilled water was used throughout.

Methods

Biscuits preparation

A commercial and two finer jet milled samples of both barley and rye flours partially replaced wheat flour at 0, 10, 20, 30 and 40%. Biscuits with 100% wheat flour were used as control.

The ingredients used for the biscuit dough were 100 g of flour, 28.3 g of white sugar, 12.5 g of water, 35.3 g of vegetable fat, 1.1 g of salt and 1.3 g of baking soda. Initially, fat was stirred for 3 min at medium speed in a household mixer. Sugar, salt and baking soda were then added and mixed in order to obtain a creamed mixture. Subsequently, water was added and a homogeneous mixture was obtained following mixing at medium speed. Finally, the required amount of flour was progressively added and the mixture was stirred for 4–5 min.

The batter was rolled to a thickness of 5 mm, cut to the desired diameter of 6 cm with a biscuit die and transferred to a lightly greased aluminum baking tray. The biscuits were baked at 180 °C for 10 min in a preheated baking oven. Then, they were cooled to room temperature and sealed in air tight containers until needed. For porosity measurements, biscuits with a diameter of 0.5 cm were also prepared.

Physical properties of biscuits

The weight, width, thickness and spread ratio of biscuits were measured. For thickness measurements, 6 biscuits were put on top of each other and the average thickness was determined. For diameter determination, 6 biscuits were placed next to each other with the help of a scale. Then the biscuits were rotated by 90° and their total diameter was measured again. The final diameter was the

Table 1 Mean particle size and characteristics of flours

Flour samples	Botanical origin	Flour	Mean particle size (μm)
W	Wheat	Commercial	173.87 ± 1.17
R	Rye	Commercial	100.91^{a} ± 2.39
R1	Rye	Jet milled at 90% (4.2 kg/h)	54.59^{b} ± 0.16
R2	Rye	Jet milled at 70% (1.33 kg/h))	35.66^{c} ± 0.56
B	Barley	Commercial	181.65^{a} ± 3.32
B1	Barley	Jet milled at 90% (3.35 kg/h)	42.99^{b} ± 1.22
B2	Barley	Jet milled at 70% (1.17 kg/h)	31.34^{c} ± 1.12

*Mean values followed by the same letters within flours of the same botanical origin are not significantly different ($P > 0.05$)

average of these two measurements divided by six. Spread ratio was calculated by dividing the average value of diameter by the average value of thickness. The average weight of 6 biscuits was noted.

Textural measurement of biscuits

The three point break method was used for the measurement of the fracture strength of the biscuits, using an Instron Universal machine (Instron 1011, Massachusetts, U.S.A.). The biscuits were supported across two beams (5 cm long, 6 cm high) spaced at 3 cm apart. The cutting beam (5 cm long, 4.65 cm high) was brought down from above at a constant speed of 10 mm/min until the sample snapped. The force required to break the biscuits was recorded. An average of 6 biscuits was tested.

True density, bulk density and porosity measurements

Porosity measurements were performed on the biscuits with the diameter of 0.5 cm by means of a gas pycnometer (Stereopycnometer SPY-3, Quantachrome, Syosset, N.Y., U.S.A.) with helium as the displacement fluid. True and bulk density and porosity were determined as described by Drakos et al. (2017). The values reported are the mean of three measurements per biscuit formulation.

Colour analysis of biscuits

A Minolta colorimeter (CR-200, Minolta Company, Ramsey, NJ, USA) was used for the measurement of the L^*, α^* and b^* parameters of the CIELAB system at three different surface locations of each biscuit. The values reported here are the mean of three measurements. The total colour difference (ΔE^*) was calculated by the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta \alpha^*)^2 + (\Delta b^*)^2} \quad (1)$$

Free soluble phenolics content and antioxidant activity

Biscuits were converted into powder using a laboratory mill. Then, 2 g of biscuit powder were extracted three times with 80% aqueous ethanol: twice with 10 mL of ethanol and once with 5 mL. Each step of extraction lasted 10 min. The suspensions were centrifuged at 6200 × g for 10 min and the supernatants were collected and combined. The final volume was brought to 25 mL with 80% aqueous ethanol. The extracts were stored at − 20 °C until used for the determination of total phenolics content and antioxidant activity of the biscuits following the procedures and the equations described by Drakos et al. (2017).

Statistical analysis

Analyses of variance (ANOVA) and least significant difference tests (LSD) were carried out on the data in order to determine significant differences among the samples. The significant level was $P < 0.05$ throughout the study. Analysis of data was carried out with the statistical software package Statistica v.8.0 for Windows.

Results and discussion

Tables 2 and 3 present the values for weight and spread ratio for biscuits with rye and barley flours, respectively. Statistics showed (Table 4) that all factors, with the exception of the interaction between flour substitution and botanical origin of the substitute flour, had a significant effect on the weight of the biscuits. In addition, flour substitution, the interaction between botanical origin of the substitute flour and particle size as well as the three way interaction were significant for spread ratio.

The first set of biscuits was prepared with wheat-rye composite flours. For all rye flours, the level of substitution was not statistically significant for both weight and spread ratio (Table 2). Their spread ratio and weight were ∼ 10.6 and ∼ 11 g, respectively. When barley flour was used (Table 3), the level of substitution was significant only for biscuits with B2. 30 and 40% B2 biscuits had statistically greater weight and lower spread ratio than the remaining B2 biscuits. Overall, for B2 biscuits, weight varied from ∼ 11 to ∼ 12.2 g and spread ratio from ∼ 10.6 to ∼ 9.8.

Several works on biscuits prepared with composite flours can be found in literature. The general trend reported is that wheat flour substitution is accompanied by an increase in weight and a decrease in spread ratio (e.g. Hooda and Jood 2005; Zucco et al. 2011). In some cases, the decrease is only seen at higher substitution levels (e.g. Arshad et al. 2007). There are two main approaches for explaining the reduction in spread ratio. The first suggests that all the ingredients of a biscuit that can absorb water during baking affect spread ratio due to their competition for the available water (Fuhr 1962). Among the ingredients, protein and damaged starch are of great importance (Pareyt and Delcour 2008). According to the second view, composite flours, due to the different water absorption capacities of their ingredients, form aggregates. As a result, the number of hydrophilic sites that can interact with water increases (McWatters 1978). The flours of the present study differ in their water absorption capacity and their damaged starch content but not on their protein content (Drakos et al. 2017). Thus, we can assume that starch is the dominant factor for the discrepancies reported.

Table 2 Properties of biscuits with rye flours

Flours	Substitution (%)	Spread ratio (diameter/width)	Weight (g)	Porosity	True density (g/mL)	Bulk density (g/mL)	Breaking force (N)	[L*]	[ΔE*]	Soluble phenolics content (mg gallic acid/g)	DPPH radical scavenging activity (% inhibition)
R	0	$10.71^{a} \pm 0.43$	$11.49^{a} \pm 0.26$	$0.33^{a} \pm 0.04$	$1.12^{a} \pm 0.00$	$0.75^{a} \pm 0.05$	$20.89^{a} \pm 0.01$	$78.88^{a} \pm 0.49$	$34.17^{a} \pm 0.05$	$0.30^{a} \pm 0.01$	$17.55^{a} \pm 1.31$
	10	$11.29^{a,b} \pm 0.53$	$10.70^{a} \pm 0.56$	$0.30^{b} \pm 0.04$	$1.10^{a} \pm 0.07$	$0.77^{a} \pm 0.08$	$25.44^{b} \pm 0.06$	$74.47^{b} \pm 0.83$	$33.83^{a} \pm 0.25$	$0.69^{b,c} \pm 0.12$	$17.71^{a} \pm 0.02$
	20	$11.84^{b} \pm 0.38$	$10.62^{a} \pm 0.66$	$0.33^{a,b} \pm 0.01$	$1.12^{a} \pm 0.01$	$0.75^{a} \pm 0.00$	$30.44^{a,c} \pm 2.23$	$70.3^{c} \pm 0.26$	$36.08^{b} \pm 0.16$	$0.47^{d} \pm 0.06$	$23.44^{b} \pm 0.38$
	30	$11.18^{a,b} \pm 0.92$	$11.31^{a} \pm 0.25$	$0.31^{a,b} \pm 0.00$	$1.11^{a} \pm 0.02$	$0.76^{a} \pm 0.01$	$19.37^{c} \pm 2.27$	$68.66^{d} \pm 0.20$	$37.23^{c} \pm 0.68$	$0.72^{b} \pm 0.10$	$31.69^{c} \pm 0.71$
	40	$10.49^{a} \pm 0.53$	$11.04^{a} \pm 0.79$	$0.31^{a,b} \pm 0.04$	$1.11^{a} \pm 0.02$	$0.76^{a} \pm 0.06$	$16.25^{d} \pm 1.94$	$66.39^{e} \pm 0.34$	$39.25^{d} \pm 0.67$	$0.56^{c,d} \pm 0.02$	$29.68^{d} \pm 0.32$
R1	0	$10.71^{a} \pm 0.43$	$11.49^{a,b} \pm 0.26$	$0.33^{a} \pm 0.04$	$1.12^{a} \pm 0.00$	$0.75^{a,b} \pm 0.05$	$20.89^{a} \pm 0.01$	$78.88^{a} \pm 0.49$	$34.17^{a} \pm 0.05$	$0.30^{a} \pm 0.01$	$17.55^{a} \pm 1.31$
	10	$10.63^{a} \pm 1.09$	$11.65^{a} \pm 0.29$	$0.36^{a} \pm 0.02$	$1.11^{a,b} \pm 0.00$	$0.71^{a} \pm 0.03$	$25.87^{b} \pm 0.02$	$71.04^{b} \pm 0.76$	$37.52^{b} \pm 0.80$	$0.67^{b} \pm 0.04$	$17.94^{a} \pm 0.63$
	20	$10.50^{a} \pm 0.88$	$11.29^{b} \pm 0.14$	$0.28^{b} \pm 0.01$	$1.10^{b} \pm 0.01$	$0.79^{b} \pm 0.00$	$20.91^{a} \pm 0.75$	$66.05^{c} \pm 0.01$	$40.13^{c} \pm 0.15$	$0.65^{b} \pm 0.04$	$22.92^{b} \pm 0.29$
	30	$10.71^{a} \pm 0.93$	$11.58^{a,b} \pm 0.00$	$0.23^{c} \pm 0.01$	$1.12^{a} \pm 0.01$	$0.86^{c} \pm 0.02$	$18.70^{a} \pm 0.40$	$63.21^{d} \pm 0.54$	$41.72^{d} \pm 0.33$	$0.76^{b,c} \pm 0.10$	$24.01^{b} \pm 2.05$
	40	$10.82^{a} \pm 0.85$	$11.78^{a} \pm 0.08$	$0.19^{d} \pm 0.04$	$1.10^{b} \pm 0.01$	$0.88^{c} \pm 0.05$	$15.13^{c} \pm 2.21$	$61.22^{e} \pm 0.90$	$43.15^{e} \pm 1.34$	$0.82^{c} \pm 0.08$	$28.71^{c} \pm 0.57$
R2	0	$10.71^{a} \pm 0.43$	$11.49^{a,c} \pm 0.26$	$0.33^{a} \pm 0.04$	$1.12^{a} \pm 0.00$	$0.75^{a} \pm 0.05$	$20.89^{a} \pm 0.01$	$78.88^{a} \pm 0.49$	$34.17^{a} \pm 0.05$	$0.30^{a} \pm 0.01$	$17.55^{a} \pm 1.31$
	10	$12.75^{b} \pm 0.96$	$10.47^{b} \pm 0.49$	$0.28^{a} \pm 0.13$	$1.08^{b} \pm 0.02$	$0.78^{a,b} \pm 0.12$	$17.63^{b} \pm 0.92$	$69.72^{b} \pm 1.00$	$38.72^{b} \pm 1.53$	$0.34^{b} \pm 0.01$	$17.67^{a} \pm 1.14$
	20	$10.88^{a} \pm 0.15$	$12.16^{c} \pm 0.74$	$0.21^{b} \pm 0.03$	$1.07^{b} \pm 0.02$	$0.85^{b} \pm 0.05$	$20.57^{b} \pm 0.12$	$66.29^{c} \pm 0.48$	$40.31^{b} \pm 1.07$	$0.34^{b} \pm 0.02$	$22.76^{b} \pm 1.38$
	30	$10.69^{a} \pm 0.03$	$11.52^{a,c} \pm 0.11$	$0.29^{a} \pm 0.02$	$1.10^{c} \pm 0.01$	$0.78^{a,b} \pm 0.02$	$26.55^{c} \pm 1.88$	$63.41^{d} \pm 0.41$	$41.96^{b} \pm 1.20$	$0.42^{c} \pm 0.00$	$24.42^{b,c} \pm 0.47$
	40	$11.03^{a} \pm 0.55$	$11.05^{a,b} \pm 0.27$	$0.29^{a} \pm 0.02$	$1.11^{a.c} \pm 0.01$	$0.78^{a,b} \pm 0.01$	$18.05^{a,b} \pm 0.48$	$60.99^{e} \pm 1.74$	$43.97^{b} \pm 1.28$	$0.44^{d} \pm 0.01$	$26.79^{c} \pm 0.91$

*Mean values followed by the same letters within the same flour are not significantly different ($P > 0.05$)

Table 3 Properties of biscuits with barley flours

Flours	Substitution (%)	Spread ratio (diameter/width)	Weight (g)	Porosity	True density (g/mL)	Bulk density (g/mL)	Breaking force (N)	[L*]	[ΔE*]	Soluble phenolics content (mg gallic acid/g)	DPPH radical scavenging activity (% inhibition)
B	0	$10.71^{a} \pm 0.43$	$11.49^{a,c} \pm 0.26$	$0.33^{a,c} \pm 0.04$	$1.12^{a,b} \pm 0.00$	$0.75^{a,b} \pm 0.05$	$20.89^{a} \pm 0.01$	$78.88^{a} \pm 0.49$	$34.17^{a} \pm 0.05$	$0.30^{a,c} \pm 0.01$	$17.55^{a} \pm 1.31$
	10	$12.75^{b} \pm 0.96$	$10.47^{b} \pm 0.49$	$0.30^{a} \pm 0.06$	$1.12^{a,b} \pm 0.02$	$0.78^{a} \pm 0.04$	$20.27^{a} \pm 1.80$	$73.92^{b} \pm 0.94$	$35.84^{a} \pm 0.85$	$0.54^{b} \pm 0.14$	$20.00^{a,b} \pm 3.64$
	20	$10.88^{a} \pm 0.15$	$12.16^{c} \pm 0.74$	$0.32^{a} \pm 0.05$	$1.10^{a} \pm 0.02$	$0.75^{a,b} \pm 0.07$	$14.60^{b,c} \pm 2.38$	$73.80^{b} \pm 0.98$	$33.22^{a} \pm 0.59$	$0.51^{b} \pm 0.04$	$24.15^{b} \pm 1.41$
	30	$10.69^{a} \pm 0.03$	$11.52^{a,c} \pm 0.11$	$0.40^{b} \pm 0.02$	$1.14^{b} \pm 0.03$	$0.68^{c} \pm 0.01$	$17.08^{b} \pm 0.19$	$74.16^{b} \pm 1.16$	$32.43^{a} \pm 1.83$	$0.53^{b} \pm 0.02$	$24.68^{b} \pm 1.49$
	40	$11.03^{a} \pm 0.55$	$11.05^{a,b} \pm 0.27$	$0.37^{b,c} \pm 0.02$	$1.12^{a,b} \pm 0.02$	$0.71^{b,c} \pm 0.01$	$14.25^{c} \pm 0.16$	$74.57^{b} \pm 0.25$	$31.30^{a} \pm 0.09$	$0.43^{b,c} \pm 0.07$	$22.27^{a,b} \pm 0.10$
B1	0	$10.71^{a} \pm 0.43$	$11.49^{a} \pm 0.26$	$0.33^{a} \pm 0.04$	$1.12^{a} \pm 0.00$	$0.75^{a} \pm 0.05$	$20.89^{a,b} \pm 0.01$	$78.88^{a} \pm 0.49$	$34.17^{a} \pm 0.05$	$0.30^{a,b} \pm 0.01$	$17.55^{a} \pm 1.31$
	10	$10.95^{a} \pm 0.10$	$11.56^{a} \pm 0.56$	$0.29^{a} \pm 0.06$	$1.05^{b} \pm 0.02$	$0.74^{a} \pm 0.04$	$20.08^{a} \pm 0.30$	$73.34^{b} \pm 1.43$	$35.66^{b} \pm 2.01$	$0.27^{b} \pm 0.03$	$16.49^{a,b} \pm 0.21$
	20	$10.35^{a} \pm 0.82$	$11.71^{a} \pm 0.16$	$0.31^{a} \pm 0.09$	$1.08^{c} \pm 0.01$	$0.75^{a} \pm 0.09$	$20.17^{a,b} \pm 3.49$	$70.0^{c} \pm 0.76$	$37.75^{c} \pm 0.94$	$0.41^{c} \pm 0.03$	$14.78^{b} \pm 1.11$
	30	$10.10^{a} \pm 0.18$	$11.67^{a} \pm 0.56$	$0.29^{a} \pm 0.03$	$1.09^{c} \pm 0.01$	$0.78^{a} \pm 0.03$	$17.38^{b,c} \pm 1.70$	$68.86^{d} \pm 1.54$	$38.14^{c} \pm 2.10$	$0.33^{a} \pm 0.01$	$18.07^{a} \pm 0.58$
	40	$10.68^{a} \pm 1.07$	$11.16^{a} \pm 0.01$	$0.21^{b} \pm 0.04$	$1.09^{c} \pm 0.02$	$0.86^{b} \pm 0.05$	$16.62^{c} \pm 0.58$	$67.24^{e} \pm 1.05$	$39.08^{c} \pm 0.98$	$0.38^{c} \pm 0.00$	$24.92^{c} \pm 0.54$
B2	0	$10.71^{a,c} \pm 0.43$	$11.49^{a} \pm 0.26$	$0.33^{a} \pm 0.04$	$1.12^{a} \pm 0.00$	$0.75^{a} \pm 0.05$	$20.89^{a} \pm 0.01$	$78.88^{a} \pm 0.49$	$34.17^{a} \pm 0.05$	$0.30^{a} \pm 0.01$	$17.55^{a} \pm 1.31$
	10	$12.01^{a,b} \pm 0.78$	$10.62^{b} \pm 0.13$	$0.29^{a} \pm 0.02$	$1.11^{a,b} \pm 0.02$	$0.78^{a,b} \pm 0.04$	$23.96^{b} \pm 2.19$	$70.46^{b} \pm 0.69$	$38.49^{b} \pm 0.31$	$0.34^{b} \pm 0.01$	$17.71^{a} \pm 1.92$
	20	$12.35^{b} \pm 0.83$	$10.84^{b} \pm 0.10$	$0.31^{a} \pm 0.02$	$1.10^{a,b} \pm 0.01$	$0.74^{a} \pm 0.04$	$15.63^{c} \pm 0.53$	$67.8^{c} \pm 2.23$	$38.96^{b} \pm 1.62$	$0.36^{b} \pm 0.00$	$13.76^{b} \pm 0.89$
	30	$11.39^{a} \pm 1.23$	$12.11^{c} \pm 0.01$	$0.25^{b} \pm 0.00$	$1.10^{a,b} \pm 0.02$	$0.82^{b} \pm 0.02$	$14.29^{c} \pm 1.25$	$65.37^{d} \pm 0.72$	$40.99^{c} \pm 0.45$	$0.40^{c} \pm 0.03$	$18.37^{a} \pm 0.34$
	40	$9.75^{c} \pm 0.32$	$12.24^{c} \pm 0.01$	$0.25^{b} \pm 0.05$	$1.08^{b} \pm 0.01$	$0.81^{b} \pm 0.06$	$19.54^{b} \pm 0.58$	$65.55^{d} \pm 0.01$	$40.32^{d} \pm 0.03$	$0.49^{d} \pm 0.02$	$30.53^{c} \pm 1.15$

*Mean values followed by the same letters within the same flour are not significantly different ($P > 0.05$)

Table 4 Significant main effects and interaction for evaluation of biscuits prepared at various levels of wheat flour substitution (flour) by barley or rye flours (origin) differing in their particle size (size)

Factor	Weight	Spread ratio	Porosity	True density	Bulk density	Hardness	[L*]	[ΔE*]	Phenolics	Antioxidant activity
Flour	*	*	*	*	*	*	*	*	*	*
Origin	*	NS	*	NS	*	*	*	*	*	*
Size	*	NS	*	*	*	NS	*	*	*	*
Flour × origin	NS	NS	*	NS	NS	*	*	*	*	*
Flour × size	*	NS	*	NS	*	*	*	*	*	*
Origin × size	*	*	NS	*	NS	*	*	*	*	*
Flour × origin × size	*	*	*	*	*	*	*	*	NS	*

NS no significant effect ($P > 0.05$); *$P < 0.05$

The next quality parameter evaluated was texture. The breaking strength of biscuits, which correlates with their hardness, was measured and the results for rye and barley flour formulations are shown in Tables 2 and 3, respectively. According to ANOVA (Table 4), all factors, except of the particle size of rye and barley flours, were significant for hardness. Among biscuits with flour of the same botanical origin, both the wheat flour substitution level and particle size of the substitute flour were statistically significant. Overall, the substitution of wheat flour by rye or barley flour resulted in softer biscuits. That was more evident at the two greater substitutions i.e. 30 and 40%. The breaking force for the control biscuit was ∼ 21 N and it was reduced to ∼ 15–∼ 19 N for the various composite biscuits. These results are in good agreement with literature. Gupta et al. (2011) studied several properties of biscuits supplemented with barley flour and reported that the incorporation of barley flour led to biscuits that required significantly decreased force in order to break. Similar results were reported for other composite blends of wheat flour with sorghum and oat flours (Chavan and Kadam 1993) as well as corn and potato flours (Singh et al. 2003). Regarding particle size, literature reports that finer flours led to harder biscuits (e.g. Dayakar Rao et al. 2016). Similar findings are reported in the present work, for most of the biscuits studied and especially those with barley. Once again, a possible explanation for our observations can be the competition for available water. Apart from protein and damaged starch, a finer particle can absorb more water than a coarser one (e.g. Protonotariou et al. 2016). Moreover, the physicochemical and granular properties of starch can also contribute to the observed behaviour. According to Singh et al. (2003), the starch granules of the various flours show different swelling behaviours that can result in the formation of air zones of different volumes. Thus, the fracture force of the biscuits can vary depending on the flour used. Kaur et al. (2014) reported that breaking strength can be positively correlated, among others, to lactic acid and sodium carbonate retention capacities. Solvent retention capacity tests are conducted in order to evaluate the suitability of flour for the production of a specific bakery product (Duyvejonck et al. 2012). Regarding the barley and rye flours of the present study, both lactic acid and sodium carbonate retention capacities increased with decreased particle size (Drakos et al. 2017). Bearing that in mind, our findings did not support the correlation between hardness and lactic acid and sodium carbonate retention capacities.

Porosity and true and bulk densities are important parameters for determining storage, transportation and packaging of biscuits. Their corresponding values are shown in Tables 2 and 3, for rye and barley biscuits, respectively. For all three parameters, and within biscuits with flour of the same botanical origin, only particle size was statistically significant. For all composite flours, apart from those with the commercial ones (i.e. R and B), bulk density increased with substitution level. The bulk density of the control biscuit was 0.75 g/mL and decreased to 0.88 and 0.86 g/mL at 40% wheat flour substitution for R1 and B1 biscuits, respectively. In addition, true density did not exhibit great differences for the wheat-rye flours (∼ 1.11 g/mL), whereas it showed a tendency to decrease when the jet milled barley flours were used (1.11–1.08 g/mL). In most composite flours, porosity decreased with flour substitution. Porosity values ranged from 0.19 to 0.40. As the commercial wheat, barley and rye flours have similar particle sizes, our findings are rather expected. The lower particle size of the jet milled flours can explain the observed porosity values.

The colour characteristics of biscuits were also studied and the corresponding values for [L*] and total colour difference [ΔE*] are presented in Tables 2 and 3, for rye and barley biscuits, respectively. According to the statistical results (Table 4), all factors and all interactions were significant for both colour parameters. For all composite flours, [ΔE*] increased with wheat flour substitution. Biscuits with 100% wheat flour were the lighter of all ([L*] ≈ 79). Furthermore, for all wheat-rye and wheat-barley flours, the biscuits became darker as the level of wheat flour's substitution increased. For example, at 40% flour substitution [L*] varied from ~ 61 to ~ 75, among composite flour biscuits. In agreement with our findings, Gupta et al. (2011) studied biscuits with partial replacement of wheat by barley flour and reported that their colour changed with the incorporation of barley flour from pale cream to golden brown. The increased substitution level also led to darker biscuits when wheat flour was combined with several pulse flours (Zucco et al. 2011) or corn and potato flours (Singh et al. 2003).

Within biscuits of the same composite flour, whiteness (i.e. [L*]) decreased with flour substitution. Biscuits with 40% of all three rye flours exhibited the lower [L*] values (66.39, 61.22 and 60.99 for R, R1 and R2 biscuits, respectively). The colour of the flour is affected by its botanical source (Torbica et al. 2012) and its content of pigments. Rye flour is darker than the other two flours and thus, darker biscuits are prepared when great amounts of rye flour are incorporated in the dough.

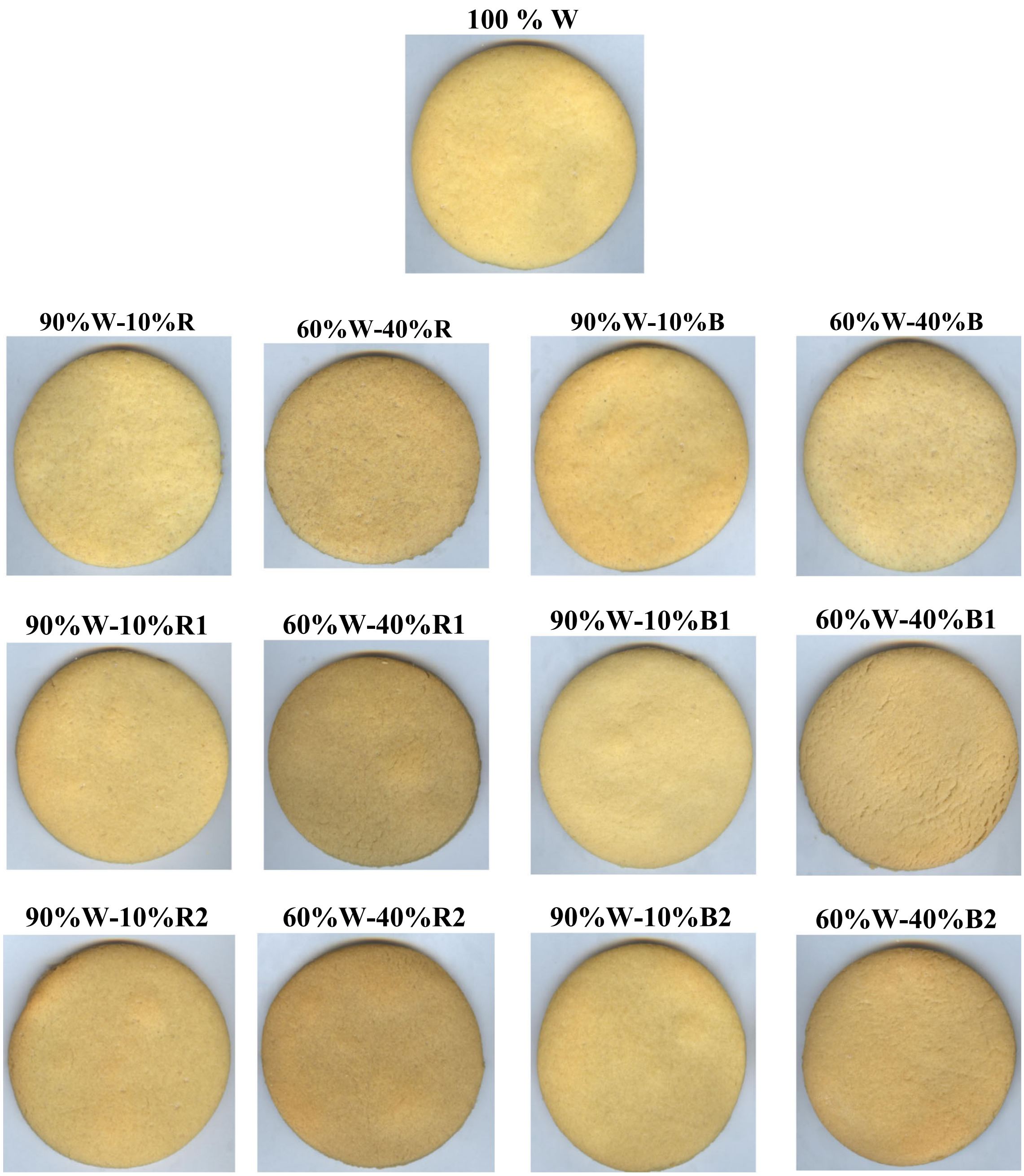

Fig. 1 Photos of biscuits prepared by substitution of wheat flour by commercial and jet milled rye and barley flours at 10% and 40%. Biscuit with 100% wheat flour is also shown (control)

Moreover, during baking, starch dextrinisation and non–enzymatic browning reactions (Maillard and sugar caramelisation) take place (Chevallier et al. 2000). Thus, the baking process itself contributes to the colour of a biscuit. Our findings are further confirmed by the photos of selected biscuits, shown in Fig. 1.

The total phenolics content and the antioxidant activity of biscuits were also evaluated (Tables 2 and 3). Based on the statistical analysis (Table 4), all factors and interactions were significant for antioxidant activity. For the phenolic content, only the three way interaction did not have a significant effect. The incorporation of barley and rye flour increased the phenolic content of the biscuits, which was more evident for flours R and R1. The phenolic content of the control biscuit was 0.30 mg gallic acid/g whereas biscuits with R and R1 exhibited values up to 0.7-0.8 mg gallic acid/g. Moreover, composite flour biscuits showed greater antioxidant activity than the control, especially at the two greater substitutions. Antioxidant activity varied from ∼ 17.6 (control biscuit) to ∼ 31(biscuit with 40% B2 and 30% R). The formation of aggregates during the extraction process might explain the lower phenolic content values of barley biscuits. Furthermore, the composition of the phenolic compounds varies among the flours and some of the compounds may decompose and volatilise during baking. No good correlation between phenolic content and antioxidant activity was found. This can be attributed to the fact that the antioxidant activity of individual phenolic compounds depends on the donor-proton capacity and thus, it can vary (Rice-Evans et al. 1996).

Overall, the partial replacement of wheat flour by barley and rye flours was not statistically significant for the weight and the spread ratio of the biscuits. However, the substitution resulted in softer and darker biscuits, with greater total phenolics content and antioxidant activity compared to biscuits with 100% wheat. Porosity and bulk and true densities were affected by the particle size of the substitute flours.

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