



Article The Effect of Inulin on the Physical and Textural Properties of Biscuits Containing Jet Milled Barley Flour

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Abstract: The quality properties of biscuits with partial replacement of sugar by inulin (at 0, 10, 20 and 30%) and wheat flour by barley flour (at 0, 10, 20, 30 and 40%) were studied. A commercial and a jet milled finer barley flour were used. For all flour substitutions, elevated amounts of inulin led to increased weight and decreased spread ratio of the biscuits. Inulin biscuits, within the same flour composition, were softer than those with no inulin but inulin's concentration was not statistically important. The incorporation of barley flour resulted in reduced lightness, more evident in the case of the finer barley flour. Inulin also affected the lightness of the biscuits. Phenolic content was affected by the presence of barley flour but no clear trend of inulin's effect was detected. The interactions between barley substitution, inulin substitution and particle size of the barley flour were important for all studied properties, i.e., weight, spread ratio, hardness, total phenolic content and color.

Keywords: biscuits; inulin; barley flour; jet mill; physical and textural properties



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1. Introduction

The major constituents of biscuits are typically wheat flour, sucrose and fat, making these products a rather energy dense cereal food [1]. Over the last years, the consumers' need for healthy, natural and functional products has led to the development of new products with increased nutritive value and functionality. This is achieved by altering their nutritive composition. In the case of biscuits, attempts are towards protein or dietary fiber enrichment, replacement of fat and sugar, increase of antioxidant capacity and improvement of prebiotical characteristics [2].

Barley is the fourth major cereal crop in the world and mainly utilized in malting and brewing and as animal feed [3]. However, lately, as barley has a high content of dietary fiber and a high proportion of soluble fiber, especially β -glucan, it is used in the formulation of new human food products. Furthermore, it has a high phenolic content and its antioxidant activity is greater than that of rice and wheat [4]. Studies have shown that the presence of β -glucan and phenolic compounds have the potential to lower cholesterol and blood glucose levels [5]. Moreover, its proteins are a rich source of the limiting essential amino acids and thus, barley is used as protein diet fortification [6].

Inulin is a fructooligosacharide (FOS) composed of fructose units bonded together by β -(2-1) linkages with a degree of polymerization (DP) varying from 2–60 [7]. It can be found in several vegetables, fruits and cereals (e.g., artichoke, onion, garlic, leek, banana, wheat and barley). Industrially it is obtained by chicory roots [8]. Recently, FOS, in general, and inulin, in particular, have become widely used in the formulation of innovative healthy foods. Inulin has a reduced caloric value and it is used as a substitute of fat and sucrose allowing an improvement of both taste and texture [9]. Moreover, it acts as a dietary fiber [10].

In the present study, inulin was used as sucrose replacer. Moreover, as the use of composite flour for biscuit production seems to have several advantages [11], biscuits were prepared with barley flour as partial substitute for wheat flour. Furthermore, two types

of barley flours differing in their particle size were used. The finer one resulted from jet milling. Jet milling is a fluid energy impact-milling technique generally used for producing ultrafine powders [4]. The effect of flour substitution, inulin substitution and particle size of the barley flour on the physical and textural characteristics of the biscuits was determined. Given the connection between health and food, our aim was to prepare healthy biscuits with increased nutritive value by reducing their sucrose content and enriching them with dietary fiber coming from both inulin and barley flour.

2. Materials and Methods

2.1. Materials

Commercial soft wheat (W) and barley (B) flours were kindly donated by Loulis Mills S.A. (Keratsini, Greece). Barley flour (B) was subjected to further pulverization 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 giving flour JMB with a lower particle size than B ($42.99 \pm 1.22 \mu m$ and $181.65 \pm 3.32 \mu m$, respectively). Vegetable fat (Nea Fytini, Elais-Uniliver, Athens, Greece), white sugar (Hellenic Sugar Industry S.A., Thessaloniki, Greece), salt and baking soda were bought from a local supermarket. The inulin sample (I) used was "BeneoTM GR inulin" (ORAFTI Group, Tienen, Belgium) with a minimum average DP of 10. 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.

2.2. Methods

2.2.1. Biscuit Preparation

Two barley flours differing in their particle size and inulin were incorporated in the biscuit formulation as partial replacers of wheat flour and sugar, respectively. Thus, wheat flour was replaced at 0, 10, 20, 30 and 40% and sugar at 0, 10, 20 and 30%. Biscuits with wheat flour and no inulin were considered as the control.

The recipe for the biscuit dough (based on 100 g of flour) was 35.3 g of fat, 28.3 g of sugar, 12.5 g of water, 1.3 g sodium bicarbonate and 1.1 g salt. The formulation of the studied biscuits is presented in Table 1. For the preparation of the dough, fat was mixed for 3 min at medium speed. Sugar, inulin, salt and baking soda were then added and mixed in order to obtain a creamed mixture. Water was then added and mixed at medium speed till a homogeneous mixture was obtained. Finally the required amount of flour was progressively added and the mixture was mixed for 4–5 min.

Wheat Flour (W) (g)	Barley Flour [Commercial (B) or Jet Milled (JMB)] (g)	Sucrose (g)	Inulin (I) (g)	Fat (g)	Water (g)	Sodium Bicarbonate (g)	Salt (g)
100	0	28.3 25.47 22.64 19.81	0 2.83 5.66 8.49				
90	10	28.3 25.47 22.64 19.81	0 2.83 5.66 8.49	_			
80	20	28.3 25.47 22.64 19.81	0 2.83 5.66 8.49	- 35.3	12.5	1.3	1.1
70	30 40	28.3 25.47 22.64 19.81	0 2.83 5.66 8.49				
60		28.3 25.47 22.64 19.81	0 2.83 5.66 8.49				

Table 1. Formulations of biscuits.

The batter was sheeted to a thickness of 5 mm with the help of a rolling pin. Biscuits were cut to the desired diameter of 6 cm with a biscuit die and transferred to a lightly greased aluminum baking tray. They were baked at 180 °C for 10 min in a preheated baking oven (Rohnson, Model R200, China). Then, they were cooled to room temperature and packed in airtight containers for further analysis.

2.2.2. Physical Properties of Biscuits

The weight, width, thickness and spread ratio of all biscuits was measured. Diameter was measured by placing 6 biscuits 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 average of these two measurements divided by six. Thickness was measured by putting 6 biscuits on top of each other and taking average thickness. The weight was the average weight of 6 biscuits. Spread ratio was calculated by dividing the average value of diameter by the average value of thickness.

2.2.3. Texture Measurement of Biscuits

The fracture strength of the biscuits was determined by the three point break method using an Instron Universal machine (Instron 1011, Norwood, MA, USA). The biscuits were put on two beams (5 cm long, 6 cm high). Their distance was 3 cm. 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 peak force, representing the fracture strength, was recorded. An average of 6 biscuits was tested.

2.2.4. Color Analysis of Biscuits

Surface color was measured at three different locations of each biscuit using a Minolta colorimeter (CR-200, Minolta Company, Ramsey, NJ, USA). The values reported for the L^* , α^* and b^* parameters of the CIELAB system were the mean of three measurements (D65 illuminant, 8 mm diameter measuring area). The whiteness [*WIE**] of the biscuits was calculated by the following equation:

$$WIE = 100 - \sqrt{(100 - L^*)^2 + \alpha^{*2} + b^{*2}}$$
(1)

The total color difference $[\Delta E^*]$ was also calculated using the following equation:

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

2.2.5. Soluble Phenolic Content of Biscuits

Biscuits were converted into powder with the means of 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 $6200 \times g$ 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 they were used for the colorimetric determination of the total phenolic content as described by Drakos et al. (2017) [4]. The determination of total phenolic content was achieved by comparison to a calibration curve constructed by gallic acid. The results were expressed as mg equivalents of gallic acid per g of the samples.

2.3. Statistical Analysis

Analyses of variance (ANOVA) and least significant difference tests (LSD) were carried out on the data in order to determine significant differences between 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.

3. Results and Discussion

Physical characteristics are very important for the quality of a biscuit. Weight, width, thickness and spread ratio of all biscuit formulations were measured. As the changes in width and thickness are reflected to spread ratio, Table 2 presents the corresponding results for weight and spread ratio for all studied biscuits.

				Weight (g)					Spread Ratio (Diameter/Width)					
	Inulin	Barley Flour						Barley Flour						
Flour	(%)	0%	10%	20%	30%	40%	0%	10%	20%	30%	40%			
В	0 10 20 30	$\begin{array}{c} 11.58\ ^{a}\pm 0.71\\ 12.72\ ^{b}\pm 1.02\\ 12.73\ ^{b}\pm 0.44\\ 11.57\ ^{a}\pm 0.41 \end{array}$	$\begin{array}{c} 11.72 \ a,b \ \pm \ 1.15 \\ 12.40 \ b \ \pm \ 0.92 \\ 12.70 \ b \ \pm \ 0.22 \\ 11.33 \ a \ \pm \ 0.63 \end{array}$	$\begin{array}{c} 12.20 \ ^{a} \pm 1.47 \\ 12.69 \ ^{a} \pm 1.01 \\ 12.48 \ ^{a} \pm 0.49 \\ 12.99 \ ^{a} \pm 0.49 \end{array}$	$\begin{array}{c} 10.99 \ a \pm 1.15 \\ 13.21 \ b \pm 0.96 \\ 12.18 \ c \pm 0.56 \\ 12.75 \ b,c \pm 0.55 \end{array}$	$\begin{array}{c} 9.82 \ ^{a} \pm 1.39 \\ 12.80 \ ^{b} \pm 0.58 \\ 12.31 \ ^{b} \pm 0.44 \\ 13.06 \ ^{b} \pm 0.66 \end{array}$	$\begin{array}{c} 11.08 \ ^{a} \pm 0.11 \\ 10.16 \ ^{b} \pm 0.30 \\ 8.32 \ ^{c} \pm 0.32 \\ 9.13 \ ^{d} \pm 0.22 \end{array}$	$\begin{array}{c} 11.26 \ ^{a} \pm 0.20 \\ 8.25 \ ^{b} \pm 0.10 \\ 8.55 \ ^{b} \pm 0.07 \\ 8.35 \ ^{b} \pm 0.17 \end{array}$	$\begin{array}{c} 10.13 \ ^{a} \pm 0.15 \\ 8.49 \ ^{b} \pm 0.25 \\ 7.44 \ ^{c} \pm 0.21 \\ 8.68 \ ^{b} \pm 0.18 \end{array}$	$\begin{array}{l} 9.43 \ ^{a} \pm 0.08 \\ 8.03 \ ^{b} \pm 0.11 \\ 8.51 \ ^{b} \pm 0.46 \\ 8.56 \ ^{b} \pm 0.22 \end{array}$	$\begin{array}{c} 10.83 \ ^{a} \pm 0.24 \\ 8.56 \ ^{b} \pm 0.26 \\ 9.21 \ ^{c} \pm 0.30 \\ 7.42 \ ^{d} \pm 0.05 \end{array}$			
ЈМВ	0 10 20 30	$\begin{array}{c} 11.58\ ^{a}\pm 0.71\\ 12.72\ ^{b}\pm 1.02\\ 12.73\ ^{b}\pm 0.44\\ 11.57\ ^{a}\pm 0.41 \end{array}$	$\begin{array}{c} 11.47\ ^{a}\pm 0.44\\ 11.12\ ^{a}\pm 1.28\\ 12.58\ ^{b}\pm 0.52\\ 12.01\ ^{a,b}\pm 0.32 \end{array}$	$\begin{array}{c} 11.12 \ ^{a} \pm 0.99 \\ 14.02 \ ^{b} \pm 0.70 \\ 12.50 \ ^{c} \pm 0.57 \\ 12.05 \ ^{c} \pm 0.52 \end{array}$	$\begin{array}{c} 11.29\ ^{a}\pm 0.68\\ 12.99\ ^{b}\pm 0.53\\ 13.33\ ^{b}\pm 0.46\\ 13.37\ ^{b}\pm 0.44\end{array}$	$\begin{array}{c} 12.60 \ a,b \ \pm \ 1.09 \\ 12.32 \ a \ \pm \ 0.19 \\ 12.96 \ a,b \ \pm \ 0.45 \\ 13.08 \ b \ \pm \ 0.35 \end{array}$	$\begin{array}{c} 11.08\ ^{a}\pm 0.11\\ 10.16\ ^{b}\pm 0.30\\ 8.32\ ^{c}\pm 0.32\\ 9.13\ ^{d}\pm 0.22 \end{array}$	$\begin{array}{c} 9.59 \ ^{a} \pm 0.20 \\ 10.36 \ ^{b} \pm 0.12 \\ 7.94 \ ^{c} \pm 0.28 \\ 8.24 \ ^{c} \pm 0.18 \end{array}$	$\begin{array}{c} 9.99 \ a,b \ \pm \ 0.13 \\ 8.24 \ b,c \ \pm \ 0.58 \\ 11.72 \ a \ \pm \ 1.10 \\ 7.42 \ c \ \pm \ 0.41 \end{array}$	$\begin{array}{c} 9.70\ ^{a}\pm 0.02\\ 8.54\ ^{b}\pm 0.19\\ 10.10\ ^{a}\pm 0.38\\ 6.81\ ^{c}\pm 0.21\end{array}$	$\begin{array}{c} 11.11 \ ^{a} \pm 0.44 \\ 8.60 \ ^{b} \pm 0.27 \\ 11.59 \ ^{a} \pm 0.40 \\ 7.56 \ ^{c} \pm 0.19 \end{array}$			

Table 2. Weight and spread ratio of the biscuits.

Values of each column with different superscripts within the same barley flour are significantly different (p < 0.05).

Initially, biscuits with 100% wheat flour (W) and increasing amounts of inulin were studied. The incorporation of inulin led to an increase of the weight up to 20% replacement. Thus, at 0% inulin, biscuits weighted 11.58 g and in the presence of 20% inulin, 12.73 g. At 30% the weight decreased (11.57 g). Then, biscuits with barley flour were prepared. A commercial and a jet milled finer barley flour (B and JMB, respectively) were used. According to Table 2, and for the commercial barley flour (B), weight for 10% barley substituted biscuits increased with inulin up to 20% substitution and then at 30% it decreased. For 30 and 40% B flour incorporated biscuits, weight increased with inulin increase for all studied inulin concentrations, whereas for the 20% ones, the inulin concentration was not statistically important. On the other hand, and for all barley substitutions, the incorporation of inulin led to biscuits with lower spread ratio. For example, the spread ratio for 10% B substituted biscuits was reduced from 11.26 in the absence of inulin to 8.35 in the presence of 30% inulin.

The effect of the incorporation of the finer barley flour (JMB) on the same parameters is also shown in Table 2. The general trend is that for all flour substitutions, elevated amounts of inulin led to an increase in the biscuit weight and a decrease at their spread ratio. For example, for biscuits with 20% JMB, weight increased from 11.12 to 12.05 and spread ratio decreased from 9.99 to 7.42 in the presence of 0% and 30% inulin, respectively.

The effect of flour substitution, inulin substitution and particle size of the barley flour on all studied properties is shown in Table 3. According to it (Table 3), only the particle size of the barley flour and the interaction between inulin substitution and particle size of the barley flour did not have a significant effect on the weight of the biscuits. Regarding spread ratio the three-way and all two-way interactions were significant.

Table 3. Significant main effects and interaction for evaluation of biscuits prepared with various levels of flour substitution (flour), inulin substitution (Inulin) using barley flours differing in their particle size (Size).

Factor	Weight	Spread Ratio	Hardness	Phenolics	[L*]	[WIE*]	[ΔE*]
Flour	0.0002	0.0000	0.0740	0.0001	0.0000	0.0000	0.0000
Inulin	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Size	0.1018	0.0000	0.7094	0.4248	0.0000	0.0000	0.0000
Flour imes Inulin	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
$Flour \times Size$	0.0017	0.0000	0.0027	0.0000	0.0000	0.0000	0.0000
Inulin \times Size	0.0916	0.0000	0.0408	0.0000	0.0000	0.0000	0.0000
$Flour \times Inulin \times Size$	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Padma Ishwarya et al. (2013) [12] studied biscuits with 0–30% partial replacement of sugar by FOS. They also reported that the weight of the biscuits increased for up to 20%

incorporated inulin whereas decreased at 30% replacement. This was explained in terms of the different ability to hold water of the flour–inulin blends. The same work also reported the decrease in spread ratio with increased FOS concentration, in good agreement with our findings. Similar observations are reported by Baljeet et al. (2010) [13].

Regarding spread ratio, Tangkanakul et al. (1995) [14] suggested that an increase in fiber content can lead to decreased spread ratio. In the present study, biscuits were prepared using a composite wheat–barley flour. As already mentioned in the Introduction, barley flour is richer in dietary fiber than wheat. Furthermore, McWatters (1978) [15], when studying composite flours, reported that flours fortified with components which had different water absorption capacities had lower spread ratios. He attributed this observation to the formation of aggregates within the composite flours with more hydrophilic sites available to compete for the limited free water in the biscuit dough. The water absorption capacity of the flours used in the present study is 68.04% for wheat and 96.13% and 123.34% for B and JMB, respectively [4].

Moreover, the spread ratio is connected to the protein and the damaged starch content of the flours [16]. A higher protein content is related to a higher number of hydrophilic sites that compete for the limited free water. Rapid partitioning of free water by these hydrophilic sites results in higher sugar concentrations in the water phase, higher internal dough viscosity and thus, limited cookie spread [17]. For similar reasons, greater damaged starch content is connected to greater water retention of the flour and thus, decreased spread ratio. Regarding the barley flours used in the present work, the jet milled one presents higher protein (JMB: 10.56%; B: 9.52%) and damaged starch content (JMB: 7.05%; B: 4.72%) than the commercial one [4]. W flour has a protein content of 10.74% and a damaged starch content of 1.93%.

The next evaluated quality parameter was texture. The breaking strength of the biscuits, which correlates with their hardness, was measured and the results for all biscuit formulations are shown in Table 4. Based on the ANOVA (Table 3), inulin substitution had a main effect on hardness. Hardness was also affected by the three-way and all two-way interactions.

				Breaking Force (N)				5	Soluble Phenolic Con (mg GAE/g)	tent				
Flour	Inulin (%)			Barley Flour				Barley Flour						
		0%	10%	20%	30%	40%	0%	10%	20%	30%	40%			
В	0 10 20 30	$\begin{array}{c} 13.95\ ^{a}\pm 3.51\\ 5.88\ ^{b}\pm 0.40\\ 4.10\ ^{b}\pm 0.67\\ 4.87\ ^{b}\pm 0.95\end{array}$	$\begin{array}{c} 12.50 \ ^{a} \pm 1.02 \\ 7.63 \ ^{b} \pm 1.28 \\ 5.21 \ ^{c} \pm 0.84 \\ 5.87 \ ^{c} \pm 1.46 \end{array}$	$\begin{array}{c} 15.46 \ ^{a} \pm 5.84 \\ 4.54 \ ^{b} \pm 0.98 \\ 6.20 \ ^{b} \pm 0.88 \\ 5.97 \ ^{b} \pm 1.38 \end{array}$	$\begin{array}{c} 22.38\ ^{a}\pm 4.66\\ 4.23\ ^{b}\pm 0.63\\ 5.00\ ^{b}\pm 1.48\\ 5.02\ ^{b}\pm 1.19\end{array}$	$\begin{array}{c} 14.89 \ ^{a} \pm 2.63 \\ 6.46 \ ^{b,c} \pm 0.95 \\ 7.48 \ ^{b} \pm 0.92 \\ 4.55 \ ^{c} \pm 1.02 \end{array}$	$\begin{array}{c} 0.479\ ^{a}\pm 0.003\\ 0.490\ ^{a}\pm 0.048\\ 0.567\ ^{a}\pm 0.096\\ 0.534\ ^{a}\pm 0.065\end{array}$	$\begin{array}{c} 0.609 \ a \pm 0.061 \\ 0.621 \ a \pm 0.007 \\ 0.660 \ a \pm 0.028 \\ 0.860 \ b \pm 0.124 \end{array}$	$\begin{array}{c} 0.451 \ a \pm 0.012 \\ 0.700 \ b \pm 0.075 \\ 0.661 \ b \pm 0.091 \\ 0.660 \ b \pm 0.024 \end{array}$	$\begin{array}{c} 1.082 \ a \pm 0.017 \\ 0.721 \ b \pm 0.027 \\ 0.830 \ c \pm 0.006 \\ 0.747 \ b \pm 0.002 \end{array}$	$\begin{array}{c} 0.597 \ ^{a} \pm 0.045 \\ 0.745 \ ^{a} \pm 0.062 \\ 0.705 \ ^{a} \pm 0.096 \\ 0.755 \ ^{a} \pm 0.023 \end{array}$			
јмв	0 10 20 30	$\begin{array}{c} 13.95 \ ^{a} \pm 3.51 \\ 5.88 \ ^{b} \pm 0.40 \\ 4.10 \ ^{b} \pm 0.67 \\ 4.87 \ ^{b} \pm 0.95 \end{array}$	$\begin{array}{c} 21.04 \ ^{a} \pm 3.75 \\ 7.92 \ ^{b} \pm 2.39 \\ 4.90 \ ^{c} \pm 0.85 \\ 2.98 \ ^{c} \pm 0.79 \end{array}$	$\begin{array}{c} 19.19 \ ^{a} \pm 3.56 \\ 4.95 \ ^{b} \pm 0.46 \\ 4.38 \ ^{b} \pm 0.67 \\ 3.87 \ ^{b} \pm 0.63 \end{array}$	$\begin{array}{c} 13.91 \ ^{a} \pm 2.02 \\ 6.63 \ ^{b} \pm 0.98 \\ 6.73 \ ^{b} \pm 1.65 \\ 6.44 \ ^{b} \pm 0.91 \end{array}$	$\begin{array}{c} 6.38 \ ^{a}\pm 1.30 \\ 8.92 \ ^{b,c}\pm 2.71 \\ 5.58 \ ^{a}\pm 0.65 \\ 7.40 \ ^{a,c}\pm 0.83 \end{array}$	$\begin{array}{c} 0.479\ ^{a}\pm 0.003\\ 0.490\ ^{a}\pm 0.048\\ 0.567\ ^{a}\pm 0.096\\ 0.534\ ^{a}\pm 0.065\end{array}$	$\begin{array}{c} 0.718 \ ^{a} \pm 0.144 \\ 0.706 \ ^{a} \pm 0.003 \\ 0.779 \ ^{a} \pm 0.086 \\ 0.595 \ ^{a} \pm 0.046 \end{array}$	$\begin{array}{c} 0.809\ ^{a}\pm 0.000\\ 0.599\ ^{b}\pm 0.041\\ 0.716\ ^{c}\pm 0.040\\ 0.822\ ^{a}\pm 0.020\end{array}$	$\begin{array}{c} 0.782 \ ^{a} \pm 0.051 \\ 0.480 \ ^{b} \pm 0.006 \\ 0.559 \ ^{b,c} \pm 0.077 \\ 0.658 \ ^{c} \pm 0.066 \end{array}$	$\begin{array}{c} 0.713 \ ^{a} \pm 0.050 \\ 0.634 \ ^{a} \pm 0.004 \\ 1.292 \ ^{b} \pm 0.050 \\ 0.721 \ ^{a} \pm 0.019 \end{array}$			

Table 4. Hardness and soluble phenolic content of biscuits.

Values of each column with different superscripts within the same barley flour are significantly different (p < 0.05).

Biscuits with inulin, within the same flour composition, were softer than those with no inulin but the concentration of inulin was not statistically important. For example, hardness for biscuits with 10% B decreased from 12.50 N to 5.87 N when inulin was added at a concentration of 30%. Our results are in good agreement with the works of Padma Ishwarya et al. (2013) [12] and Handa et al. (2012) [18] who also studied the textural properties of FOS-enriched biscuits. They suggested that the observed reduction in breaking strength can be attributed to the ability of inulin to bind more water; thus, resulting in softer biscuits. At the same time, the level of flour substitution was important only for the biscuits with the fine barley flour (JMB).

The biscuits with the highest JMB flour substitution (40%) in the absence of inulin were the softer ones (6.38 N). Literature reports that hardness increased when the flour's particle size was decreased since a flour of finer particles necessitates more water to absorb in order to prepare the dough [19–21], thus, limiting the available water for the other ingredients

like protein and starch [22]. In our case, composite flours differing in their particle size as well as their composition and physicochemical properties were used. These differences may influence the association between protein and starch resulting in biscuits with varying hardness [23].

At the same time, our measurements showed that the substitution of wheat flour by barley flour was not statistically important. On the contrary, literature reports that the incorporation of barley flour into wheat flour led to biscuits wherethe force required to break them was significantly decreased [24]. Similar findings were also reported for other composite flours like sorghum-wheat and oat-wheat flours [25]. Most probably this diverse behavior can be attributed to the different composition and physicochemical properties of the flours used in the present study.

The total phenolic content of the biscuits was also evaluated (Table 4). Based on the ANOVA (Table 3), only the particle size of the barley flour did not have a significant effect on this parameter. All two-way and the three-way interactions were found to be important. In the absence of inulin, the addition of the rich in phenolics barley flour more or less resulted in greater phenolic content. For example, wheat flour substitution by 10% B flour increased the total phenolic content of the biscuits from 0.479 to 0.609 mg GAE/g. The work of Gupta et al. (2011) [24] has also reported similar findings. Regarding biscuits with 100% W, the incorporation of inulin had no statistically important effect on the phenolic content (~0.5 mg GAE/g). For both barley flours, no clear trend can be detected when increasing inulin for the same flour substitution. These findings can be connected to the behavior of the unstable and reactive phenolic compounds such as their interactions with the other components of the biscuit formulation as well as their decomposition and volatilization due to the high temperatures of the baking process.

Color is another important factor for the acceptability of a baked product by the consumers. The whiteness index [WIE*] combines all three parameters of the CIELAB into a single term and is used along with $[\Delta E^*]$ in the present study to compare the color of the biscuits. The greater the whiteness index value, the whiter the biscuit. Whiteness index, $[\Delta E^*]$ and $[L^*]$ for all studied biscuits are presented in Table 5. The statistical analysis showed a significant three-way interaction for all three color parameters (Table 3). The biscuits with 100% W, both in the presence and absence of inulin, were the lighter of all biscuits ([L*]~81). The incorporation of barley flour resulted in reduced lightness which was more evident in the case of the JMB ([L*]~71–77). The presence of inulin also affected the lightness of the biscuits. Regarding [WIE*], the incorporation of the finer barley flour resulted in less-white biscuits. In the presence of 30% inulin [WIE*] was 62.51 and 59.98 for biscuits with 100% W and biscuits substituted by 40% JMB flour, respectively. [ΔE^*] was greater when the finer flour was used in the recipe, e.g., in the presence of 0% inulin [ΔE^*] was ~30 and ~34 for biscuits with B and JMB flour, respectively. The incorporation of inulin also affected [ΔE^*] with the main trend being that inulin's concentration increased [ΔE^*]. Our measurements were further confirmed by the photos of selected biscuits varying in their formulation (Figure 1). From the photos, it became obvious that the incorporation of barley, apart from leading to darker biscuits, also led to surface cracking.

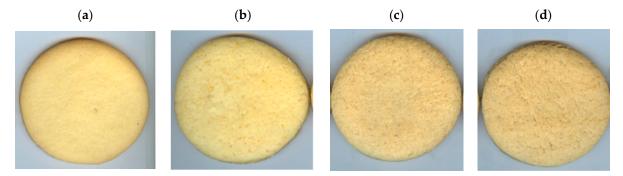


Figure 1. Photos of biscuits with (**a**) 100% wheat flour—0% inulin, (**b**) 100% wheat flour—30% inulin, (**c**) 60% wheat flour—40% barley flour (JMB)—30% inulin and (**d**) 60% wheat flour—40% barley flour (JMB)—30% inulin.

				[L]					[WIE]		
	Inulin			Barley Flour	Barley Flour						
Flour	(%)	0%	10%	20%	30%	40%	0%	10%	20%	30%	40%
	0	$81.87 \stackrel{a}{-} \pm 0.38$	79.96 ^a ± 0.41	77.53 ^a ± 0.30	$76.35\ ^{a}\pm 0.33$	$75.51\ ^{a}\pm 0.63$	$64.52 \stackrel{a}{_{-}} \pm 0.41$	$66.43 \stackrel{a}{.} \pm 0.91$	$66.17 \stackrel{a}{_{-}} \pm 0.47$	$65.03 \ ^{a} \pm 0.38$	$65.70 \stackrel{a}{}{} \pm 0.51$
_	10	$81.34 \text{ a,b} \pm 0.58$	$78.97 \frac{b}{\pm} \pm 0.50$	$76.50 \text{ b} \pm 0.57$	76.39 ^a ± 0.79	$75.64^{a} \pm 0.50$	$65.74^{b} \pm 0.16$	$63.64^{b} \pm 0.14$	63.57 ± 0.85	$65.16 \ a \pm 0.69$	$64.58 b \pm 0.14$
В	20	$81.88 \stackrel{a}{\pm} \pm 0.50$	$79.22 b \pm 0.47$	$76.04 \text{ b} \pm 0.53$	$76.09 \text{ a,b} \pm 0.28$	75.06 $a,b \pm 0.49$	$63.02 ^{\text{c}} \pm 0.67$	$64.33 b \pm 0.23$	$63.65 \text{ b} \pm 0.45$	$64.51 \stackrel{a}{\pm} \pm 0.78$	$63.35 \text{ c} \pm 0.32$
	30	$81.31 \text{ b} \pm 0.33$	77.14 ^c \pm 0.40	$76.50^{\ b} \pm 0.40$	$75.55 b \pm 0.52$	$74.52^{\ b} \pm 0.50$	$62.51 \text{ c} \pm 0.76$	62.42 ^c \pm 0.86	$62.71 \text{ c} \pm 0.91$	$62.53^{\ b} \pm 0.76$	$62.21 \text{ d} \pm 0.86$
	0	$81.87\ ^{a}\ \pm\ 0.38$	$75.65\ ^{a}\pm 0.33$	$73.34\ ^{a}\pm 0.48$	$73.14\ ^{a}\pm 0.38$	$71.10\ ^{a}\pm 0.70$	$64.52\ ^{a}\pm 0.41$	$61.60\ ^{a}\pm 0.24$	$61.72\ ^{a}\ \pm\ 0.39$	$62.61\ ^{a}\ \pm\ 0.24$	$61.48\ ^{a}\ \pm\ 0.71$
	10	81.34 ^{a,b} ± 0.58	$76.98 b \pm 0.51$	$74.02^{b} \pm 0.27$	$72.19^{b} \pm 0.79$	71.70 $^{\rm a}$ \pm 0.58	$65.74^{b} \pm 0.16$	$62.44 \text{ b,c} \pm 0.52$	$62.48 b \pm 0.27$	$61.27 \text{ b} \pm 0.58$	60.96 $^{a} \pm 0.44$
JMB	20	$81.88 \stackrel{a}{=} \pm 0.50$	$77.19^{b} \pm 0.18$	72.20 ^c ± 0.89	$71.76^{b} \pm 0.69$	71.84 $a \pm 1.51$	63.02 ^c ± 0.67	$62.96 \text{ b} \pm 0.28$	$59.56 \text{ c} \pm 0.51$	$60.70 \text{ b} \pm 0.67$	61.13 ^a ± 0.89
	30	$81.31 \text{ b} \pm 0.33$	$77.29^{b} \pm 0.37$	73.37 ^{a,b} ± 0.33	72.45 ^{a,b} ± 1.07	71.83 $^{\rm a}$ \pm 0.24	$62.51 \text{ c} \pm 0.76$	$62.22 ^{\text{c}} \pm 0.69$	59.53 c \pm 0.55	$59.87 \text{ c} \pm 0.88$	$59.98 b \pm 0.43$
				[ΔE]							
	Inulin	Barley Flour									
Flour	(%)	0%	10%	20%	30%	40%					
	0	$31.15 \ ^{a} \pm 0.41$	$29.16\ ^{a}\pm 0.91$	$29.42 \ ^{a} \pm 0.47$	$30.57 \ ^{a} \pm 0.38$	$29.91 \ ^{a} \pm 0.52$					
	10	$29.88 b \pm 0.16$	$32.28^{b} \pm 0.54$	$32.02 b \pm 0.85$	$30.43 \ a \ \pm \ 0.70$	$31.01 \text{ b} \pm 0.14$					
В	20	32.68 ^c ± 0.67	$30.92 \text{ c} \pm 0.46$	$31.95 b \pm 0.46$	$31.08 \ ^{a} \pm 0.78$	$32.26 \text{ c} \pm 0.33$					
	30	$33.18 \text{ c} \pm 0.77$	$34.21 \ d \ \pm 1.26$	$32.90\ ^{c}\pm 0.92$	$33.07 b \pm 0.76$	$33.40 \ d \ \pm 0.87$					
	0	$31.15 \ ^{a} \pm 0.41$	$34.03 \ ^{a} \pm 0.24$	$34.12\ ^{a}\pm 0.46$	$33.02 \ ^{a} \pm 0.24$	$34.18 \ ^{a} \pm 0.71$					
	10	$29.88 b \pm 0.16$	$32.62^{b} \pm 0.94$	$33.04^{b} \pm 0.34^{c}$	$34.36 \text{ b} \pm 0.59$	$34.68\ a\ \pm\ 0.44$					
JMB	20	32.68 ^c ± 0.67	$32.65^{b} \pm 0.28$	36.06 ^c ± 0.51	$34.93^{b,c} \pm 0.67$	34.51 ^a ± 0.90					
		33.18 ^c ± 0.77	$33.40^{a} \pm 0.70^{a}$	$36.10 \text{ c} \pm 0.55$	35.89 ^c ± 1.42	$35.66 b \pm 0.43$					

Table 5. [L*], [WIE*] and $[\Delta E^*]$ color characteristics for biscuits.

Values of each column with different superscripts within the same barley flour are significantly different (p < 0.05).

Our findings are supported by the work of Gupta et al. (2011) [24] who studied biscuits with partial replacement of wheat by barley flour. According to them, the color of the biscuits changed with the incorporation of barley flour from pale cream to golden brown. Moreover, they also reported surface cracking of the biscuits with barley flour. Darker biscuits with an increase of the flour substitution level were also reported by Zucco et al. (2011) [21]. The color of the biscuits is generated from the baking process. It can be attributed to non-enzymatic browning reactions (Maillard), starch dextrinization and sugar caramelization [26]. Maillard reactions are connected to the protein content of the flour. Thus, the greater the protein content, the darker the biscuit. Moreover, inulin has a higher dextrose equivalence than sucrose and thus, stronger Maillard reactions occur and more color during baking is produced [12]. The impact of the flour particle size on the observed color can be attributed to the greater protein content of the flour.

4. Conclusions

In the present work, the incorporation of barley flour and inulin in biscuit formulation was selected in order to fulfill the consumers' demand for a superior nutrition as both of them consist of health-related compounds like fibers and proteins. According to our findings, the presence of increased amounts of inulin led to increased weight and decreased spread ratio of the biscuits. Moreover, within the same flour composition, inulin biscuits were softer than those with no inulin. Lightness was affected by both the presence of barley flour and inulin. The incorporation of barley flour was also significant for the total phenolic content. Overall, the incorporation of barley flour and inulin in the recipe led to promising results regarding the production of health-promoting biscuits. However, the level of substitution for both barley flour and inulin was significant for the formulation of organoleptically-accepted biscuits as it affected the color and the strength of the biscuits. Thus, a compromise between nutritional value and organoleptic acceptance should take place by keeping a low level of substitution so as not to produce dark and soft biscuits.

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