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Gluten free Maize Cookies Prepared with High-amylose Starch: *In Vitro* Starch Digestibility and Sensory Characteristics

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Abstract

In an effort to improve gluten-free (GF) cookies overall nutritional aspects, as a means of decreasing the glycemic index and increasing the resistant starch content, experimental GF maize cookies were formulated with a normal amylose GF maize flour mix (NAM) and increasing levels of high amylose maize starch flour (HAM) represented by substitution ratio of 0%, 25% and 50% on a total flour (NAM+HAM) basis. Chemical composition, *in vitro* starch digestibility and sensory evaluation were carried out. Dietary fibre, total starch and amylose contents increased ($p<0.05$) when the level of HAM increased in the recipe for GF cookies. Both resistant starch and slowly digestible starch increased ($p<0.05$), whereas rapidly digestible starch and available starch levels decreased ($p<0.05$) when the level of HAM increased in the formulation. Slower rate of starch hydrolysis (up to minus 66.0%; $p<0.05$) and lower predicted glycemic index (up to minus 35.0%; $p<0.05$) were recorded when HAM increased in the composite. No differences were reported in the sensory profile and in the overall acceptability of GF maize cookies formulated with increasing levels of HAM. Taking together, present *in vitro* findings suggest that the partial substitution of NAM with HAM contributed to formulate GF maize cookies with slowly digestible starch properties without affecting overall sensory attributes.

Keywords: Gluten-free; High amylose; Predicted glycemic index; Resistant starch; Sensory evaluation; Cookie

Introduction

Celiac disease is one of the most common food induced disorders caused by the ingestion of gluten containing grains in genetically susceptible individuals [1]. Being the only efficacious treatment the total elimination of gluten, the main raw materials permitted in the production of Gluten-Free (GF) foods are native starches, non-wheat flours (e.g., maize, rice, sorghum, pseudo-cereals) and commercial GF blends [1]. However, several GF foods based on aforementioned ingredients exhibit lower nutritional quality, lower content of resistant starch (RS) and higher glycemic index (GI) than their gluten containing counterparts [2-4], mainly due to the type and source of carbohydrates (starch and dietary fibre), the food matrix viscosity along with the total absence of the gluten network [1,5].

However, international dietary guidelines encourage the consumption of slowly digestible carbohydrates and suggest that starchy foods should contain at least 14.0% of RS on total starch [6]. The RS fraction represents the sum of starch plus starch digestion products not degraded in the small intestine of human subjects, but fermented in the large intestine favouring butyrate production [7]. Several health benefits are associated with RS, including hypoglycaemic effects, prevention of colorectal cancer, lower plasma cholesterol and triglyceride concentrations and inhibition of fat accumulation [8]. The GI was introduced to classify different carbohydrate-rich foods with respect to their effect on post-meal glycaemia, foods being divided into low (<55), medium (55-69) and high (>70) GI [2]. The consumption of lower GI foods can positively influence post-prandial and insulin responses and can be beneficial for prevention and control of obesity and metabolic risk factors [9].

As a consequence, research has been conducted to improve the overall nutritional characteristics of GF products [1,5]. Within this perspective cookies could represent a potentially nutritious GF snack through the selection of ingredients. The improvement of GF cookie nutritional aspect, as a means of decreasing the GI while increasing the RS contents, could be achieved by the partial replacement of Normal Amylose (NA) with High Amylose (HA) starches. Promising results have been obtained both *in vitro* and *in vivo*, revealing that the use of HA sources contributed to reduce the GI of several gluten containing bakery products [3,10,11]. It is recognised that digestive enzymes slowly digest amylose, whereas amylopectin is rapidly digested due to its branched structure [12,13]. However, to the best of our knowledge, a broad investigation of starch digestibility of GF cookies prepared using HA flours in the recipe is currently missing. Therefore, the aim of this study was to evaluate overall *in vitro* starch digestibility of GF cookies prepared with increasing levels of HA maize starch flour (HAM) in substitution of NA maize flour mix (NAM). Sensory analysis was also conducted, since the substitution of base flours might impair sensory characteristics. The investigation is based on the hypothesis that the utilization of HAM in GF maize cookie formulation could reduce the GI while increasing the RS content.

Materials and Methods

Ingredients, recipes and baking conditions

The HAM (native Amylo-maize starch N-400; 61.0-65.0% amylose according to the manufacturer) was obtained from Roquette Italia

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SpA (Sardigliano Alessandria, Italy). All GF ingredients (food grade) employed in the current evaluation were acquired in local supermarkets and stored depending on individual requirements. Preparation of experimental cookies followed the procedure of Giuberti et al. [3] with minor modifications. The NAM (Mix C; Dr. Schär AG/SpA, Postal, Italy; containing NA maize starch, NA maize flour and locust bean gum; 22.0-25.0% amylose according to the manufacturer) was substituted with HAM (on a total flour basis) to formulate GF control cookies containing 0% HAM (0-CK) and experimental GF cookies containing 25% HAM (25-CK) and 50% HAM (50-CK) (Table 1). Briefly, butter was creamed, mixed with liquid ingredients and then added to flours. Ingredients were then combined with a domestic blender (Kitchen Aid, Model K5SSWH, and St. Joseph, Mich., U.S.A.) for 6 min to obtain homogeneous batter. The batter was laminated by a pasta roller attachment at 0.7 cm height, allowed to rest for 30 min a 4°C, cut with a circular mould (5 cm diameter) and baked with a household oven (RKK 66130, Rex International, Pordenone, Italy) at a temperature of 180°C for 20 min. Once baked, cookies were allowed to cool for 2 h and kept in separate airtight plastic bags at room temperature until analysis. Three GF cookie batches of 0-CK, 25-CK and 50-CK were produced on the same day.

Chemical composition

All samples were dried at 55°C for 24 h in a forced-air oven and then ground through a 1-mm screen using a laboratory mill (Retsch grinder model ZM1; Brinkman Instruments, Rexdale, ON, Canada). Analyses were performed according to AOAC [14] for dry matter (DM; method 930.15), ash (method 942.05), crude protein (method 976.05) and crude lipid (method 954.02 without acid hydrolysis). Enzymatic quantifications of dietary fibre (Megazyme assay kit K-INTDF 02/15), total starch (Megazyme assay kit K-TSTA 07/11), total amylose (Megazyme assay kit K-AMYL 07/11) and free sugars (Megazyme assay kit K-SUFRG 06/14) were also carried out. For each treatment, batches were analysed in triplicate.

Determination of starch fractions

Starch fractions as rapidly digestible starch (RDS) and slowly digestible starch (SDS) were determined by controlled enzymatic hydrolysis [15]. The glucose released from samples after 20 min and 120 min of incubation was measured colorimetrically and converted to RDS and SDS indexes with the equations proposed by Englyst et al. [16]. The RS was quantified with the direct method detailed by Goñi et al. [17]. Potentially available starch content was determined by the subtracting the difference of total starch by resistant starch. For each treatment, batches were analysed in triplicate.

In vitro starch digestion

The multi-enzymatic protocol described by Giuberti et al. [18] was employed to evaluate the starch hydrolysis of experimental GF cookies "as eaten". Samples (800 mg of available starch) were weighed accordingly in 50 mL tubes and pre-treated with a 0.05 M HCl solution containing pepsin (5 mg/mL; P-7000, Sigma-Aldrich® Co., Milan, Italy) for 30 min at 37°C under agitation. Then, the pH was adjusted to 5.2 by adding 0.1 M sodium acetate buffer prior to the addition of an enzyme mixture containing pancreatin (about 7500 FIP-U/g; 7130, Merck KGaA, Darmstadt, Germany), amyloglucosidase (about 300 U/mL; A-7095, Sigma-Aldrich® Co., Milan, Italy) and invertase (about 300 U/g; I-4504, Sigma-Aldrich® Co., Milan, Italy). Aliquots were carefully taken from each tube at 0 (prior to the addition of the enzyme mixture simulating the pancreatic phase), 15, 30, 60, 90, 120 and 180 min after the enzyme addition, then absolute ethanol was added and the amount of released glucose was determined colorimetrically [18]. The percentage of hydrolysed starch at each time interval was calculated using a factor of 0.9 [18]. Batches were analysed in triplicate.

Ingredients (g/100 g)	Substitutions with HAM flour		
	Cookie 0-CK	Cookie 25-CK	Cookie 50-CK
NAM	52.5	39.4	26.3
HAM	0	13.1	26.3
Whole egg	18	18	18
Cream butter	12.5	12.5	12.5
Water	17	17	17

Table 1: Gluten-free maize cookie formulations used in the present evaluation. NAM: normal amylose gluten-free maize mix; HAM: high amylose maize starch flour; 0-CK, control cookie formulated with 0:100 HAM: NAM; 25-CK, formulated with 25:75 HAM: NAM; 50-CK, formulated with 50:50 HAM: NAM.

Calculations

The hydrolysis index (HI) was derived from the ratio between the area under the hydrolysis curve (0-180 min) of each product (0-CK, 25-CK and 50-CK) and the corresponding area of the reference sample (white wheat bread) as a percentage over the same period. From the HI obtained *in vitro,* the pGI value was calculated using the empiric formula pGI=8.198+0.862 × HI proposed by Granfeldt et al. [19].

To describe starch hydrolysis kinetics, a first-order exponential model was applied: $C_t=C_0+C_{\infty-0}(1-e^{-kt})$, where C_t is the starch hydrolysed at time t (g/100 g dry starch), C_0 is the starch solubilised in the buffer at 0 min (g/100 g dry starch), C_∞ is the equilibrium percentage of starch hydrolysed at infinite time (g/100 g dry starch), $C_{\infty-0}$ is C_∞ minus C_0 (g/100 g dry starch), k is the rate constant (min^{-1}) and t is the chosen time (min) [20]. For the purpose of data fitting, values were obtained by the Marquardt method using the PROC NLIN procedure of SAS 9.3 (SAS Inst. Inc, Cary, NC., USA).

Sensory evaluation

An eight-member trained panel (4 male and 4 female, 23-37 years old) with a long-time experience in sensory evaluation of bakery products conducted the test. Analyses were performed in a laboratory equipped with individual sensory booths under white light. Each panelist evaluated all the samples prepared for each treatment in one session. The GF cookies were offered in random order labelled with three-digit random codes and quality test was carried out using a nine point scoring scale [21]. The panellists had to specify their preference for "appearance", "structure", "flavour", "taste" and "overall acceptability" of each cookie sample. Appearance was assessed in terms of surface colour, roughness and presence of fractures, whereas the evaluation of structure included hardness at first chew, dryness and adhesiveness [22]. A score of 5 or below was considered a limit of acceptability for all sensory attributes tested. The sensory analysis was conducted 24 h after the baking procedure. Water was provided to cleanse the palate between evaluations.

Statistical analyses

Normal distribution of data was verified by the Shapiro-Wilk test. Data were analysed as a completely randomised design using 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 on the j^{th} subject (GF cookie batch) assigned to treatment i, μ is the overall mean, α_i is the fixed effect of HAM flour substitution to NAM flour (i=0%, 25% and 50%), and e_{ij} is the residual error. Orthogonal contrasts were *post-hoc* carried out to study the linear response of fixed effect. Experimental unit was the GF cookie batch. Significance was declared at $p<0.05$.

Results and Discussion

Chemical composition of experimental gluten-free maize cookies

The chemical composition of experimental GF maize cookies was influenced by HAM addition (Table 2). In particular, total starch and amylose contents increased (p<0.05) ranging from 68.8 g/100 g DM to 71.1 and 73.0 g/100 g DM and from 17.4 g/100 g DM to 26.1 and 33.4 g/100 g DM, respectively, whereas crude protein content decreased (p<0.05) from 11.3 g/100 g DM to 10.1 and 8.6 g/100 g DM when the level of HAM increased in the recipe of cookies. These differences are related to the relative amount of HAM added in each GF maize cookie formulation, as already reported for cookies prepared from wheat flour in combination with banana starch [23]. In addition, dietary fibre content increased (p<0.05) ranging from 4.9 g/100 g DM to 5.5 and 6.8 g/100 g DM for control, 25-CK and 50-CK. Greater amount of fibre of GF bakery products are considered beneficial, since a general low intake of this food component has been reported for celiac population [24]. No differences (p>0.05) were reported for moisture, crude lipids and free sugars, on average being 3.8 g water/100 g food, 8.1 g/100 g DM and 0.9 g/100 g DM, respectively. With the exception of soft types, the moisture content of cookies should be below 5% after baking, resulting in good storage stability [25].

Starch fraction content of experimental gluten-free maize cookies

Based on the rate and extent of enzymatic digestion, dietary starch is commonly classified into RDS, SDS and RS fractions. From a physiological point of view, the RDS fraction induces a fast increase in blood glucose and insulin levels, whereas SDS is slowly digested throughout the small intestine, resulting in a slow and prolonged release of glucose into the blood stream [26]. Lastly, the RS fraction escapes digestion in the small intestine, thus reaching the large intestine where can be substrate for microbial fermentation [7]. As presented in Table 2, starch fractions were strongly influenced by HAM addition: both RS and SDS increased (p<0.05) ranging from 3.6 g/100 g DM to 7.9 and 12.7 g/100 g DM and from 16.2 g/100 g DM to 24.0 and 26.4 g/100 g DM, whereas RDS and available starch levels decreased (p<0.05) ranging from 49.0 g/100 g DM to 39.2 and 33.9 g/100 g DM and from 65.2 g/100 g DM to 63.2 and 60.3 g/100 g DM for control, 25-CK and 50-CK, respectively. Similarly, gluten-containing cookies partially substituted with banana starch showed lower RDS and higher SDS than control samples [23]. In addition, higher amounts of RS have been reported for maize and wheat breads made from HA genotypes when compared with corresponding food products prepared with NA flours, RS being positively related with amylose content [10,27,28]. During baking, the native structure of starch undergoes a characteristic re-organization commonly know as gelatinisation, which increases the accessibility of starch to hydrolysing enzymes due to swelling of starch and loss of the crystalline structure [28]. However, higher amylose content can result in incomplete gelatinisation process. This because amylose, being a more compact molecule compared to amylopectin, can reduce the degree of starch swelling during gelatinisation, thus leading to higher SDS and RS levels [26,29]. In addition, upon cooling, starch undergoes a re-association process commonly known as retrogradation, leading to a tightly packed crystalline structure slowly hydrolysed by digestive enzyme [28]. Evidences suggest that retrograded starch is primarily amylose because the relatively short and linear amylose chains can re-associated in a quicker way than amylopectin chains [30].

Starch hydrolysis and predicted glycemic index of gluten-free maize cookies

Previous studies have involved both *in vivo* and *in vitro* methods to estimate the effect of starch composition on the rate and extent of starch digestion. However, *in vitro* approaches have advantages over *in vivo* studies, being viable, rapid and cost effective alternatives for predicting the likely *in vivo* response of new-developed products [13]. As reported in Table 3 control cookies were characterised by high HI and pGI values (103 and 97, respectively; calculated using white wheat bread as reference), in line with previous *in vivo* findings [2]. However, when HAM increased in the composite, both HI and pGI decreased (p<0.05) from 81 to 64 and from 78 to 63 for CK-25 and CK-50, respectively. The lower pGI calculated for HAM-enriched GF cookies could be related to respective SDS and RS contents, these fractions being associated with a decreased *in vitro* glycemic response [18]. Analogously, bread produced by the substitution of HAM for a part of NA wheat flour showed a lower *in vivo* GI than the control bread (60 *vs.* 100, respectively) [31]. Likewise, Granfeldt et al. [10] reported a marked decrease in the pGI of arepa maize bread made from HAM (i.e., 50) when compared to NA maize arepas (i.e., 81).

Parameters	Substitutions with HAM flour			√MSE	*p*-value
	Cookie 0-CK	Cookie 25-CK	Cookie 50-CK		
Chemical composition (g/100 g dry matter)					
Moisture [a)]	3.9	3.8	3.7	0.57	n.s.
Total starch	68.8	71.1	73.0	1.04	<0.05
Crude protein	11.3	10.1	8.6	0.41	<0.05
Crude lipid	8.1	8.2	8.0	0.81	n.s.
Dietary fibre	4.9	5.5	6.8	0.49	<0.05
Ash	1.2	0.9	0.7	0.07	<0.05
Free sugars	1.1	1.0	0.8	0.1	n.s.
Amylose	17.4	26.1	33.4	0.88	<0.05
Starch fractions (g/100 g dry matter)					
Resistant starch	3.6	7.9	12.7	0.89	<0.05
Rapidly digestible starch	49.0	39.2	33.9	1.29	<0.05
Slowly digestible starch	16.2	24.0	26.4	1.39	<0.05
Available starch	65.2	63.2	60.3	1.51	<0.05

Table 2: Chemical composition and starch fraction contents of gluten-free maize cookies added with different substitution levels of high amylose maize starch flour. NAM: normal amylose gluten-free maize mix; HAM: high amylose maize starch flour; 0-CK, control cookie formulated with 0:100 HAM:NAM; 25-CK; formulated with 25:75 HAMS:NAM; 50-CK, formulated with 50:50 HAMS:NAM; n.s; not significant (p>0.05). [a)]g water/100 g food.

Parameters	Substitutions with HAM flour			√MSE	*p*-value
	Cookie 0-CK	Cookie 25-CK	Cookie 50-CK		
HI [a)]	103	81	64	6.0	<0.05
pGI [b)]	97	78	63	5.1	<0.05
k (min^{-1})	0.15	0.07	0.05	0.021	<0.05

Table 3: Hydrolysis index of starch (HI), in vitro predicted glycemic index (pGI) and rate of starch hydrolysis (k) of gluten-free maize cookies added with different substitution levels of high amylose maize starch flour. NAM: normal amylose gluten-free maize mix; HAM: high amylose maize starch flour; 0-CK, control cookie formulated with 0:100 HAM: NAM; 25-CK, formulated with 25:75; HAM: NAM; 50-CK; formulated with 50:50 HAM: NAM. (A) Calculated using white wheat bread as reference (HI=100). (B) Calculated with the equation pGI=8.198+0.862 × HI (pGI white wheat bread=96) [19].

For gluten-containing cookies, the substitution of a part of NA wheat flour with HAM contributed to formulate cookies with favourably lower pGI values (about 30%) than control products [3]. Lastly, slower ($p<0.05$) k values were calculated when the HAM level increased in the GF cookie formulation, being 0.15 min^{-1}, 0.07 min^{-1} and 0.05 min^{-1} for CK-0, CK-25 and CK-50, respectively. The consumption of foods with slowly digestible starch properties may be helpful in controlling and preventing hyperglycaemia-related disorders, such us diabetes and cardiovascular diseases [26].

Overall, present *in vitro* findings suggest that the use of HA ingredients could contribute to formulate GF cookies with slowly digestible starch properties, with no alteration by the baking process. For instance, at the highest level of substitution, experimental GF maize cookies were characterised by a lower GI if compared to many commercial available cookies and breakfast cereals [2]. It must be pointed out that the different amylose content, despite having a significant role in reducing rate and extent of starch digestion, is not the only factor that can contribute to explain present findings, since other physicochemical properties of starch have been reported to influence enzymatic starch digestion [32]. In particular, starch granules exhibit different types of crystalline domains that resist enzymatic hydrolysis to different degrees [33]. Normal amylose starches exhibit the A-type power diffraction pattern, which has an open structure that renders starches highly digestible, whereas HA starches give the so-called B-pattern, which is more resistant to digestion by pancreatic amylase due to a close-packed arrangement [34]. In addition, the absence of granule pores and channels in HA starches when compared to NA sources can result in substantially lower digestion potentials, since digestion is effected by a surface erosion mechanism [32,35]. Lastly, the increasing amount of dietary fibre found in HAM-enriched GF cookies, along with the possible formation of amylose-lipid complexes during cooking, could have contributed to further reduce the accessibility of amylase to hydrolyse the starch [36,37].

Sensory evaluation of gluten-free maize cookies

None of the panellists reported any sense of fullness or nausea during evaluation. The mean sensory evaluation scores for "appearance", "structure", "flavour", "taste" and "overall acceptability" of GF maize cookies are presented in Table 4. Overall, selected sensory attributes were positive and not impaired by HAM addition. On average, sensory scores of 5.7, 7.1, 6.8 and 7.1 were recorded for "appearance"; "structure"; "flavour", and "taste", respectively. The mean overall acceptability scores were 7.6 for 0-CK, 7.9 for 25-CK and 7.8 for 50-CK and were not influenced ($p>0.05$) by HAM addition. These results may be expected, since GF flours used in the present evaluation were all maize based. Evidences suggest that the addition of HAM did not significantly alter the sensory profile of baked food products made with flours from different botanical origins. For instance, the replacement up to 20% of total starch content with HAM in the recipe of GF breads did not influence the sensory properties of the final products [4]. Similarly, no difference in the overall acceptability of gluten containing cookies was reported when wheat flour was replaced with increasing percentage of HAM [3].

Parameters	Substitutions with HAM flour			√MSE	*p*-value
	Cookie 0-CK	Cookie 25-CK	Cookie 50-CK		
Appearance	5.7	5.7	5.6	1.41	n.s.
Structure	7.2	7.0	7.2	1.55	n.s.
Flavour	6.7	6.9	6.7	1.27	n.s.
Taste	7.1	7.0	7.2	1.35	n.s.
Overall acceptability	7.6	7.9	7.8	1.56	n.s.

Table 4: Sensory evaluation scores of gluten-free maize cookies added with different substitution levels of high amylose maize starch flour. Values expressed on a 1-9 point scoring scale. NAM: normal amylose gluten-free maize mix; HAM: high amylose maize starch flour; 0-CK; control cookie formulated with 0:100 HAM:NAM; 25-CK; formulated with 25:75 HAM:NAM; 50-CK; formulated with 50:50 HAM:NAM; n.s., not significant ($p>0.05$).

Conclusions

Differences in the chemical composition were observed in gluten-free cookies prepared with increasing levels of high amylose maize starch. Lower protein and higher dietary fibre and starch contents were achieved when the level of high amylose maize starch increased. Noticeable differences were obtained considering the *in vitro* starch digestion of gluten-free cookies. Higher resistant starch and slowly digestible starch contents, along with lower rapidly digestible starch and predicted glycemic index values were reported when high amylose maize starch increased in the composite. Taking together, these changes indicated that the partial substitution of normal amylose gluten-free flour with high amylose maize starch contributed to formulate gluten-free maize cookies with likely slowly digestible starch properties, without affecting selected sensory attributes.

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