Use of Underexploited Flours for the Reduction of Glycaemic Index of Gluten-Free Biscuits: Physicochemical and Sensory Characterization

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Abstract

The type and the amount of starch present in bakery products affect their glycaemic index. The control of glycaemic index in our diet can be relevant to reduce the risk of type 2 diabetes and heart diseases. The use of alternative flours to rice, maize, and their starches may improve the nutritional quality of gluten-free baked goods by reducing the glycaemic index. This work aimed at the formulation of satisfactory gluten-free biscuits by using underexploited flour mixes (from cereals, pseudocereals, and legumes), at the evaluation of their physical properties, starch digestibility and subsequent estimation of the glycaemic index. The presence of chickpea flour increased biscuit hardness and gluten-free flours darkened biscuit surface. The greatest differences in starch composition and its digestion were found between gluten-free samples and wheat-containing control. Experimental biscuits had also a lower predicted glycaemic index compared to commercial products, whereas experimental formulations presented similar values between each other. Consumers' acceptance and sensory profiling by Check All That Apply questionnaire were carried out. All formulations but one were above the acceptance threshold (50); crumbliness and easiness to swallow were drivers of acceptance, whereas sandiness exerted a negative impact on liking score.

Keywords Gluten free · Biscuits · Starch digestibility · Predicted glycaemic index · CATA

Introduction

People affected by celiac disease and other gluten-related disorders are forced to follow a strict gluten-free (GF) diet; the consumption of GF products is pivotal for them. Moreover, a part of population decides to follow a GF diet as lifestyle choice. Despite the growing awareness towards a conscious nutrition and demand for healthier products, the nutritional quality of GF products still remains a major concern (Stantiall & Serventi, 2017).

A topic of rising interest in food research is the glycaemic index (GI) of conventional and GF bakery. A low GI diet can be helpful in reducing the risk of developing type II diabetes, obesity, cardiovascular disease, and some cancers (Garsetti et al., 2005; Takao et al., 2016). Nowadays, numerous studies are aimed at the investigation or reduction of GI of pasta, bread, and other bakery products (Brennan & Samyue, 2004; Di Cairano et al., 2021; Lal et al., 2021; Scazzina et al., 2013; Sethupathy et al., 2020; Trevisan et al., 2019). Celiac disease is associated with high incidence of type I diabetes; therefore, it is important to maintain a good glycaemic

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control whilst adhering to the GF diet (Berti et al., 2004). The use of specific ingredients, such as slowly digestible carbohydrates, fibres, and phenolic-rich ingredients (Lee & Bae, 2020), and the employ of specific processing technology, represents a strategy for the reduction of the GI.

Biscuits are a convenient food, inexpensive, ready to eat, and accessible for large part of the population (Nagi et al., 2012; Sulieman et al., 2019). Valitutti et al. (2017) showed that celiac disease patients prefer biscuits and crackers to bread as carbohydrate source compared to the general population. Hence, biscuits represent an ideal product to be improved by using new ingredients so as to provide healthier products to people following a GF diet. GF biscuits are mostly made by rice and maize flours and starches (Di Cairano et al., 2018; do Nascimento et al., 2013; Gallagher, 2009). In example, only less than 5% of GF biscuits on the Italian market contains buckwheat flour. Less than 1% contains whole millet and sorghum flours, whereas no lentil or chickpea flour was present in Italian GF biscuits at moment of data collection (Di Cairano et al., 2018). However, these flours even when employed do not represent the main ingredients in the recipes. It has been reported that the use of more nutrient dense flours could improve the nutritional quality of GF products (Cannas et al., 2020; Di Cairano et al., 2018; Foschia et al., 2016; Martínez-Villaluenga et al., 2020). The use of ingredients rich in rapidly digestible starch and with a low phenolic content can contribute to the increase of blood glucose level (Di Cairano et al., 2020; Moraes et al., 2015; Sun & Miao, 2020).

GF biscuits, precisely for the flours they are mainly made of, are characterized by a mild and neutral taste and have a less distinctive flavour compared to their gluten-containing counterparts (Badiu et al., 2014; Sandri et al., 2017). The use of less common flours like the ones aforementioned may significantly affect biscuit taste. Indeed, the use of rice flour can be advantageous for the bland taste, but the employ of other flours could give the product a “too strong” taste (Gallagher, 2009). When dealing with the formulation of new products, it is fundamental to meet consumers’ tastes. The best way to understand consumer preference is using consumer data (Tarancón et al., 2013). In this context, Check All That Apply questionnaire can be helpful to collect information regarding consumers’ perception of foods, which is relevant for the characterization and improvement of the final product. Moreover, knowing consumer acceptance and drivers of liking and disliking can give valuable hints for the marketability of a product.

It is common opinion that further research is needed to develop more palatable and nutritious bakery products (Cappelli et al., 2020; Conte et al., 2019; Di Cairano et al., 2018; Xu et al., 2020). Investigations on the use of buckwheat (Dapčević Hadnadev et al., 2013; Kaur et al., 2015; Molinari et al., 2018), sorghum (Cayres et al., 2020; Omoba et al., 2015), millet (Adebiyi et al., 2017; Devisetti et al., 2015;

Sharma et al., 2016), and lentil and other legume flours in biscuit and cakes (Gularte et al., 2012; Mancebo et al., 2015; Sparvoli et al., 2016) have been documented. Nevertheless, not all of them investigate the digestibility of starch or include sensory evaluation. To our best knowledge, none of the work available in literature deals with the employ of flour mixes including the aforementioned flours, in the view of the reduction of GI of gluten-free biscuits without neglecting their sensory evaluation. Based on these premises, this work looks at the formulation of acceptable GF biscuits with underexploited flours, mainly obtained by buckwheat, millet, sorghum, lentils, and chickpeas, which are rarely found in commercial GF biscuits.

The effect of the use of buckwheat, millet, sorghum, lentils, and chickpea in biscuit formulations was evaluated, taking into account physical properties, starch digestibility, predicted glycaemic index (pGI), liking score, and sensory profile by using the Check All That Apply (CATA) questionnaire.

Materials and Methods

Flour Mixes and Ingredients

Six formulations of GF biscuits were prepared by mixing cereal, pseudocereal, and legume flours along with a control biscuit based on wheat. Type 0 soft wheat (Molino Pivetti, Italy), buckwheat (Molino Filippini, Italy), whole millet (Molino Filippini), chickpea (Terre di Altamura, Italy), sorghum (Molino Filippini), and lentil (Terre di Altamura) flours were employed. Flour mixes were prepared by combining and mixing the flours together, according to the percentages reported in Table 1. The mixes

Table 1 Flour mixes employed in the formulations of gluten-free biscuits

Sample	Flours		
C	Soft wheat100%	-	-
1A	Buckwheat 50%	Millet 50%	-
1B	Buckwheat 50%	Millet 30%	Chickpea 20%
1C	Buckwheat 50%	Millet 30%	Lentil 20%
2A	Buckwheat 50%	Sorghum 50%	-
2B	Buckwheat 50%	Sorghum 30%	Chickpea 20%
2C	Buckwheat 50%	Sorghum 30%	Lentil 20%

were realized on the basis of preliminary trials. Flours, inulin (Fibruline, Cosucra, Belgium), soy lectin, and leavening powders (BicarFood, Solvay, Belgium; ammonium bicarbonate, Esseco, USA) were supplied by Di Leo Pietro s.p.a (Italy). Sunflower oil (Olio Basso, Italy), eggs (Ovomont, Italy), and salt were purchased in a local supermarket.

Functional Properties of Flour Mixes

Water and oil absorption capacity (WAC and OAC, respectively) were determined as reported by Kaur et al. (2015). Ten millilitres of distilled water or refined soybean oil was added to 1 g of flour and kept at 20 °C for 30 min. Then, the samples were centrifuged at 2000g for 10 min and the supernatant decanted. WAC and OAC are the weight of the gel obtained after removal of the supernatant per unit weight of original dry solids.

The supernatant of WAC was decanted into an evaporating dish and dried at 105 °C until constant weight in order to determine water solubility index (WSI) (Chauhan et al., 2016). WSI is the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample. Measurements were taken in triplicate.

Bulk density (BD) was determined as reported by Chauhan et al. (2016). Ten grams of flour was placed in a 25-ml graduated cylinder and the cylinder was gently tapped ten times from a height of 7–8 cm. The final volume of the flour was measured and expressed as grammes per millilitre. Measurements were taken in triplicate.

Biscuit Making

The ingredients were mixed using a Kenwood KMX750RD (Kenwood Ltd., UK). In the first mixing phase, sugar (19.07%), oil (8.18%), and soy lecithin (0.77%) were mixed for 3 min at speed 2. Then, eggs (13.55%), ammonium bicarbonate (0.11%), and water (0.80%) were added and mixed for three more minutes. Finally, flour mix (56.22%), inulin (1.10%), sodium hydrogen carbonate (0.11%), and salt (0.11%) were added and mixed for another 3 min at minimum speed. The dough was wrapped in a plastic film and left to rest for 10 min; then, it was sheeted with a wooden rolling pin, and after that, its height was adjusted to 6 mm with a regulating rolling pin. Biscuits were cut in a round shape with a biscuit cutter of 4 cm diameter. Then, biscuits were baked at 200 °C in a domestic oven (De Longhi, Italy) for 11 min. Two replicates for each formulation were made. After cooling, biscuits were sealed in polypropylene plastic bags for a week before analysis and stored at 20 ± 2 °C.

Physical Properties of Biscuits: Geometric Indexes, Colour, and Breaking Point

Diameter and thickness of 5 biscuits were measured with a calliper. The spread ratio (SR) was calculated dividing biscuit diameter by height (Zoulias et al., 2000).

Breaking point was measured with a force gauge (Lutron FG-5000A) using a chisel head adapter on five biscuits for each sample. The force required to break the biscuit was recorded. Results were expressed in Newton (N). The test was conducted on 6 biscuits for each formulation.

Colour was measured by using a Minolta Chroma meter CR-300 with a D 65 illuminant, and expressed as colour L^* (lightness), a^* (redness), and b^* (yellowness) values using CIELab parameters. Measurements were made in 3 different positions on 5 biscuits for each sample. The whiteness index which mathematically combines lightness and yellow-blue into a single term was calculated according to Eq. 1 (Hsu et al., 2003).

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

In Vitro Starch Digestibility

Total, Non-Resistant and Resistant Starch Content

Resistant starch (RS) and total starch (TS) were determined according to AACC method 32–40.01 (AACC, 2009) using the kit, K-RSTAR (Megazyme International Ireland Ltd., Wicklow, Ireland). Measurements were taken at least in duplicate.

In Vitro Starch Digestion Rate and Predicted Glycaemic Index

In vitro starch digestion rate was evaluated according to the method reported by Molinari et al. (2018) based on Goñi et al. (1997). One hundred milligrams of minced biscuits were added with 10 ml of HCl-KCl buffer and 0.2 ml of pepsin (P7000, Sigma-Aldrich) solution (1 g in 10 ml HCl-KCl buffer) and incubated at 40 °C for 1 h. Then, the solution was brought up to 25 ml with sodium maleate buffer (0.2 M, pH 6.9) and 5 ml (2.6U/5 ml) of α -amylase (A3176, Sigma-Aldrich) solution was added. Samples were incubated at 37 °C for 180 min with constant stirring. One-milliliter aliquots were taken at 0, 30, 60, 90, 120, and 180 min. Immediately after the withdrawal, the aliquot was incubated at 100 °C to inactivate the enzyme. Each test was cooled in ice and kept cold if not used immediately. The aliquots were centrifuged at 2500g at 4 °C. Five hundred microlitres of supernatant were brought up to 2 ml with sodium acetate buffer (0.2M, pH 4.75). Then, 60 μ l of amyloglucosidase (E-AMGDF, Megazyme) was added to the sample and incubated at 60 °C

for 45 min. The glucose content was measured with glucose oxidase/peroxidase reagent (K-GLUC, Megazyme) by incubating 0.1 ml of solution with 3 ml of mixture for 20 min at 40 °C. The absorbance was read at 510 nm. Glucose content was multiplied by 0.9 to calculate starch amount. The rate of starch digestion was expressed as grams of starch hydrolyzed per 100-g sample at 0, 30, 60, 90, 120, and 180 min. The hydrolysis index (HI) was calculated as the ratio between the area under the hydrolysis curve (0–180 min) of the raw flours and the area of reference sample (white bread). The area under the curve was calculated with the trapezoidal rule. The pGI was calculated according to the Eq. 2 (Goñi et al., 1997). Measurements were taken at least in duplicate.

$$\text{pGI} = 39.71 + 0.549 \times \text{HI} \quad (2)$$

Consumer Sensory Profiling—Liking Score and CATA Analysis

Biscuits were subjected to sensory evaluation by a panel of 76 consumers aged between 20 and 65 and recruited among students and staff of the University of Basilicata. The evaluation was performed in individual booths under white light at room temperature. Before sensory evaluation, all participants signed an informed consent form.

The six biscuits were served all together in a white plastic dish in a randomized balanced order. Each sample was associated to a random three digit code. At least 1-min rest between each sample was required to restore the neutrality of mouth by rinsing it with water. Consumers were asked to express their liking score on a general labeled magnitude scale (gLMS). The labels of the conventional gLMS (Bartoshuk et al., 2003) were changed to collect hedonic ratings: 0—“the most unpleasant you can imagine” and 100—“the most pleasant you can imagine”.

Biscuits were also evaluated using the CATA questionnaire. The list of terms comprised 18 attributes covering texture and mouthfeel

sensations (“easy to chew”, “hard to chew”, “easy to swallow”, “hard to swallow”, “mealy”, “hard”, “crumbly”, “sandy”, “crispy”, “adhesive”) and flavour/taste and after taste (“sweet”, “salty”, “persistent”, “legume flavour”, “roasted flavour”, “cereal flavour”, “biscuit flavour”, “off flavour”). Sensory sessions were conducted by using FIZZ software (BioSystem, France).

Statistical Analysis

Statistical analysis was carried out in Excel 2013 (Microsoft Office, USA), using XLSTAT Premium Version (2019.4.2, Addinsoft, France).

One-way ANOVA was adopted to compare variable means. It was conducted both on all samples and on samples grouped according to the cereal and legume flours contained in the recipe. ANOVA was followed by Tuckey HSD test ($p < 0.05$) or Games-Howell test when homogeneity of variance assumption was not fulfilled. Sensory data were analyzed using the “CATA analysis” tool in XLSTAT software. Cochran’s Q test followed by a critical difference procedure (Shenskin) was applied to differentiate samples on the basis of their attributes. Penalty lift analysis was carried to see the mean impact of the attributes on liking score.

Results and Discussion

Functional Properties of Flour Mixes

Functional properties represent a characteristic of food materials reflecting the interactions among food components. Functional properties of flour mixes used for biscuit making are reported in Table 2. WAC of the flour mixes ranged between 0.64 and 1.39 g/g. Wheat flour showed the lowest value whereas 1C (buckwheat, millet, and lentil) the highest. WAC represents the capacity of the flour to bind with water and may help to retain a softer texture in bakery products (Du et al.,

Table 2 Functional properties of flour mixes

Sample	Water absorption capacity (g/g)	Water solubility index (g/g)	Oil absorption capacity (g/100 g)	Bulk density (g/ml)
C	0.64 ± 0.01 ^c	4.13 ± 0.27 ^d	1.04 ± 0.02 ^a	0.68 ± 0.01 ^{ab}
1A	1.20 ± 0.02 ^b	7.49 ± 0.16 ^b	0.93 ± 0.06 ^a	0.71 ± 0.03 ^a
1B	1.26 ± 0.02 ^b	8.97 ± 0.21 ^a	0.96 ± 0.06 ^a	0.69 ± 0.00 ^a
1C	1.17 ± 0.01 ^b	5.26 ± 0.09 ^d	0.99 ± 0.06 ^a	0.70 ± 0.01 ^a
2A	1.39 ± 0.03 ^a	8.21 ± 0.60 ^{ab}	1.01 ± 0.08 ^a	0.64 ± 0.01 ^b
2B	1.34 ± 0.07 ^{ab}	8.90 ± 0.69 ^{ab}	0.93 ± 0.08 ^a	0.71 ± 0.01 ^a
2C	1.28 ± 0.04 ^{ab}	4.25 ± 0.05 ^d	1.03 ± 0.19 ^a	0.71 ± 0.03 ^a

Data are expressed as mean ± standard deviation. Values followed by the same letter are not significantly different ($p < 0.05$). C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)

Fig. 1 Gluten-free biscuits and wheat control biscuit. C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20). For the coloured version, please refer to the online version

2014). Among GF flour mixes, samples containing millet flour had a higher WAC ($p < 0.05$), confirming the trend reported in a previous study (Di Cairano et al., 2020). The major presence of fibre in millet flour (as in technical sheet, data not reported) explains the major capacity of water absorption of mixes containing it.

WSI ranged between 4.13 g/100 g of control sample and 8.90 g/100 g of 2B (buckwheat, sorghum, and chickpea). Flour mixes containing chickpea flour tend to have a higher WSI. OAC ranged between 0.93 and 1.04 g/100 g, but no significant differences were found between samples. All the flour mixes showed the same capacity of binding with oil. OAC was not significantly affected neither by cereal employed nor by legume flours. Since fats play an important role in flavour retention, OAC may have an impact on product flavour (Kaur et al., 2015). Also, BD values resulted to be very similar between each other. Cereal flours had not effect on BD, whereas legume flours increased bulk density, but only lentil flour showed a significant impact on BD ($p < 0.05$). The different granulometry of the flours (data not shown) had a major impact on BD.

Physical Properties of Biscuits: Geometric Indexes, Colour, and Breaking Point

All the biscuits maintained their round shape during cooking and had a pleasant appearance (Fig. 1).

Most of the formulations showed similar thickness and diameter values, meaning that they had a similar rising ability during cooking. SR which is the ratio between diameter and thickness was similar for all the formulations. Millet containing samples had lower SR values (Table 3), probably due to a higher presence of fibres (data not shown) which lower SR values. Nevertheless, looking at data by grouping them according to cereal flour, it appeared that SR was not affected by cereal flour employed ($p < 0.05$). On the contrary, the use of chickpea instead of lentil flour led to higher SR value. Generally, high SR values are considered desirable (Chauhan et al., 2016).

Breaking point (Table 3) ranged between 21.56 and 46.93 N. The use of millet flour rather than sorghum flour did not have a significant effect on breaking point ($p > 0.05$), whereas it clearly appeared that the presence of chickpea flour contributed to biscuit hardness ($p < 0.05$) more than lentil or absence of legume flours. Together with baking conditions, quantity and protein content of ingredients can influence hardness (Adeola & Ohizua, 2018), but no specific trend related to protein content of the flour employed and breaking point was recorded. Breaking point was positively related ($r = 0.958$, $p < 0.05$) to the number of times that the attribute “hard” was selected by consumers, suggesting a good correlation between the CATA answers and instrumental analysis. The CATA analysis results are reported in “Liking and sensory profiling with Check All That Apply questionnaire”.

Colour analysis (Table 3) allowed to highlight the differences between wheat flour-based biscuits and GF formulations. Indeed, C, the control sample based on wheat flour, presented a lighter colour, less red, and more yellow compared to all other formulations. GF biscuits had lower lightness

Table 3 Geometric indexes, colour, and breaking point of gluten-free biscuits

	Thickness (cm)	Diameter (cm)	Spread ratio	L^*	a^*	b^*	Whiteness index	Breaking point (N)
C	1.10 ± 0.07^b	4.71 ± 0.04^a	4.30 ± 0.30^a	76.76 ± 1.45^a	1.88 ± 1.09^d	32.37 ± 1.31^a	60.08 ± 1.63^a	28.67 ± 6.33^{bc}
1A	1.25 ± 0.13^b	4.50 ± 0.04^b	3.63 ± 0.38^{bc}	62.54 ± 0.95^e	5.85 ± 1.09^a	27.44 ± 1.31^{cd}	53.19 ± 1.63^c	28.36 ± 5.13^{bc}
1B	1.17 ± 0.03^b	4.77 ± 0.05^a	4.08 ± 0.08^{ab}	66.17 ± 0.72^c	3.75 ± 0.29^c	26.55 ± 0.63^d	56.82 ± 0.65^b	46.93 ± 3.90^a
1C	1.43 ± 0.07^a	4.53 ± 0.05^b	3.17 ± 0.12^c	63.23 ± 1.19^{de}	4.84 ± 0.54^b	28.06 ± 1.40^c	53.48 ± 1.47^c	26.04 ± 2.52^c
2A	1.25 ± 0.08^b	4.80 ± 0.07^{ab}	3.85 ± 0.21^{ab}	66.56 ± 1.28^c	4.26 ± 0.69^{bc}	28.03 ± 1.17^c	56.15 ± 1.52^b	21.56 ± 3.25^c
2B	1.23 ± 0.03^b	4.79 ± 0.06^a	3.88 ± 0.04^{ab}	68.16 ± 1.13^b	2.88 ± 0.66^d	27.91 ± 1.19^c	57.55 ± 1.33^b	34.24 ± 6.65^{ab}
2C	1.23 ± 0.10^b	4.56 ± 0.13^a	3.73 ± 0.32^b	64.14 ± 0.99^d	4.34 ± 0.72^{bc}	29.83 ± 0.98^b	53.14 ± 1.07^c	31.66 ± 2.50^{abc}

Data are expressed as mean $\pm$ standard deviation. Values followed by the same letter are not significantly different ($p < 0.05$). C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)

values that means a darker surface. This characteristic can be attributed to GF flours, such as buckwheat one, which have a dark brownish colour compared to wheat flour (Schober et al., 2003). Considering only GF formulations, biscuits with sorghum flour had higher lightness values compared to millet-containing ones. Millet flour, being a whole flour, was actually darker compared to other flours. Sorghum biscuits also presented lower a^* values and higher b^* values. Samples with sorghum flour showed also a higher whiteness index. The presence or absence of legume flours also affected the colour of the finished biscuit; lentil-containing biscuits resulted to be lighter than all other formulations. Colour is one of the attributes that determines the acceptability of bakery products (Zucco et al., 2011); it can be affected by the colour of raw material, but its formation during the baking stage is mainly attributed to reducing sugars that react with the amino acids present in the flour via Maillard reaction or caramelization.

In Vitro Starch Digestibility

Total, Non-Resistant and Resistant Starch Content

Control biscuit had the highest starch content (50.59%), whereas total starch of GF samples ranged between 33.77 and 38.27 g/100 g dwb (Table 4). Apart from control biscuit, total starch content was similar for all the formulations. Sample 2B (buckwheat, sorghum, and chickpea) had the highest RS content; other samples had similar amount of RS. However, considering only GF formulations and grouping them on the basis of the flours contained, RS content appeared to be significantly affected by legume flour type. Indeed, samples containing chickpea showed a slightly higher RS content ($p < 0.05$) than samples containing lentil or no legume flours. Also, millet flour gave a major contribution to the amount of RS present in the final sample than sorghum flour. From a nutritional standpoint, the presence of RS is beneficial for human health, since it is not

Fig. 2 Area under the curve for the in vitro starch hydrolysis at 180 min of white bread (reference sample—std) wheat control biscuit and gluten-free biscuits. Different letters indicate significantly different values. C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)

digested in the small intestine and does not contribute to glucose release, and it is fermented in the colon, acting like a soluble fibre (Graça et al., 2021). However, it would be desirable to increase RS content of the newly developed biscuits. Indeed, supplementation of bakery products with RS showed a reduction of in vitro glycaemic index (Cervini et al., 2021; Kahraman et al., 2019)

Starch Digestion Rate and Predicted Glycaemic Index

During in vitro digestion, the starch present in control formulation was the most hydrolyzed (Fig. 2), followed by starch present in sorghum biscuits and then the starch in millet formulations. Indeed, grouping sample according to the type of cereal used, the area under the curve (AUC) of formulations with millet was significantly lower than the ones with sorghum ($p < 0.05$). This could be explained by the higher

Table 4 Resistant, non-resistant starch, hydrolysis index, and predicted glycaemic index of gluten-free biscuits

Sample	Nonresistant starch (g/100 g dwb)	Resistant starch (g/100 g dwb)	Total starch (g/100 g dwb)	Resistant starch (g/100 g total starch)	Hydrolysis index	Predicted glycaemic index
C	50.59 ± 2.12 ^a	0.40 ± 0.00 ^b	50.99 ± 2.12 ^a	0.78 ± 0.03 ^c	56.94 ± 1.73 ^a	70.97 ± 0.95 ^a
1A	38.07 ± 0.60 ^b	0.30 ± 0.03 ^b	38.37 ± 0.57 ^b	0.78 ± 0.08 ^c	30.39 ± 1.33 ^c	56.39 ± 0.73 ^c
1B	38.27 ± 2.70 ^b	0.36 ± 0.06 ^b	38.63 ± 2.77 ^b	0.93 ± 0.10 ^c	28.80 ± 0.42 ^c	55.52 ± 0.23 ^c
1C	35.68 ± 3.52 ^b	0.36 ± 0.04 ^b	36.04 ± 3.48 ^b	1.40 ± 0.21 ^c	27.97 ± 4.32 ^c	55.07 ± 2.31 ^c
2A	35.80 ± 2.08 ^b	0.40 ± 0.10 ^b	36.20 ± 1.98 ^b	1.10 ± 0.33 ^{bc}	42.76 ± 1.55 ^b	63.18 ± 0.85 ^b
2B	35.37 ± 0.58 ^b	0.73 ± 0.01 ^a	36.09 ± 0.57 ^b	2.01 ± 0.05 ^a	35.92 ± 0.81 ^{bc}	59.43 ± 0.44 ^{bc}
2C	33.73 ± 2.82 ^b	0.50 ± 0.02 ^b	34.23 ± 2.84 ^b	1.47 ± 0.08 ^b	41.34 ± 0.07 ^b	62.40 ± 0.04 ^b

Data are expressed as mean ± standard deviation. Values followed by the same letter are not significantly different ($p < 0.05$). C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)

content of amylose of millet flour compared to sorghum, whose starch is constituted by a higher amount of amylopectin (Di Cairano et al., 2020). Amylopectin is more digestible than amylose, having a large surface area available for enzymatic attack (Singh et al., 2010). With regard to legume flours, samples containing chickpea had a lower AUC than samples with no legume or with lentil flours. Generally, legume starches are less digestible than cereal starches due to the high presence of amylose, proteins, and antinutrients which hinder the action of digestive enzymes (Singh et al., 2010). The AUC of biscuit formulations was very low if compared to the AUC of white bread. Starch present in biscuits, due to the low moisture, is not fully gelatinized, and this implies a lower availability to enzymatic hydrolysis, unlike that of bread (Garsetti et al., 2005). Dividing the AUC of each sample by the AUC of reference sample (white bread), the hydrolysis index was obtained. From the hydrolysis index, the GI according to Eq. 2. pGI of GF formulations ranged between 55.07 and 63.18 (Table 4) was estimated. Biscuits containing millet had lower pGI values compared to sorghum containing formulations, and control biscuit based on wheat flour had the highest pGI (70.97). Millet flour has a higher amount of fibre; indeed, fibre contributes to lowering of glycaemic response both in vivo and in vitro (Brennan & Tudorica, 2008; Kahraman et al., 2019; Scazzina et al., 2013). pGI was also determined for two commercial GF biscuits so as to make a comparison with experimental GF samples. Biscuits selected were representative of commercial GF biscuits on Italian market; the first commercial sample was mostly made with maize flour and potato starch, the latter with potato and maize starches and maize and rice flours. They had higher pGIs, 67.03 and 76.12, respectively, compared to experimental GF formulations. In example, Molinari et al. (2018) found a reduction of pGI of gluten-free biscuits when replacing part of rice flour with buckwheat one (110.2 vs 62.8). Buckwheat flour was the main flour employed in our GF biscuits, and compared to rice flour, itself can already give a contribution to pGI reduction (Bączek et al., 2020; Di Cairano et al., 2020; Wolter et al., 2013). Indeed, the presence of lipids, proteins, dietary fibre, rutin, phytic acid, protease inhibitor, and tannins in buckwheat reduces the availability of starch to enzymatic hydrolysis due to their interaction with starch itself or with alpha amylase (Molinari et al., 2018; Wolter et al., 2013).

Samples showing the highest pGI, such as commercial formulations, had the highest amount of starch hydrolyzed 20 min after the beginning of hydrolysis, which correspond to rapidly digestible starch; a positive correlation ($r = 0.959$, $p < 0.05$) was recorded between the latter and pGI. Indeed, rapidly digestible starch is known to greatly contribute to the rising of blood glucose level (Zhang & Hamaker, 2009). The selection of specific flours and avoiding the use of pure starches facilitated obtaining experimental biscuits with a lower pGI than that of commercial samples.

According to GI classification (Atkinson et al., 2008), our samples could be considered medium GI foods, whereas one of the commercial biscuits has to be considered high GI food. Generally, biscuits are included in medium-high glycaemic index products (Trinidad et al., 2010); Scazzina et al. (2015) reported low-medium GI for commercial GF biscuits, whereas Parker et al. (2000) reported a GI of 83 for commercial GF digestive biscuits, showing that data are often varied. However, GF food products are generally considered to have a higher GI than their gluten-containing counterparts (Berti et al., 2004; Lamacchia et al., 2014; Vici et al., 2016). The consumption of biscuits with a lower pGI may help to adhere to a low GI diet; low GI diet could contribute to prevent type II diabetes, cardiovascular, and chronic metabolic diseases (Augustin et al., 2015; Brand-Miller et al., 2009). Moreover, celiac disease and type I diabetes are often associated (Kylökas et al., 2016; Weiman et al., 2021); the reduction of GI of GF foods could contribute to a better quality of life of patients suffering from celiac disease and diabetes.

Though the data obtained from the in vitro estimation of GI should be considered cautiously, dynamics of digestion in vivo are indeed difficult to be exactly replicated in vitro. Some authors reported a lack of a strong correlation between in vivo and in vitro GI (Ferrer-Mairal et al., 2012). Nevertheless, the technique is constantly evolving to better simulate in vivo conditions and more and more researchers use in vitro study for sample screening confirming the usefulness of in vitro studies. They indeed offer the advantage of standardized conditions, lower costs, shorter analysis times, and avoiding ethical concerns (Brouns et al., 2005; Lal et al., 2021).

Liking and Sensory Profiling with Check All That Apply Questionnaire

Control sample based on wheat flour got the highest liking scores, followed by the formulation containing buckwheat and sorghum flours (2A) and buckwheat, sorghum, and lentil flours (2C). All other samples showed lower scores but similar between each other; only the sample based on buckwheat and millet flours completely differed from the others, being less preferred (Fig. 3). However, liking scores expressed for all the formulations were considered satisfying, seeing that no sample, except 1A, was below the threshold of acceptance, set at 50 “neither pleasant nor unpleasant”. Sample 1A contained 50% buckwheat and 50% millet flour; the lower liking score is consistent with results obtained by Mcsweeney et al. (2016), who found that increasing levels of millet flour decreased the liking of biscuits. This can be related to the relatively bitter taste of millet flour due to its richness in phenolic compounds (Adebiyi et al., 2017). Data were also analyzed by grouping samples according to the type of cereal flour employed. It resulted that biscuits containing sorghum flour were most

Fig. 3 Liking score (mean values, 76 consumers) of control wheat biscuit and gluten-free biscuits on a general labeled magnitude scale. In the legend, liking scores are reported as mean $\pm$ standard deviation; values followed by different letters are not significantly different ($p < 0.05$). C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20). For the coloured version, please refer to the web version of the article

liked compared to samples containing millet ($p < 0.05$), whereas no significant differences resulted by grouping samples according to legume flour employed.

The CATA questionnaire was employed to depict the profile of the biscuits. The use of untrained assessors could provide a description of the product closer to the perception of the

final consumer (Ares & Varela, 2017). Moreover, it has also been reported that description of the product by the CATA analysis is very close to that obtained by descriptive analysis with trained assessors (Mello et al., 2019).

Table 5 reports the characterization by attributes of the samples obtained through the Cochran Q test. Most of the attributes

Table 5 Characterization by attribute of GF biscuits according to Cochran's Q test

Attributes	p -value	1A	1B	1C	2A	2B	2C	C
Easy to chew	< 0.0001	0.408a	0.355a	0.474abc	0.618bc	0.461abc	0.421ab	0.658c
Hard to chew	0.055	0.105a	0.132a	0.079a	0.026a	0.039a	0.118a	0.039a
Easy to swallow	< 0.0001	0.211a	0.197a	0.237ab	0.421bc	0.316ab	0.303ab	0.566c
Hard to swallow	0.003	0.184ab	0.237b	0.171ab	0.066a	0.118ab	0.105ab	0.066a
Sweet	< 0.0001	0.184a	0.250ab	0.276ab	0.421b	0.342ab	0.329ab	0.737c
Salty	0.315	0.158a	0.118a	0.197a	0.118a	0.118a	0.092a	0.092a
Mealy	0.026	0.421b	0.355ab	0.421b	0.382ab	0.316ab	0.368ab	0.197a
Hard	< 0.0001	0.079a	0.342b	0.145a	0.026a	0.197ab	0.171ab	0.105a
Crumbly	< 0.0001	0.303ab	0.224a	0.382abc	0.579cd	0.408abc	0.461bcd	0.645d
Sandy	< 0.0001	0.553c	0.329b	0.342b	0.342b	0.368bc	0.303b	0.039a
Crispy	< 0.0001	0.158a	0.250ab	0.342abc	0.276abc	0.382bc	0.461c	0.434bc
Adhesive	0.938	0.145a	0.118a	0.118a	0.132a	0.105a	0.105a	0.092a
Persistent	< 0.0001	0.434c	0.316bc	0.316bc	0.158ab	0.145ab	0.171ab	0.118a
Legume flavour	< 0.0001	0.197b	0.329b	0.211b	0.158ab	0.250b	0.237b	0.013a
Roasted flavour	< 0.0001	0.395c	0.368bc	0.329bc	0.171ab	0.329bc	0.224abc	0.053a
Cereal flavour	0.024	0.197a	0.276a	0.197a	0.303a	0.329a	0.342a	0.158a
Biscuit flavour	0.088	0.105ab	0.105ab	0.092ab	0.092ab	0.079ab	0.053a	0.197b
Off flavour	0.282	0.132a	0.079a	0.118a	0.053a	0.079a	0.039a	0.105a

Significant attributes following the Cochran Q test ($p \leq 0.05$) are reported in bold. Frequency relative values are reported within rows, values with the same letter do not differ significantly from each other according to the Shenskin procedure ($p < 0.05$). C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)

Fig. 4 Sensory profiles of gluten-free biscuits according to CATA questionnaire. Results are expressed as % of judges selecting the attributes associated to each sample. C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20). For the coloured version, please refer to the web version of the article

checked by consumers differentiated the samples ($p < 0.05$). Control sample based on wheat flour was perceived sweeter than GF biscuits; each formulation contained the same amount of sugar but the presence of flours with a stronger taste in GF formulations made the perception of sweetness less marked. Indeed, food intrinsic factors, such as taste-aroma interactions, together with food texture can affect sweetness perception (Wang et al., 2019). Stronger sensations, i.e. “cereal flavour”, “roasted flavour”, and “legume flavour”, were associated to gluten-free biscuits. For the attribute “legume flavour”, a clear difference was recorded between control sample and GF biscuits. In example, also, Cannas et al. (2020) reported that the employ of an

alternative flour, specifically quinoa, gave a more characteristic taste to ladyfinger GF biscuits. It is interesting to observe that “legume flavour” was perceived also in formulations without chickpea or lentil flours due to a more characteristic taste of the flours employed. The sensory profile of each biscuit was depicted (Fig. 4) on the basis on attributes proposed and checked by consumers. The most selected attributes were “easy to chew”, followed by “crumbly”, “sweet”, “mealy”, and “sandy”. The first three are positive attributes, whereas the last two are generally associated to GF biscuits and considered unpleasant (Schober et al., 2003). Attributes related to texture and mouthfeel sensations depend on ingredients and compounds present in the oral cavity. In

Fig. 5 Mean drop graph, the effect of attribute on liking scores. Attributes in bold have a significant effect on liking score ($p < 0.05$). C: wheat flour (100) biscuits; 1A: buckwheat to millet (50:50); 1B: buckwheat to millet to chickpea (50:30:20); 1C: buckwheat to millet to lentil (50:30:20); 2A: buckwheat to sorghum (50:50); 2B: buckwheat to sorghum to chickpea (50:30:20); 2C: buckwheat to sorghum to lentil (50:30:20)

this case, the differences found among these attributes depend on the flours employed, their composition, granulometry, and their interaction with other ingredients. However, it would be extremely complex to explain the different behaviour of each biscuit when tasted.

Knowing the effect of each attribute on liking score gives the possibility to identify the acceptability drivers, which can help producers in the design of new products and to understand the priorities when reformulating existing products (Ares & Jaeger, 2015). By the way, Fig. 5 depicts the mean impact of each attribute on liking score. In short words, it displays the impact of each attribute on liking score. Positive attributes were “biscuit flavour”, “crumbly”, “easy to swallow”, “crispy”, and “sweet”, whereas “hard to chew”, “hard to swallow”, “legume flavour”, and “roasted flavour” had a negative impact; on the other hand, the remaining attributes had only a very slightly negative impact. According to the penalty lift analysis, the significant impact on liking score was exerted only by “crumbly” (+ 4.38), “easy to swallow” (+ 3.80), and “sandy” (− 5.08) attributes.

Some correlations were found between attributes; “adhesive”, and “mealy” and so as “adhesive” and “sandy” showed a positive correlation ($r = 0.795$ and $r = 0.840$, $p < 0.05$), whereas “adhesive” and “crispy” showed a negative correlation ($r = -0.908$, $p < 0.05$). Some data were redundant, namely that similar attributes were positively related whereas opposite attributes showed a negative correlation according to Pearson correlation test ($p < 0.05$). This can be considered an index of panel reliability.

Conclusions

This work disclosed that it is possible to produce GF biscuits with alternative flours, avoiding the use of rice and maize flours and starches, and suggests the attributes that drive consumers’ acceptance and dislike.

The use of buckwheat, millet, sorghum, chickpea, and lentil flour allowed to obtain GF biscuits with a lower pGI, compared to wheat control biscuit and two commercial biscuits. Nevertheless, to pursue the accomplishment of low GI biscuits, it will be necessary to reformulate the product by using ingredients that are able to give a major contribution to its reduction as well as it would be desirable to validate the data through an in vivo study.

The consumer test showed that all the formulations but one were above the threshold of acceptance; moreover, the fact that GF biscuits are generally characterized by a sandy mouthfeel which results unpleasant was confirmed. “Sandy” was indeed a driver of disliking of the biscuit, whereas “crumbly” and “easy to swallow” can be considered drivers of liking. This information should be taken into account to improve biscuit sensory properties.

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Data Availability Statement The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable

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Declarations

Conflict of Interest The authors declare no competing interests.

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