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Germination of proso millet (*Panicum miliaceum L.*) grains trigger biochemical changes that augment bioavailability of flower and its utility for gluten-free dietary foods

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Abstract. During past decade, there has been an active search for new sources and means, such as biologic modification of raw plant material, to produce bioavailable foods with pre-defined properties. In this study, we tested whether germination of proso millet (*Panicum miliaceum L.*) grains could be used to increase bioavailability of the flower for gluten-free diets (GFD). Our analysis demonstrated that grains of four selected cultivars had similar germination rates in different media. However, the pikes of amylolytic and proteinase activities were detected at the 2nd and 3rd days of germination, whereas the pike of lipase activity was associated with the 4th day. The highest and the lowest enzymatic activities were detected in grains germinated in whey and in NaCl, respectively. During germination, cumulative phenolic content increased up to 3.5 times reaching the highest levels by day 5. Based on these data, we produce batches of flour from grains germinated for 3 days and evaluated its utility in producing non-rising cake and shortbread pastry dough. Sensory evaluation of the baked products confirmed that flour from germinated grains could be used for substitution of the wheat flower in the dough. Collectively, our novel findings demonstrated that biochemically defined germination conditions could be used to produce proso millet flour with greater digestibility and nutritive value for the development of new GFD recipes.

Key words: proso millet, germination, enzymatic activity.

INTRODUCTION

During past decade, an incidence of gluten sensitivity diseases and behavioral eating disorders is on the rise worldwide (Fisher et al., 2014; Volta et al., 2017). As diet and nutrition are the primary treatments for these disorders, there is an active search for new sources and means to produce bioavailable foods with pre-defined properties for gluten free diet (GFD). Of particular interest is the development of new technologies utilizing biologically modified millets. Out of multiple species, proso millet (*Panicum miliaceum L.*) is one of the most common cultivated millets worldwide. The growing interest to proso millet is mostly driven by its high nutritive value, high content of calcium, iron, potassium, magnesium, phosphorous, zinc, dietary fiber, protein (Kalinova & Moudry, 2006), B-complex vitamins and sulfur-containing essential amino acids (methioninee and cysteine) (Saleh et al., 2013). Interest to this millet is also dictated by several economically important features: it is relatively drought-tolerant and produces grain with as little as 350 mm of annual rainfall (Oelke et al., 1990). It can be also cultivated in colder climates (up to 54ºN latitude) and in low pH soils (Gulati et al., 2017). Its selective cultivars can produce robust harvests and provide higher yield of flour (77.5−83.7% dry weight) as compared to wheat grains (70−75% dry weight). Currently, most of proso millet is produced in Asia (51.3%) and Africa (44.6%). Europe and Americas produce 3% and 1% of this millet, respectively. In 2017, U.S. farms produced 14.6 million bushels of proso millet for a total crop value of 44.5 million in 2017. Although in US and Europe it is mostly used as a bird and animal feed, proso millet was recently introduced as a whole grain alternative in a healthy gluten-free diet because it contains about 12% of protein, 8% of crude fiber, and 76% of total digestible nutrients (USDA−NASS, 2018). Prior studies demonstrated that germination of proso millet grains increases free amino acids and total sugars and decreased the dry weight and starch content (Parameswaran & Sadasivam, 1994). Considering that germination is associated with enzymatic hydrolysis of various biopolymers and enhanced digestibility (Andriotis et al., 2016; Diaz-Mendoza et al., 2019), in the current study, we conducted a longitudinal biochemical characterization of the germinating proso millet grains. We defined optimal conditions for germination and demonstrated that flour from biologically modified grains can be successfully used for substitution of the wheat flour in non-rising dough-based products.

MATERIALS AND METHODS

Materials

Four cultivars (Alba, Sputnik, Kazak, and Regent) of proso millet (*Panicum miliaceum L.*) were used in the study. For biochemical characterization, grains were germinated in water, 1% NaCl, 1% sucrose and whey.

Assessment of enzymatic activities

To provide basic biochemical characterization, amylolytic, proteinase and lipase activities were measured. Amylolytic activity was assessed by spectrophotometry-based quantitation of the unconverted starch using acidic iodine solution according to standard protocol (Barrera et al., 2016). Proteolytic activity was analyzed by measuring low molecular weight peptides and amino acids after hemoglobin hydrolysis in highly acidic (pH 3.0), low acidic (pH 5.3), neutral (pH 7.0) and highly alkaline (pH 9.0) conditions. After completion of reaction, proteolysis was terminated and non-hydrolyzed hemoglobin was precipitated with trichloroacetic acid. Free amino acids and peptides were then measured by spectrophotometer as described previously (Galleschi et al., 2002). Lipase activity was defined by the speed of the enzymatic hydrolysis of the triacylglycerides of vegetable oil, which was assessed by lipase-mediated release of fatty acids and quantified by titration as described previously (Rose & Pike, 2006). Assessment of cumulative phenols was done by spectrophotometry using Folin-Denis reagent according to standard protocol (Padda & Picha, 2007).

Sensory evaluation

Organoleptic assessment was conducted according to the State Standard #31986−2012 'Method of organoleptic assessment of the quality of products for public nutrition′ Standardinform 2014. Briefly, evaluation was done using standard duo-trio test. The testers were given three samples. One testing sample was marked as a reference sample; the other two samples were given coded. The testers determined which sample corresponds to the reference sample and which one is different from it. Baked products sensory profile was described by appearance, color, smell, texture, and taste, which were graded by each taster (scores from 1 to 5 including decimals, with 5 being the highest). Then, average and cumulative scores were calculated. Deviation from the reference sample was evaluated based on t-test.

Statistical analysis

Comparison of changes in enzymatic activities and in phenolic content was done using two-tail t-test. Changes were considered significant when $p < 0.05$. For the sensory evaluation, the average score for each evaluative point was calculated

RESULTS AND DISCUSSION

Germination increases enzymatic activity in proso millet grains

During germination, activation of various enzymes in grains leads to hydrolysis of different biopolymers making them more assessable for digestive system. These enzymes also influence on the quality of the baked products. Thus, amylases are necessary for even crumb structure and a high loaf volume; lipases modify natural lipids to strengthen the dough, and proteases weaken gluten to give plastic properties required in dough for biscuits. To define conditions supporting activation of these enzymatic activities in germinating proso millet, we tested several germinating media including water, 1% NaCl, 1% sucrose and whey. Four different cultivars were germinated for 5 days in these media. With 24 h intervals, samples (100 g) of germinating grains were collected and processed for the assessment of amylolytic, proteinase and lipase activities.

Changes in amylase activity in grains germinated in different media followed similar trend: enzymatic activity was significantly increased by day 3 (Table 1) following a drop to a starting levels within 2 additional days. Germination of grains in sucrose and whey provided the highest activation of the amylolytic activity.

Proteinase and lipase activities in germinating grains were also rising during germination and reached their maximum by day 3. On the contrary to amylase activity, both proteinase and lipase activities sustained at higher levels, albeit some decrease was observed after the 3^{rd} day (Tables 2, 3). Comparison of averages of all cultivars demonstrated that germination in whey provided the highest activation of all enigmatic activities. All observed changes were statistically significant. Besides assessing enzymatic activities, changes in cumulative phenolic content were also evaluated in grains germinated in water. As shown in Table 4, the overall concentration of phenols increased during germination reaching its maximum by day 5. At day 4, the highest level of cumulative phenols was detected in cultivar Sputnik, whereas at day 5 – in Regent and Alba (Table 4). Overall, germination increased phenolic content levels in all cultivars 2–3.5 times within 5 days. These finding are in agreement with prior data showing higher phenolic content in germinating canary seeds and rye grains (Liukkonen et al., 2003).

Table 1. Changes in amylase activity in germinating grains (units ± SD)

Germinating media	Days	Cultivars				p-value*
		Sputnik	Regent	Kazak	Alba	
Water	1	1.81 ± 0.01	1.67 ± 0.01	1.69 ± 0.01	1.58 ± 0.01	
	2	2.52 ± 0.01	2.31 ± 0.01	2.46 ± 0.01	1.99 ± 0.01	
	3	2.99 ± 0.01	3.09 ± 0.01	2.99 ± 0.01	2.93 ± 0.01	$p < 0.05$
	4	1.99 ± 0.01	1.48 ± 0.01	1.81 ± 0.01	1.58 ± 0.01	
	5	1.00 ± 0.01	1.29 ± 0.01	1.10 ± 0.01	1.24 ± 0.01	
1% NaCl	1	1.43 ± 0.01	1.67 ± 0.01	1.84 ± 0.01	1.67 ± 0.01	
	2	2.54 ± 0.02	2.52 ± 0.02	2.43 ± 0.02	2.40 ± 0.02	
	3	2.67 ± 0.03	2.66 ± 0.03	2.67 ± 0.03	2.64 ± 0.02	$p < 0.05$
	4	1.75 ± 0.01	1.63 ± 0.01	1.54 ± 0.01	1.69 ± 0.01	
	5	1.34 ± 0.01	1.29 ± 0.01	1.28 ± 0.01	1.28 ± 0.01	
1% Sucrose	1	1.43 ± 0.01	1.64 ± 0.01	1.81 ± 0.01	1.54 ± 0.01	
	2	1.84 ± 0.01	1.87 ± 0.01	2.08 ± 0.02	1.81 ± 0.01	
	3	2.99 ± 0.03	3.05 ± 0.03	2.96 ± 0.03	3.14 ± 0.03	$p < 0.05$
	4	1.66 ± 0.01	1.46 ± 0.01	1.78 ± 0.01	1.58 ± 0.01	
	5	1.08 ± 0.01	1.28 ± 0.01	1.19 ± 0.01	1.37 ± 0.01	
Whey	1	1.49 ± 0.01	1.25 ± 0.01	1.46 ± 0.01	1.28 ± 0.01	
	2	2.52 ± 0.02	2.52 ± 0.02	2.55 ± 0.02	2.05 ± 0.02	
	3	3.05 ± 0.03	3.16 ± 0.03	2.96 ± 0.03	3.31 ± 0.03	$p < 0.05$
	4	1.55 ± 0.01	1.87 ± 0.01	1.66 ± 0.01	1.84 ± 0.01	
	5	1.28 ± 0.01	1.28 ± 0.01	1.31 ± 0.01	1.40 ± 0.01	

*For all cultivars, increase in the enzymatic activity achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Table 2. Changes in protease activity in germinating grains (units ± SD)

Germinating media	Days	Cultivars				p-value*
		Sputnik	Regent	Kazak	Alba	
Water	1	0.243 ± 0.017	0.231 ± 0.016	0.206 ± 0.014	0.244 ± 0.017	
	2	0.252 ± 0.018	0.244 ± 0.017	0.230 ± 0.016	0.250 ± 0.018	
	3	0.278 ± 0.019	0.275 ± 0.019	0.290 ± 0.020	0.281 ± 0.020	$p < 0.05$
	4	0.276 ± 0.019	0.235 ± 0.017	0.278 ± 0.019	0.244 ± 0.017	
	5	0.255 ± 0.018	0.220 ± 0.015	0.249 ± 0.017	0.233 ± 0.016	
1% NaCl	1	0.176 ± 0.012	0.199 ± 0.014	0.196 ± 0.014	0.191 ± 0.013	
	2	0.195 ± 0.014	0.235 ± 0.017	0.225 ± 0.016	0.226 ± 0.016	
	3	0.238 ± 0.016	0.258 ± 0.018	0.258 ± 0.018	0.254 ± 0.018	$p < 0.05$
	4	0.229 ± 0.016	0.234 ± 0.016	0.239 ± 0.017	0.245 ± 0.017	
	5	0.220 ± 0.015	0.220 ± 0.015	0.225 ± 0.015	0.233 ± 0.016	
1% Sucrose	1	0.249 ± 0.017	0.246 ± 0.017	0.226 ± 0.016	0.265 ± 0.019	
	2	0.260 ± 0.018	0.268 ± 0.019	0.265 ± 0.019	0.279 ± 0.020	
	3	0.278 ± 0.019	0.273 ± 0.019	0.293 ± 0.020	0.298 ± 0.020	$p < 0.05$
	4	0.268 ± 0.018	0.265 ± 0.019	0.276 ± 0.019	0.280 ± 0.019	
	5	0.251 ± 0.018	0.228 ± 0.016	0.250 ± 0.018	0.265 ± 0.019	
Whey	1	0.253 ± 0.018	0.266 ± 0.019	0.244 ± 0.017	0.274 ± 0.019	
	2	0.269 ± 0.019	0.278 ± 0.019	0.290 ± 0.020	0.304 ± 0.020	
	3	0.279 ± 0.019	0.285 ± 0.020	0.304 ± 0.020	0.320 ± 0.020	$p < 0.05$
	4	0.240 ± 0.017	0.245 ± 0.017	0.284 ± 0.020	0.300 ± 0.020	
	5	0.235 ± 0.016	0.231 ± 0.016	0.256 ± 0.018	0.260 ± 0.018	

*For all cultivars, increase in the enzymatic activity achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Our data showing an increased enzymatic activities in germinating grains supports prior studies showing that germination is associated with high mineral content of foxtail millet (Coulibaly, 2011) and with increased anti-oxidant activity of the finger millet (*Eleusina coracana*) (Abioye et al., 2018). Moreover, our studies demonstrated that germination for 3 days is optimal for the induction of the enzymes and that longer germination reduces proteolytic, amylase, and lipase activities. The importance of these novel findings is emphasized by fact that these enzymes increase bioactivity, nutritional value, and the overall quality of baked products. Of particular interest to further development of the technology is the observed increase in cumulative phenolic content in germinated proso millet grains.

Table 3. Changes in lipase activity in germinating grains (units ± SD)

Germinating media	Days	Cultivars				*p* -value*
		Sputnik	Regent	Kazak	Alba	
Water	1	0.40 ± 0.02	0.53 ± 0.02	0.38 ± 0.02	0.42 ± 0.02	$p < 0.05$
	2	0.45 ± 0.02	0.60 ± 0.02	0.42 ± 0.02	0.45 ± 0.02	
	3	0.90 ± 0.03	1.04 ± 0.03	0.86 ± 0.03	0.80 ± 0.02	
	4	0.95 ± 0.03	1.02 ± 0.03	0.86 ± 0.03	0.76 ± 0.03	
	5	0.80 ± 0.03	0.90 ± 0.03	0.80 ± 0.03	0.60 ± 0.02	
1% NaCl	1	0.44 ± 0.02	0.55 ± 0.02	0.40 ± 0.02	0.43 ± 0.02	$p < 0.05$
	2	051 ± 0.02	0.62 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	
	3	1.08 ± 0.03	0.96 ± 0.03	0.88 ± 0.03	0.86 ± 0.03	
	4	1.02 ± 0.03	0.92 ± 0.03	0.82 ± 0.03	0.80 ± 0.03	
	5	0.82 ± 0.03	0.90 ± 0.03	0.75 ± 0.03	0.70 ± 0.03	
1% Sucrose	1	0.38 ± 0.02	0.48 ± 0.02	0.39 ± 0.02	0.40 ± 0.02	$p < 0.05$
	2	0.40 ± 0.02	0.51 ± 0.02	0.41 ± 0.02	0.42 ± 0.02	
	3	0.84 ± 0.03	0.78 ± 0.03	0.30 ± 0.03	0.77 ± 0.03	
	4	0.80 ± 0.03	0.75 ± 0.02	0.78 ± 0.02	0.75 ± 0.02	
	5	0.67 ± 0.02	0.60 ± 0.02	0.68 ± 0.02	0.66 ± 0.02	
Whey	1	0.46 ± 0.02	0.51 ± 0.02	0.40 ± 0.02	0.42 ± 0.02	$p < 0.05$
	2	0.52 ± 0.02	0.56 ± 0.02	0.47 ± 0.02	0.45 ± 0.02	
	3	1.16 ± 0.03	1.12 ± 0.03	0.92 ± 0.03	0.84 ± 0.03	
	4	1.10 ± 0.03	1.08 ± 0.03	0.90 ± 0.03	0.80 ± 0.03	
	5	0.98 ± 0.03	0.96 ± 0.03	0.72 ± 0.02	0.72 ± 0.02	

*For all cultivars, increase in the enzymatic activity achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Table 4. Changes in cumulative phenols in germinating grains

Day of germination	Cumulative phenols in cultivars (mg kg^{-1})				*p*-value*
	Sputnik	Kazak	Regent	Alba	
Dry grain	525 ± 7	550 ± 7	575 ± 8	525 ± 6	$p < 0.05$
1	425 ± 6	462 ± 6	545 ± 8	450 ± 5	
2	460 ± 6	470 ± 7	562 ± 8	500 ± 5	
3	1,550 ± 17	1,025 ± 11	775 ± 7	700 ± 6	
4	1,700 ± 18	1,125 ± 12	950 ± 12	850 ± 7	
5	1,250 ± 13	1,145 ± 12	1,850 ± 19	1,820 ± 19	

*For all cultivars, increase in cumulative phenols achieves statistical significance ($p < 0.05$) at day 3 as compared to starting point.

Polyphenols are important antioxidants (Kadiri, 2017), which at low concentration can delay or prevent oxidation of an oxidizable substrates and could reduce risk of cardiovascular disease and cancer (Kris-Etherton et al., 2002). They can also mitigate oxidative stress on DNA. Because amounts of cumulative phenols in germinating proso millet (up to 1,850 μg g^{-1}) are compatible and even higher than in bran layer (1,258–3,157 μg g^{-1}) and whole wheat grains (168–459 μg g^{-1}) (Vaher et al., 2010), flour from germinating proso millet could be used in design of selective diets with high antioxidant capacity.

Flour from germinating proso millet grains could be used in non-rising dough for wheat flower substitution.

Based on the obtained data, we produce a batch of flour from Sputnik and Regent grains germinated in water for 3 days. Germination in water was selected because it showed a median rise of all enzymatic activities and cumulative phenolic content, as compared to other media. The resultant flour was used for partial or complete substitution of the wheat flour in non-rising cake and shortbread dough. Following preparation, the final products were assessed and graded by 10 independent tasters for visual appearance, color, smell, texture, and taste. Products prepared from the wheat flour according to the same recipe were used as a reference control. Based on this evaluation, all shortbread pastry with 30% and 50% substitution of wheat flour did not substantially deviate from control samples and were graded similarly to the wheat flour-based products. Cakes will 50% substitution also did not substantially deviate from wheat-based products. Despite lower scores for several parameters, all products, particularly shortbreads, prepared from 100% substituted flour, were evaluated as compatible with wheat flour-based products (Table 5).

Table 5. Sensory assessment results of partial or complete substitution of the wheat flour in non-rising cake and shortbread dough

Assessed parameters	Ratio of wheat/germinated proso millet flower in cake // shortbread flour			
	100/0	70/30*	50/50	0/100
Appearance	5.0	// 4.8	4.0 // 5.0	4.0 // 3.8
Color	5.0	// 4.5	4.7 // 4.9	4.3 // 4.9
Smell	5.0		4.8 // 4.8	4.0 // 4.2
Taste	5.0	// 4.8	4.8 // 4.9	4.5 // 4.6
Structure	5.0	// 4.7	4.7 // 4.9	4.3 // 4.0
Combined Score*	25.0	// 23.5	23.9 // 24.5	21.7 // 21.5

* 70/30 ratio was used only for shortbread flour; ** all scores reflect deviations from the control sample and not the quality of the product; presented scores are the averages of scores from all testers.

The utility of biologically modified millet flours in traditional millet-based foods (e.g. instant fura) were previously tested (Amadou et al., 2011). In this study, besides providing biochemical characterization, we also test the utility of the flour from germinating proso millet for partial and complete substitution of the wheat flower in traditionally wheat-based non-rising dough products. Our evaluation confirmed that this flour could be successfully used for partial replacement of wheat flour in a cake and shortbread dough and complete replacement – in a cake dough without substantial change in basic sensory characteristics. Out of all, typical wheat flour smell was missing in products with 100% substitution and products crumbled easily. Yet, these drawbacks did not reduce the overall appeal of the baked products, particularly shortbread pastries.

CONCLUSIONS

Collectively, our studies demonstrated that germination of proso millet grains for 3 days is optimal for activation of enzymatic activities to increase bioavailability of the flour which could be successfully used for partial and complete substitution of wheat flour in wheat flour-based recipes. Our findings provide the basis for the further development of the technology for the preparation of healthy and nutritional food products such as complementary food products, composite flours, GFD products, and infant formula.

REFERENCES

Abioye, V., Ogunlakin, G., Taiwo, G.A. 2018. Effect of Germination on Anti-oxidant Activity, Total Phenols, Flavonoids and Anti-nutritional Content of Finger Millet Flour. *Journal of Food Processing & Technology* **9**, 719.

Amadou, I., Le, G.-W., Gbadamosi, O.S. 2011. Millet-based raditional Processed Foods and Beverages − A Review. *Sereal Food World* **56**, 115−121.

Andriotis, V.M., Rejzek, M., Rugen, M.D., Svensson, B., Smith, A.M. & Field, R.A. 2016. Iminosugar inhibitors of carbohydrate-active enzymes that underpin cereal grain germination and endosperm metabolism. *Biochem. Soc. Trans.* **44**, 159−165.

Barrera, G.N., Tadini, C.C., Leon, A.E. & Ribotta, P.D. 2016. Use of alpha-amylase and amyloglucosidase combinations to minimize the bread quality problems caused by high levels of damaged starch. *J. Food Sci. Technol.* **53**, 3675−3684.

Coulibaly, C. 2011. Evolution of Energetic Compounds, Antioxidant Capacity, Some Vitamins and Minerals, Phytase and Amylase Activity During the Germination of Foxtail Millet. *American Journal of Food Technology* **6**, 40−51.

Diaz-Mendoza, M., Diaz, I. & Martinez, M. 2019. Insights on the Proteases Involved in Barley and Wheat Grain Germination. *Int. J. Mol. Sci.* **20**.

Fisher, M.M., Rosen, D.S., Ornstein, R.M., Mammel, K.A., Katzman, D.K., Rome, E.S., Callahan, S.T., Malizio, J., Kearney, S. & Walsh, B.T. 2014. Characteristics of avoidant/restrictive food intake disorder in children and adolescents: a 'new disorder' in DSM-5. *J. Adolesc. Health.* **55**, 49−52.

Galleschi, L., Capocchi, A., Ghiringhelli, S., Saviozzi, F., Calucci, L., Pinzino, C, & Zandomeneghi, M. 2002. Antioxidants, free radicals, storage proteins, and proteolytic activities in wheat (Triticum durum) seeds during accelerated aging. *J. Agric. Food Chem.* **50**, 5450−5457.

Gulati, P., Li, A., Holding, D., Santra, D., Zhang, Y. & Rose, D.J. 2017. Heating Reduces Proso Millet Protein Digestibility via Formation of Hydrophobic Aggregates. *J. Agric. Food Chem.* **65**, 1952−1959.

Kadiri, O. 2017. A review on the status of the phenolic compounds and antioxidant capacity of the flour: Effects of cereal processing. *International Journal of Food Properties* **20**, S798−S809.

Kalinova, J. & Moudry, J. 2006. Content and quality of protein in proso millet (Panicum miliaceum L.) varieties. *Plant Foods. Hum. Nutr.* **61**, 45−49.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. & Etherton, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **113**(Suppl 9B), 71S−88S.

Liukkonen, K.-H., Katina, K., Wilhelmsson, A., Myllymaki, O., Lampi, A.-M., Kariluoto, S., Piironen, V., Heinonen, S.-M., Nurmi, T., Adlercreutz, H., Peltoketo, A., Pihlava, J.-M., Hietaniemi, .-M. & K. Poutanen. 2003. Process-induced changes on bioactive compounds in whole grain rye. *Proceedings of the Nutrition Society* **62**, 117−122.

Oelke, E.A., Oplinger, E.S., Putnam, D.H.,Durgan, B.R., Doll, J.D. & Undersander, D.J. 1990. Millets:Alternative Field Crop Manual. CAPAP and the Minnesota Extension Service.

Padda, M.S. & Picha, D.H. 2007. Methodology Optimization for Quantification of Total Phenolics and Individual Phenolic Acids in Sweetpotato (Ipomoea batatas L.) Roots. *Journal of Food Science* **72**, C412−C416.

Parameswaran, K.P. & Sadasivam, S. 1994. Changes in the carbohydrates and nitrogenous components during germination of proso millet, Panicum miliaceum. *Plant. Foods. Hum. Nutr.* **45**, 97−102.

Rose, D.J. & Pike, O.A. 2006. A simple method to measure lipase activity in wheat and wheat bran as an estimation of storage quality. *Journal of the American Oil Chemists' Society* **83**, 415−419.

Saleh, A.S., Zhang, Q.,Chen, J. & Andshen, Q. 2013. Milletgrains:nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safaty* **12**, 218−295.

Vaher, M., Matso, K., Levandi, T., Helmja, K. & Kaljurand, M. 2010. Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry* **2**, 76−82.

Volta, U., Caio, G., Karunaratne, T.B., Alaedini, A. & De Giorgio, R. 2017. Non-coeliac gluten/wheat sensitivity: advances in knowledge and relevant questions. *Expert Rev Gastroenterol Hepatol* **11**, 9−18.