



Research article

Nutritional, physical and sensory characteristics of gluten-free biscuits incorporated with a novel resistant starch ingredient



Mariasole Cervini^a, Antonello Frustace^b, Guillermo Duserm Garrido^b, Gabriele Rocchetti^b, Gianluca Giuberti^{b,*}

^a Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134, Verona, Italy

^b Department for Sustainable Food Process (DiSTAS), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy

ARTICLE INFO

Keywords:

Gluten-free
Resistant starch
Hydrolysis index
Dietary fiber
Biscuits

ABSTRACT

Gluten-free (GF) biscuits were prepared by replacing part of a GF flour mix (GFM) with 0, 15, 30 and 45 g/100 g (total flour) with a novel resistant starch-rich ingredient obtained from annealed white sorghum starch (RSWS). The chemical composition, physical characteristics, in vitro starch digestion and sensory evaluation of biscuits were considered. The chemical composition of samples was influenced by the addition of the RSWS. The highest total dietary fibre and RS contents ($p < 0.05$) were measured in 45-RSWS biscuits. The starch hydrolysis index values decreased when the level of RSWS increased in the composite. With regard to quality parameters, the use of RSWS influenced the hardness of the biscuits, and the highest value obtained for 45-RSWS. Some of the selected sensory attributes, along with the overall acceptability score, were negatively influenced by the RSWS addition, even if all remained above the limit of acceptability. The use of RSWS in GF biscuit formulation can contribute towards the creation of food products likely having slowly digestible starch properties, and this can be achieved without drastically compromising on the quality and sensory attributes.

1. Introduction

The consumer demand for gluten-free (GF) bakery foods is growing (Padalino et al., 2016). However, commercially available GF cereal-based products frequently presented inferior nutritional quality in comparison to the gluten-containing equivalents (Giuberti and Gallo 2018). One of the most used strategies for ameliorating the nutritional profile of GF baked foods is the partial substitution of commonly used GF flours (e.g., maize and rice) with novel nutrition-dense ingredients (Ciudad-Mulero et al., 2020). Among these, the use of flours containing an appreciable amount of resistant starch (RS) for the enrichment of GF foods is gaining prominence (Foschia et al., 2017; Kiumarsi et al., 2019; Raungrusmee et al., 2020).

By definition, the RS fraction is a certain fraction of starch that does not degrade in the small intestine of healthy subjects; rather, it ferments in the large intestine favouring a series of health-related benefits (Fuentes-Zaragoza et al., 2011; Birt et al., 2013). Nutritional indications suggest that about 10–20% of daily complex carbohydrate intake needs to be in the form of RS for realizing the associated physiological benefits (Birt et al., 2013; Dupuis et al.,

2014). However, food processing (i.e., cooking) can destroy most forms of RS, making them unsuitable as food ingredients (Roman and Martinez, 2019). This necessitates the production of RS-rich ingredients with a higher level of resistance to food processing. So far, several attempts have been made to generate heat-stable forms of RS through physical, chemical and enzymatic treatments of native starches from different botanical sources (Giuberti et al., 2017; Jiang et al., 2020; Raungrusmee et al., 2020).

Promising results have been reported for the subsection of isolated white sorghum starch to annealing (Giuberti et al., 2019). The authors reported that the annealing process applied to sorghum starch proved effective in increasing the amount of RS (+25%) and its heat stability (+83%) with respect to the native starch form. Annealing is the hydrothermal physical modification of starch conducted at different water content levels (from >60% w/w to about 40% w/w) and at temperatures above the glass transition and below the gelatinisation temperatures (Tester and Debon, 2000). It is considered as an effective technique for modifying starch digestion and generating certain fractions of starch that are resistant to digestion. This is done through internal structural rearrangements within the amorphous and

* Corresponding author.

E-mail address: gianluca.giuberti@unicatt.it (G. Giuberti).

crystalline domains of the starch with enhanced stability (Magallanes-Cruz et al., 2017; Giuberti et al., 2019).

Several RS-rich ingredients are currently available in the market, but as new ingredients emerge, so does the need to explore their functionality and their effects on formulation. Moreover, few papers deal with the RS enrichment in biscuits. Aparicio-Saguilán et al. (2007) evaluated the effect of a novel RS-rich powder (RSRP) from autoclave-treated lintnerised banana starch on wheat-based biscuits. The authors reported that the use of RSRP contributed to the final biscuit's RS content, but there was no difference in terms of preference between the RSRP-substituted biscuits and the control sample. Similar results have been reported for GF biscuits formulated with blends of rice flour and different RS ingredients obtained from modified waxy rice starch (Giuberti et al., 2017) and for GF sugar-snap cookies containing native banana starch as rice flour's replacement (Roman et al., 2019). However, the nutritional and physicochemical roles of the RS-rich ingredient from annealed sorghum starch (RSWS) in GF food formulation have not been fully addressed.

This study aimed to evaluate the effect of RSWS inclusion in GF biscuits by taking into consideration the chemical, physical and the textural characteristics along with the *in vitro* digestibility of the starch. Sensory analysis was also conducted, since the substitution of base flours can impair certain sensory attributes.

2. Materials and methods

2.1. Raw materials, recipes and baking conditions

The novel RSWS was obtained from isolated white sorghum starch through annealing (Giuberti et al., 2019). In brief, the sorghum starch was isolated from commercial dehulled white sorghum grains and then dispersed in an excess of distilled water (ratio of 1:4 w/v starch to water) in a sealed container and left at 50 °C for 24 h. The resulting RSWS ingredient was recovered after centrifugation (4000 rpm; 15 min), oven dried at 40 °C (final moisture content was 9%) and finely ground (0.5-mm screen). The RS content of the RSWS was 53.9 g/100g of dry matter (DM), which is in line with previous findings (Giuberti et al., 2019). All other GF ingredients were acquired from local markets.

The gluten-free biscuits were formulated by replacing a commercial GF flour mix (GFM; Mix It! Universal; Dr. Schär, Bolzano, Italy) with 0, 15, 30 and 45 g/100 g total flour basis of the RSWS ingredient, which yielded CTR, 15-RSWS, 30-RSWS and 45-RSWS samples, respectively. The recipe consisted of the following: 240 g of combined flours, 120 g of whole eggs, 60 g of skimmed milk, 50 g of creamed butter, 1 g of salt and 1 g of sodium bicarbonate (total dough weight was 472 g). The ingredients were mixed (Kitchen Aid, Model K5SSWH, St. Joseph, Mich., USA) for 9 min at intermediate speed. The resulting dough was kept at 4 °C for 60 min, laminated by a pasta roller attachment at 0.5 cm height, cut into circular shapes (4 cm in diameter) and baked (180 ± 4 °C for 20 ± 1 min) in a household oven (RKK 66130, Rex International, Italy). For each recipe, three batches were created on the same day.

2.2. Chemical composition of biscuits

The biscuits were finely ground (0.5-mm screen). The samples were scrutinised for DM (method 930.15), ash (method 942.05), crude protein (method 976.05), crude lipid (method 954.02 without acid hydrolysis) and total starch (method 996.11) (AOAC, 2000). The total dietary fibre content was assessed enzymatically (Megazyme assay kit K-INTDF 02/15, which includes RS and non-digestible oligosaccharides). A commercial assay kit (K-RSTAR 02/17, Megazyme International, Wicklow, Ireland) was used for the quantification of RS. Briefly, a 16-h pancreatic α -amylase (10 mg/mL) and amyloglucosidase (3 U/mL) hydrolysis was performed at 37 °C. Ethanol was added, and the samples were centrifuged. The resultant pellet (containing the RS fraction) was dissolved in 2 M KOH by vigorously stirring over an ice/water bath using a magnetic stirrer for 25 min, and the solution was neutralized with 1.2 M sodium

acetate buffer. Starch was hydrolysed to glucose with amyloglucosidase (0.1 mL: 3300 U/mL) at 50 °C for 30 min with intermittent mixing on a vortex mix. The concentration of D-glucose was measured at 510 nm. For each treatment, each batch was analysed in triplicate.

2.3. Physical and textural characteristics of biscuits

The thickness and diameter were evaluated by using a Vernier calliper at 5 different places of 10 randomly selected biscuits. The ratio of diameter/thickness was used to calculate the spread ratio. The surface colour of the samples (CIELAB system colour space; L^* , a^* , b^*) was measured using a Minolta CR410 Chroma Meter (Konica Minolta Co., Japan) with reference to the D65 standard illuminant and a visual angle of 10. Five readings were taken for each batch. The total colour difference (ΔE^*) was calculated as follow (Equation 1):

$$\Delta E_{s-c}^* = [(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2]^{1/2} \quad (1)$$

where s = RSWS biscuits; subscript c = CTR biscuits. A ΔE^* value >3 was used to indicate whether the differences in colour between two different samples were perceivable by the human eye (Laguna et al., 2011).

Textural characteristics were conducted 24 h after the baking, and a TA-XT2i Texture Analyser (Stable Micro Systems, UK) was used. The hardness (as the maximum value of fracture force) of the samples was measured via a 3-point bending test using a 3-point bending rig, a trigger force of 25 g, and a load cell of 50 kg. The pre-test, test, and post-test speeds were 1.5, 2, and 10 mm/s, respectively. The compression distance was 10 mm and the distance between the two bottom supports was 22 mm. The Texture Export Exceed Release 2.54 (Stable Micro System) was used to acquire the maximum peak force as fracture force (N) (Sharma et al., 2016). For each batch, 5 biscuits were tested.

2.4. *In vitro* starch digestion

The biscuits were digested through a 2-step (i.e., gastric and pancreatic phases) static *in vitro* starch digestion procedure (Giuberti et al., 2015). The samples were passed through a meat mincer to mimic mastication, weighed (800 mg of total starch), inserted in glass tubes containing glass balls and hydrolysed with a 0.05 M HCl solution (5 mL) containing pepsin (5 mg/mL; Sigma P-7000, Sigma-Aldrich® Co., Milan, Italy) for 30 min at 37 °C under agitation. For the pancreatic phase, the pH was increased to 5.2 using 20 mL of 0.1 M sodium acetate buffer, and an enzyme mixture with an amylase activity of about 7000 U/mL [pancreatin (7500 FIP-U/g; Merck 7130, Merck KGaA, Darmstadt, Germany), amyloglucosidase (300 U/mL; Sigma A-7095, Sigma-Aldrich® Co., Milan, Italy) and invertase (300 U/g; Sigma I-4504, Sigma-Aldrich® Co., Milan, Italy)] was added. Aliquots were taken every 30 min for up to 180 min during the pancreatic phase. Absolute ethanol was added, and the amount of the released glucose was determined colourimetrically with a glucose oxidase kit (GODPOD 4058, Giese Diagnostic snc, Rome, Italy). The percentage of digested starch was calculated using a factor of 0.9. The values were plotted on a graph vs. time, and the area under the hydrolysis curve (AUHC; 0–180 min) was measured. The starch hydrolysis index (HI) value was calculated by taking into consideration the AUHC of each GF biscuit and the corresponding area of fresh white wheat bread (Giuberti et al., 2015). For each treatment, the batches were analysed in triplicate.

2.5. Sensory analysis

A twenty-member (voluntary) panel (11 male and 9 female, 24–42 years old) conducted the test. The panellists were staff and graduate students from our department. Prior to the sensory test, 10 h of panel training was conducted for them in several sessions over a week. A laboratory equipped with individual sensory booths under white light was used. The samples, labelled with random three-digit codes, were

randomly offered to the panellists 24 h after baking. The test was carried out in one session using a nine-point hedonic scale. Appearance (i.e., surface colour, roughness and presence of fractures), structure (i.e., hard at first chew, dryness and adhesiveness), flavour and taste were considered (Meilgaard et al., 2006). The panellists were also asked to comment on the overall acceptability. For all sensory attributes, a score of 5 was considered as the limit of acceptability (Meilgaard et al., 2006). Fresh water was provided between evaluations. The form used in the sensory test is included here as Figure 1. The research was approved by the Ethical Committee of Policlinico Universitario Agostino Gemelli (authorisation 5289/15). A written informed consent was obtained from each participant before starting the study.

2.6. Statistical analyses

Normal distribution of data was verified by the Shapiro-Wilk test. Data were analysed as a completely randomised design with the GLM procedure of SAS 9.3 (SAS Inst. Inc., Cary, N.C., USA) according to the model: $Y_{ij} = \mu + \alpha_i + e_{ij}$, where Y_{ij} is the dependent variable in the j^{th} subject (GF biscuit batch) assigned to treatment i , μ is the overall mean, α_i is the fixed effect of RSWS substitution level to GFM flour, and e_{ij} is the residual error. Experimental unit was the batch. Significance was set at $p < 0.05$.

3. Results and discussion

3.1. Chemical composition and resistant starch content of gluten-free biscuits

The chemical composition of the experimental GF biscuits was influenced by the addition of the RSWS ingredient (Table 1).

Biscuits are a baked product characterised by a low final water content. Irrespective of the formulation, the experimental GF biscuits were characterised by a similar moisture content, having 4.3 g water/100g of product on average. Similar results were obtained by Laguna et al. (2011) for short-dough wheat-based biscuits formulated with an RS-rich

ingredient. In addition, the final moisture contents obtained in the present study resemble those recorded by Bello-Pérez et al. (2004) for wheat-based biscuits prepared with native corn or banana RS. For good storage stability, dry biscuits should have a moisture content lower than the 5 % after baking (Giuberti et al., 2015). Total starch and crude protein content decreased ($p < 0.05$) as the inclusion level of RSWS increased in the recipe, ranging from 64.7 g/100 g DM to 55.8 g/100 g DM and from 10.3 g/100 g DM to 6.9 g/100 g DM, respectively, for CTR and 45-RSWS biscuits. In general, GF biscuits are characterised by high starch and low protein content, and evidently, their actual levels depend on the formulation employed. The total dietary fibre content increased ($p < 0.05$) with the increasing levels of RSWS in the recipe, and the highest value was obtained for 45-RSWS biscuits (i.e., 14.0 g/100 g DM; $p < 0.05$). Similar results were reported by Giuberti et al. (2017) for GF rice-based biscuits formulated with different RS-rich powders obtained from hydrothermally treated waxy rice starch. The current nutritional guidelines state that the term ‘total dietary fibre’ includes carbohydrate polymers that are not hydrolysed within the small intestine of humans. Therefore, the RS fraction should be included as a dietary fibre component (Betteridge, 2009). Consequently, the increase in the total dietary fibre content following RSWS addition is related to the analytical procedure employed in the current evaluation, which measures the total dietary fibre by taking into account the RS and non-digestible oligosaccharides (McCleary, 2007; McCleary et al., 2015). Previous studies showed a low dietary fibre intake for people following a strict GF-diet (Taetzsch et al., 2018). From a nutritional perspective, GF bakery products with high dietary fibre contents can therefore be considered beneficial (Lamacchia et al., 2014).

The RS fraction is considered as a functional dietary component that helps maintain metabolic and colonic health (Sajilata et al., 2006; Ma et al., 2018). The intake of RS in Europeans ranges from 3 to 9 g/person/day, but it has been observed that having 6–12 g of RS in a meal has beneficial effects on postprandial glucose and the insulin levels in humans (Murphy et al., 2008). The RS content of CTR biscuits was 1.1 g/100 g DM, which is in line with previous findings (Giuberti et al., 2017). The RS content in the RSWS-substituted biscuits was markedly

PANEL ID _____ DATE _____ TIME _____ SESSION _____

SAMPLE CODE _____

CONSIDERING THE FOLLOWING CHARACTERISTICS, INDICATE YOUR OPINION BY CHECKING THE BOX [x].

	Like				Indifferent	Dislike			
Appearance	Like extremely	Like very much	Like moderately	Like slightly	Neither like or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Structure	Like extremely	Like very much	Like moderately	Like slightly	Neither like or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Flavour	Like extremely	Like very much	Like moderately	Like slightly	Neither like or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Taste	Like extremely	Like very much	Like moderately	Like slightly	Neither like or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Overall, how much do you like or dislike this biscuit?

Like				Indifferent	Dislike			
Like extremely	Like very much	Like moderately	Like slightly	Neither like or dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Figure 1. Form used in the sensory test of samples.

Table 1. Chemical composition and resistant starch content (g/100g dry matter) and in vitro starch hydrolysis index (HI) of gluten-free biscuits formulated with a resistant starch-rich ingredient derived from annealed white sorghum starch (RSWS)¹.

Parameters	Experimental biscuits				p value
	CTR	15-RSWS	30-RSWS	45-RSWS	
Moisture ^{2, ns}	4.2 ± 0.10	4.3 ± 0.16	4.2 ± 0.05	4.3 ± 0.21	0.922
Total starch	64.7 ± 0.47 ^d	62.1 ± 0.25 ^c	59.8 ± 0.50 ^b	55.8 ± 0.52 ^a	<0.05
Crude protein	10.3 ± 0.18 ^d	9.1 ± 0.16 ^c	8.1 ± 0.06 ^b	6.9 ± 0.06 ^a	<0.05
Crude lipid ^{ns}	16.3 ± 0.14	16.4 ± 0.11	16.0 ± 0.08	16.2 ± 0.24	0.827
Total dietary fibre	2.2 ± 0.17 ^a	6.5 ± 0.19 ^b	10.1 ± 0.21 ^c	14.0 ± 0.20 ^d	<0.05
Ash ^{ns}	0.9 ± 0.07	1.0 ± 0.05	1.0 ± 0.04	1.1 ± 0.05	0.698
Resistant starch	1.1 ± 0.05 ^a	5.7 ± 0.06 ^b	9.2 ± 0.11 ^c	12.3 ± 0.13 ^d	<0.05
Starch HI ³	88.3 ± 2.23 ^d	76.4 ± 1.64 ^c	67.3 ± 1.78 ^b	59.5 ± 1.96 ^a	<0.05

CTR: control gluten-free biscuits prepared with 100 % commercial gluten-free flour mix (GFM). 15-RSWS: gluten-free biscuits prepared by replacing 15 g/100g w/w of GFM with RSWS. 30-RSWS: gluten-free biscuits prepared by replacing 30 g/100g of GFM with RSWS. 45-RSWS: gluten-free biscuits prepared by replacing 45 g/100g of a GFM with RSWS. Values within lines with different superscripts differed ($p < 0.05$). ns superscript within one line denotes means were not significantly different ($p \geq 0.05$).

¹ Three batches' replicates were produced for each recipe and analyzed in triplicate.

² g water/100 g food.

³ Calculated using white wheat bread as reference (HI = 100 by definition).

higher, and the greatest value was recorded for 45-RSWS samples (i.e., 12.3 g/100 g DM; $p < 0.05$). The findings indicated that the RSWS ingredient obtained from annealed white sorghum starch proved effective in increasing the RS content in GF biscuits. However, it is difficult to compare these findings with literature because this is the first time that an RS-rich ingredient obtained from annealed sorghum starch has been employed in GF biscuit formulation. Comparable RS contents were obtained by Laguna et al. (2011) for wheat-based short-dough biscuits formulated with 60 g/100 g w/w of a thermally modified RS obtained from an ae-VII maize hybrid. In addition, the present findings suggest that the baking process does not alter this indigestible component, thus corroborating previous indications (Giuberti et al., 2019). In particular, Giuberti et al. (2019) reported that RSWS is characterised by higher thermal stability compared to the native white sorghum starch. Similarly, Chung et al. (2009) obtained an increase in the RS levels in annealed gelatinised corn, pea and lentil starches; this suggests that starch chain interactions, formed during the annealing process, are not disrupted during gelatinisation, and this restricts the accessibility of the starch chains to the starch-hydrolysing enzymes.

3.2. Physical and textural characteristics of gluten-free biscuits

The substitution of RSWS did not statistically change the diameter, thickness and spread ratio values of biscuits, being 41.5 mm, 8.2 mm and 5.1 mm on average, respectively (Table 2). Comparable spread ratio

values have been reported for GF biscuits made from a flour blend of minor millers (Sharma et al., 2016) and for GF rice biscuits containing 50 g/100 g w/w of different RS-rich preparations obtained from waxy rice starch (Giuberti et al., 2017). The present findings suggest that in GF biscuit formulation, the addition of high doses of RS-rich materials does not substantially impair the physical characteristics of biscuits, which is in line with previous findings (Nugraheni et al., 2017). However, it must be pointed out that in the gluten-containing system, the spread ratio decreases linearly with the gluten level due to a diluting effect on the wheat protein (Pareyt and Delcour, 2008). Further studies are needed to explore the suitability of RSWS for use in the gluten-containing system.

Colour is related to the physicochemical characteristics of ingredients and baking conditions. According to the values obtained for L^* , RSWS-substituted biscuits became more luminous ($p < 0.05$) than the CTR, as the level of RSWS increased in the formulation. In addition, the b^* value decreased ($p < 0.05$) with increasing levels of RSWS, with the lowest values being obtained for 45-RSWS (i.e., 24.1; $p < 0.05$). In terms of the total colour difference, the ΔE^* of all the RSWS biscuits (equation 1) was >3 when compared to CTR (i.e., ΔE^* 15-RSWS versus CTR = 7.01; ΔE^* 30-RSWS versus CTR = 13.2 and ΔE^* 45-RSWS versus CTR = 21.1). This means that, as perceived by the human eye, all the RSWS were different in colour with respect to the CTR, i.e., all of them were visibly paler than the CTR. Laguna et al. (2011) have already reported that wheat-based biscuits formulated with an increased amount of an RS-rich ingredient are whiter on the upper surface, which is in line with the findings

Table 2. Physical and textural characteristics of gluten-free biscuits formulated with a resistant starch-rich ingredient derived from annealed white sorghum starch (RSWS)¹.

Parameters	Experimental biscuits				p value
	CTR	15-RSWS	30-RSWS	45-RSWS	
Diameter (mm) ^{ns}	41.3 ± 0.10	41.2 ± 0.09	42.3 ± 0.21	41.3 ± 0.11	0.788
Thickness (mm) ^{ns}	8.3 ± 0.02	8.2 ± 0.07	8.2 ± 0.04	8.0 ± 0.10	0.771
Spread ratio (D/T) ^{ns}	5.2 ± 0.05	5.1 ± 0.12	5.1 ± 0.11	5.0 ± 0.10	0.701
L^* (lightness)	68.3 ± 1.21 ^a	74.4 ± 1.38 ^b	79.7 ± 1.00 ^c	87.1 ± 1.33 ^d	<0.05
a^* (redness-greenness) ^{ns}	2.4 ± 0.01	2.3 ± 0.02	2.3 ± 0.01	2.2 ± 0.02	0.981
b^* (yellowness-blueness)	34.1 ± 1.54 ^d	30.3 ± 1.33 ^c	26.6 ± 1.91 ^b	24.1 ± 1.04 ^a	<0.05
Hardness (N)	61.2 ± 0.44 ^a	64.9 ± 0.68 ^b	67.7 ± 0.59 ^c	70.6 ± 0.77 ^d	<0.05

CTR: control gluten-free biscuit prepared with 100 % commercial gluten-free flour mix (GFM). 15-RSWS: gluten-free biscuit prepared by replacing 15 g/100g w/w of GFM with RSWS. 30-RSWS: gluten-free biscuit prepared by replacing 30 g/100g of GFM with RSWS. 45-RSWS: gluten-free biscuit prepared by replacing 45 g/100g of GFM with RSWS. Values within lines with different superscripts differed ($p < 0.05$). ns superscript within one line denotes means were not significantly different ($p \geq 0.05$).

¹ For each recipe, three batches' replicates were produced and analyzed in triplicate.

obtained for GF food products (Giuberti et al., 2017). The lower protein content of biscuits due to the RSWS inclusion may have contributed towards the reduction of the Maillard-type reactions during baking (Uthumporn et al., 2005; Pourmohammadi et al., 2018). Similarly, the crust of RS-supplemented breads is characterised by lower b^* colour values as the level of RS increases in the formulation (Ozturk et al., 2009).

The fracture force of the biscuits was significantly affected by the increase in the RSWS content. In particular, 45-RSWS biscuits had the greatest hardness value, whereas the CTR had the lowest one (i.e., 70.6 N versus 61.2 N, respectively; $p < 0.05$). The hardness of biscuits depends on the composition of flours and the interactions among the ingredients during baking (Fustier et al., 2008). The present findings are in agreement with the study by Norhidayah et al. (2014), showing that biscuits containing greater amounts of RS are characterised by the highest hardness value. This could be due to the fact that some of the starch granules that remain in the native form on baking do not form a continuous structure, and this leads to an increase in hardness (Norhidayah et al., 2014). In addition, in GF biscuits, greater levels of fibre can make compact the structure of the food (Giuberti and Gallo, 2018). For GF bread, Korus et al. (2009) reported the increase in the hardness of the crust as a function of the RS-rich ingredient and its relative amount in the recipe. Similarly, Kiumarsi et al. (2019) found that the hardness values increased by about 50% when an RS-rich ingredient from phosphorylated corn starch was used in GF-bread formulation; this was probably due to an increase in the relative crystallinity, which promoted a structural rearrangement of the starch matrix into a highly ordered structure.

3.3. In vitro starch digestion of gluten-free biscuits

The *in vitro* starch digestion curves are depicted in Figure 2. Starch from white wheat bread was *in vitro* digested to a higher extent for the entire incubation period, in line with previous findings (Jia et al., 2020). The experimental biscuits exhibited a decrease in the extent of the *in vitro* starch digestion to up to 120 min of incubation as the level of RSWS increased in the recipe. Consistently, different starch HIs were calculated (Table 1).

The starch HI of the CTR biscuits was 88.3, in line with similar GF food products (Giuberti et al., 2017). The substitution of part of the GFM

with increasing levels of RSWS decreased ($p < 0.05$) the starch HI of the samples. In particular, the lowest HI was recorded for 45-RSWS (i.e., 59.5; $p < 0.05$). From a nutritional standpoint, the ability to control starch digestion is of great importance if one is aiming to design food products with desired nutritional characteristics. This could be of particular interest to diabetic patients and/or overweight individuals following a strict GF diet (Krupa-Kozak and Lange, 2019). This is because several GF products are characterised by higher glycaemic response as a consequence of the use of starches and/or refined flours low in fibre and high in rapidly digested starch fractions (Roman et al., 2019). Similar results were reported by Aparicio-Saguilán et al. (2007) for wheat-based biscuits prepared by adding an RS-rich ingredient obtained from autoclave-treated lintnerised banana starch. For GF snap-cookies, Roman et al. (2019) showed a significant reduction in *in vitro* starch digestion kinetics when 30% of the rice flour was replaced by an RS-rich ingredient from native banana starch. The decrease in the HI values following RSWS inclusion could be connected to the different behaviour of the starch system during cooking (Sajilata et al., 2006). Accordingly, it was recently reported that the presence of RS in food products can affect the susceptibility of the available starch fractions to digestion, due to the encapsulation of gelatinised starch between layers of RS that have greater resistance to enzyme hydrolysis (Tian and Sun, 2020). Similar results have been reported for GF noodles formulated with native autoclaved RS from rice (Raungrusmee et al., 2020). In particular, the authors indicated that the use of this ingredient helps formulate GF noodles with a slower digestion rate and lower glycaemic index compared to the control sample. In addition, the possible influence of the product's compactness (i.e., hardness) could also partially preserve the starch's granular structural integrity during cooking and/or modulate the *in vitro* accessibility of enzymes to starch to a certain extent; this might have contributed towards the reduction of the starch HI of the samples (Sparvoli et al., 2016; Pellegrini et al., 2020).

3.4. Sensory evaluation of gluten-free biscuits

None of the panellists reported any adverse effects during the test. Some of the selected sensory attributes were influenced by the RSWS addition ($p < 0.05$), but all remained above the limit of acceptability (i.e., 5) (Table 3). In novel food product formulation, maintaining the

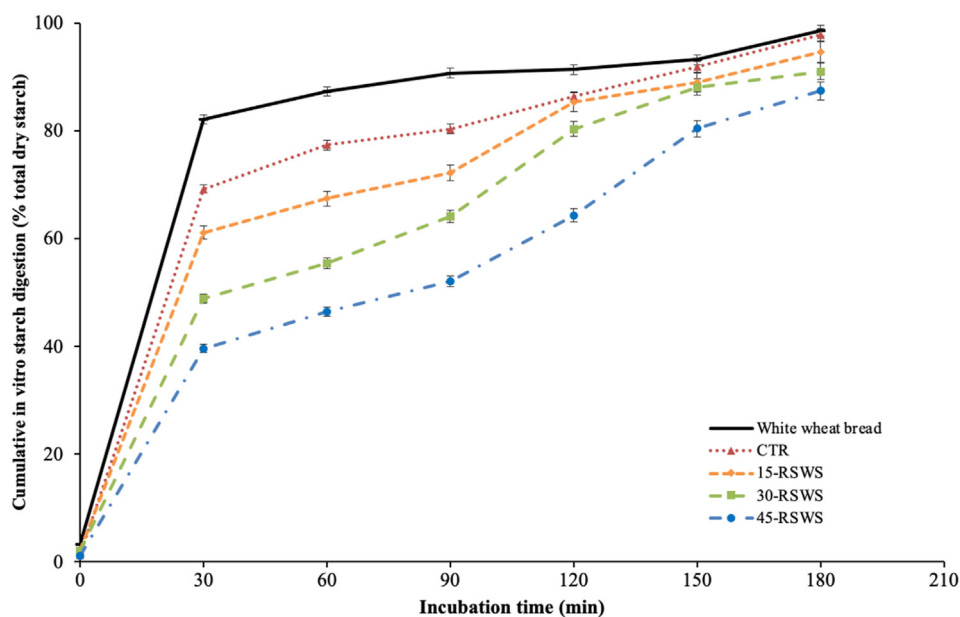


Figure 2. In vitro starch digestion of gluten-free biscuits prepared with resistant starch from annealed white sorghum starch (RSWS) at different levels. CTR, control biscuits formulated with 0:100 RSWS: gluten-free flour mix (GFM); 15-RSWS, formulated with 15:85 RSWS:GFM; 30-RSWS, formulated with 30:70 RSWS:GFM; 45-RSWS, formulated with 45:55 RSWS:GFM. White wheat bread used as reference.

Table 3. Sensory evaluation scores of gluten-free biscuits formulated with different levels of a resistant starch-rich ingredient derived from annealed white sorghum starch (RSWS).

Parameters	Experimental biscuits				p value
	CTR	15-RSWS	30-RSWS	45-RSWS	
Appearance	7.2 ± 1.02 ^c	6.3 ± 1.00 ^b	5.8 ± 0.89 ^a	5.7 ± 0.80 ^a	<0.05
Texture	7.4 ± 0.74 ^c	6.0 ± 0.81 ^b	5.9 ± 0.67 ^b	5.2 ± 0.62 ^a	<0.05
Flavour ^{ns}	6.8 ± 0.81	6.7 ± 0.77	6.6 ± 1.01	6.6 ± 0.83	0.822
Taste ^{ns}	6.4 ± 0.55	6.6 ± 0.61	6.5 ± 0.44	6.4 ± 0.49	0.899
Acceptability	7.1 ± 0.54 ^c	6.8 ± 0.41 ^b	6.7 ± 0.43 ^b	5.4 ± 0.31 ^a	<0.05

Values expressed on a 1-9-point scoring scale. CTR: control gluten-free biscuit prepared with 100 % commercial gluten-free flour mix (GFM). 15-RSWS: gluten-free biscuit prepared by replacing 15 g/100g w/w of GFM with RSWS. 30-RSWS: gluten-free biscuit prepared by replacing 30 g/100g of GFM with RSWS. 45-RSWS: gluten-free biscuit prepared by replacing 45 g/100g of GFM with RSWS. Values within lines with different superscripts differed ($p < 0.05$). ns superscript within one line denotes means were not significantly different ($p \geq 0.05$).

product's sensory characteristics is of great importance. In particular, CTR biscuits obtained the highest score ($p < 0.05$) for "appearance" (i.e., 7.2; $p < 0.05$), and the 45-RSWS biscuits received the lowest score for "texture" (i.e., 5.2; $p < 0.05$). On the contrary, similar sensory scores were recorded for "flavour" and "taste", which were 6.6 and 6.5 on average, respectively. This may be due to the neutral flavour of the RSWS ingredient; it did not modify these two sensory attributes even when its level of inclusion was the highest in the recipe. However, the mean of the overall acceptability scores decreased ($p < 0.05$) with the increase of RSWS in the recipe, being 7.1 for CTR, 6.8 for 15-RSWS, 6.7 for 30-RSWS and 5.4 for 45-RSWS. Since both textural parameters and sensory attributes can influence consumers' overall acceptance of a certain food product, the reported changes in colour, hardness, appearance and texture due to RSWS inclusion could explain the present findings (Kiu-marsi et al., 2019). Despite this decrease in the overall acceptability, it must be pointed out that this descriptor remained above the limit of acceptability (i.e., 5). In sensory evaluation, a food product with a score of more than five for overall acceptability can be considered a good-quality product (Meilgaard et al., 2006). An overall decrease in sensory acceptance has been previously reported in short-dough wheat-based biscuits formulated with increasing levels of an RS-rich ingredient from maize starch (Laguna et al., 2011). Similarly, Nugraheni et al. (2017) showed that the addition of a high amount (i.e., up to 12 g/100 g total flour base) of a novel RS-rich ingredient obtained from autoclaved and cooled *Maranta arundinacea* flour contributes towards the reduction of the overall acceptability of the newly developed GF biscuits.

4. Conclusions

Four different GF biscuits were formulated using 100% of a commercially available GF flour mix and its blends with 15%, 30% and 45% w/w of an RS-rich ingredient obtained by subjecting white sorghum starch to annealing. Differences in the chemical composition were reported in GF biscuits prepared with increasing levels of the RS-rich ingredient. Lower protein and higher dietary fibre content were obtained when the level of RSWS increased in the recipe. The findings proved that the RSWS ingredient was effective in increasing the RS content in GF biscuits and suggested that the baking process does not alter this indigestible component. In addition, lower starch HI values were reported when the level of RSWS increased in the composite. With regard to quality parameters, the use of RSWS influenced the hardness of GF biscuits, and the highest value was obtained at the greatest RSWS inclusion level. Some of the selected sensory attributes, along with the overall acceptability score, were negatively influenced by RSWS addition, but they all remained above the limit of acceptability. To sum up, the present findings indicate that the partial substitution of commercial flour with RSWS in GF biscuit formulation can help in the creation of food products likely having slowly digestible starch properties and higher total dietary fibre content, and this can be achieved without drastically compromising on the selected sensory attributes.

For confirming the present in vitro results, in vivo trials are strongly warranted.

Declarations

Author contribution statement

Mariasole Cervini, Antonello Frustace, Guillermo Duserm Garrido: Performed the experiments; Analyzed and interpreted the data.

Gabriele Rocchetti: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gianluca Giuberti: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

The data that has been used is confidential.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Aparicio-Saguilán, A., Sáyago-Ayerdi, S.G., Vargas-Torres, A., Tovar, J., Ascencio-Otero, T.E., Bello-Pérez, L.A., 2007. Slowly digestible cookies prepared from resistant starch-rich lintnerized banana starch. *J. Food Compos. Anal.* 20 (3-4), 175–181.
- Association of Official Analytical Chemists (AOAC), 2000. *Official Methods of Analysis*, seventeenth ed. Gaithersburg, MD.
- Bello-Pérez, L.A., Sáyago-Ayerdi, S.G., Méndez-Montealvo, G., Tovar, J., 2004. In vitro digestibility of banana starch cookies. *Plant Foods Hum. Nutr.* 59, 79–83.
- Betteridge, V., 2009. Dietary fibre: an evolving definition? *Nutr. Bull.* 34 (2), 122–125.
- Birt, D.F., Boylston, T., Hendrich, S., Jane, J.-L., Hollis, J., Li, L., McClelland, J., Moore, S., Phillips, G.J., Rowling, M., Schalinske, K., Scott, M.P., Whitley, E.M., 2013. Resistant starch: promise for improving human health. *Adv. Nutr.* 4 (6), 587–601.
- Chung, H.-J., Liu, Q., Hoover, R., 2009. Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydr. Polym.* 75 (3), 436–447.
- Ciudad-Mulero, M., Barros, L., Fernandes, A., Ferreira, I.C.F.R., Jesús Callejo, M., Cruz Matallana-González, M., Fernández-Ruiz, V., Morales, P., Carrillo, J.M., 2020. Potential health claims of durum and bread wheat flours as functional ingredients. *Nutrients* 12 (2), 504.
- Dupuis, J.H., Liu, Q., Yada, R.Y., 2014. Methodologies for increasing the resistant starch content of food starches: a review. *Compr. Rev. Food Sci. Food Saf.* 13 (6), 1219–1234.

- Foschia, M., Horstmann, S.W., Arendt, E.K., Zannini, E., 2017. Legumes as functional ingredients in gluten-free bakery and pasta products. *Annu. Rev. Food Sci. Technol.* 8, 75–96.
- Fuentes-Zaragoza, E., Sánchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernández-López, J., Pérez-Alvarez, J.A., 2011. Resistant starch as prebiotic: a review. *Starch - Stärke* 63 (7), 406–415.
- Fustier, P., Castaigne, F., Turgeon, S.L., Biliaderis, C.G., 2008. Flour constituent interactions and their influence on dough rheology and quality of semi-sweet biscuits: a mixture design approach with reconstituted blends of gluten, water-solubles and starch fractions. *J. Cereal. Sci.* 48 (1), 144–158.
- Giuberti, G., Gallo, A., 2018. Reducing the glycaemic index and increasing the slowly digestible starch content in gluten-free cereal-based foods: a review. *Int. J. Food Sci. Technol.* 53 (1), 50–60.
- Giuberti, G., Gallo, A., Cerioli, C., Fortunati, P., Masoero, F., 2015. Cooking quality and starch digestibility of gluten free pasta using new bean flour. *Food Chem.* 175, 43–49.
- Giuberti, G., Marti, A., Fortunati, P., Gallo, A., 2017. Gluten free rice cookies with resistant starch ingredients from modified waxy rice starches: nutritional aspects and textural characteristics. *J. Cereal. Sci.* 76, 157–164.
- Giuberti, G., Marti, A., Gallo, A., Grassi, S., Spigno, G., 2019. Resistant starch from isolated white sorghum starch: functional and physicochemical properties and resistant starch retention after cooking. *A Comparative Study. Starch - Stärke* 71 (7–8), 1800194.
- Jia, M., Yu, Q., Chen, J., He, Z., Chen, Y., Xie, J., Nie, S., Xie, M., 2020. Physical quality and in vitro starch digestibility of biscuits as affected by addition of soluble dietary fiber from defatted rice bran. *Food Hydrocolloids* 99, 105349.
- Jiang, F., Du, C., Jiang, W., Wang, L., Du, S.-K., 2020. The preparation, formation, fermentability, and applications of resistant starch. *Int. J. Biol. Macromol.* 150, 1155–1161.
- Kiumarsi, M., Shahbazi, M., Yegenehzad, S., Majchrzak, D., Lieleg, O., Winkeljann, B., 2019. Relation between structural, mechanical and sensory properties of gluten-free bread as affected by modified dietary fibers. *Food Chem.* 277, 664–673.
- Korus, J., Witeczak, M., Ziobro, R., Juszczyk, L., 2009. The impact of resistant starch on characteristics of gluten-free dough and bread. *Food Hydrocolloids* 23, 988–995.
- Krupa-Kozak, U., Lange, E., 2019. The Gluten-free diet and glycaemic index in the management of coeliac disease associated with Type 1 diabetes. *Food Rev. Int.* 35, 587–608.
- Laguna, L., Salvador, A., Sanz, T., Fiszman, S.M., 2011. Performance of a resistant starch rich ingredient in the baking and eating quality of short-dough biscuits. *LWT* 44 (3), 737–746.
- Lamacchia, C., Camarca, A., Picascia, S., Di Luccia, A., Gianfrani, C., 2014. Cereal-based gluten-free food: how to reconcile nutritional and technological properties of wheat proteins with safety for celiac disease patients. *Nutrients* 6, 575–590.
- Ma, Z., Yin, X., Hu, X., Li, X., Liu, L., Boye, J.I., 2018. Structural characterization of resistant starch isolated from Laird lentils (*Lens culinaris*) seeds subjected to different processing treatments. *Food Chem.* 263, 163–170.
- Magallanes-Cruz, P.A., Flores-Silva, P.C., Bello-Perez, L.A., 2017. Starch structure influence its digestibility: a review. *J. Food Sci.* 82 (9), 2016–2023.
- McCleary, B.V., 2007. An integrated procedure for the measurement of total dietary fibre (including resistant starch), non-digestible oligosaccharides and available carbohydrates. *Anal. Bioanal. Chem.* 389, 291–308.
- McCleary, B.V., Sloane, N., Draga, A., 2015. Determination of total dietary fibre and available carbohydrates: a rapid integrated procedure that simulates in vivo digestion. *Starch - Stärke* 67 (9–10), 860–883.
- Meilgaard, M., Civille, G.V., Carr, B.T., 2006. *Sensory Evaluation Techniques*, fourth ed. CRC Press, Boca Raton, USA.
- Murphy, M.M., Douglass, J.S., Birkett, A., 2008. Resistant starch intakes in the United States. *J. Am. Diet Assoc.* 108 (1), 67–78.
- Norhidayah, M., Noorlaila, A., Nur Fatin Izzati, A., 2014. Textural and sensorial properties of cookies prepared by partial substitution of wheat flour with unripe banana (*Musa x paradisiaca* var. *Tanduk* and *Musa acuminata* var. *Emas*) flour. *Int. Food Res. J.* 21 (6), 2133–2139.
- Nugraheni, M., Sutopo, Purwanti, S., Handayani, T.H.W., 2017. Development of gluten-free cookies rich in resistant starch type 3 from *Maranta arundinacea*. *Pakistan J. Nutr.* 16 (9), 659–665.
- Ozturk, S., Koksel, H., Ng, P.K.W., 2009. Farinograph properties and bread quality of flours supplemented with resistant starch. *Int. J. Food Sci. Nutr.* 60 (6), 449–457.
- Padalino, L., Conte, A., Del Nobile, M.A., 2016. Overview on the general approaches to improve gluten-free pasta and bread. *Foods* 5 (4), 87.
- Pareyt, B., Delcour, J.A., 2008. The role of wheat flour constituents, sugar, and fat in low moisture cereal based products: a review on sugar-snap cookies. *Crit. Rev. Food Sci.* 48 (9), 824–839.
- Pellegrini, N., Vittadini, E., Fogliano, V., 2020. Designing food structure to slow down digestion in starch-rich products. *Curr. Opin. Food Sci.* 32, 50–57.
- Pourmohammadi, K., Abedi, E., Hashemi, S.M.B., Torri, L., 2018. Effects of sucrose, isomalt and maltodextrin on microstructural, thermal, pasting and textural properties of wheat and cassava starch gel. *Int. J. Biol. Macromol.* 120, 1935–1943.
- Raungrusmee, S., Shrestha, S., Sadiq, M.B., Anal, A.K., 2020. Influence of resistant starch, xanthan gum, inulin and defatted rice bran on the physicochemical, functional and sensory properties of low glycemic gluten-free noodles. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 126, 109279.
- Roman, L., Sahagun, M., Gomez, M., Martinez, M.M., 2019. Nutritional and physical characterization of sugar-snap cookies: effect of banana starch in native and molten states. *Food Funct.* 10, 616–624.
- Roman, L., Martinez, M.M., 2019. Structural basis of resistant starch (RS) in bread: natural and commercial alternatives. *Foods* 8 (7), 267.
- Sajilata, M.G., Singhal, R.S., Kulkarni, P.R., 2006. Resistant starch - a review. *Compr. Rev. Food Sci. Food Saf.* 5 (1), 1–17.
- Sharma, S., Saxena, D.C., Riar, C.S., 2016. Nutritional, sensory and in-vitro antioxidant characteristics of gluten free cookies prepared from flour blends of minor millets. *J. Cereal. Sci.* 72, 153–161.
- Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., Fortunati, P., Daminati, M.G., Cominelli, E., Bollini, R., 2016. Exploitation of common bean flours with low antinutrient content for making nutritionally enhanced biscuits. *Front. Plant Sci.* 7, 27, 00928.
- Taetzsch, A., Das, S.K., Brown, C., Krauss, A., Silver, R.E., Roberts, S.B., 2018. Are gluten-free diets more nutritious? An evaluation of self-selected and recommended gluten-free and gluten-containing dietary patterns. *Nutrients* 10 (12), 1881.
- Tester, R.F., Debon, S.J.J., 2000. Annealing of starch - a review. *Int. J. Biol. Macromol.* 27 (1), 1–12.
- Tian, S., Sun, Y., 2020. Influencing factor of resistant starch formation and application in cereal products: a review. *Int. J. Biol. Macromol.* 149, 424–431.
- Uthumporn, U., Woo, W.L., Tajul, A.Y., Fazilah, A., 2005. Physico-chemical and nutritional evaluation of cookies with different levels of eggplant flour substitution. *CyTA - J. Food* 13 (2), 220–226.