Functional properties and predicted glycemic index of gluten free cereal, pseudocereal and legume flours

Maria Di Cairano[a]*, Nicola Condelli[a], Marisa Carmela Caruso[a], Alessandra Marti[b], Nazarena Cela[a], Fernanda Galgano[a]

*corresponding author: Maria Di Cairano - maria.dicairano@unibas.it

[a]School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale Dell'Ateneo Lucano 10, 85100, Potenza, Italy

[b] Department of Food, Environmental, and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via Celoria, 2, 20133 Milan, Italy

email addresses: nicola.condelli@unibas.it, marisa.caruso@unibas.it, alessandra.marti@unimi.it, nazarena.cela@unibas.it, fernanda.galgano@unibas.it

keywords: flours, gluten free, glycemic index, pasting properties, functional properties

Declarations of interest: none

Abstract

Most of gluten free (GF) bakery products available on the market are made by a restricted number of grains. Flours and starches from rice and maize are mainly used. For this reason, people affected by celiac disease frequently suffer of nutritional deficiencies and have high intake of some nutrients. The use of a wider range of GF flours, rich in nutrients and phytochemicals, may improve the nutritional quality of GF products.

This study aimed at the characterization of twelve GF flours obtained from cereals, pseudocereals and legumes for what concerns their functional properties, starch composition, phenolic and flavonoid content, and their effect on glycemic index (GI), that was estimated in vitro. In particular, starch composition had an influence on predicted glycemic index (pGI). pGI and damaged starch were positively related, whereas flavonoid, amylose and resistant starch (RS) contents were negatively related to pGI. In general, for all the parameters considered, cereals, except for rice flour, showed similar behavior, so as legumes. On the contrary, pseudocereals presented quite different characteristics between each other, due to their different botanical origin. The knowledge of starch composition, its relationship with GI, and functional properties could contribute to the selection of flours for healthier GF bakery products.

1. Introduction

Around 1% of world population is affected by celiac disease (Fasano and Catassi 2012) and 5% is estimated to be affected by a gluten related disorder (Elli et al. 2015). To the date, the only cure available is the adherence to a strict gluten-free (GF) diet. Besides people who have pathologies related to gluten ingestion, a part of consumers decides to follow a GF diet as lifestyle choice. Hence, a growth of GF market was recorded in recent years and is still expected in years to come. The offer of GF bakery products, such as biscuits, cakes and bread substitutes, is often not very healthy, due to the low protein and high fat content. Many consumers have a rising awareness towards the consumption of healthier products. Thus, there is the need for researchers and industries to answer consumers' demand. Many studies showed that it is possible to offer healthier GF bakery (Pellegrini and Agostoni 2015; Stantiall and Serventi 2017).

GF products are mainly based on refined flour or starches (Gallagher, Gormley, and Arendt 2004). In example, it has been reported that the Brazilian GF products are mostly made with rice flour and cassava fecula and most of breads are made with refined rice flour and starches (do Nascimento et al. 2013; Santos, Aguiar, and Capriles 2018). GF biscuits present on the Italian market are mainly made of rice and maize flours and starches (Di Cairano et al. 2018)**.** These flours and starches have a neutral taste, can be employed in a variety of formulations but they lack in vitamins, minerals and fibers and they may contribute to a raise of the glycemic index (GI) because of their richness in rapidly digestible starch. Indeed, it has been seen that GF products generally tend to have a higher GI than their gluten containing counterparts (Vici et al. 2016).

The selection and the employment of more nutrient dense flours can improve the quality of GF products. Different authors studied the use of cereal, pseudocereal and legume flours in the production of GF pasta and baked products obtaining a positive effect on product quality without affecting sensory properties (Campo et al. 2016; Sharma, Saxena, and Riar 2016).

While reformulating baked products with the aim of enhancing their nutritional profile, the functional properties of the flours cannot be neglecting in order to assure the proper behavior during the production and to keep the desired texture/structure in the final product. The knowledge of functional properties of flours, interaction between GI and starch composition and their richness in phytochemicals could help the selection of raw materials for production of healthier GF products. To our knowledge, few studies report this information on such different number of flours. In particular, functional properties, pasting properties, starch fractions and phenolic and flavonoid content were studied. In this context, the aim of this study was to compare the nutritional and functional properties of twelve different GF cereal, pseudocereals and legume flours. Moreover, correlations between flour characteristics and pGI were explored to understand the potential impact of these grains of starch quality.

2. Material and methods

2.1. Flour samples

Commercial cereal, pseudocereal and legume flours were analyzed. Whole brown millet (Molino Filippini, Italy), whole oat flakes (THERMAR 63, Lameri SpA, Italy), rice (CareRice TQ, Caremoli SpA, Italy), sorghum (Molino Favero, Italy) and white teff (Molino Favero) flours were analyzed for cereal group; amaranth (Molino Favero), buckwheat (Careflour Buckwheat F, Caremoli) and quinoa (CareFlour Quinoa, Caremoli) flours were studied for pseudocereal group and chickpea (Terre di Altamura, Italy), green lentil (Terre di Altamura, Italy), red lentil (Cereal Veneta, Italy) and yellow pea (CareFlour YP yellow pea CF, Caremoli SpA) flour flours were analyzed for legume group. All analysis were made on raw flours; flours were stored in sealed plastic bags at ambient temperature and in the dark (20°C).

2.2. Functional properties

2.2.1. Water absorption capacity, oil absorption capacity and water solubility index

Water and oil absorption capacity (WAC and OAC) were determined according to Kaur et al. (2015). One gram of flour was mixed with 10 ml of distilled water or refined soybean oil and kept at 20 °C for 30 minutes. Then, the samples were centrifuged at 2000g for 10 minute and the supernatant decanted. WAC and OAC are the weight of the sample after removal of the supernatant per unit weight of original dry solids.

To determine water solubility index (WSI), the supernatant of WAC was decanted into an evaporating dish and dried at 105°C until constant weight (Chauhan, Saxena, and Singh 2015). WSI is the weight of dry solids expressed as a percentage of the original weight of sample. Measurements were taken in triplicate.

2.2.2. Bulk density

Bulk density (BD) was determined as reported in Chauhan et al. (2015). Ten grams of flour were placed in a 25 ml graduated cylinder which was gently tapped ten times from a height of 7-8 cm. The final volume of the flour was measured and bulk density was expressed as g/ml. Measurements were taken in triplicate.

2.3. Pasting properties

Pasting properties of the flours were determined using a Micro Visco-Amylo-Graph, MVAG, (Brabender GmbH., Duisburg, Germany) according to the procedure of Marengo et al. (2017). Twelve grams of flour were dispersed in 100 ml of distilled water, scaling both sample and water weight on a 14% flour moisture basis. The suspensions were subjected to the following temperature profile: heating from 30 up to 95°C, holding at 95°C for 20 minutes and cooling from 95 to 30°C with a heat/cooling rate of 3°C/min. The following parameters were considered: beginning of gelatinization (BG) (temperature at which an initial increase in viscosity occurs), maximum viscosity (MAXV) (MAXV reached during the analysis), peak temperature (PT) (temperature at the MAXV), breakdown (BKD) (difference between the MAXV and the viscosity reached at the end of the holding period) and setback (SB) (difference between the final viscosity at 30°C and the viscosity reached at the end of the holding period). BG and PT were expressed in °C, MAXV, BKD and SB were expressed in Brabender Units. Measurements were taken in duplicate.

2.4. Starch characterization

2.4.1. Resistant starch and total starch content

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

2.4.2. Damaged starch

Damaged starch (SDAM) content was evaluated according to AACC Method 76-31.01 using K-SDAM assay kit (Megazyme). Measurements were taken in duplicate.

2.4.3. Amylose/amylopectin content

Amylose content was measured by using K-AMYL assay kit (Megazyme). In brief, starch samples were dispersed by heating in dimethyl sulfoxide and then amylopectin was precipitated with Con A solution. Supernatant (amylose) was hydrolyzed to glucose with α-amylase and amyloglucosidase and measured with a glucose oxidase/peroxidase reagent mixture at 510 nm (Cary 1E UV-VIS spectrophotometer, Varian e Agilent, Milano, Italy). TS aliquot was determined on the precipitated starch solution after the incubation with amyloglucosidase and α-amylase. Amylose content was calculated as ratio between glucose freed from the Con A supernatant and TS and expressed as percentage. Measurements were taken in duplicate.

2.5. *In vitro* starch digestion rate and predicted glycemic index

In vitro starch digestion rate was evaluated to calculate the pGI. The method of Molinari et al. (2018) based on Goñi et al. (1997) was employed. One hundred milligrams of raw flour were added with 10 ml of HCl-KCl buffer and 0.2 ml of pepsin (P7000, Sigma-Aldrich) solution (1 g in 10 ml HCl-KCl buffer) and incubated at 40°C for one hour. Then, the solution was brought up to 25 ml with sodium maleate buffer (0.2 M, pH 6.9) and 5 ml (2.6U/5ml) of α-amylase (A3176, Sigma-

Aldrich) solution were added. Samples were incubated at 37°C for 180 min with constant stirring. One-milliliter aliquots were taken at 0, 30, 60, 90, 120 and 180 min. Immediately after the withdrawal the aliquot was incubated at 100°C to inactivate the enzyme. Each test was cooled in ice and kept cold if not used immediately. The aliquots were centrifuged at 2500*g* at 4°C. Five hundred µl of supernatant were brought up to 2 ml with sodium acetate buffer (0.2M, pH 4.75). Then, 60 µl of amyloglucosidase (E-AMGDF, Megazyme) were added to the sample and incubated at 60°C for 45 minutes. The glucose content was measured with glucose oxidase/peroxidase reagent (K-GLUC, Megazyme) by incubating 0.1 ml of solution with 3 ml of mixture for 20 min at 40°C. The absorbance was read at 510 nm. Glucose content was multiplied by 0.9 to calculate starch amount. The rate of starch digestion was expressed as the percentage of TS hydrolyzed at 0, 30, 60, 90, 120 and 180 min. The hydrolysis index (HI) was calculated as the ratio between the area under the hydrolysis curve (0-180 min) of the raw flours and the area of reference sample (white bread). The pGI was calculated according to the equation: $pGI = 39.71 + 0.549 \times HI$ (Goñi et al., 1997).

2.6. Phenolic and flavonoid contents

2.6.1. Methanolic extract

A methanolic extract was prepared according to Molinari et al. (2018). Twenty-five ml of methanol: water solution (80:20) were added to 1 g of flour. The mixture was shaken for 8 hours at room temperature and in the dark. Then, the extract was centrifuged at 1000*g* for 10 min and the supernatant was recovered and used for determination of total phenolic and flavonoid contents. Extractions were made in duplicate.

2.6.2. Total phenolic and flavonoid content

The Folin Ciocalteu method (Singleton and Rossi 1965) was applied to determine total phenolic content as reported in (Molinari et al. 2018). Slight modifications were applied to optimize the reaction conditions. In brief, 0.25 ml of extract were added with 4 ml of deionized water, 0.25 ml of Folin Ciocalteu Reagent and 0.5 ml of Na_2CO_3 saturated solution. The samples were incubated at 40°C for 30 min and the absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalent/g flour.

Total flavonoid content was determined using the $AlCl_3$ method, as described by Ferri, Gianotti, and Tassoni (2013). In brief, 0.4 ml of distilled water were added with 0.5 ml of extract. Then 30 µl of $NaNO_2$ (5%) were added. After 5 minutes 30 µl of $AlCl_3$ 10% were added. The reaction was stopped after 6 min incubation by adding 200 µl of NaOH 1M. The absorbance at 415 nm was measured. Results were expressed as mg quercetin equivalent/g flour. Measurements were taken in triplicate.

2.7. Statistical analysis

Statistical analysis was performed using R software (3.6.0, R Core Team) and XLSTAT Premium Version (2019.4.2, Addinsoft). Principal Component Analysis (PCA) was performed to visualize differences among samples**.** One way analysis of variance (ANOVA) followed by Tukey HSD post hoc test ($p<0.05$) was employed. When ANOVA assumptions were not fulfilled, Kruskal-Wallis non parametric test was employed followed by Dunn test ($p<0.05$). Correlation between variables was determined using Pearson’s coefficient ($p<0.05$).

3. Results and discussion

3.1. Functional properties

Table 1 summarizes water absorption capacity, oil absorption capacity, water solubility index and bulk density results. Functional properties are strongly affected by the composition of the flour and reflect the interactions between flour components. WAC represents the ability of a product to bind water. Variations in WAC may depend on the structure of proteins and on the presence of hydrophilic carbohydrates (Kaur et al. 2015). WAC ranged between 1.05 and 1.77 g/g. Pea flour showed the highest WAC, whereas chickpea and green lentil flour the lowest. WAC may affect the texture of bakery products; the use of flours with a higher WAC may help to retain a soft texture (Du et al. 2014). The type of proteins and the presence of non-polar side chains, may also have an effect on OAC. OAC is the ability of a product to bind with oil. In this study, no significant differences were found between OAC of different grains**.** Fats play and important role in sensory properties of foods, OAC affects the mouthfeel and capacity to retain the flavour (Kaur et al. 2015).

WSI is an indicator of starch degradation, in fact WSI and SDAM were slightly negatively related ($r = -0.410$, $p<0.05$). WSI data were little variable, but a trend can be highlighted: legume and pseudocereal flours showed a higher WSI than cereal flours. BD data were variable and flours belonging to the same group showed different results; that may be related to the heterogeneous granulometry of the flours (data not shown). The presence of lipids, which may act as adhesives in agglomeration of flour particles (Joshi, Liu, and Sathe 2015) as well as milling conditions can affect the granulometry. BD gives an indication of flour heaviness (Adebiyi et al. 2017). Kaur et al. (2015) recorded an enhancement in sensory scores of GF biscuits due to the improvement of WAC and OAC after the addition of gums; functional properties can have a direct effect on final product characteristics.

3.2. Pasting properties

Pasting properties represent the variation of the viscosity of the starch, in excess of water, during heating and cooling treatments under controlled conditions. The MVAG profiles of the grains (data not shown) are similar to what has been reported so far in the literature. Specifically, pseudocereals and legumes did no show the peak of viscosity which is typical of cereals. On the contrary, in pseudocereals and pulses viscosity reached a plateau or even continued to increase during the holding phase at 95 °C (data not shown), suggesting that starch did not reach the maximum gelatinization degree. This behavior is due to a different molecular arrangement among grains, with pseudocereals and legumes having a more compact starch structure that limits its swelling and gelatinization.

Table 2 presents the main indices extracted from the MVAG profiles. Pasting properties depend on starch characteristics and interactions between starch and other components. BG ranged between 50 and 80 °C, with amaranth and oat flours showing the lowest values and sorghum the highest. BG showed a slight positive correlation with amylose content ($r = 0.489$, $p<0.05$) and RS content ($r = 0.505$, $p<0.05$). This data is consistent with Varavinit et al. (2003): amylopectin plays a major role in starch granule crystallinity, whereas the presence of amylose lowers the melting temperature of crystalline regions and the energy for starting gelatinization (Flipse et al. 1996). Starch with higher amylose content has more amorphous regions, lowering gelatinization temperature.

Legume flours reached lower values of viscosity compared to all other samples. Pseudocereal and cereal flours showed similar behavior. In particular, buckwheat flour and rice flours showed the highest values in viscosity. MAXV was negatively related ($r = -0.760$, $p<0.05$) with RS content. An higher level of fiber acts as filler, diluting starch content and consequently reducing its viscosity (Korus et al. 2017). Proteins due to their interaction with starch restrict starch swelling and can reduce viscosity. Also fat can reduce the starch swelling as it absorbs water and inhibits interactions between starch molecules (Duta and Culetu 2015). All the flours reached their MAXV at temperature higher than 90°C; only amaranth flour reached its maximum at 75°C. It seems that amaranth starch swallows and gelatinizes at lower temperature than all other flours considered. However, no significant differences were recorded between PT of amaranth, rice and oat flours.

Legume flours had low breakdown values. BKD is the difference between the MAXV and the viscosity at the end of the holding period. Hence, that indicates a good paste stability and a strong shearing resistance of legume flours. This is of great interest to predict starch behavior during processing. Rice and buckwheat flour had the highest BKD values whereas all other flours showed intermediate values between them and legume flours. BKD had a significant correlation with MAXV ($r = 0.809$, $p<0.05$), also reported by Kuo et al. (2001). A positive correlation was also recorded between MAXV and SB values ($r = 0.757$, $p<0.05$). SB represents the tendency to retrograde of the starch: the higher the SB value, the higher the tendency to retrograde. Legume flours showed the lowest SB values whereas buckwheat flour presented the highest values**.** This data is consistent with Kuo et al. (2001), who reported a high positive correlation between MAXV and SB value. Predicting starch retrogradation tendency is important in bakery, especially in those rich in starch, such as GF products.

3.3. Starch characterization and predicted glycemic index

3.3.1. Total starch, resistant starch and damaged starch content

Total, non resistant, resistant, damaged starch and amylose content of GF cereal, pseudocereal and legume flours are reported in table 3. Starch content and its fractions may significantly vary according to the botanical source. TS content ranged from 26.0 to 84.5 g/100g. Cereal and pseudocereal flours had the highest starch content whereas legume flours the lowest.

RS content ranged between 0.1 and 11.8 g/100g of TS with legume flours showing the highest content between the flours considered. Indeed, it is well known that legume flours are a good source of RS. Cereal and pseudocereal flours showed similar values, but it emerged that millet flour and sorghum flour had higher values than all flours of the same groups. Rice, oat and buckwheat flour showed the lowest values.

The SDAM represents a physical modification to the native starch due to milling or other processing conditions. Damaged starch is easily hydrolyzed by α-amylase. Indeed, a slight positive correlation ($r = 0.362$, $p<0.05$) was found between SDAM and pGI. Rice flour and amaranth flour had the highest SDAM content, whereas quinoa flour the lowest. This may depend on the different resistance that the endosperm shows during milling.

3.3.2. Amylose content

Amylose content ranged between 6.8 and 32.0 g/100g of TS with millet flour showing the highest content and quinoa flour the lowest. However, data are quite homogeneous with few significant differences between flours belonging to cereal, pseudocereal and legume flours. The ratio between amylose and amylopectin, the molecules constituting starch, can be very variable, depending on the botanical source and growing conditions. It is of technological importance, in particular bread staling is related to it, but it also plays a nutritional role, affecting starch characteristics (Hager et al. 2012). Amylopectin is more subject to hydrolysis, hence, their ratio can have an impact on starch digestion and consequently on GI. In this study, a slight negative correlation was found between amylose content and pGI ($r = -0.368$, $p<0.05$).

3.3.3. Starch digestion rate and predicted glycemic index

GI is strictly related to the rate and extent of starch digestion; indeed, reducing sugars are released during digestion of the starch with an increase in sugar blood level. Equations have been developed to estimate GI starting from in vitro hydrolysis of starch (Goñi, Garcia-Alonso, and Saura-Calixto 1997; Granfeldt et al. 1992). Cereal, pseudocereal and legume flours presented pGI values from low to high according to Atkinson et al. (2008), with values ranging from 46.4 to 76.1 (Table 3). Rice flour had a different pGI from all other cereal flours and quinoa flour presented the highest value. Quinoa is generally considered a low glycemic index grain (Gordillo-Bastidas and Díaz-Rizzolo 2016); the intact grain may have a different behavior compared to the flour. Legume flours, presented the lowest pGI values. The pGI reflects the rate of starch digestion. The higher the digestion rate, the higher HI and consequently pGI. It must be mentioned that cooking affects the rate of starch digestion since starch granules gelatinize and are more available for enzymatic hydrolysis, with an increase in glycemic response. Moreover, the starch digestion rate also depends on the degree and type of food processing. In this study, the raw flours were characterized with the aim of evaluating the rate of digestion of the starch in its raw form, aware of the fact that uncooked starch is quite resistant to the digestion and that the dynamics of starch digestion may change when the flours are employed in combination with other ingredients to realize a food product. Further investigation would be useful in order to compare the digestion rate of raw and cooked flour and to see how the use of different ingredients in different food products affects the rate of starch digestion. On the basis of the hydrolysis rate, starch is classified in rapidly digestible, slowly digestible and RS (Englyst et al. 1996). The higher the non-RS content the higher the GI. However, foods with a higher slowly digestible starch content contribute to a moderate release of glucose in blood, because slowly digestible starch is less subject to hydrolysis. A negative correlation ($r = -0.626$, $p<0.05$) was found between pGI and RS content. RS is not digested and hydrolyzed in reducing sugars; for this reason, the amount of RS does not contribute to an increase of the glycemic response.

pGI doesn’t strictly depend only on starch composition. The presence of specific compound may have an effect on hydrolytic enzymes. In example, the presence of certain phenolic compounds may inhibit the action of α-amylases, with a resulting lower GI.

3.4. Total phenolic and flavonoids content

Polyphenols, secondary plant metabolites, can affect nutritional and sensory properties. Rice flour, the most employed flour in GF bakery so far, resulted to be the poorest in phenolic compounds. Instead, buckwheat showed the highest total phenolic content followed by quinoa, millet, teff and green lentil (Figure 1). Buckwheat presented also the highest flavonoid content. In different cases flavonoid content resulted to be higher than phenolic content; that could depend on the reference standard employed. Moraes et al. (2015) found significant negative correlations between pGI and phenolic and flavonoid content. In this study, only a slight negative correlation was found between flavonoid content and pGI (r = -0.442, $p<0.05$).

3.5. Principal Component Analysis

PCA was applied to visualize the variation in the properties among flours and the correlations between parameters (Figure 2). The first and the second principal components described 35.09% and 20.88% of variance, respectively. All cereal flours, except rice, presented similarities; also legumes are located very close on the graph. Instead, pseudocereal flours are quite dissimilar between each other, with buckwheat flour located on the opposite quadrant compared to the other pseudocereal flours. That can easily be explained by the fact that the flours grouped as pseudocereal flours do not belong to the same family; “pseudocereal” is not a real botanical classification.

The loading plot (Figure 1 b) provided information about correlations between the measured parameters. The parameters whose curves lie close are positively correlated; whereas curves located in the opposite directions indicate parameters with negatively related

Buckwheat differenced from amaranth and quinoa for OAC, phenolic compounds and SB value. On the other hand, legumes differenced from most of the other grains for their water solubility index, RS and BG.

4. Conclusions

The use of nutrient dense flours may contribute to the improvement of gluten free bakery product quality. However, technological and sensory challenges to which GF production subject industries and researchers must not be forgotten.

GI of bakery products, and GF in particular, is a trending research theme. Legume flours showed the higher RS content and also pGI values. Rice flours, widely employed in GF bakery had the highest pGI. However, further investigations would be useful in order to evaluate the differences in pGI of both raw and cooked or processed flours as well as the effect of each flour on final food products.

Concerning other parameters, cereal flours, except for rice, showed similar results; so as legume flours. Pseudocereals, which are not a botanical family were more dissimilar. Surely, a study with a more substantial number of samples could better confirm the results obtained.

Acknowledgements

Authors would like to thank Daniele Rivolta and Caremoli spa (Monza, Italy), Di Leo Pietro spa (Matera, Italy) and Giuseppe Visci and Andriani spa (Andria, Italy) and for kindly providing the flours.

Fundings

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

References

Adebiyi, Janet Adeyinka, Adewale Olusegun Obadina, Oluwafemi Ayodeji Adebo, and Eugenie Kayitesi. 2017. "Comparison of Nutritional Quality and Sensory Acceptability of Biscuits Obtained from Native, Fermented, and Malted Pearl Millet (*Pennisetum glaucum*) Flour." Food Chemistry 232: 210–17.

Atkinson, Fiona, Kaye Foster-Powell, and Jennie C Brand-Miller. 2008. "Glycemic Load Values : 2008." Diabetes care 31(12): 2281–83.

Di Cairano, Maria et al. 2018. “Focus on Gluten Free Biscuits: Ingredients and Issues.” Trends in Food Science & Technology 51: 203–12. http://www.sciencedirect.com/science/article/pii/S0924224418301924.

Campo, Eva et al. 2016. “Impact of Sourdough on Sensory Properties and Consumers’ Preference of Gluten-Free Breads Enriched with Teff Flour.” Journal of Cereal Science 67: 75–82. http://dx.doi.org/10.1016/j.jcs.2015.09.010.

Chauhan, Arti, D. C. Saxena, and Sukhcharn Singh. 2015. “Total Dietary Fibre and Antioxidant Activity of Gluten Free Cookies Made from Raw and Germinated Amaranth (*Amaranthus* Spp.) Flour.” LWT - Food Science and Technology 63(2): 939–45. http://dx.doi.org/10.1016/j.lwt.2015.03.115.

Du, Shuang kui, Hongxin Jiang, Xiuzhu Yu, and Jay lin Jane. 2014. “Physicochemical and Functional Properties of Whole Legume Flour.” LWT - Food Science and Technology 55(1): 308–13.

Duta, Denisa Eglantina, and Alina Culetu. 2015. “Evaluation of Rheological, Physicochemical, Thermal, Mechanical and Sensory Properties of Oat-Based Gluten Free Cookies.” Journal of Food Engineering 162: 1–8. http://dx.doi.org/10.1016/j.jfoodeng.2015.04.002.

Elli, Luca et al. 2015. “Diagnosis of Gluten Related Disorders: Celiac Disease, Wheat Allergy and Non-Celiac Gluten Sensitivity.” World Journal of Gastroenterology 21(23): 7110–19.

Englyst, H N, S M Kingman, G J Hudson, and J H Cummings. 1996. “Measurement of Resistant Starch in Vitro and in Vivo.” The British journal of nutrition 75(5): 749–55.

Fasano, Alessio, and Carlo Catassi. 2012. “Celiac Disease.” New England Journal of Medicine 367(25): 2419–26. http://www.nejm.org/doi/abs/10.1056/NEJMcp1113994.

Ferri, Maura, Andrea Gianotti, and Annalisa Tassoni. 2013. “Optimisation of Assay Conditions for the Determination of Antioxidant Capacity and Polyphenols in Cereal Food Components.” Journal of Food Composition and Analysis 30(2): 94–101. http://dx.doi.org/10.1016/j.jfca.2013.02.004.

Flipse, E., C. J.A.M. Keetels, E. Jacobsen, and R. G.F. Visser. 1996. “The Dosage Effect of the Wildtype GBSS Allele Is Linear for GBSS Activity but Not for Amylose Content: Absence of Amylose Has a Distinct Influence on the Physico-Chemical Properties of Starch.” Theoretical and Applied Genetics 92(1): 121–27.

Gallagher, E., T. R. Gormley, and E. K. Arendt. 2004. “Recent Advances in the Formulation of Gluten-Free Cereal-Based Products.” Trends in Food Science and Technology 15(3–4): 143–52.

Goñi, Isabel, Alejandra Garcia-Alonso, and Fulgencio Saura-Calixto. 1997. “A Starch Hydrolysis Procedure to Estimate Glycemic Index.” Nutrition Research 17(3): 427–37.

Gordillo-Bastidas, E, and DA Díaz-Rizzolo. 2016. “Quinoa (*Chenopodium quinoa* Willd), from Nutritional Value to Potential Health Benefits: An Integrative Review.” Journal of Nutrition & Food Sciences 06(03): 1–10.

Granfeldt, Yvonne, I Björck, A Drews, and Juscelino Tovar. 1992. “An in Vitro Method Based on Chewing to Predict Metabolic Responses to Starch in Cereal and Legumes Products.” European Journal of Clinical Nutrition 46(May 2014): 649–60.

Hager, Anna-Sophie et al. 2012. “Nutritional Properties and Ultra-Structure of Commercial Gluten Free Flours from Different Botanical Sources Compared to Wheat Flours.” Journal of Cereal Science 56: 239–47.

Joshi, Aditya U, Changqi Liu, and Shridhar K Sathe. 2015. “Functional Properties of Select Seed Flours.” LWT - Food Science and Technology 60: 325–31.

Kaur, Maninder, Kawaljit Singh Sandhu, Amit Pal Arora, and Aruna Sharma. 2015. “Gluten Free Biscuits Prepared from Buckwheat Flour by Incorporation of Various Gums: Physicochemical and Sensory Properties.” LWT - Food Science and Technology 62(1): 628–32. http://dx.doi.org/10.1016/j.lwt.2014.02.039.

Korus, Anna et al. 2017. “Evaluation of the Quality, Nutritional Value and Antioxidant Activity of Gluten-Free Biscuits Made from Corn-Acorn Flour or Corn-Hemp Flour Composites.” European Food Research and Technology 243(8): 1429–38.

Kuo, Bo Jein, Mei Chu Hong, and Fu Sheng Thseng. 2001. “The Relationship between the Amylographic Characteristics and Eating Quality of Japonica Rice in Taiwan.” Plant Production Science 4(2): 112–17.

Marengo, Mauro et al. 2017. “Macromolecular Traits in the African Rice *Oryza glaberrima* and in Glaberrima/Sativa Crosses, and Their Relevance to Processing.” Journal of Food Science 82(10): 2298–2305.

Molinari, Romina et al. 2018. “Tartary Buckwheat Malt as Ingredient of Gluten-Free Cookies.” Journal of Cereal Science 80: 37–43.

Moraes, Érica Aguiar et al. 2015. “Sorghum Flour Fractions: Correlations among Polysaccharides, Phenolic Compounds, Antioxidant Activity and Glycemic Index.” Food Chemistry 180: 116–23.

do Nascimento, Amanda Bagolin, Giovanna Medeiros Rataichesck Fiates, Adilson dos Anjos, and Evanilda Teixeira. 2013. “Analysis of Ingredient Lists of Commercially Available Gluten-Free and Gluten-Containing Food Products Using the Text Mining Technique.” International Journal of Food Sciences and Nutrition 64(2): 217–22. http://www.tandfonline.com/doi/full/10.3109/09637486.2012.718744.

Pellegrini, Nicoletta, and Carlo Agostoni. 2015. “Nutritional Aspects of Gluten-Free Products.” Journal of the Science of Food and Agriculture 95(12): 2380–85. http://doi.wiley.com/10.1002/jsfa.7101 (November 7, 2017).

Santos, Fernanda G., Etiene V. Aguiar, and Vanessa D. Capriles. 2018. “Analysis of Ingredient and Nutritional Labeling of Commercially Available Gluten-Free Bread in Brazil.” International Journal of Food Sciences and Nutrition 70(5): 562–69. https://doi.org/10.1080/09637486.2018.1551336.

Sharma, Seema, Dharmesh C. Saxena, and Charanjit S. Riar. 2016. “Nutritional, Sensory and in-Vitro Antioxidant Characteristics of Gluten Free Cookies Prepared from Flour Blends of Minor Millets.” Journal of Cereal Science 72: 153–61. http://dx.doi.org/10.1016/j.jcs.2016.10.012.

Singleton, V L, and Joseph A Rossi. 1965. “Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents.” American Journal of Enology and Viticulture 16(3): 144 LP – 158.

Stantiall, Sophie E., and Luca Serventi. 2017. “Nutritional and Sensory Challenges of Gluten-Free Bakery Products: A Review.” International Journal of Food Sciences and Nutrition 69(4): 1–10. https://doi.org/10.1080/09637486.2017.1378626.

Varavinit, Saiyavit et al. 2003. “Effect of Amylose Content on Gelatinization, Retrogradation and Pasting Properties of Flours from Different Cultivars of Thai Rice.” Starch/Staerke 55(9): 410–15.

Vici, Giorgia, Luca Belli, Massimiliano Biondi, and Valeria Polzonetti. 2016. “Gluten Free Diet and Nutrient Deficiencies: A Review.” Clinical Nutrition 35(6): 1236–41. http://dx.doi.org/10.1016/j.clnu.2016.05.002.

Tables

Table 1 Functional properties of gluten free flours obtained from cereal, pseudo cereal and legume flours

	Flour	**Water absorption capacity (g/g)***	**Water solubility index (g/100g)***	**Oil absorption capacity (g/g)***	**Bulk density (g/ml)****
Cereals	*Millet*	1.18±0.02^{ab}	2.92±0.13^{ab}	1.38±0.03^{a}	0.67±0.02^{d}
	Oat	1.59±0.07^{ab}	3.37±0.34^{abc}	1.41±0.02^{a}	0.50±0.01^{f}
	Rice	1.51±0.04^{ab}	1.21±0.00^{a}	1.65±0.11^{a}	0.73±0.02^{bc}
	Sorghum	1.35±0.03^{ab}	5.32±0.14^{abc}	1.46±0.11^{a}	0.72±0.01^{bc}
	Teff	1.36±0.04^{ab}	5.09±0.20^{abc}	1.64±0.09^{a}	0.76±0.01^{b}
Legumes	*Chickpea*	1.05±0.05^{a}	26.02±1.63^{c}	1.71±0.04^{a}	0.55±0.01^{e}
	Lentil	1.09±0.02^{a}	24.55±1.34^{bc}	1.44±0.11^{a}	0.72±0.02^{bc}
	Pea	1.77±0.06^{b}	12.69±0.19^{abc}	1.70±0.02^{a}	0.67±0.00^{d}
	Red lentil	1.38±0.20^{ab}	20.55±0.71^{abc}	1.56±0.08^{a}	0.84±0.01^{a}
Pseudo cereals	*Amaranth*	1.22±0.06^{ab}	10.22±0.14^{abc}	1.66±0.30^{a}	0.57±0.01^{e}
	Buckwheat	1.45±0.04^{ab}	6.22±0.14^{abc}	1.06±0.02^{a}	0.69±0.00^{cd}
	Quinoa	1.27±0.08^{ab}	11.02±0.38^{abc}	1.75±0.10^{a}	0.54±0.02^{ef}

Data are expressed as mean±sd; Values followed by the same letter in the same column are not significantly different ($p<0.05$) according to *Kruskal Wallis test followed by Dunn test and **ANOVA followed by Tuckey HSD test.

Table 2 Pasting properties of gluten free cereal, pseudocereal and legume flours

	Flour	**Beginning of gelatinization (°C)***	**Maximum viscosity (BU)***	**Temperature at peak (°C)***	**Breakdown (BU)****	**Setback (BU)***
Cereals	*Millet*	68±7^{ab}	283±60de	92±3^{cde}	68±17de	127 ±45de
	Oat	53±1^{a}	486±4^{bc}	91±0.2^{efg}	231±14^{b}	490±11^{f}
	Rice	65±1^{ab}	559±3^{ab}	90±0.0^{fg}	334±4^{a}	387±3^{f}
	Sorghum	80±0.1^{b}	283±3^{ef}	95±0.1^{a}	81±1^{c}	520±2^{e}
	Teff	72.±0.4^{ab}	319±1^{d}	93±0.1^{def}	115±1^{d}	436±4^{f}
Legumes	*Chickpea*	71±0.3^{ab}	210±6^{g}	95±0.0^{cde}	30±4^{fg}	98±1^{b}
	Lentil	73±0.4^{ab}	141±3^{i}	95±0.0^{bcd}	7±3^{g}	66±3^{a}
	Pea	65±2.3^{ab}	201±9^{gh}	95±0.0^{bcd}	7±3^{g}	117±11^{a}
	Red lentil	75±0.2^{b}	171±13^{hi}	95±0.3^{ab}	12±0.5^{g}	122±29^{b}
Pseudo cereals	*Amaranth*	5±14^{a}	270±6^{f}	75±0.2^{g}	55±1^{ef}	123±2^{c}
	Buckwheat	55±12 ^{ab}	623±49^{a}	95±0.6^{abc}	126±18^{c}	811±17^{g}
	Quinoa	60±0.3^{ab}	351±1^{c}	95±0.1^{ab}	64 ±1de	20±5^{cd}

Data are expressed as mean±sd; Values followed by the same letter in the same column are not significantly different ($p<0.05$) according to *Kruskal Wallis test followed by Dunn test and **ANOVA followed by Tuckey test.

Table 3 Starch fractions, hydrolysis index and predicted glycemic index of cereal, pseudocereal and legume flours

	Flour	Total starch (g/100g dw)**	Non resistant starch (g/100 g dw)*	Resistant starch (g/100g total starch dw)*	Damaged starch (g/100 g)**	Amylose (g/100 g Starch)*	Hydrolysis index**	Predicted glycemic index**
Cereals	*Millet*	37.7±0.48^{c}	36.4±0.27^{e}	3.0±1.4^{de}	3.7±0.06^{c}	32.0±3.1^{b}	29.14±0.95^{c}	55.42±0.73^{c}
	Oat	39.5±0.83^{c}	39.3±0.85^{de}	0.5±0.06^{g}	1.5±0.02^{g}	15.2±3.8^{ab}	28.04±0.67^{c}	54.83±0.51^{c}
	Rice	84.5±1.7^{a}	71.1±1.7^{a}	0.2±0.02^{g}	6.8±0.12^{a}	17.6±5.3^{ab}	56.53±5.79^{b}	70.20±4.4^{b}
	Sorghum	55.6±0.87^{b}	54.1±1.1^{b}	2.8±0.48^{ef}	3.5±0.01^{c}	23.8±2.3^{ab}	22.05±2.0^{c}	51.60±1.5^{c}
	Teff	50.7±1.5^{b}	50.1±1.5^{bc}	1.2±0.10^{gf}	3.0±0.02^{d}	25.5±1.4^{ab}	28.57±0.90^{c}	55.12±0.69^{c}
Legumes	*Chickpea*	26.0±1.0^{d}	24.4±1.0^{f}	6.3±0.29^{b}	2.1±0.10^{e}	17.2±2.2^{ab}	9.76±0.62^{f}	44.98±0.47^{f}
	Lentil	49.3±3.3^{b}	46.67±3.2^{bcd}	5.8±0.09^{bc}	2.1±0.04^{e}	12.7±3.3^{ab}	16.76±0.80^{def}	48.74±0.60^{def}
	Pea	51.5±0.3^{b}	49.1±0.40^{bc}	4.6±0.30^{cd}	1.0±0.02^{h}	24.7±0.54^{ab}	24.62±4.1^{def}	47.69±4.3^{def}
	Red lentil	49.1±0.87^{b}	43.3±1.1^{cde}	11.8±0.62^{a}	1.2±0.03^{h}	24.9±4.7^{ab}	12.46±0.15^{ef}	46.43±0.11^{ef}
Pseudocereals	*Amaranth*	37.9±2.3^{c}	37.5±2.7^{e}	1.1±0.94^{g}	6.1±0.13^{b}	13.2±0.4^{ab}	48.97±4.3^{b}	66.12±3.3^{b}
	Buckwheat	53.3±6.7^{b}	53.1±6.7^{b}	0.5±0.12^{g}	1.8±0.05^{f}	9.0±1.9^{a}	20.47±0.37^{cde}	50.74±0.28^{cde}
	Quinoa	56.3±6.4^{b}	50.6±6.5^{bc}	0.37±0.05^{g}	0.18±0.00^{i}	6.85±2. 5^{a}	69.24±3.6^{a}	77.06 ±2.8^{a}

Data are expressed as mean±sd; Values followed by the same letter in the same column are not significantly different ($p<0.05$) according to *Kruskal Wallis test followed by Dunn test and **ANOVA followed by Tuckey test

Figure Captions

Figure 1

Total phenolic and flavonoid content of gluten free cereal, pseudocereal and legume flours.

Figure 2

Principal component analysis of gluten free cereal, pseudocereal and legume flours. a) score plot describing the variation among flours (cereals (●), pseudocereals(●) and legumes (●)). b) loading plot describing the relationship between parameters (BD, bulk density; BG, beginning of gelatinization; BKD, breakdown; HI, hydrolysis index; MAXV, maximum viscosity; NRS, non resistant starch; OAC, oil absorption capacity; P, temperature at the peak; pGI, predicted glycemic index; RS, resistant starch; SB, setback; SDAM, damaged starch; TFC, total flavonoid content; TPC, total phenolic content; TS, total starch; WAC, water absorption capacity; WSI, water solubility index)

Figures

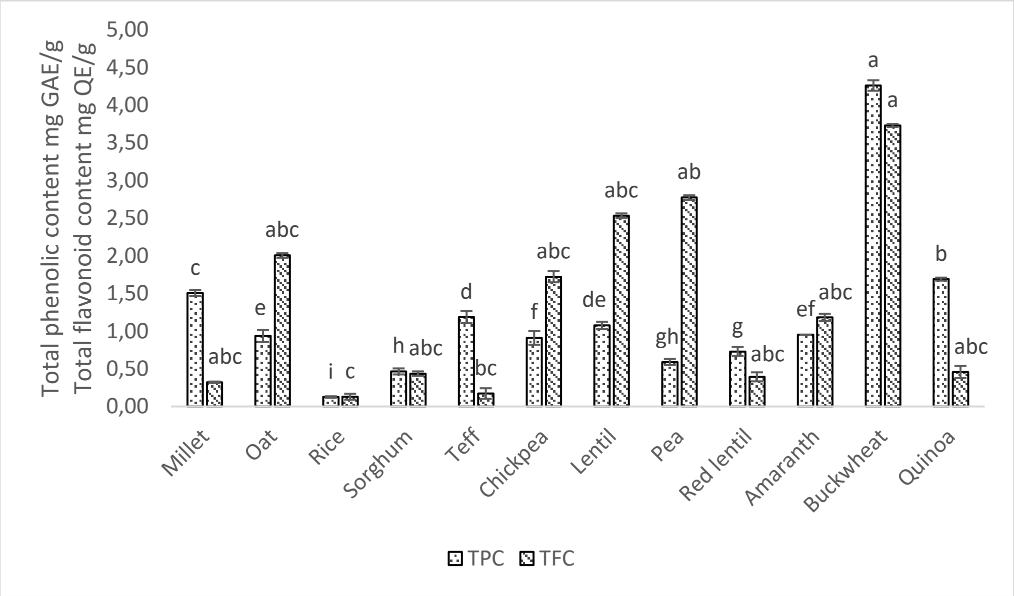

Figure 1 Total phenolic content (TPC) and total flavonoid content (TFC) of gluten free cereal, pseudocereal and legume flours. Different letters indicate significant differences (p<0.05) according to ANOVA followed by Tuckey HSD test (TPC) and Kruskal Wallis test followed by Dunn test.

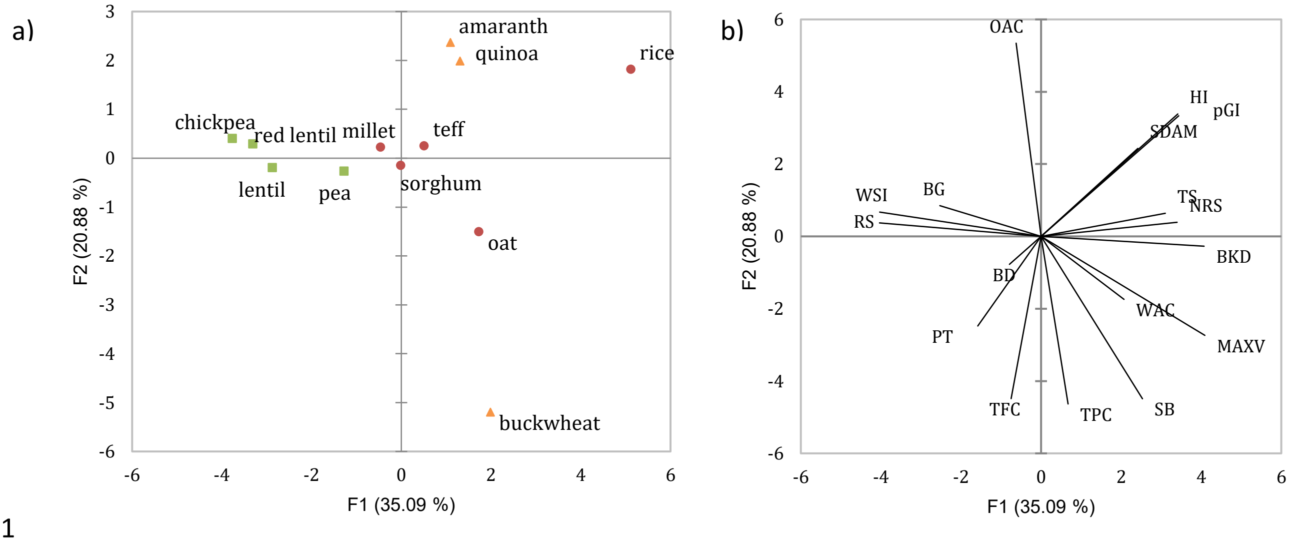

Figure 2 Principal component analysis of gluten free cereal, pseudocereal and legume flours. a) score plot describing the variation among flours (cereals (●), pseudocereals(▲), legumes (■)). b) loading plot describing the relationship between parameters (BD, bulk density; BG, beginning of gelatinization; BKD, breakdown; HI, hydrolysis index; MAXV, maximum viscosity; NRS, non resistant starch; OAC, oil absorption capacity; PT, temperature at the peak; pGI, predicted glycemic index; RS, resistant starch; SB, setback; SDAM,

damaged starch; TFC, total flavonoid content; TPC, total phenolic content; TS, total starch; WAC, water absorption capacity; WSI, water solubility index) ***<u>Color should be used online only</u>***