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ORIGINAL ARTICLE

Expected glycemic impact and probiotic stimulating effects of whole grain flours of buckwheat, quinoa, amaranth and chia

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Abstract Chia, amaranth, quinoa and buckwheat grains have been widely used in food formulations because of their high and balanced nutritional properties. Since all grains are not equally nutritious, there is a requirement for comparing the health-related effects and processing performance of a variety of whole grains. The expected glycemic index (eGI) flours of chia was determined to be quite low, and flours except quinoa can be classified as low GI foods. The highest resistant starch (RS) content (4.76 g/100 g) was found in amaranth flour, and it was followed by buckwheat (1.27 g/100 g). The amaranth had the highest stimulation effect on the growth of probiotics and increased the count of *L. acidophilus* and *B. bifidum* as 4.57 and 2.26 log CFU/ml, respectively. Moreover, chia flour showed a positive effect on the growth of *L. acidophilus* whereas it negatively affected *B. bifidum* compared to the control. A significant correlation was detected between rapidly available glucose content and eGI. On the other hand, a significant relationship between RS and the growth rate of probiotics was reported.

Keywords Pseudocereals · Glycemic index · Resistant starch · Probiotic

Abbreviations

eGI	Expected glycemic index
RS	Resistant starch
WGF	Whole grain foods
GOPOD	Glucose oxidase/peroxidase
AUC	Area under the curve
HI	Hydrolysis index
RAG	Rapidly available glucose
SAG	Slowly available glucose
GG	Glycemic glucose
MRS	Man Rogosa sharp broth
CFU	Colony forming unit

Introduction

Today, the widely accepted term of glycemic index (GI) is the total rise in a person's blood glucose level after consumption of food. The diets containing high GI foods are associated with risk factors of type 2 diabetes, obesity, hyperlipidemia and cancer. Since numerous studies have demonstrated positive effects of low glycemic diet, more attention is paid to formulate whole grain foods (WGF) for the aim of decreasing GI. Moreover, FDA declared that diets containing a high amount of WGF might minimize the risk of cardiovascular disease and cancers (Laparra and Haros 2018).

Recent studies have revealed that WGFs are important in preventing diabetes by slowing digestion and absorption of carbohydrates thus they create a depletion effect on GI (Laparra and Haros 2018). The studies generally focused on the development of fibre enriched foods by replacements of wheat flour with whole grains as fibre sources (Bae et al. 2013). The new sources of grains are an essential task from a nutritional point of view. For instance,

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WGF of pseudocereals and chia may be an attractive additive to decrease postprandial glucose level of blood and to improve food quality with their high bioactive content.

In recent years, interest and demand for pseudocereals have increased steadily. They have gained importance as alternative raw material instead of traditional and widely used for human nutrition (Haros and Schoenlechner 2017). Pseudocereals are not a member of the *Gramineae* family, but their seeds can be easily milled into flour owing to cereal-like starchy endosperm (Skrabanja et al. 2001). Buckwheat, amaranth and quinoa are classified as pseudocereals, and they are widely used in food formulations due to their high nutritional qualities. Buckwheat, the most known member of pseudocereals, has an annual production of 3 million tonnes (Haros and Schoenlechner 2017). It is a rich source of proteins, dietary fibres, antioxidants, phenolic compounds, vitamins, essential minerals and resistant starch (RS). There are many studies showed that nutrients such as proteins, flavonoids, and thiamin-binding proteins of buckwheat had positive effects on controlling cholesterol, blood pressure, and serum glucose level (Bae et al. 2016; Vujic et al. 2014).

Amaranth is an important protein source with its high content of essential amino acids such as lysine, methionine and cysteine which present limitedly in most cereal grains (Capriles et al. 2008; Caselato-Sousa and Amaya-Farfan 2012; Vujic et al. 2014). Furthermore, its high nutritional value and agronomic potential were demonstrated by the United States National Academy of Sciences (Caselato-Sousa and Amaya-Farfan 2012). Quinoa, having similar properties with amaranth, is also recommended with its ideal protein balance by the FAO. There is an increasing demand to quinoa in functional food applications due to its nutrient availability. Chia is the most promising crop, and its global production has increased mainly due to its popularity throughout the World. The chia seed oil has been reported as the most abundant natural source of the essential fatty acid of α-linolenic [ALA; 18:3(n-3)] (Menga et al. 2017).

Pseudocereals are considered as balanced nutritional sources with proven functional properties by various studies (Haros and Schoenlechner 2017). Chia, amaranth, quinoa and buckwheat are suitable to use in food formulation for people with celiac disease (Giuberti and Gallo 2018). Zevallos et al. (2014) reported that celiac patients safely tolerated 50 g of quinoa without any deterioration in health condition. Furthermore, a strong association with diabetes mellitus and celiac disease was reported since both of these diseases result from similar human leukocyte antigens (Berti et al. 2004; Wolter et al. 2014). Therefore, the use of pseudocereals in the food formulations can be an excellent way to maintain glycemic control and development of foods for patients who have celiac disease. On the other hand, gluten-free products are generally characterised by the low nutritional quality when compared with their gluten-containing counterparts (Giuberti and Gallo 2018). So, it may be worthwhile improving the formulation of these products by WGF of buckwheat, quinoa, amaranth and chia having balanced essential amino acid and polyunsaturated fatty acids composition.

Starch digestibility is affected by various factors such as nature and structure of starch source, protein and lipid interactions, the presence of antinutrients or enzyme inhibitors and process conditions. Therefore, a careful selection of grains is needed (Vujic et al. 2014). Since all grains are not equally nutritious, there is a requirement for comparing the health-related effects and processing performance of a variety of whole grains. For this reason, more systematic studies are needed for better understanding the potential of WGF as functional ingredients (Bae et al. 2013). However, there has been no report comparing the glycemic impacts of chia, amaranth, quinoa and buckwheat in the literature. The effects of pseudocereals on GI and probiotic microorganisms are still limited when consumed as whole grains. Therefore, the objectives of this study were to investigate and compare the expected glycemic impact and probiotic stimulating effects of four WGF of pseudocereals and demonstrate functional food properties.

Materials and methods

Materials

In this study, chia (*Salvia hispanica* L., from Argentina), amaranth (*Amaranthus hypochondriacus*, from India), quinoa (*Chenopodium quinoa Wild,* from Peru) and buckwheat (*Fagopyrum esculentum,* from Russia) seeds were obtained a local exporter. The broken parts and and foreign materials were removed. After that, the grains were milled with a laboratory scale hammer mill (MF 10, IKA, Staufen, Germany) equipment with 500-micron sieve.

Lactobacillus acidophilus LA-5 and *Bifidobacterium bifidum* BB-12 (Chr. Hansen, Horsholm, Denmark) were used as probiotic bacteria. Pepsin (Sigma, Taufkirchen, Germany), pancreatic α-amylase, amyloglucosidase and glucose peroxidase (GOPOD) (Megazyme, Wicklow, Ireland) were used for the determination of the starch digestibility and eGI.

In vitro starch digestion and expected Glycemic Index (eGI)

The expected glycemic index (eGI) of grains was determined according to Goni et al. (1997) with some modifications. The analysis was conducted after defatting of chia, amaranth, quinoa and buckwheat flours with hexane. Each sample (50 mg) was mixed with 10 mL of HCl–KCl solution (pH 1.5). Then 0.2 mL pepsin solution (porcine, 200 U/mL) was added to sample tubes and incubated at 40 °C for 1 h in shaking water bath. After incubation, sample volumes were adjusted to 25 mL of Tris-Maleate buffer (pH 6.9), and 5 mL pancreatic α-amylase solution (2.6U in the tris-maleate buffer) was added. This solution was incubated at 37 °C for 180 min. During this second incubation period, aliquots of 1 mL were taken every 30 min from 0 to 180 min. To inactivate the enzyme, these aliquots were kept at 100 °C for 5 min and stored at 4 °C until the end of the incubation time. After incubation completed, 3 mL of 0.4 M sodium acetate buffer (pH 4.75) and 60µL pancreatic amyloglucosidase were added to each aliquot and samples were incubated at 60 °C for 45 min to convert digested starch to glucose. An aliquot (0.1 mL) was enriched with 3 mL GOPOD (glucose oxidase/peroxidase) and left for final incubation at 45 °C for 20 min. Finally, the glucose concentration was measured at 510 nm, and the measured glucose level was converted into starch by multiplying for 0.9. The rate of starch digestion was expressed as the percentage of total starch hydrolysis at different times of experiment (0, 30, 60, 90, 120 and 180 min). By plotting the starch content which was hydrolysed against time, a nonlinear starch hydrolysis curve was obtained. Therefore, the first order kinetic equation (Eq. 1) was applied to describe the kinetics of starch hydrolysis;

$$C = C_{\infty}\left(1 - e^{-kt}\right) \tag{1}$$

C = Concentration of each time, C_{∞} = Concentration at equilibrium, k = Kinetic constant, t = Time.

A nonlinear model was used to calculate the area under the curve (AUC) (Eq. 2).

$$AUC = C_{\infty}(t_f - t_0) - \frac{C_{\infty}}{k}\left(1 - e^{-k(tf - t0)}\right) \tag{2}$$

C_{∞} = Concentration at equilibrium, t_f = The end time of the experiment, t_0 = The start time of the experiment, k = Kinetic constant.

The hydrolysis index (HI) was calculated as the relation between the AUC of flours and standard material from control (white bread). Finally, eGI was calculated by Eq. 3 and convert to glucose reference by Eq. 4.

$$eGI = 39.71 + 0.549(HI) \tag{3}$$

$$eGI(white\,bread) = 1.43 \times eGI(glucose) \tag{4}$$

Determination Of Rapidly Available Glucose (RAG), Slowly Available Glucose (SAG) and Glycemic Glucose (GG)

The amount of RAG and SAG were measured as a marker of starch degradation by digestive enzymes. The level of RAG, SAG and GG of chia, amaranth, quinoa and buckwheat flours were calculated from the data, obtained in glycemic index analysis, according to Englyst et al. (2003). RAG and SAG values were the glucose released at 30 min and between 30 and 180 min, respectively. GG was calculated as the total of RAG and SAG.

Determination of RS

RS content of WGFs were determined following the AACC (2010) method of 32–40 using of RS analysis kit (K-RSTAR, Megazyme Int. Wicklow, Ireland). For the experiment, 100 mg of each pseudocereal flour was weighted and incubated with 4 mL solution containing pancreatic α-amylase (3U/mg) and amyloglucosidase (300U/mL) at 37 °C for 16 h with the 100-rpm agitation in a water bath. After incubation, 4 mL ethanol was added to tubes, and centrifuged at 4500 rcf for 5 min. The supernatant was removed, and the precipitate was washed twice with 8 mL ethanol solution (50%, v/v) and samples were centrifuged. After that, sample tubes were held in a vacuum oven (VO200, Memmert, Germany) at 40 °C for 30 min for removal of residual water. For the dissolution of RS, 2 mL KOH (2 M) was added and stirred in an ice bath for 20 min. After mixing, 8 mL sodium acetate buffer (1.2 M) and 0.1 mL amyloglucosidase (3300 U/mL) were added and incubated at 50 °C for 30 min. The sample tubes were centrifuged, and an aliquot of 0.1 mL was left a second incubation period for 20 min after the addition of 3 mL GOPOD. After incubation, the absorbance was measured at 510 nm, and the hydrolysed resistant starch level was calculated with Eq. 5.

$$Resistant\ starch = \Delta E \times \left(\frac{F}{W}\right) \times 9.27 \tag{5}$$

ΔE = The absorbance of the sample, F = The absorbance obtained for 100 µg of D-glucose standard, W = Dry weight of the sample.

Bacterial growth ın the presence of pseudocereal flours

Before using, freeze-dried probiotic strains were cultured with Man Rogosa Sharp broth (MRS) at 37 °C for 18 h and stored in 20% glycerol solution at − 18 °C for further analysis. MRS Broth was enriched separately using 1% chia, amaranth, quinoa and buckwheat flour as the carbon source and pH value was adjusted to 6.2 with 0.1 N NaOH for *L. acidophilus*. To growth of *B.bifidum,* MRS Broth was enriched with 0.05% L-cysteine, and pH value was adjusted to 6.5 with 0.1 N NaOH after addition of 1% each pseudocereal flour. Following the autoclaving and cooling of broths, probiotic cultures (3–4 log CFU) were individually inoculated and incubated at 37 °C for 18 h. The counts of *L. acidophilus* and *B.bifidum* were enumerated on MRS agar at 37 °C for 72 h in anaerobic conditions. The MRS medium without any addition was used as a negative control.

Statistical analysis

The analyses were conducted in three parallel. The factorial randomised block design was used as an experimental design. The data subjected to analysis of variance, and proper mean separation was conducted using Duncan's Multiple-Range Test. All statistical calculations were performed by SAS Statistical Software. The correlation tests between RAG, SAG, GG, eGI and RS and probiotic stimulating effect were evaluated by XLSTAT.

Results and discussion

Starch digestibility and eGI value

HI and eGI values of WGFs was given in Fig. 1 and Table 1, respectively. The hydrolysis rate of chia was lower than 5%, and it was significantly lower than amaranth (30–40%), buckwheat (20–60%) and quinoa (50–60%). Similar with starch hydrolysis rates, the lowest eGI value was also measured in the chia as 28.53. It might be explained with lower total starch and higher dietary fibre contents of chia compared to other pseudocereal flours. The quinoa, amaranth and buckwheat were reported to have high carbohydrate content as 60–74% while it was nearly 42% for chia (Haros and Schoenlechner 2017; Munoz et al. 2013). Moreover, the dietary fibre content of quinoa, amaranth and buckwheat were 10%, 11% and 7%, respectively, while it was nearly 40% for chia (Lamothe et al. 2015; Reyes-Caudillo et al. 2008; Steadman et al. 2001).

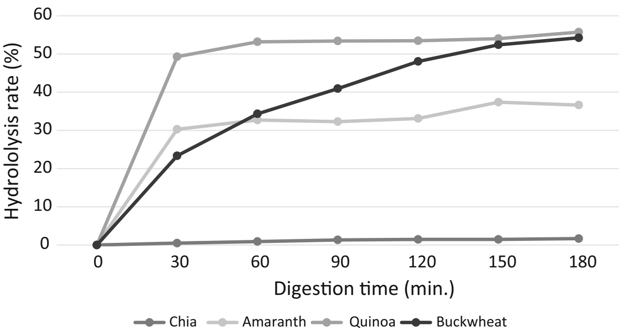

Fig. 1 Hydrolysis rate of pseudocereal whole grain flours

Previous studies demonstrated that the glycemic effect of foods depends on several different factors such as amylose/amylopectin ratio, origin and structure of starch granule, food texture and particle size, the physical entrapment of starch molecules within food and food processing techniques (Englyst et al. 2003; Laparra and Haros 2018; Wolter et al. 2014). Moreover, the fatty acid content of food also affects the starch digestion by forming starch-lipid or starch-fatty acid complexes. According to Takahama and Hirota (2010), fatty acids may be bound in hydrophobic helical structure of amylose and inhibits its digestion. Thus, the high fatty acid content of the chia seed may also be the reason for the low eGI. Apart from fatty acids, other micronutrients of chia may show reducing the effect on in vitro rate of starch hydrolysis. In previous studies, polyphenols and antinutritional factors of buckwheat seemed to have a lowering effect on starch digestibility (Wolter et al. 2013; Skrabanja et al. 2001; Vujic et al. 2014).

Nowadays, the increase in obesity and health problems encourage people to consume foods labelled as low GI value. Foods are classified in three groups according to their GI as low (GI < 55), medium ($56 < \mathrm{GI} < 69$), and high (GI > 70) concerning glucose as reference (Vujic et al. 2014). When consumed a high GI food, it breaks down quickly during digestion and releases glucose rapidly into the bloodstream, but this effect is shown more slowly in a low GI food. According to this classification, WGF of buckwheat, amaranth and chia were classified in low GI foods and quinoa was medium GI food.

This finding was in accordance with the results of Priyanka et al. (2018) who classified quinoa as medium GI food with the GI value of 63.37. From a nutritional viewpoint, these four flours can be used in food formulations to modulate the glycemic impact of the final product. Many previous studies are demonstrating that the eGI of foods decreased with the addition of different pseudocereal flours. Vujic et al. (2014) reported that the buckwheat and amaranth flour incorporation into the biscuit formulation resulted in a slightly lower GI value. In another study, the GI value of noodle could decrease to 65 by addition of buckwheat flour (Bae et al. 2016). The postprandial glucose control effects of pseudocereals were also supported by different in vivo studies (Laparra and Haros 2018; Skrabanja et al. 2001).

Table 1 Expected glycaemic impact value, glucose fractions and resistant starch content of pseudo-cereal samples (g/100 g)

Grain	eGI	RAG	SAG (g/100 g)	GG (g/100 g)	RS (g/100 g)
Chia	28.53 ± 0.07^{D}	1.43 ± 0.01^{D}	0.95 ± 0.07^{C}	2.38 ± 0.07^{C}	0.08 ± 0.01^{C}
Amaranth	47.65 ± 0.84^{C}	29.63 ± 1.55^{B}	6.94 ± 1.36^{B}	36.57 ± 1.01^{B}	4.76 ± 0.71^{A}
Quinoa	61.50 ± 0.13^{A}	54.05 ± 0.21^{A}	3.39 ± 1.15^{C}	57.44 ± 1.13^{A}	0.23 ± 0.04^{C}
Buckwheat	52.35 ± 0.80^{B}	23.61 ± 0.96^{C}	31.22 ± 0.82^{A}	54.82 ± 1.71^{A}	1.27 ± 0.05^{B}

GI Glycemic index; *RAG* Rapid available glucose content, *SAG* Slow available glucose content; *GG* Glycemic glucose; *RS* Resistant starch. The data are presented as basis on dry matter mean values ± standard errors (n = 3). The superscript letters, in the same column, indicate that are significantly different by Duncan's multiple range test ($P < 0.05$)

RAG, SAG and GG

Glycemic glucose fractions are classified as two main groups as RAG and SAG to reflect the release and absorption rate of consumed glucose (Englyst et al. 2003). These values may also be named as rapidly digestible starch (RDS, digested in first 20 min) and slowly digestible starch (SDS, digested between 20 and 120 min). RDS causes a sudden rise in blood glucose levels, whereas SDS results in a gradual increase in blood glucose levels and both of them are important in the prevention of diabetes and cardiovascular diseases (Bae et al. 2016; Vujic et al. 2015).

The RAG value of chia flour was 1.43 g/100 g, which was significantly ($p < 0.01$) lower than amaranth (29.63 g/100 g), quinoa (54.05 g/100 g) and buckwheat (23.61 g/100 g) (Table 1). In previous studies, foods containing low RAG and high SAG reported decreasing postprandial glucose level (Bae et al. 2016). As expected, chia flour had the lowest RAG and SAG values in comparison to pseudocereal flours. This result might be attributed to limited starch content of chia. As previously mentioned, the nature of starch has a fundamental role in the rate of its digestibility and absorption (Vujic et al. 2014).

Moreover, chia contains high fatty acids that can have a protective effect against α-amylase via reducing the available surface area through blocking active sites. A similar finding was also reported for soy that has high protein and lipid content (Vujic et al. 2014). It was reported that the addition of chia flour in pasta formulation decreased the RAG and SAG value to 18.43 and 3.12%, respectively (Menga et al. 2017).

The highest RAG value was determined in quinoa flour. Haros and Schoenlechner (2017) attributed this result to high amylopectin and low amylose content of quinoa. Additionally, low RAG value of buckwheat could also be related with its high amylose content reported as the level of 18–47% (Haros and Schoenlechner 2017). It has been known that amylose molecules contain more hydrogen bonds making them less accessible to α-amylase than amylopectin molecules which has a branch structure (Vujic et al. 2014; Wolter et al. 2014). Namely, high amylopectin content of grains seems to play a direct role on GI. Similarly, Xiao et al. (2017) determined the RAG and SAG value of Tartary buckwheat flour as 39.9, and 29.9%, respectively. The incorporation of buckwheat flour in noodles resulted in lower RAG and higher SAG values (Bae et al. 2016). On the other hand, the values reported in this study were in accordance with Capriles et al. (2008) who found RAG content of raw amaranth seeds as 30.7%. Vujic et al. (2015) determined the RAG and SAG content in biscuits produced by buckwheat and amaranth flour were 15.55–21.22% and 15.51–20.16%, respectively.

RS content

RS is known as non-digestible starch fractions, and it helps to grow probiotics forming the gut microflora. By fermentation of RS in the large intestine, several metabolically active short-chain fatty acids, important to the maintenance of gastrointestinal health, are formed.

The RS content of WGFs was presented in Table 1. Amaranth flour had the highest RS content, and it was followed by buckwheat flour. Although chia was announced as a good source of dietary fibre, the RS content was the lowest. The high dietary fibre content of chia may source from containing mucilage (Felisberto et al. 2015). Additionally, low GI properties of chia may come from the absence of starch rather than RS content. Such assumptions were confirmed with our eGI results, and as expected, the amaranth having the highest RS content provided the second lowest eGI.

The obtained results were in agreement with the RS content of Tartary buckwheat flour (Molinari et al. 2018). Wolter et al. (2014) determined the RS content of bread produced using buckwheat and quinoa as 2.01% and 0.96%, respectively. Linsberger-Martin et al. (2012) compared the RS content of wheat and pseudocereal, and they determined that RS content of wheat, quinoa and amaranth were 0.39, 0.18 and 1.29%, respectively. The properties of RS may be changed in varieties of cereals.

From a nutritional point of view, RS is important for human health to control of blood glucose level and diabetes. According to EFSA rules to bear the claim; high carbohydrate baked foods should contain at least 14% of total starch as RS (Linsberger-Martin et al. 2012). Therefore, foods with high amounts of RS could be recommended.

Probiotic stimulating effect

The increases in the growth rate of *L. acidophilus* LA-5 and *B. bifidum* BB-12 were given in Fig. 2. Higher bacterial counts of *L. acidophilus* were obtained in the medium supplemented with amaranth flour ($p < 0.01$), and it was followed by chia, quinoa and buckwheat. As expected, control medium, not containing pseudocereal flour, showed the low supportive effect *on L. acidophilus.* From the results, it could be drawn that each flour presented a selective effect on the growth of *L. acidophilus*.

Similar with *L. acidophilus* population, the highest growth rate of *B. bifidum* was also observed in amaranth added medium. Controversially, the control medium provided more sustained growth of *B. bifidum* compared to chia supplemented medium. A possible explanation for this finding may be the absence of nutrients in chia for the proliferation of *B. bifidum.* Bearing in mind that, the selective fermentation of a substrate is vigorously strain dependent. The results of Vieira et al. (2017) demonstrated that the stimulus effect might not be extrapolated to all members of a genus or cluster since the *B. longum* BB-46 strain did not show the same growth as the other Bifidobacterium strains in the presence of amaranth. To the best of our knowledge, the probiotic stimulation of chia has not been studied previously, and the results indicated that chia flour showed a positive effect on *L. acidophilus* whereas it negatively affected *B. bifidum* growth when compared to control medium. On the other hand, the detected growth rate of *L. acidopilus* was higher than that of *B. bifidum*, and this result revealed that the cells of *L. acidophilus* were more adaptable than *B. bifidum* to pseudocereal as a substrate. Consequently, the data of the present study reinforced the fact that WGF of pseudocereals, especially amaranth, stimulated the growth of probiotic strains by showing prebiotic effect.

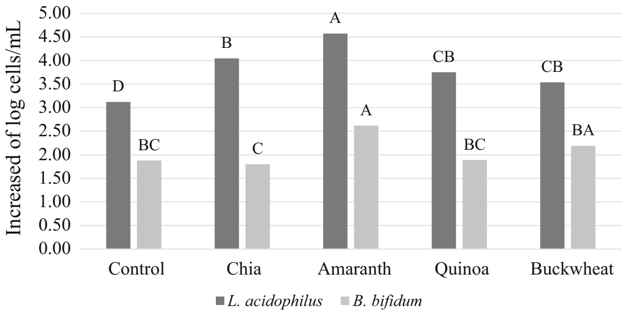

Fig. 2 Viable numbers of probiotic bacteria in media supplemented with different pseudocereal flours

Most of the studies investigating the effects of pseudocereals on probiotic bacteria have been conducted with buckwheat, and there are very limited studies on probiotic effects of other members. On the other hand, in a study of experimental rat models, *L. plantarum, Bifidobacterium spp* and *B. lactis* only found in buckwheat-fed diets (Prestamo et al. 2003). Gullon et al. (2016) determined that media containing quinoa and amaranth caused an increment of total cell count of colonic microflora up to 0.96 and 0.88 log-fold after 48 h of fermentation. Overall, the use of high dietary fibre-containing grains as a substrate for probiotic bacteria would be a good alternative for the development of new synbiotic food formulations.

Correlation of results

The Pearson correlation test results obtained from analysis of eGI and probiotic stimulating effect were presented in Table 2. As can be seen in the table, there was a significant ($p < 0.01$) correlation between RAG, GG and eGI. GG also significantly ($p < 0.01$) correlated with GI. Moreover, RAG is positively correlated with eGI since it is highly responsible for the postprandial rise in blood glucose concentration due to its rapid digestion and absorption in the small intestine (Englyst et al. 2003). Vujic et al. (2014) also reported a positive correlation between eGI and RAG values.

On the other hand, in most of the studies, RS was also reported having a decreasing role on eGI. However, no significant relationship was observed between RS and eGI in the present study. This finding was similar to Wolter et al. (2014) who found no correlation between RS content and hydrolysis indices for wheat sourdough bread and they explained these results with the negligible level of resistant starch content. Englyst et al. (2003) declared that the positive correlation between RS and eGI was difficult to explain on its own, as the RS fraction was not absorbed in the small intestine and therefore cannot elicit a glycemic response.

Although RS was not correlated with eGI, a significant ($p < 0.05$) correlation between RS and the growth rate of *L. acidophilus* and *B. bifidum* was determined. It could be concluded that RS content of grains had the stimulating effect on *L. acidophilus* and *B. bifidum.*

Table 2 Pearson correlation coefficients of pseudocereals

Variables	RAG	SAG	GG	eGI	RS
RAG					
SAG	− 0.033				
GG	0.834**	0.524			
eGI	0.939**	0.311	0.972**		
RS	0.095	0.049	0.108	0.103	
L. acidophilus	− 0.314	− 0.534	− 0.562	− 0.487	0.636*
B. bifidum	0.100	0.466	0.342	0.247	0.650*

GI Glycemic index; *RAG* Rapid available glucose content; *SAG* Slow available glucose content; *GG* Glycemic glucose; *RS* Resistant starch. Values in bold are different from 0 with a significance level alpha = 0.05

** < 0.01, * < 0.05,

Conclusion

Recently, food scientists have focused on the development of healthier food formulations by using whole grains since their positive effects on the glycemic index. Pseudocereals are considered as "superfood" with their reasonably well-balanced nutrient composition. In this study, the glycemic impact and probiotic stimulating effects of whole grain flours of chia, amaranth, quinoa and buckwheat were investigated.

The lowest glycemic index, RAG, SAG and GG value was determined in whole grain flours of chia. All pseudocereal flours except quinoa (medium GI food) were classified in low GI foods. The resistant starch content of amaranth flour was higher than other pseudocereal flours. Among the four pseudocereal flours, amaranth presented the most stimulus effect on the growth of probiotic strains. Additionally, chia flour showed a positive effect on the growth of *L. acidophilus* whereas it negatively affected *B. bifidum*. A significant correlation was detected between RAG, GG and GI, whereas no correlation was observed in RS and GI. On the other hand, a significant correlation between RS and the growth rate of *L. acidophilus* and *B. bifidum* was determined.

In conclusion, this study presents new perspectives for the development of symbiotic food formulations with pseudocereals and low glycemic index values. Additionally, this developed food formulation can be a good alternative for patients with celiac disease.

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Author contributions CCU, CM and SAT performed experiments; SAT conducted data analysis and write the draft. ME; edited and revised paper.

Data availability Data available on request from authors.

Declarations

Conflict of interest The authors declare no conflict of interest.

Consent to participate All authors have approved and reviewed the final manuscript.

Consent to publication All authors have approved and reviewed the final manuscript.

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