Digestibility, starch morphology and nutritive value of rusks made from wheat flour with addition of proso

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Running title: Proso lowers glycemic index and increases nutritive properties of rusks

Abstract:

Background and objectives: “Modern-diseases” that are result of “high sugar, low nutrient” diet are becoming more prevalent. Incorporation of “traditional” gluten-free cereals, like proso, in “everyday” food, such as rusks, would allow diabetics and people looking to increase fiber and nutrient content to include rusks in their diet.

Findings: Compared to wheat rusks, rusks with addition of proso showed higher content of microelements and essential amino acids. Also, higher content of dietary fiber and smaller and more regularly arranged starch granules increased digestibility of rusks with addition of proso. Additionally, rusks with addition of proso showed better antioxidative properties and had lower glycemic index when compared to wheat rusks.

Conclusions: Incorporation of proso flour in rusk production, could be beneficial not only to people suffering from diabetes, but also as way of improving general well-being. However, due to the decreased water absorption and therefore greater hardness and fracturability, our results suggested that addition of proso flour should be capped at 20 %.

Significance and novelty: To our knowledge, this is the first description of effects on different additions of proso on digestibility, starchmorphology, glycemic index and nutritive and antioxidative properties of rusks.

Keywords: starch, rheological properties, proso, glycemic index; *in vitro* digestion

1. Introduction

Due to the changes in lifestyle and consequent impaired function of immune system incidence of diabetes mellitus is rising in the recent years (Zhou et al., 2018). Additionally, content of starch and gluten in often consummated cereals (rice, wheat, corn and barley) significantly impacts condition of patients with diabetes mellitus. However, “gluten-free” cereals that also have high content of crude fiber and minerals, such as proso, could serve as healthy alternative to wheat(Das et al., 2019; Panghal et al., 2019; Romero et al., 2017).

Several factors affect digestibility of starch and nutritive value of cereal products. The biggest influence is exerted by: ratio of resistant to digestible starch, type and geometry of starch granules and interactions of starch with lipids and proteins (Rahman et al., 2007; Annor et al., 2017). Starch is composed of linear or slightly branched amylose and branch amylopectin and high amylose content is directly correlated with formation of resistant forms of starch and thus with slower digestibility of starch (Rahman et al., 2007).

Glycemic index (GI) was developed to allow for classification of food based on their level of blood glucose after meal.GI of bakery products depends not only on type(s) of cereal, but also from the processing conditions (Lau et al., 2015).For example, Marangoni and Poli (2008) and Priyadarshini et al. (2021) showed that oven-baked products, such as biscuits, exhibited lower GI. Thus, it is to be expected, that rusks will have different GI index compared to other bakery products. Additionally, content of dietary fibers could be important characteristics, since it influences lower levels of glucose in the blood (Maragoni and Poli, 2008).

Proso and wheat significantly differ in both nutritive value and content of vitamins, dietary fibers and type of proteins and fatty acids. In average, proso has 12% less starch, 3 times more crude fiber and 1.2 times more vitamins from B complex (Das et al., 2019). Also, content of prolamin is higher in proso (compared to wheat) which contributes not only to high essential amino acid index of proso t , but also impacts its solubility, surface hydrophobicity and emulsifying properties. Additionaly, content of calcium, iron, phosphorus, dietary fibers and polyphenols in proso is high (Kalina and Moudry, 2006).

Aim of this work is to determine nutritive, rheological and antioxidative properties, as well as glycemic index of rusks with addition of proso flour (in different amounts) and to establish connection between starch structure and changes in digestibility.

2. Materials and methods:

2.1. Materials: All chemicals, except of ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid), enzymes and bile salts, were purchased from Merck (Germany) and were p.a, grade. ABTS, Trolox and enzymes and bile salts (p.a. grade) used for *in vitro* digestion and amyloglucosidase were purchased from Sigma-Aldrich (USA).

2.2. Preparation of rusks: Wheat flour used in this work was “Type 400” with particles diameter 150 μm (“Aleksandrija”, Serbia). Proso flour was obtained from species *Panicum miliaceum L*. Four types of rusks were made:. “0% R” rusks were made entirely from wheat flour; while in “10% R”, “20% R” and “30% R” 10 %, 20 %and 30 % (w/w) of wheat flour was substituted with proso flour, respectively. Rusks were made by the following procedure: dough was prepared in a spiral mixer (Fimar 18/S 22 Ltr Spiral Dough Mixer). Fermentation was done at 40 °C (40 min for 0% R, 45 min for 10% R and 20% R and 50 min for 30% R) in a fermentation chamber (Lievi Real, Italy). Baking was done at 250 °C (40 min for wheat rusk, 45 min for 0% R, 10% R and 20% R and 50 min for 30% R) in an oven (Minel, Germany). After stabilization (8h), rusks were dried for 35 min at 180°C.

2.3. Characterization of the dough: Rheological properties of dough were determined according to standard ICC methods (no. 114/1, 115/1) using Brabender farinograph and extensograph (Duisburg, Germany). Thermo-mechanical properties were monitored according to manufacturer instruction using a Mixolab apparatus (Chopin Technologies, France). Mass of measured dough was 75 g.

2.4. Texture analysis of rusks: Texture Analyzer (Stable Micro Systems, Surrey, UK) was used to measure force in compression following the three-point bending protocol. Measurements were done according to following parameters: max. load- 30 kg with 3-point bending rig as a fixture; probe test speed -5 mm/s; travelling distance- 8 mm; trigger force- 50 g, the length between the supports -36 mm. In this test, maximal force at the point of fracture was measured as an indicator of sample hardness whereas the anvil distance at fracture point characterized the fracturability of the sample.

2.5. Sensory analysis of rusks: Sensory properties of rusks were marked by 5 examiners, using grade system (with 1 being very negative and 5 being very positive).

2.6. Determination of starch granule type and morphology: Prior to analysis, samples were coated with gold film (Bal-Tec sample sputter/coater 050; t = 100 s; I = 30 mA). Starch granule type and morphology were analyzed by SEM (scanning electron microscopy) (Model JSM 6390, Jeol, USA).

2.7. Determination of mineral content, content of dietary fibres and starch: Content of zinc, potassium, calcium, sodium, magnesium, copper and iron was determined using standardized SRPS EN 13805: 2008 method (“Foodstuffs - Determination of trace elements - Pressure“).Content of dietary fibers was measured using standardized AOAC 985.29 method (“Total dietary fibers in foods, enzymatic-gravimetric method”), with kit Megazim K-TDFR

12/05. Starch content was measured using standardized method ISO 10520: 1997 ("Native starch – Determination of starch content – Ewers polarimetric method").

2.8. Determination of content of amylose and amylopectin: Contents of amylose and amylopectin amylose were determined spectrophotometrically using iodine reagent according to the method by Jarvis and Walker (1993).

2.9. *In vitro* digestion procedure: Enzymatic *in vitro* digestion, mimicking gastric and intestinal phases of digestion, was performed according to procedure by Chandrasekara and Shahidi (2012). Briefly, 1 g of previously ground sample was placed in Erlenmeyer flask and 15 ml of distilled H_2O and 10 mL of 0.85% (w/v) sodium chloride solution. Erlenmeyer flask was closed and sample was incubated in orbital shaker at 37 °C for 10 min at 120 rpm. After 10 min, 1 mL of porcine α-amylase (50 units/mL, EC 232-565-6) diluted in 20 mM sodium phosphate buffer (pH 6.9) containing 1 mM calcium chloride was added. After 5 min, 4.5 mL of 0.15 M hydrochloric acid was added and sample was shaken for 2 min (37 °C, 120 rpm). After desired pH value (2.5) was reached, 1 ml of pepsin (20 mg/mL, EC 232-629-3) dissolved in 20 mM hydrochloric acid was added. Then sample was incubated for 2 h (37 °C, 120 rpm) to imitate conditions present during gastric phase of digestion. Afterwards, 4 mL of bile salt (150 mg/mL), dissolved in 0.15 M sodium bicarbonate, 4 mL of porcine pancreatin (18.75 mg/mL, EC 232-468-9) diluted in 0.15 M sodium bicarbonate and 1 mL of porcine mucin (75 mg/mL, EC 282-010-7) dissolved in distilled water were added and sample was incubation was incubated for 3 h (intestinal phase of digestion).

2.10. Determination of glycemic index: Supernatants recovered from enzymatic digestion were centrifuged at 14000 rpm for 10 min. Total starch (TS) was determined according to procedure by Goni et al. (1997). In short, after centrifugation, sample was dispersed in 2M KOH (3 ml) by shaking (30 min, room temperature). Afterwards, Na-acetate buffer (pH=4.75) was added (3 ml) and the sample was treated by amyloglucosidase (E.C. 3.2.1.3; 30-60 U/mg, 100 µl) for 45 minutes at 60°C in the orbital shaker. Determination of percentage of hydrolyzed total starch were taken at six different times (20, 40, 60, 90, 120, 150 and 180 min). Starch was measured as glucose, using a glucose oxidase assay (GOD-PAP reagent, Sigma-Aldrich). Glucose content was measured according to manufacturer instructions. Content of glucose was transformed to starch content using conversion factor 0.9. Hydrolysis index (HI) of starch was calculated as the ratio between the area under hydrolysis curve (AUC) for rusk and the AUC for white bread, expresses as a percentage. GI was calculated using equation given by Ferrer-Mairal et al. (2012):

$$eGI = 0.862 + calcHI + 8.198$$

where eGI is expected GI and calcHI is calculated hydrolysis index.

2.11. Determination of protein composition and amino acid analysis: Total protein content was measured using Kjedhal method (1883) with conversion factor 6, 25. Determination of amino acid composition was performed using standardized ISO 13903: 2011 (“Animal feeding stuffs – Determination of amino acid content”) on apparatus ICS 5000 (Thermo scientific, US) with column AminoPac PA10 guard. Sample mass was 50 mg.

2.12. Determination of antioxidative properties: Antioxidative properties were determined by ABTS assay using procedure by Re et al. (1999). Trolox was used as a standard and results are expressed in µmol Trolox equivalents/g.

2.13. Statistical analysis: All measurements were done in triplicate and results are expressed as average value ± SD (standard deviation). To access differences between samples, Tukey test was used. Results of were analyzed in SPSS software version 24 (IBM, Armonk, New York, United States). All statistical analyses were done at significance level $p < 0.05$.

3. Results and discussion

3.1. Characterization of dough: As could be seen from Table S1, water absorption decreased proportionally with addition of proso flour. These results are in accordance with the findings of Vijayakumar & Mohankumar (2009) who observed decrease in water absorption in millet-wheat-soy composite doughs with increased doses of millet. Given that the differences in total starch content in wheat (63-74 % of dry mass) and in proso (63-68 % of dry mass) (Lullien-Pellerin, 2020) are relatively small, observed differences could be explained by "entrapment" of water molecules in "loose" protein network (Kalinova and Moudry, 2006; Ortolan and Steel, 2018; Li et al., 2020).. Unlike in wheat where gliadins and glutenins are linked via non-covalent bonding (Ortolan and Steel, 2018), in proso prolamins , albumins, globulins and glutelins () don't form gluten-like network (Kalinova and Moudry, 2006), leaving more space for water molecules. Similar results were reported by Bojnanska et al. (2021) for doughs with addition of legume flour and Culetu et al. (2019) for dough with addition of soryz. Weaker protein network could also explain shorter development time, decreased dough stability and higher degree of softening of dough with added millet flour. However, dough with added 30 % of millet flour had same degree of softness as dough made with only wheat flour. Although this should be further examined, it is possible that different type of starch granules were present and/or that new bonds were formed between proso proteins and starch (Li et al., 2020). Weakening of the protein network can also be observed through decrease in C2 value with addition of millet flour (Table S2), which is a consequence of gluten dilution (Bojnanska et al., 2021). Similarly to results by Onipe et al. (2017), we found that water absorption values were negatively correlated with R/E (resistance/extensibility) ratio (Table S1). These findings can be attributed to gluten dilution (Onipe et al., 2017). Lower extensibility of doughs with addition of proso (Table S1) was also due to gluten dilution and presence of dietary fibers in proso. These results are in accordance with results obtained for bread with addition of finger millet flour (Panghal et al., 2019).

Both C3 value (gelatinization ability) and C4 value (reflection of amylase activity during heating stage) monitor changes in dough viscosoelastic properties resulting from changes in starch structure. Viscosity increased with addition of millet flour (Table S2), which suggests stronger bonds in starch granule, higher amylopectin/amylose ratio in native millet starch and association of starch granule. These findings are in accordance with the results reported by Shimray et al. (2012) and Panghal et al.(2019). C5 values (representative of starch retrogradation) decreased with addition of millet flour, which suggest that millet starch is susceptible to retrogradation. This susceptibility could be explained by geometry and characteristics of starch granule in proso (further discussed in section 3.3).

3.2. Texture profile analysis of rusks: Results of textural analysis showed that toast bread supplemented with millet flour tended to be more fragile and less hard, especially at 30% supplementation level (Table S3). These results are consistent with results reported by Shimray et al. (2012) for biscuits with addition of finger millet flour. Proso had different geometry and type of starch granules compared to wheat (Figures 1 and 2), different composition of proteins, higher content of dietary fibers (Table 2) and lower amylose/amylopectin ratio, which is in accordance with results by Lullien-Pellerin (2020), Das et al. (2021) and Kalinova and Moudry (2006). Aoki et al. (2020) and Zhou et al. (2021) showed that all of these factors contribute to increased fracturability and lower hardness thus difference in hardness between wheat rusks and rusks with addition of proso is probably joined effect of all these parameters. .

3.3. Starch granule type and morphology: Starch granules in wheat and proso flour were of different size and shape (Figure 1). Starch granules in wheat flour were significantly larger than starch granules in proso flour, with size range 14-30 μm (Figure 1 B and D). Additionally, unlike granules in proso flour, starch granules in wheat flour were spherical and immersed in protein network. These results are consistent with results obtained by Verma et al. (2018). Size of starch granules in proso flour was in range 2 – 10 μm (Fig. 1 A and 1 C). Two shapes of granules were present – round and polygonal. This is not unusual, since both shape and size of starch granules varies depending on type of millet (Verma et al., 2018). Since granules were regularly placed, orderly structure resembling honeycomb was observed. SEM images of dough made with 10% of proso flour (Fig 2B) showed increased number of small, round granules (compared to proso flour) and decreased number of large, spherical granules (compared to wheat flour, Fig. 2A). This increase in number of small, round starch granules was even more pronounced in case of dough made with 20 % of proso flour (Fig 2C) and dough made with 30 % of proso flour (Fig 2D). Alongside with decrease in number of large, spherical wheat starch granules percentage of "sticky" protein network was also decreasing in samples with higher percentage of proso flour (probably due to the lack of gluten in proso) (Kalinova and Moudry, 2006). Observed deviation from round and spherical form of starch granules might be explained by effect of heat during preparation of rusks (Yang et al., 2019).

3.4. Difference in mineral composition between wheat and proso flour: Content of microelements (one of parameters of nutritive value) in the proso flour was significantly higher than in the wheat flour (Table 1). While wheat flour contained more macroelements, namely Ca and Na, proso flour had significantly more microelements: Fe, Zn, Cu, as well as almost three-times higher concentration of Mg. Due to involvement of Zn, Fe and Mg in stimulation of immune response (such as increase in number of T helper and natural killer cells, interferon expression, etc.), inclusion of proso in diet would result in lower risk of infection and boost in immunity (Gombart et al., 2020).

3.5. Content of water, ash, dietary fiber and starch in flours and rusks: Content of ash in proso flour was2.28 times higher compared to ash content in wheat flour (Table 2). Higher ash content was a consequence of higher mineral content in proso (Table 1). Moisture content in wheat and proso flour showed no significant differences. Proso flour contained more than 3 times higher amount of dietary fiber compared to wheat flour (Table 2). This is consistent with results of Das et al. (2019) and given the role that dietary fiber play in prevention of diseases affecting digestive and cardiovascular system (Zhou et al., 2021), rusks incorporating proso represent better choice for people struggling with digestion or cardiovascular health. Amount of total starch in wheat and proso flour was similar (Table 2). Therefore, there was no significant difference in amount of total starch in rusks (Table 2). Lower amount of total starch in rusks compared to both proso and wheat flower could be explained by degradation of starch during thermal processing (Lullien-Pellerin, 2020).

3.6. Content of amylose and amylopectin: Although total amount of starch in wheat and proso flour was similar, proso flour contained less amylose compared to wheat flour (Table 2). This is agreement with results reported by Kalinova and Moudry, 2006 and Das et al. (2019). Lower amount of amylose in proso flour could explain why rusks made with higher ratio of proso flour had lower amylose content (Table 2). Due to lack of branching, amylose have tendency to form complexes, especially with lipids, during thermal processing (Zhou et al., 2021) . Therefore, higher content of amylose would result in lower digestibility, which is in agreement with results by Zhu et al. (2011) who observed higher amount of amylose in resistant and slowly digestible starches. This tendency for amylose-lipid complex formation coupled with lower amylose content in proso could explain variability in rusks' digestibility.

3.7. Sensory properties of rusks: Although smell was marked as similar for wheat and proso rusks, other parameters (appearance, texture and taste) showed more variability (Table 3). 10 % rusks were ranked the highest for appearance, while 20% rusks were deemed as the tastiest. Overall, texture of rusks with addition of proso was more appealing than texture of wheat rusks (Table 3). These data suggest that rusks with addition of proso ranked better in terms of consumer satisfaction compared with wheat rusks.

3.8. Protein content and content of essential amino acids: Both wheat and proso flour had similar protein content (11.52 % (w/w) and 11.06 % (w/w), respectively), which could explain why there was no statistically significant difference in protein content between rusks (Table 4). These findings are in agreement with data reported in literature (Kalinova and Moudry, 2006). However, content of certain essential amino acid showed variation when rusks with addition of proso flour were compared to wheat rusks (Table 4). Content of histidine, leucine, phenylalanine and methionine was higher in all rusks made with addition of proso flour, while content of isoleucine was higher in 20 % and 30 % rusks (compared to rusks made entirely from wheat flour). Observed differences could be explained by higher content of essential amino acids (namely, leucine, isoleucine and methionine) in proso flour compared with wheat flour (Kalinova and Moudry, 2006; Das et al., 2019). Since diet with high essential amino acid content was proven to increase muscle protein anabolism, influence insulin concentration, improves mitochondrial function and modulates lipid metabolism (Anthony et al., 2013), incorporation of nutritively more valuable rusks with addition of proso could significantly improve overall health.

3.9. Glycemic index (GI): Starch hydrolysis rate of wheat rusks was higher compared to both 20 % rusks and 30 % rusks (46.8 and 50.9 in 180th minute of digestion for 20 % rusks and 30 % rusks, respectfully) (Figure 3). This results are similar to those reported by McSweeney et al. (2017) for muffin, couscous, extruded snack, and porridge with proso flour. Several factors might contribute to slower digestibility of rusks with addition of proso: 1. more regular, crystalline structure of starch granules in proso leads to the slower digestibility and lower GI (Figures 1 and 2) (de la Rosa-Millán, 2017); 2. Higher amylose content compared to wheat (Table 2) could cause slower digestibility, due to the possibility of formation of amylose-protein and/or amylose-lipid complexes (Zhu et al., 2011); 3. Presence of slowly-digestibly and resistant starch form in proso decreases its GI (Bangar et al., 2021; Kumar et al., 2023). Given that starch hydrolysis rate and GI were the lowest for 20 % rusks, we could conclude that diet including these rusks would be beneficial for diabetic patients.

3.10. Antioxidative properties: Addition of proso flour improved antioxidative properties of rusks. Antioxidative capacities of rusks were: for 0% R – 2.89 ± 0.05 μmol TE (Trolox equivalents)/g; for 10% R – 2.94 ± 0.02 μmol TE/g; for 20% R – 3.02 ± 0.03 μmol TE/g; for 30% R – 2.93 ± 0.04 μmol TE/g. This improvement was particularly evident in 20 % rusks which corresponds to our previous results where antioxidative properties were assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Poleksic et al., 2018) and is consistent with results obtained by Aydin (2022). However, 30% rusks had lower antioxidative capacity than 20 % rusks. Irondi et al. (2022) and Jimenez-Pulido et al. (2022) found that formation of starch-protein and/or lipid-protein complexes diminishes protein content, which, in turn, decreases antioxidative capacity.. However, further experiments are necessary to verify this hypothesis.

4. Conclusion

Rusks with addition of proso flour had lower glycemic index compared with rusks made entirely of wheat flour. Addition of proso also increased content of microelements (namely Fe, Zn, Cu and Mg), essential amino acids (in particular leucine, isoleucine and methionine), dietary fiber and improved antioxidant properties. Lower digestibility of rusks with addition of proso flour compared to wheat results could be consequence of presence of more regular and smaller starch particles and higher content of amylose; however, further experiments are needed to conform this hypothesis. Our results suggest that consumption of rusks with addition of proso could improve overall health, especially of people suffering from diabetes.

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6. Conflict of Interest:

Autors declare no conflict of interest.

7. Supplementary Material:

Table S1: Rheological properties of dough with different added content of millet flour; Table S2: Thermo-mechanical properties of dough with different added content of millet flour; Table S3: Texture profile analysis of rusks with different added content of millet flour.

8. Author Contributions:

Authors MP, DV, VR and VR conceived experimental design. Authors MP, DV, MS, EB, BF, LJD, VP, VR and VR preformed experimental and statistical analysis. Authors MP and VR interaction wrote original draft. Authors BF, LJD, VP, VR, MD and BVR helped to design experiments, analysed and discussed data, and helped to write the final manuscript.

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Tables

Table 1: Difference in mineral composition between wheat and proso flour*

Sample	Mineral content (mg/kg) (on dry basis)						
	Ca	K	Na	Mg	Fe	Zn	Cu
Wheat flour	796 ± 2.8^{a}	1520 ± 3.4^{a}	190 ± 1.1^{a}	431 ± 3.6^{a}	1.55 ± 0.6^{a}	4.67 ± 0.4^{a}	7.30 ± 0.5^{a}
Proso flour	230 ± 1.3^{b}	1668 ± 2.2^{b}	176 ± 1.4^{b}	1210 ± 4.4^{b}	4.38 ± 0.9^{b}	20.7 ± 0.8^{b}	25.6 ± 1.3^{b}

*Different letters at the same column means that differences between samples were statistically significant (determined by Tukey test at $p < 0.05$)

Table 2: Content of ash, water, dietary fibers, total starch, amylose and amylopectin in flours and rusks (on dry basis)*

Sample	Moisture (% - w/w)	Ash (% - w/w)	Dietary fiber (% - w/w)	Total starch (% - w/w)	Amylose (% - w/w)	Amylopectin (% - w/w)
wheat flour	11.21 ± 1.33^{a}	0.45 ± 0.05^{a}	1.82 ± 0.2^{a}	72 ± 13^{a}	13.6 ± 1.52^{a}	58.4 ± 6.1^{a}
proso flour	10.30 ± 1.28^{b}	1.03 ± 0.17^{b}	7.12 ± 0.95^{b}	70 ± 16^{a}	8.8 ± 1.09^{b}	61.2 ± 7.3^{b}
10% R	6.41 ± 1.09^{c,d}	2.36 ± 0.32^{c}	3.08 ± 0.32^{c}	66 ± 12^{b}	11.2 ± 1.78^{c}	54.8 ± 4.9^{c}
20% R	6.12 ± 1.11^{c}	2.25 ± 0.27^{c}	3.91 ± 0.47^{d}	66 ± 9^{b,c}	10.1 ± 1.22^{c,d}	55.9 ± 5.8^{c}
30% R	6.28 ± 1.04^{c}	2.46 ± 0.19^{c,d}	4.07 ± 0.55^{d}	66 ± 11^{b}	9.4 ± 1.16^{d,e}	56.6 ± 6.2^{a,c}

* 0% R- rusks made entirely from wheat flour, 10% R - rusks made with 10 % (w/w) proso flour, 20% R - rusks made with 20 % (w/w) proso flour, 30% R - rusks made with 30 % (w/w) proso flour; Different letters at the same column means that differences between samples were statistically significant (determined by Tukey test at $p < 0.05$)

Table 3: Sensory properties of rusks

Sample Properties	0% R	10% R	20% R	30% R

Appearance	3.65 ± 0.14^{a}	3.9 ± 0.13^{b}	3.35 ± 0.14^{c}	3.42 ± 0.12^{a,c}
Texture	3.9 ± 0.13^{a}	4.3 ± 0.21^{b}	4.4 ± 0.22^{b}	3.95 ± 0.21^{a}
Smell	4.3 ± 0.2^{a}	4.35 ± 0.14^{a}	4.1 ± 0.13^{a,b}	4.2 ± 0.27^{a,b}
Taste	4.45 ± 0.12^{a}	4.5± 0.05^{a}	4.7 ± 0.11^{b}	4.55 ± 0.1^{a}

* 0% R- rusks made entirely from wheat flour; 10% R - rusks made with 10% (w/w) proso flour, 20% R - rusks made with 20% (w/w) proso flour, 30% R - rusks made with 30 % (w/w) proso flour; Different letters at the same row means that differences between samples were statistically significant (determined by Tukey test at $p < 0.05$)

Table 4: Protein content and content of essential amino acids in rusks*

Sample / Properties	0% R	10% R	20% R	30% R
Proteins (%- w/w)	11.21 ± 0.1^{a}	11 ± 0.1^{a}	11.11 ± 0.1^{a}	11.06 ± 0.1^{a}
Lysine (mg/g)	0.205 ± 0.064^{a}	0.223 ± 0.014^{a}	0.207 ± 0.034^{b}	0.170 ± 0.059^{c}
Valine (mg/g)	0.336 ± 0.056^{a}	0.389 ± 0.058^{b}	0.397 ± 0.035^{b,c}	0.384 ± 0.051^{b}
Threonine (mg/g)	0.329 ± 0.022^{a}	0.358 ± 0.025^{b}	0.382 ± 0.025^{c}	0.365 ± 0.031^{c,d}
Histidine (mg/g)	0.158 ± 0.016^{a}	0.209 ± 0.018^{b}	0.230 ± 0.025^{c}	0.194 ± 0.030^{a,b}
Leucine (mg/g)	0.512 ± 0.067^{a}	0.760 ± 0.027^{b}	0.904 ± 0.072^{c}	0.786 ± 0.062^{b}
Isoleucine (mg/g)	0.234 ± 0.054^{a}	0.297 ± 0.022^{a,b}	0.341 ± 0.037^{c}	0.333 ± 0.028^{c}
Phenylalanine (mg/g)	0.506 ± 0.082^{a}	0.612 ± 0.079^{b}	0.676 ± 0.089^{c}	0.639 ± 0.097^{b,c}
Methionine (mg/g)	0.112 ± 0.022^{a}	0.214 ± 0.024^{b}	0.232 ± 0.043^{c}	0.247 ± 0.058^{c}

* 0% R- rusks made entirely from wheat flour; 10% R - rusks made with 10% (w/w) proso flour, 20% R - rusks made with 20% (w/w) proso flour, 30% R - rusks made with 30 % (w/w) proso flour; Different letters at the same row means that differences between samples were statistically significant (determined by Tukey test at $p < 0.05$)

Figures captions

Figure 1: SEM images of starch granules in wheat flour and proso flour. A-starch granules in proso flour (magnification-1000x); B-starch granules in wheat flour (magnification-1700x); C-starch granules in proso flour (magnification-4500x); D-starch granules in wheat flour (magnification-2000 x)

Figure 2: SEM images of starch granules in dough made with addition of different amount of proso flour. A-starch granules in dough made from wheat flour (magnification-2000x); B-starch granules in dough made with 10% proso flour (magnification-1500x); C-starch granules in dough made with 20% proso flour (magnification-1000x); D-starch granules in dough made with 30% proso flour (magnification-1000 x)

Figure 3: Starch hydrolysis curves for rusk made entirely of wheat flour (marked as 0 % (w/w)), rusks made with 20 % (w/w) proso flour (marked as 20 %) and rusks made with 30 % (w/w) proso flour (marked as 30 %). Due to the insufficient amount of sample, starch hydrolysis curve for rusks made with 10 % (w/w) proso flour was not shown

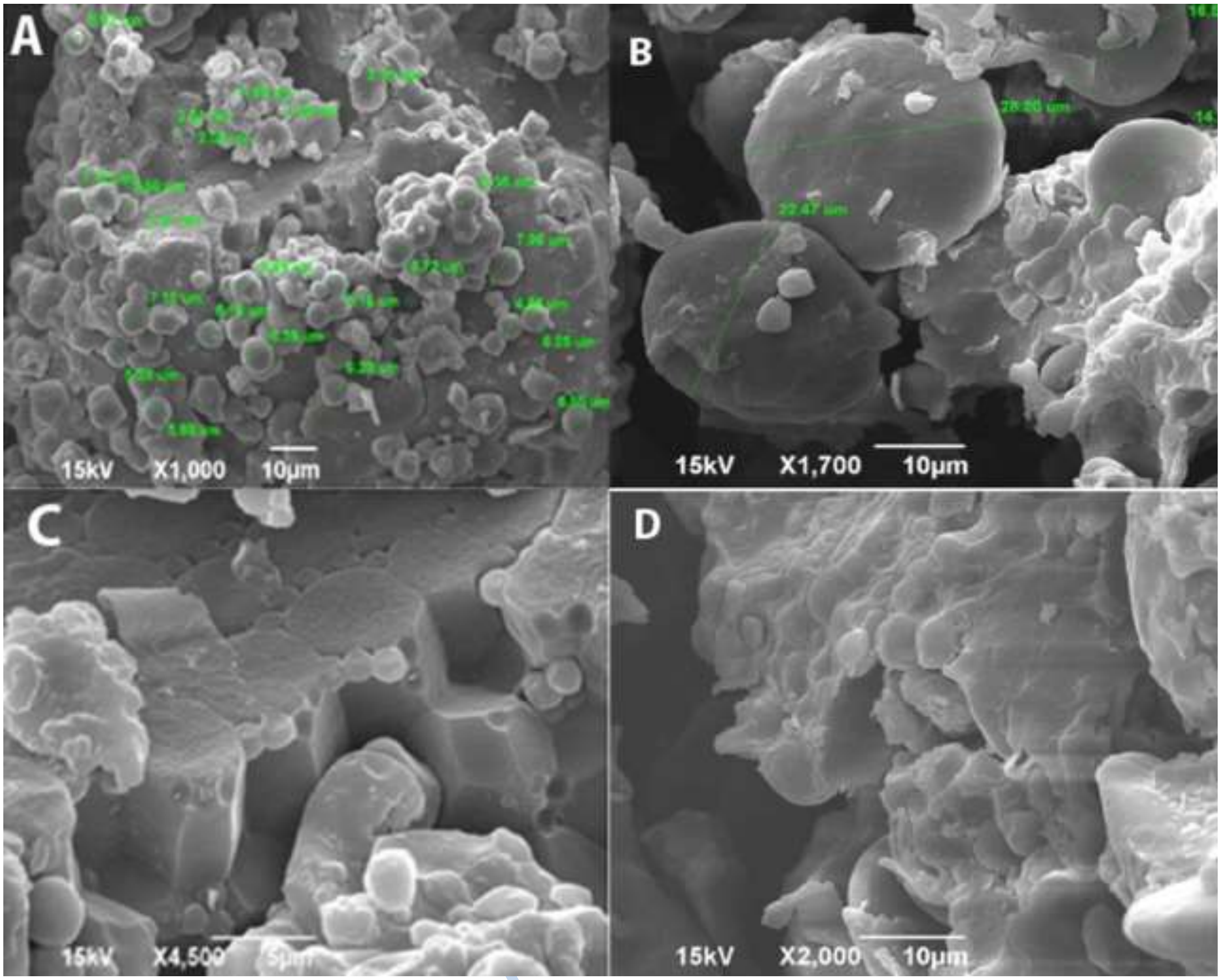
A
B
28.20 µm
22.47 µm
C
D
15kV X1,000 10µm
15kV X1,700 10µm
15kV X4,500 5µm
15kV X2,000 10µm

Figure 1

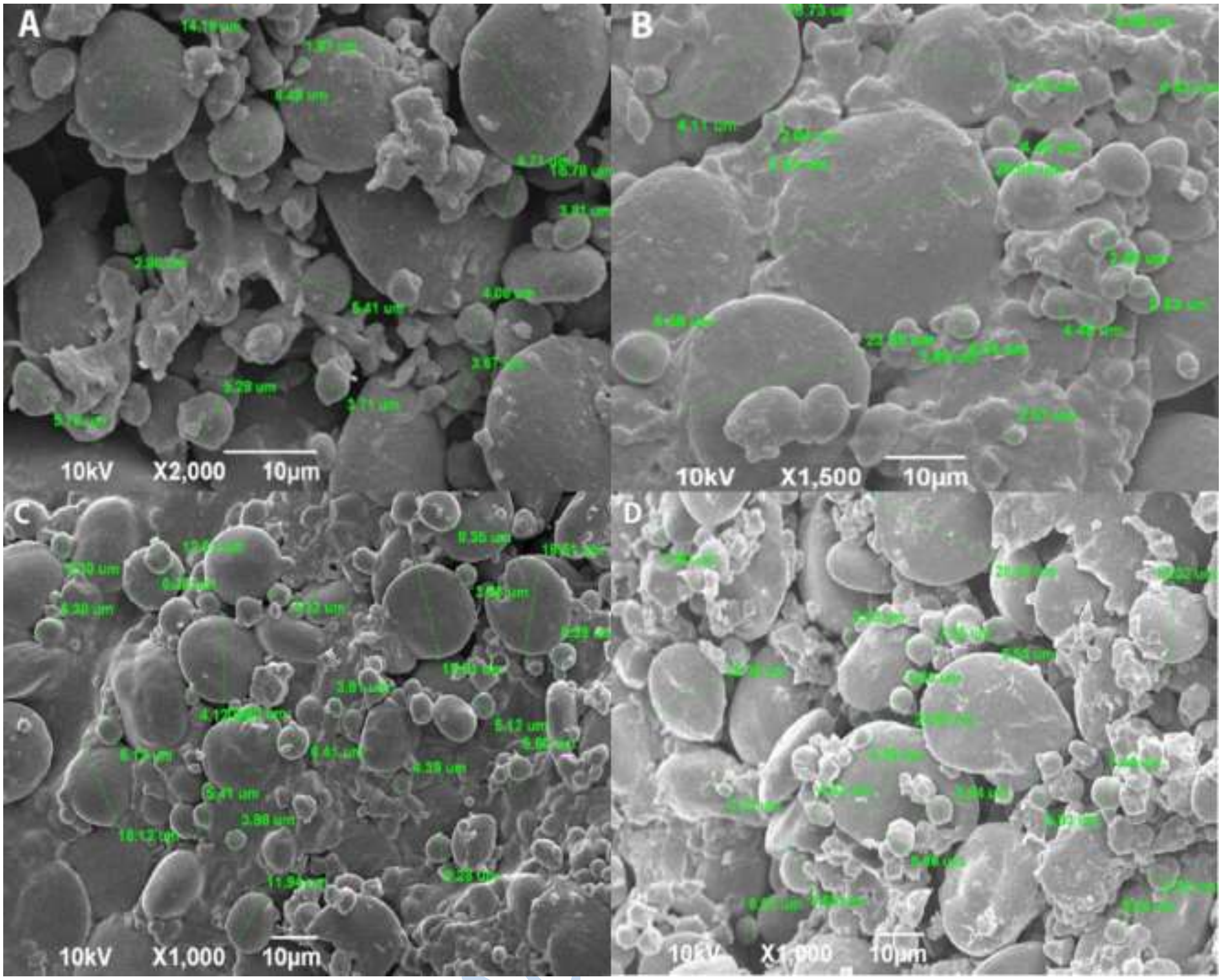
A
10kV X2,000 10μm
B
10kV X1,500 10μm
C
10kV X1,000 10μm
D
10kV X1,000 10μm

Figure 2

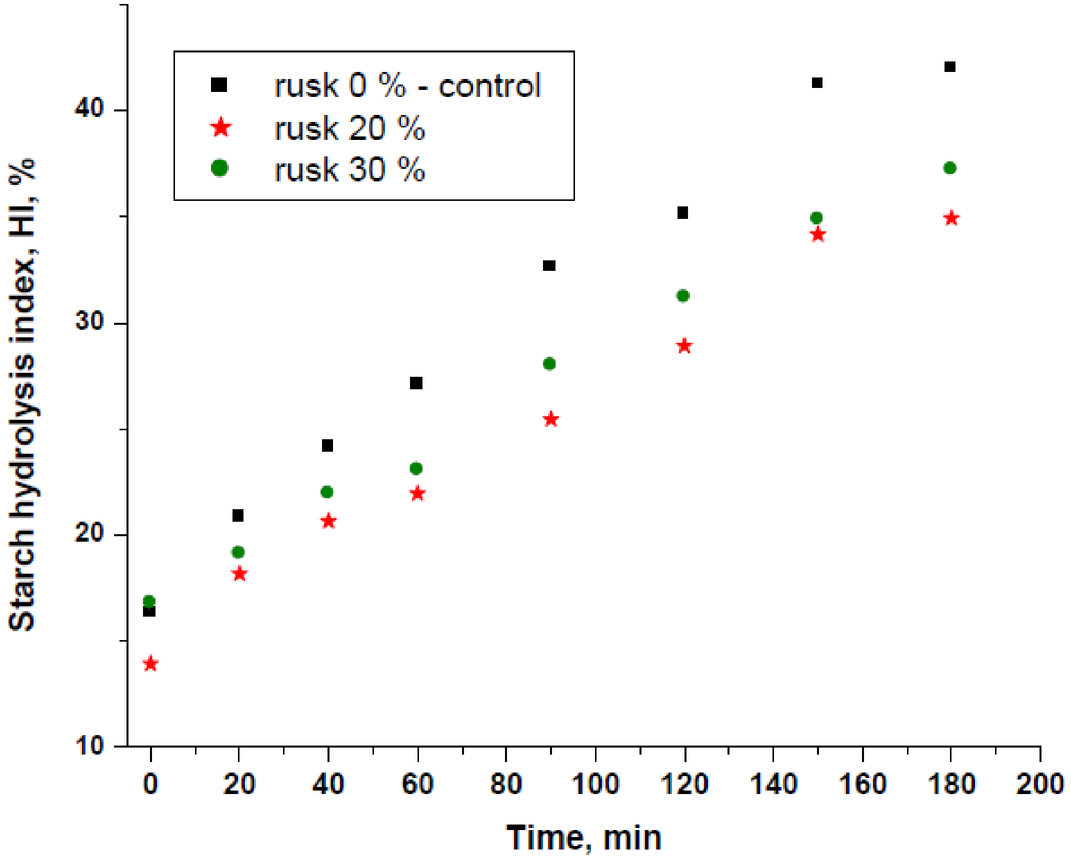
■ rusk 0 % - control
★ rusk 20 %
● rusk 30 %
Starch hydrolysis index, HI, %
10
20
30
40
0
20
40
60
80
100
120
140
160
180
200
Time, min

Figure 3