Cooking quality, digestibility, and sensory properties of proso millet pasta as impacted by amylose content and prolamin profile

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Abstract

As part of ongoing efforts to promote millet as a double crop for the American Midwest, four Minnesota-grown proso millet varieties were selected for fresh gluten-free pasta production and compared to commercially available fresh gluten-free and wheat pasta. Raw and cooked pasta were analyzed for starch and protein content, color, and carotenoids. Cooked pasta was assessed for cooking quality, *in-vitro* starch and protein digestibility, and sensory quality. Millet pasta contained less rapidly digestible starch than commercial gluten-free pasta; however, millet and commercial gluten-free pasta had lower protein digestibility than wheat pasta. Sensory panelists detected more graininess and starchiness in millet samples than in commercial pasta. Millet varieties differed in amylose content and prolamin profile, and both factors influenced pasta properties. Pasta with more amylose and high-molecular weight prolamins had lower cooking loss and lower stickiness scores. Higher amylose contents also corresponded to higher firmness and chewiness among millet pasta samples. The millet sample with the lowest amylose and prolamin content yielded pasta of the lowest quality. Results indicated that select proso millet varieties may be suitable for fresh pasta, yet quality improvement is warranted by recipe or processing optimizations.

1. Introduction

Millets exhibit positive agronomic and nutritional characteristics, are well suited for various climates and in crop rotation with other grains, while being resistant to certain pests and diseases (Saleh, Zhang, Chen, & Shen, 2013). Additionally, millets have garnered attention due to being gluten-free (GF) with low glycemic index (Saleh et al., 2013; Annor, Tyl, Marcone, Ragaee, & Marti, 2017).

Accordingly, efforts have been made to provide consumers with millet-based foods such as bread (Schoenlechner, Szatmari, Bagdi, & Tömösközi 2013), cookies (Sharma, Saxena, & Riar, 2016), and snacks (Deshpande & Poshadri, 2011). However, millet flour alone does not yield pasta of desirable quality (Jalgaonkar, & Jha, 2016), whereas a combination of flours allows balancing sensory deficits of millets and helped compensate for technological challenges (Jalgaonkar, & Jha, 2016).

Current food use of millet is limited in North America and Europe. A concerted effort along the production chain, from farmer to consumer, is needed to promote millet-based foods. In a previous study, we evaluated Minnesota-grown proso millet (*Panicum miliaceum*) for compositional, nutritional and functional characteristics (Tyl, Marti, Hayek, Anderson, & Ismail, 2018). Distinct differences among varieties included amylose to amylopectin ratio and carotenoid content (Tyl et al., 2018) which have been shown to influence pasta quality (Marti & Pagani 2013, Marti, D'Egidio, & Pagani, 2016). Therefore, the objective of this study was to assess the suitability of different proso millet varieties for production of GF pasta in terms of cooking quality, nutritional value, and sensory properties. In particular, we evaluated the impact of amylose content and prolamin profiles on the quality of fresh millet-based pasta.

2. Materials and Methods

2.1 Materials

Proso millet varieties (Dawn, Earlybird, Horizon, Snobird, Sunrise, and Sunup) were grown as double crops in two locations (Lamberton and Waseca, MN) and harvested in fall 2015. Decortication and chemical composition data were reported previously (Tyl et al., 2018). Decorticated millet samples were milled into flour (particle size ≤0.25 mm) with a Cyclone Sample Mill (UDY Corporation, Boulder, CO). Commercial fresh wheat pasta (Fettuccine Buitoni; Buitoni Pasta Company North America, Solon, OH, US;) and fresh GF pasta (Egg Fettuccine; RP's pasta company, Madison, WI, US) were used as controls.

All reagents used were of reagent grade or higher. Pancreatin (4xUSP specifications), pepsin (3,200 - 4,500 U/mg protein), lutein and zeaxanthin standards were obtained from Sigma-Aldrich (St. Lois, MO). Test kits for total and resistant starch and glucose oxidase/peroxidase (GOPOD) reagent for starch digestibility were obtained from Megazyme (Wicklow, Ireland). Broad range molecular weight marker, Laemmli buffer, 10X Tris/Glycine/SDS running buffer, and 4-15% TRIS-HCl gels were from BioRad (Hercules, CA). High-performance liquid chromatography (HPLC) grade solvents and other reagent grade chemicals were purchased from Sigma-Aldrich and Fisher (Waltham, MA).

2.2 Prolamin profile in millet flours

Prolamins were extracted and profiled using sodium dodecyl sulfite polyacrylamide gel electrophoresis (SDS-PAGE) according to the method reported by Tatham, Gilbert, Fido, & Shewry (2000). Prolamin extracts were dissolved in Laemmli buffer with the addition of 5% β-mercaptoethanol, boiled, and centrifuged at 13,000 x g for 10 min. An aliquot (5 µL; 125 µg protein loaded) of each sample's supernatant was loaded onto a 4-15% gradient gel and

electrophoresed at 200 V for 50 min. Gels were stained with a Coomassie blue stain for 1 h at room temperature, de-stained overnight, and scanned on a Bio-RadGel Dox XR system using Quantity One software.

2.3 Pasta preparation

Pasta recipes consisted of 41-46 g decorticated millet flour, 16 g potato starch, 0.2 g salt, 0.8 g guar gum, 28 g liquid eggs, and 15 g water (dough basis). The recipe was developed based on pre-trials. Potato starch and eggs were deemed necessary for a cohesive that could easily be sheeted and dough would not disintegrate upon cooking. The amount of flour was adjusted for different samples to improve dough handling. Dry ingredients were mixed, then liquid ingredients were added. Dough was kneaded manually for 5 min until a smooth consistency was reached. A KitchenAid Classicplus (KitchenAid, St. Joseph, MI, USA) was used to yield sheets of 1 mm thickness that were made into 3-4 cm long fettuccine. Two pasta batches were prepared from each millet variety (E-L, Earlybird cv. grown at Lamberton; H-L, Horizon cv. grown at Lamberton; S_r-L, Sunrise cv. grown at Lamberton; S_r-W, Sunrise cv. grown at Waseca). Pasta samples were cooked in boiling distilled water for the optimum cooking time (OCT), evaluated by tasting the pasta every 15 seconds until uniform al dente consistency. For OCT determination, two cooking trials were performed for each pasta batch.

For the determination of carotenoids, as well as starch and protein content and digestibility, sample aliquots were frozen using liquid nitrogen, lyophilized and ground with mortar and pestle to particle size ≤0.5 mm. For sensory analysis, fresh pasta was prepared in batches scaled up to 350 g, divided into 15 g portions, and stored at -20 °C. The pasta samples were thawed, then cooked as reported above until the OCT and served to panelists within one hour of cooking.

2.4 Chemical analyses

Moisture content was determined in duplicate using a moisture analyzer (MB35, Ohhaus, Parsippany, NJ). Starch in uncooked and cooked pasta were measured in triplicate with amyloglucosidase/α-amylase digestion followed by GOPOD derivatization and spectrophotometric quantification as described by AACCI method 76-13.01. Protein content was determined following AACCI Dumas combustion method 46-30.01, using 6.25 as the protein conversion factor. Carotenoids in raw and cooked pasta were analyzed in triplicate with high-performance liquid chromatography as described by Tyl et al. (2018), without modification.

2.5 Pasta quality

2.5.1 Color

The color of uncooked and cooked pasta was assessed using a Chroma Meter CR-221 (Minolta Camera Co., Osaka, Japan). Results were averages of five determinations for each uncooked pasta batch (two batches, i.e., two true replicates). For cooked samples, five determinations were carried out on two independently cooked samples from each batch.

2.5.2 Cooking loss and water absorption

Pasta cooking losses were assessed following AACCI method 66-50.01, using a 1:20 pasta:water ratio. Two samples from each pasta batch were cooked, and each of these cooked samples was analyzed in triplicate for cooking loss and water absorption. Cooking loss was calculated by difference between the content of starch or protein in uncooked and cooked pasta

2.5.3 Firmness

Cooked pasta was assessed for firmness (N) by measuring the maximum cutting stress following AACCI method 66-50.01, using a Texture Analyzer (TA.XT2, Stable Micro systems, UK), equipped with a 5 kg weigh beam and a metallic blade. A test speed and a post-test speed of 600

mm/min of 10.2 mm/min was used, and the crosshead was set to stop cutting when reaching a distance of 0.5 mm from the bottom plate. Cooked pasta samples were rested for 10 minutes, and then firmness was assessed on 5 sets of 7 strands from each cooked sample.

2.6 *in-vitro* starch digestibility

The *in vitro* starch digestibility of the cooked samples was measured following Englyst, Kingman, & Cummings (1992), with modifications reported by Annor, Marcone, Bertoft, & Seetharaman (2013), and a reduced sample size (0.2 g of lyophilized pasta). Pasta samples were digested with a mixture of pancreatin, invertase and amyloglucosidase, and liberated glucose assessed using the GOPOD assay, following the method reported by Annor et al (2013). Available starch was classified into rapidly digestible starch (RDS) and slowly digestible starch (SDS). RDS and SDS values were expressed as percentage of raw pasta. Resistant starch (RS) content of cooked pasta was assessed following AACCI method 32-40.01. Analyses were carried out in triplicate on the two independently cooked samples from each pasta batch.

2.7 *in-vitro* protein digestibility (IVPD)

The IVPD of lyophilized pasta (0.12 g) underwent two sequential digestion with pepsin and pancreatin as outlined by Pasini et al. (2001). First samples were shaken (1 h, 37 °C) with 4 mL of 0.2 N HCl containing 1.5 mg/mL pepsin (pepsin : protein ratio 1:30). Then, an aliquot (2.3 mL) of a pH 7.6 solution of 1.15 mL 1 M boric acid, 1.15 mL 0.5 N NaOH and 0.49 mg pancreatin was added (pancreatin : protein ratio 1:21). After shaking (1 h, 37 °C), the digestion was stopped by adding 6.7 mL of 20% (w/v) trichloroacetic acid. After standing for 1 h at room temperature, samples were centrifuged (8000 x *g*, 10 min), supernatants were lyophilized, and their nitrogen contents assessed by Dumas (protein conversion factor of 6.25). Sample blanks were prepared

without enzymes and analyzed concomitantly to correct for non-protein nitrogen. The *in vitro* protein digestibility was calculated as follows:

$$in\ vitro \text{ protein digestibility (\%)} = \frac{[(B-A)\times 6.25]\times C}{D}$$

Where,

A = % N in blanks; B = % N in supernatant after the digestion; C = weight of lyophilized supernatant, D = pasta sample weight x (protein content in pasta/100).

2.8 Descriptive Sensory analysis

Five training sessions were held for nine members of the Sensory Center trained panel at the University of Minnesota. Panelists adapted a lexicon (Supplement Table 1) reported previously (Cole, 1991; Janto, Pipatsattayanuwong, Kruk, Hou & McDaniel, 1998; Joyner, Jones & Rasco, 2007). The trained panel evaluated all samples in two independent testing sessions in individual booths. Serving orders were balanced using a Williams Latin square design. Panelists rated attribute intensities on a 20-point line scale from 'none' to 'intense' (Williams, 1949). Intensity ratings of taste (using nose clips) and flavor were made on a standard citric acid scale; odor ratings on the standard butanol scale. The appearance scale is shown in Supplementary Figure 1.

2.9 Statistical analyses

Millet pasta dough from each variety was prepared in duplicate. Pasta from each dough was cooked in duplicate, and each resulting sample was analyzed at least in triplicate. The two replicates of the commercial pastas (wheat and GF) consisted of pasta prepared from two different packages.

A two-way analysis of variance (ANOVA) was performed using Excel 2013, with prolamin (present versus absent) and amylose contents (high versus low) as factors for the two-way

166 ANOVA. To assess significant differences among pasta types, a one-way ANOVA (with pasta
167 type as factor) was conducted in R 3.1.0 (R Core Team, 2015), and for significant differences (P
168 ≤ 0.05) a Tukey-Kramer Honestly Significant Difference (HSD) test was performed. Differences
169 in moisture, yellowness, starch, protein, and carotenoid contents between raw and cooked pasta
170 were determined with a 2-sided t-test ($P \leq 0.05$) in Excel 2013. Sensory data were analyzed by
171 ANOVA (using SAS® PROC GLM), and Student-Neuman-Keuls multiple comparisons tests to
172 determine differences in attributes among the six pasta samples ($P < 0.05$). The attribute intensity
173 was the dependent variable; panelist, taste position, replicate, and pasta were predictors. Contrast
174 statements within the ANOVA were used to test for differences among pasta samples with
175 presence or absence of high-molecular prolamins, or between amylose levels. Relationships
176 among pasta samples and sensory attributes were summarized following Pearson-type principal
177 components analysis (PCA) (using XLSTAT®), using only attributes that significantly differed
178 among the pastas. Instrumental measurements were added as supplementary variables to the
179 PCA analysis.

180 **3. Results and Discussion**

181 **3.1 Selection of millet flours**

182 Agronomic and chemical characteristics of six millet varieties grown in two locations
183 (Lamberton and Waseca, MN, US) were reported previously (Tyl et al., 2018). Four samples
184 were selected for making fresh-pasta, based on yield, amylose content and prolamin profile.
185 Generally, varieties grown in Lamberton had higher yields and were thus preferred (Tyl et al.,
186 2018). Varieties with different amylose contents (Tyl et al., 2018) were selected to assess the
187 impact of amylose content on pasta quality. Low amylose (Earlybird from Lamberton, E-L, 7.8%
188 amylose in starch), intermediate amylose (Horizon from Lamberton, H-L, 25.1% amylose in

189 starch) and high amylose (Sunrise from Lamberton, Sr-L, 31.7% amylose in starch; and Waseca,
190 Sr-W, 35.7% amylose in starch) were selected. Usually, starches with high amylose content (>
191 25%) are preferred for the production of GF dried pasta, due to their high tendency to retrograde
192 and form a network able to withstand cooking (Marti & Pagani, 2013). However, no information
193 is available on the role of amylose content in fresh GF pasta.
194 Some varieties (H-L and Sr-L) contained high molecular weight (HMW) prolamins (50 -150
195 kDa) (Figure 1). In wheat, HMW prolamins play a major role in gluten strength and functionality
196 (Shewry, Halford, & Tatham, 1992). There are no reports on the impact of prolamin molecular
197 weight distribution on GF pasta quality. Therefore, the chosen four samples represented a
198 spectrum of amylose/prolamin make-up: low amylose/deficient in HMW prolamins (E-L),
199 intermediate amylose/contains HMW prolamins (H-L), high amylose/deficient in HMW
200 prolamins (Sr-W), and high amylose/contains HMW prolamins (Sr-L).

201 **3.2 Moisture, starch and protein content**

202 Moisture content (Table 1) of fresh millet-based pasta increased after cooking due to water
203 absorption of gelatinized starch (Marti, D'Egidio, & Pagani, 2016), yet was in the range reported
204 for fresh pasta (Pagani et al., 2007). Millet-based pasta had more starch than both commercial
205 samples (Table 1), likely due to presence of potato starch. Millet-based pasta had higher protein
206 than commercial GF pasta, although no significant differences were observed among millet
207 varieties. Starch and protein contents of the pasta followed the same trend observed in millet
208 flours (Tyl et al., 2018).

209 **3.3 Pasta color and carotenoid content**

210 All millet-based pasta had higher b* value than the GF control (Table 2), and E-L was the most
211 yellow before and after cooking. In pasta, higher yellowness (i.e. b* values) corresponds to
212 higher product quality (Marti, D'Egidio, & Pagani, 2016).

213 In fresh-pasta, carotenoids from raw materials contribute the most to yellowness. Earlybird has
214 the highest lutein and zeaxanthin levels, the two carotenoids detected in proso millet (Tyl et al.,
215 2018). Although lutein was the dominant carotenoid in proso millet flour (Tyl et al., 2018), all
216 pasta samples contained about twice as much zeaxanthin than lutein (Table 2), due to the
217 presence of eggs in the pasta dough, which contained more zeaxanthin (38.2 μg/g d.b. of
218 zeaxanthin, 6.9 μg/g d.b. of lutein) than lutein (6.9 μg/g d.b.). E-L pasta had the highest
219 zeaxanthin content among the pastas, and significantly higher lutein than all other samples
220 except for wheat. Millet pasta samples had higher amounts of zeaxanthin than wheat, which may
221 be due to differences in the amount of eggs used. GF pasta had the lowest levels of both
222 carotenoids.

223 Cooking resulted in significant ($P < 0.05$) loss in lutein content only for H-L pasta, however, the
224 observed loss was minor. The observed loss was at the low end of the range reported for loss in
225 foxtail millet kernels after cooking (Shen, Yang, Zhao, Shen, & Diao, 2015).

226 **3.4 Impact of amylose content and prolamin profile on the cooking quality of millet pasta**

227 Millet-based pasta had lower cooking loss than both controls (Table 3). While the percentage of
228 eggs in commercial wheat pasta was not stated on the package, egg protein could have hindered
229 excessive starch granule swelling and the consequent leaching of solids into the cooking water
230 (Marti et al., 2014). Our values are in the range of those reported for fresh teff-based GF pasta
231 (Hager, Lauck, Zannini, & Arendt, 2012).

232 Among the millet pastas, E-L, with the lowest amylose content, had the lowest OCT and water
233 absorption values (Table 3), likely due to faster swelling of low amylose granules (Vignaux et
234 al., 2005). Moreover, E-L sample exhibited a relatively high cooking loss and the lowest
235 firmness. The low firmness likely resulted from less retrogradation compared to other varieties,
236 as a consequence of its low amylose content.
237 Sr-L, having HMW prolamins and high amylose content, showed the highest water absorption.
238 Additionally, Sr-L together with H-L, which has HMW prolamin and intermediate amylose
239 content, required longer cooking time than the other millet pastas. Presence of HMW prolamins,
240 which can polymerize through disulfide cross-linking (Taylor, Taylor, Campanella, & Hamaker,
241 2016), may have resulted in a network capable of entrapping starch granules during cooking.
242 Regardless, no significant effect on firmness was observed, in agreement with wheat HMW
243 glutenins that increased dough strength, but not pasta firmness, suggesting the influence of other
244 factors, including starch (Sissons, Soh, & Turner, 2007).

245 **3.5 *In vitro* starch digestibility**

246 The accessibility of digestive enzymes to starch was similar in millet-based and wheat pasta
247 (Figure 2). This finding is interesting since durum wheat pasta is classified as a low glycemic
248 index product. Having a low glycemic index is an added advantage to a gluten free pasta
249 formulated with millet.
250 Millet pasta had lower RDS than the commercial GF fresh pasta, further indicating that millet
251 could be suitable for formulating GF products with low glycemic impact. The high amount of
252 protein in millet (up to 13 g/100g, Tyl et al., 2018) possibly creates a stronger network around
253 the starch, hence reducing accessibility for digestive enzymes, as observed for fresh teff pasta
254 (Hager, Czerny, Bez, Zannini, & Arendt, 2013).

255 Among the millet-based pasta, E-L and Sr-L were the only samples showing significant
256 differences in RDS (Figure 2). E-L and Sr-L had opposite characteristics: low amylose content
257 and absence of HMW prolamins (E-L), and high amylose content and presence of HMW
258 prolamins (Sr-L). High amylose content may reduce starch digestibility (Annor et al., 2017).
259 Additionally, presence of HMW prolamin may result in a stronger network around the starch,
260 hindering enzyme accessibility as is the case for wheat pasta (Colonna et al., 1990). Therefore,
261 possible explanations for differences in starch digestibility between E-L and Sr-L may be related
262 to the type of the starch–protein matrix formed in the pasta. If a ‘‘loose” structure is formed, for
263 example when LMW-glutenins are present during dough formation, starch granules are likely to
264 be more accessible to α-amylase (Aravind, Sissons, & Fellows, 2011).
265 All resistant starch values in millet pasta were lower than 2%, in agreement with resistant starch
266 content of GF dried pasta reported previously (Barbiroli et al., 2013).

267 **3.6 *In vitro* protein digestibility**

268 The protein digestibililty of the cooked millet pasta ranged between 41 and 50% (Figure 3). In
269 contrast, wheat pasta protein was almost completely digested, in line with other studies reporting
270 high protein digestibility of wheat pasta, ranging from 81% (De Marco, Steffolani, Martínez, &
271 León, 2014) to 89% (Seczyk, Swieca, Gawlik-Dziki, Luty, & Czyz, 2016). In general, millet
272 protein digestibility can be reduced by several factors, most notably the presence of tannins and
273 dietary fiber (Annor et al., 2017), which is unlikely for these samples as they were decorticated
274 and were low in phenolics and fiber content (Tyl et al., 2018). The protein digestibility, however,
275 of cooked proso millet porridge was relatively low (Gulati et al., 2017; Tyl et al., 2018). Gulati et
276 al. (2017) showed that the reduced protein digesitibility upon heating is caused by aggregate
277 formation via hydrophobic interactions, with possible involvement of surface exposure of

tryptophan residues. More work is needed to evaluate changes in protein solubility and secondary structure as affecty by processing, as these may also be associated with aggregation and could be monitored when comparing strategies to enhance protein digestibility.

3.7 Descriptive analysis

Appearance, texture and taste attributes that significantly differed among pasta samples are shown in Table 4. Other evaluated attributes can be found in supplement Table 2, and their definitions are listed in supplement Table 1.

3.7.1 Appearance

All millet samples were rated as significantly more uniform than both commercial controls, and perceived as significantly grayer. Millet samples lacking HMW prolamins were deemed more gray. Millet pasta samples were judged to be significantly more yellow than commercial GF pasta. However, except for E-L, they were rated less yellow than wheat pasta. This observation corresponds with lutein, zeaxanthin and b* values (Table 2).

3.7.2 Taste

E-L pasta scored significantly higher in bitterness and bitter aftertaste (supplement Table 2) than all other samples; none of which differed in bitterness. The commercial GF pasta was perceived as more salty than all other samples. While the exact recipe of the commercial samples is not known, higher salt levels were possibly used in their production.

3.7.3 Texture

Millet pasta was rated as more starchy, less elastic and more grainy than both controls. All gluten-free samples, including the commercial control, were less chewy than the wheat control and had lower tensile strength. Contrast analysis determined that presence of HMW prolamins in

300 millet pasta resulted in lower perceived stickiness, but higher graininess, whereas lower amylose
301 contents corresponded with lower firmness, lower chewiness, and higher stickiness. The effects
302 of amylose on firmness, chewiness and stickiness are in agreement with reported sensory
303 attributes of GF pasta made with grains other than millet (Jeong et al. 2017, Wood, 2015; Wu,
304 Meng, Yang, Tao, & Xu, 2015). E-L was significantly more sticky, but less firm and less chewy
305 than other samples. These low sensory firmness scores correspond with its low instrumental
306 firmness (Table 3). Combined with the high bitterness scores, these texture ratings suggest that
307 E-L is less suited for pasta making than the other tested proso millet varieties.

308 3.7.4 Principle component analysis

309 A principle component (PC) analysis of the sensory variables listed in Table 4 and the
310 instrumental parameters from Tables 2 and 3 as supplementary variables effectively
311 distinguished samples (Figure 4). PC1 separated commercial controls from millet pasta, whereas
312 PC2 differentiated E-L from other millet pasta samples. The commercial pastas were had higher
313 cooking loss, elasticity and tensile strength, while the millet pastas had higher starchiness,
314 graininess and uniformity. Variables that had a high negative correlation (< - 0.85) with PC1
315 included cooking loss, elasticity and tensile strength; whereas starchiness and graininess had a
316 high positive correlation (> 0.85) with PC1. PC2 had a high negative correlation (< - 0.85) with
317 the perceived yellowness as well as the instrumental CIE*b values, and as a result separated E-L
318 and W from the other samples. PC2 had a high positive correlation (> 0.85) with sensory
319 firmness and chewiness values. Their location on the PC plot indicates that E-L and wheat pasta
320 were characterized by high yellowness, low firmness and low chewiness; the other three millet
321 pastas were of intermediate yellowness and firmness, and commercial GF pasta exhibited high
322 firmness and low yellowness. Graininess, starchiness, and uniformity were characteristic for all

millet pasta, while high tensile strength and elasticity were characteristic for both commercial controls.

4. Conclusions

This study showed that proso millet is a suitable raw material for fresh pasta. Encouraging findings include lower cooking loss for proso millet pasta compared to commercial pasta, and higher carotenoids and less rapidly digestible starch compared to commercial GF pasta. While millet pastas with higher amylose contents were rated higher for several textural attributes, overall millet pasta graininess and stickiness levels warrant improvement by recipe or processing optimization. More research is needed to further characterize how millet prolamins influence the quality of pasta and other products, as well as possible interactions among proteins and those between proteins and other constituents.

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439 **Figure Captions**

440 **Figure 1.** SDS-PAGE profiling of prolamins in millet flours. M, marker; D-L, Dawn cv. grown
441 at Lamberton; D-W, Dawn cv. grown at Lamberton; E-L, Earlybird cv. grown at Lamberton; E-
442 W, Earlybird cv. grown at Waseca; H-L, Horizon cv. grown at Lamberton; H-W, Horizon cv.
443 grown at Waseca; S_b-L, Snobird cv. grown at Lamberton; S_b-W, Snobird cv. grown at Waseca;
444 S_r-L, Sunrise cv. grown at Lamberton; S_r-W, Sunrise cv. grown at Waseca; S_u-L, Sunup cv.
445 grown at Lamberton; S_u-W, Sunup cv. grown at Waseca; HMW-prolamins, high molecular
446 weight prolamins. Brackets indicate the presence of HMW-prolamins in H-L and S_r-L that were
447 used as a selection criterion for pasta production.

448 **Figure 2.** Rapid (RDS; black bars) and slowly (SDS; gray bars) digestible starch (n=3) in millet-
449 based pasta and controls (commercial wheat and gluten-free pasta). Error bars denote standard
450 error, lowercase and uppercase letters indicate differences within RDS and SDS, respectively.

451 **Figure 3**. Percent *in vitro* protein digestibility of cooked millet pasta. Error bars represent
452 standard errors, different letters indicate significant ($P < 0.05$) differences among means
453 according to Tukey's HSD test.

454 **Figure 4.** Biplot of principal components 1 and 2 showing sensory variables listed in Table 4 in
455 bold, and instrumental variables listed in Tables 2 and 3 in red).

Table 1. Moisture, starch and protein content of millet-based pasta and controls (commercial wheat and gluten-free pasta).

Type	Raw pasta			Cooked pasta		
	Moisture (g/100g)	Starch (g/100g db^)	Protein (g/100g db)	Moisture (g/100g)	Starch (g/100g db)	Protein (g/100g db)
E-L	36.9	69.9^{c}	7.7^{b*}	63.6	69.9^{b}	4.6^{b}
H-L	33.2	72.4^{b,*}	7.6^{b*}	64.2	69.3^{b}	4.2^{c}
Sr-L	34.6	78.1^{a,*}	7.6^{b*}	66.6	72.2^{a}	4.0^{d}
Sr-W	34.9	71.7^{b}	7.4^{b*}	64.5	71.7^{a}	4.1^{cd}
GF	31.5	68.9^{c,*}	6.9^{c*}	58.7	72.1^{a}	4.3^{c}
Wheat	28.2	65.6^{d}	12.2^{a*}	60.4	66.2^{c}	6.7^{a}

Means (n =3) in a column followed by different letters denote differences among pasta type, while asterisks indicate differences in an attribute between raw and cooked pasta of the same pasta type. E-L, Earlybird cv. grown at Lamberton; H-L, Horizon cv. grown at Lamberton; S_r-L, Sunrise cv. grown at Lamberton; S_r-W, Sunrise cv. grown at Waseca.

Table 2. Yellowness (*b** values) and carotenoid content of millet-based pasta and controls (commercial wheat and gluten-free pasta).

Type	Raw pasta			Cooked pasta		
	Lutein (µg/g)	Zeaxanthin (µg/g)	*b* value	Lutein (µg/g)	Zeaxanthin (µg/g)	*b* value
E-L	12.38[a]	25.12[a]	39.38[a,#]	13.24[a]	27.45[a]	31.36[a]
H-L	10.10[c]*	22.47[b]	33.42[c,#]	9.04[c]	19.78[c]	27.97[b]
Sr-L	9.37[c]	20.47[c]	34.75[b,#]	9.11[c]	20.43[c]	25.82[c]
Sr-W	11.02[b]	24.57[a]	35.22[b,#]	10.85[b]	24.42[b]	27.64[b]
GF	0.80[d]	2.60[e]	19.10[e,#]	0.83[d]	2.91[e]	11.18[d]
Wheat	13.12[a]	8.24[d]	32.22[d,#]	13.81[a]	8.87[d]	27.96[b]

Different letters after means in the same column signify differences among pasta types.
express differences in an attribute between raw and cooked pasta. E-L, Earlybird cv. grown at Lamberton; H-L, Horizon cv. grown at Lamberton; S_r-L, Sunrise cv. grown at Lamberton; S_r-W, Sunrise cv. grown at Waseca.

Table 3. Cooking quality parameters of millet-based pasta and controls (commercial wheat and gluten-free pasta).

Pasta type	Optimal cooking time (min)	Water absorption (g/100g raw pasta)	Cooking loss (g/100g raw pasta)	Firmness (N)
E-L	1.83^{e}	71.75^{c}	2.11^{d}	3.64^{e}
H-L	2.72^{b}	86.96^{b}	2.36^{c}	5.18^{c}
Sr-L	2.73^{b}	94.70^{a}	2.24^{cd}	4.37^{d}
Sr-W	2.08^{d}	84.47^{b}	1.64^{e}	4.63^{d}
GF	3.60^{a}	65.68^{d}	4.82^{a}	10.31^{a}
Wheat	2.32^{c}	84.25^{b}	3.48^{b}	5.95^{b}

Different letters in a column indicate differences among pasta types.
E-L, Earlybird cv. grown at Lamberton; H-L, Horizon cv. grown at Lamberton; S_r-L, Sunrise cv. grown at Lamberton; S_r-W, Sunrise cv. grown at Waseca.

Table 4. Mean values (over all panelists and sensory replicates; N = 9) and F and p values (from ANOVA) of appearance, taste, and texture attributes that differed significantly among all six pasta samples (column 'all 6 samples'), high and low prolamin content pastas (column 'prolamin contrasts'), high and low amylose content pastas (column 'amylose contrast'), and between millet and commercial pastas (column 'millet vs control').

Sensory Attribute	Pasta type						All 6 samples		Prolamin contrasts		Amylose contrasts		Millet vs Control	
	Sr- L	H- L	Sr- W	E- L	GF	W	F value	p value	F value	p value	F value	p value	F value	p value
Appearance														
Gray	2.8^{b}	3.6^{b}	5.9^{a}	6.0^{a}	1.2^{c}	0.7^{c}	32.8	<0.001	49.2	0.000	1.4	0.242	111.7	<0.001
Yellow	$6.8b^{c}$	5.8^{c}	5.4^{c}	8.1^{ab}	2.7^{d}	9.1^{a}	20.7	<0.001	1.0	0.310	2.9	0.091	2.0	0.165
Uniform	7.3^{ab}	9.3^{a}	7.8^{ab}	7.6^{ab}	4.9^{b}	5.6^{b}	3.1	0.012	0.5	0.465	0.9	0.349	12.1	0.001
Basic Taste														
Saltiness	1.8^{b}	1.5^{b}	1.5^{b}	2.0^{b}	3.3^{a}	1.5^{b}	7.6	<0.001	0.1	0.743	0.1	0.730	10.5	0.002
Bitterness	1.2^{b}	1.4^{b}	1.1^{b}	2.9^{a}	0.8^{b}	0.8^{b}	7.3	<0.001	6.8	0.010	11.1	0.001	11.1	0.001
Texture														
Firmness	7.7^{ab}	7.4^{ab}	6.6^{bc}	4.3^{d}	8.5^{a}	5.5^{cd}	10.4	<0.001	19.0	<0.001	7.0	0.010	1.4	0.233
Chewiness	$7.^{6b}$	7.2^{b}	7.4^{b}	4.8^{c}	9.2^{a}	7.0^{b}	9.0	<0.001	7.8	0.006	10.3	0.002	11.0	0.001
Starchiness	5.8^{a}	6.6^{a}	6.6^{a}	7.0^{a}	2.1^{b}	2.8^{b}	16.1	<0.001	0.7	0.394	1.5	0.221	76.8	<0.001
Stickiness	8.9^{b}	10.1^{b}	10.3^{b}	13.5^{a}	10.7^{b}	6.3^{c}	11.0	<0.001	10.8	0.001	8.7	0.004	12.9	0.001
Elasticity	5.5^{b}	5.5^{b}	5.1^{b}	5.4^{b}	9.9^{a}	8.7^{a}	9.6	<0.001	0.1	0.788	0.0	0.865	45.9	<0.001
Tensile strength	2.7^{b}	3.1^{b}	4.2^{b}	3.5^{b}	8.8^{a}	7.8^{a}	13.2	<0.001	1.6	0.204	0.1	0.719	62.6	<0.001
Grainy	7.9^{a}	6.8^{a}	4.9^{b}	4.9^{b}	0.5^{c}	1.1^{c}	35.7	<0.001	24.1	<0.001	0.9	0.348	151.5	<0.001

E-L, Earlybird cv. grown at Lamberton; H-L, Horizon cv. grown at Lamberton; Sr-L, Sunrise cv. Grown at Lamberton; Sr-W, Sunrise cv. Grown at Waseca; GF, commercial gluten-free pasta; W, commercial wheat pasta. Sensory ratings within a row having letter superscripts in common did not differ significantly ($P > 0.05$). Aroma, flavor, and aftertaste values can be found in supplementary table 1.

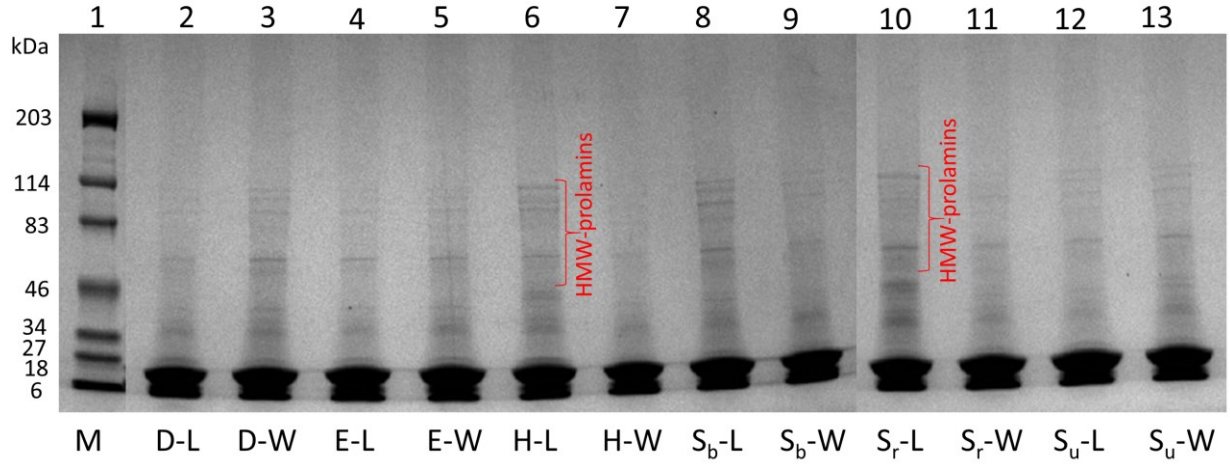
1 2 3 4 5 6 7 8 9 10 11 12 13
kDa
203
114
83
46
34
27
18
6
HMW-prolamins
HMW-prolamins
M D-L D-W E-L E-W H-L H-W S_b-L S_b-W S_r-L S_r-W S_u-L S_u-W

Figure 1.

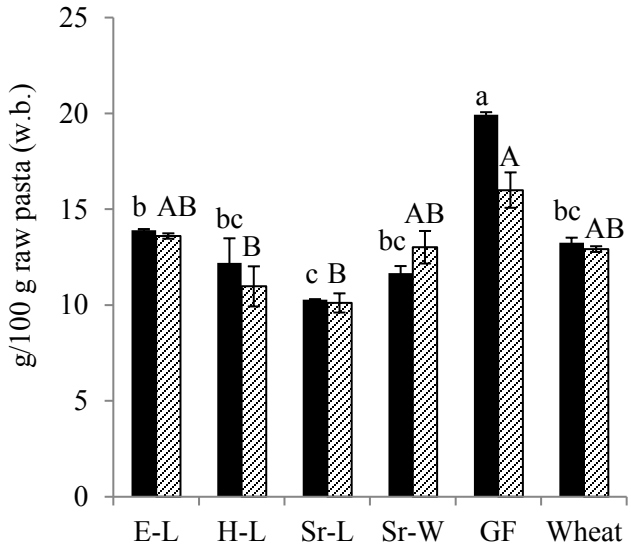
25
20
15
10
5
0
g/100 g raw pasta (w.b.)
b AB
bc B
c B
bc AB
a A
bc AB
E-L
H-L
Sr-L
Sr-W
GF
Wheat

Figure 2.

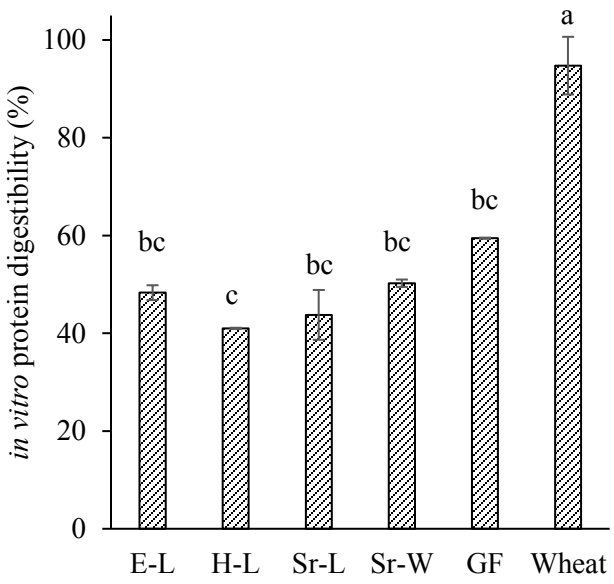
100
80
60
40
20
0
in vitro protein digestibility (%)
bc
c
bc
bc
bc
a
E-L
H-L
Sr-L
Sr-W
GF
Wheat

Figure 3.

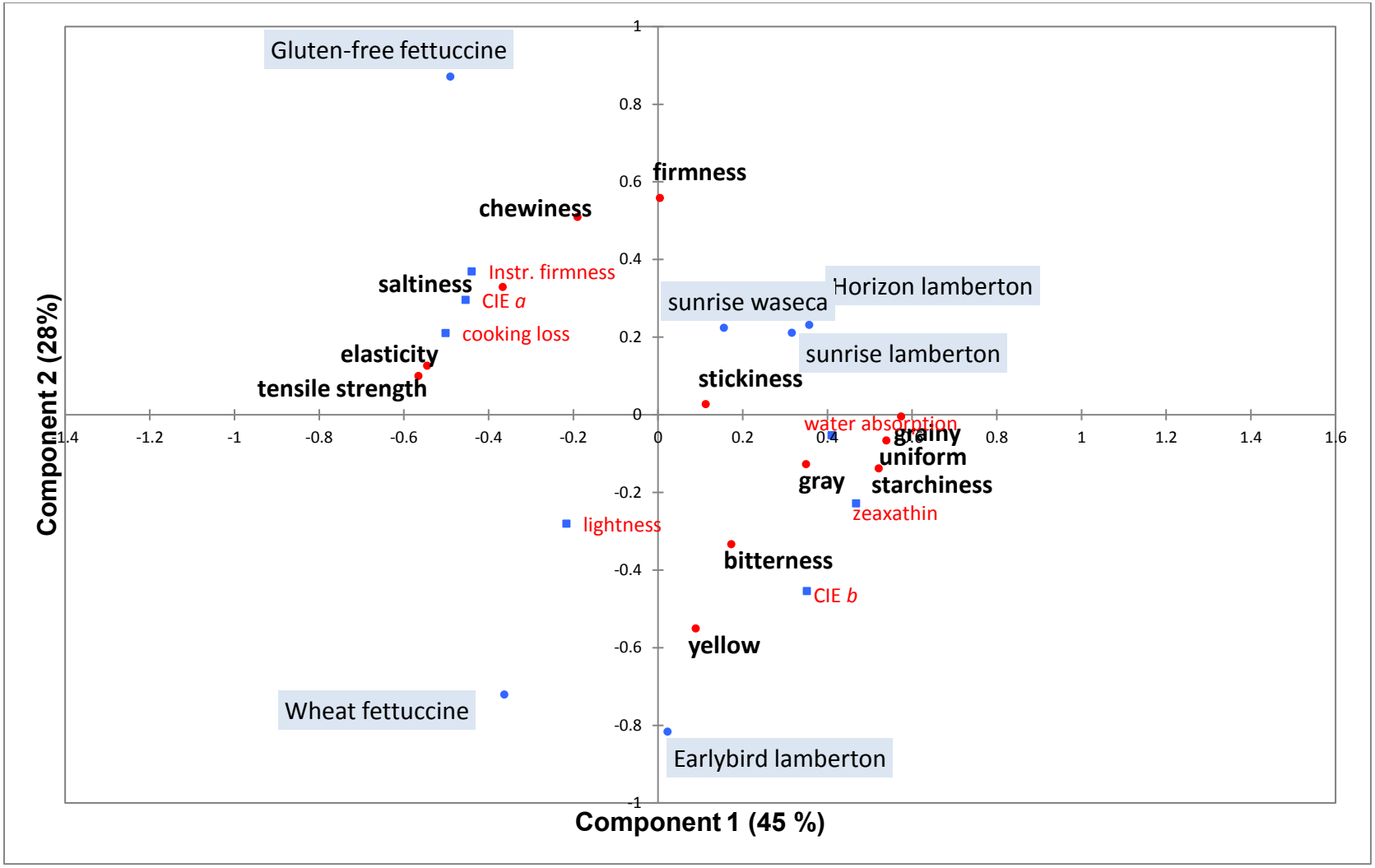
Gluten-free fettuccine
firmness
chewiness
saltiness
Instr. firmness
CIE a
cooking loss
elasticity
tensile strength
sunrise waseca
Horizon lamberton
sunrise lamberton
stickiness
water absorption
grainy
uniform
gray
starchiness
zeaxathin
lightness
bitterness
CIE b
yellow
Wheat fettuccine
Earlybird lamberton
Component 2 (28%)
Component 1 (45 %)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.4
-1.2
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6

Figure 4.