Glucose Release During *in Vitro* Digestion of Wheat

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Abstract

The research investigated the dynamics of released glucose from whole wheat flour during *in vitro* gastric and intestinal digestion. Two experiments were conducted. Experiment 1 (gastric digestion) was an enzymatic hydrolysis with a pepsin solution at pH 2.0 and 37°C for 120 minutes. In experiment 2, first step was followed by the hydrolysis with the multi-enzyme pancreatin (intestinal digestion), at pH 6.8 and 37°C for 4 h. The released glucose (RG) and the refractive index (RI) were determined from the supernatant obtained after centrifugation. Experiments showed an initial increase in the glucose released from 1 g wheat up to 0.472 mg/mL after 1 h of gastric digestion, then followed by decrease. The lowest value was recorded after 2 hours of gastric digestion (0.332 mg/mL). The concentration of the reducing sugars increased throughout the intestinal digestion from 0.549 mg/mL up to 1.990 mg/mL, as a result of starch hydrolysis to dextrins, maltose and then to glucose. The high positive correlation between RG and RI suggests that RI may be used as a simple and rapid method for estimating the released glucose during *in vitro* digestion.

Keywords: wheat, glucose, gastric digestion, intestinal digestion

1. Introduction

The predominant polymers in cereals are arabinoxylans (AX), mixed linkage (1→3; 1→4)-β-glucans (β-glucans), cellulose, and the noncarbohydrate component lignin [1,2].

Cereal grains contain various amounts of non-starch polysaccharides (NSPs), that depends on the species and tissue type [3]. The NSP is lower in wheat kernel (1.14 g kg^{-1}) than in rye (1.32 g kg^{-1}) and barley (1.67 g kg^{-1}). AXs represent the main non-cellulosic NSP component in wheat (0.6-0.8 g kg^{-1}) and rye (0.89 g kg^{-1}), while β-glucans are the predominant NSP in barley (0.76 g kg^{-1}) [4]. The molecular weight of β-glucans is higher than that of AX, and both polymers contribute to the viscosity of the extract [5].

Cereal grains are classified, according to their content of soluble NSPs, into viscous and non-viscous cereals. Rye, barley, oats, wheat and triticale are "viscous grains" as they contain considerable amounts of soluble NSPs, whereas corn, sorghum, millet and rice, which contain negligible amounts of soluble NSPs, are known as "non-viscous cereals" [6].

The endosperm of wheat grains contain 2-7% cell wall material, mostly of NSP [7]. The major components of NSPs are pentosans which are the essential structural elements of cereal cell [8]. The main pentosans are AXs, along with small amount of arabinogalactans (AG) [9]. AX constitutes a larger proportion (85%) of the NSP found in wheat grain [10].

In cereal grains with high NSP concentrations, water soluble arabinoxylans and β-glucans are responsible for increased intestinal viscosity and reduced starch, fat, and protein digestibility. Arabinoxylans account for approximately 8.8 g kg^{-1} of wheat endosperm cell wall polysaccharides, of which one third to one half is soluble in water [1].

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Exogenous carbohydrases are most commonly used when the dietary ingredients contain relatively higher amounts of fiber.

Our previous research have demonstrated that soluble NSP in wheat increased *in vivo* intestinal viscosity, which slows down the rate of diffusion of substrates, digestive enzymes, and final products of digestion, and therefore affect the digestion and utilization of nutrients [11].

The antinutritive role of arabinoxylans in wheat is explained by two mechanisms [12,13]. The first mechanism implies that the arabinoxylans in endosperm cell walls and aleurone layers are resistant to enzymatic attack in the small intestine and so prevent access of endogenous enzymes. The second mechanism stipulates that a part of the arabinoxylans in the cell walls dissolves in the small intestine and contributes to environmental viscosity. The increase in the digesta viscosity in monogastric animals decreases the nutrient diffusion [14-17], reduces the rate of the feed transit [18], and increases the microbial growth in the small intestine [19].

Consumption of whole grain cereals has been associated with a slow increase in blood glucose level compared to consumption of refined flours [20,21] even though some results suggest otherwise [22]. The mechanism through which whole grains might reduce rapid increase in blood glucose is still not clear [23]. The presence of soluble NSPs is thought to negate starch hydrolysis and glucose absorption through increased viscosity.

The aim of the study was to investigate the dynamics of released glucose from whole wheat flour during *in vitro* gastric and intestinal digestion.

2. Materials and methods

Whole meal flour samples were digested *in vitro*. *In vitro* digestion was conducted according to the method of Boisen et al. [24] with some modifications [25]. Two experiments were conducted. Experiment 1 (gastric digestion) was an enzymatic hydrolysis with a pepsin solution at pH 2.0 and 37°C for 120 minutes. In experiment 2, first step was followed by the hydrolysis with the multi-enzyme pancreatin (intestinal digestion), at pH 6.8 and 37°C for 4 h. The released glucose (RG) and the refractive index (RI) were determined from the supernatant obtained after centrifugation. The glucose content was determined using a Glucose Assay kit (GAGO-20, Sigma Chemical Company, St. Louis, USA) and a PerkinElmer UV/VIS-Lambda35 spectro-photometer. The refractive index was measured with an Abbé refractometer Krüss DR301-95.

Statistical analysis: The mean values, standard deviations and coefficients of variations were calculated. For continuous variables, the analysis of regression was made and coefficients of determination R^2 were calculated. The results were statistically analyzed using t-test. Significant differences were declared when $P \leq 0.05$.

3. Results and discussion

RI values determined in experiments performed on wheat samples subjected to *in vitro* gastric and intestinal digestion are presented in Figures 1 and 2. RI increased during pepsin digestion suggesting an increased concentration of glucose in the supernatant. In the pepsin digestion takes place mostly the breakdown of cell walls and the disruption of the starch-protein interactions. Release of the nutrients that are physically trapped inside the cell wall enables more effective use for digestion. During intestinal digestion, the substrates were exposed to enzymes that digested proteins and carbohydrates, including starch. As digestion advanced, the proportion of protein and starch macromolecules was reduced, resulting in increased RI values and lower relative viscosity values [11,26] (Figure 2).

Experiments showed an initial increase in the glucose released from 1 g wheat up to 0.472 mg/mL after 1 h of gastric digestion, then followed by decrease (Figure 3). The lowest value was recorded after 2 hours of gastric digestion (0.332 mg/mL). The concentration of the reducing sugars increased throughout the intestinal digestion from 0.549 mg/mL up to 1.990 mg/mL (Figure 4), as a result of starch hydrolysis to dextrins, maltose and then to glucose.

Determination of RI can give information on the amount of glucose released during digestion.

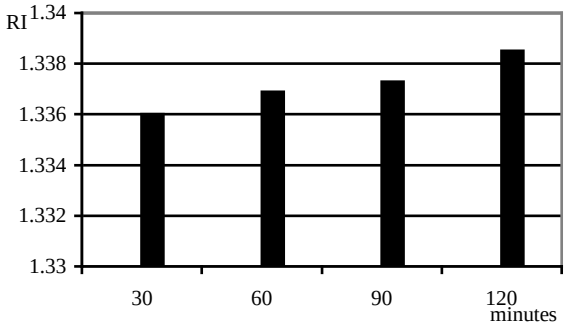

Figure 1. Dynamics of RI in gastric digestion

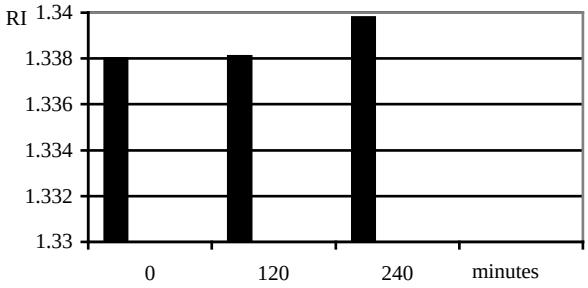

Figure 2. Dynamics of RI in intestinal digestion

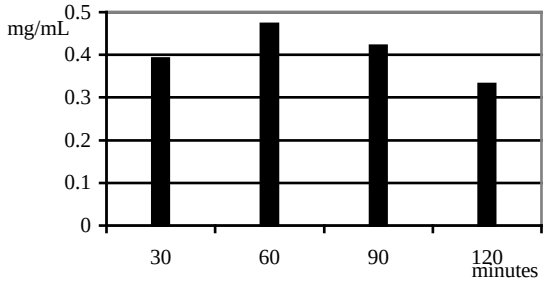

Figure 3. Dynamics of RG in gastric digestion

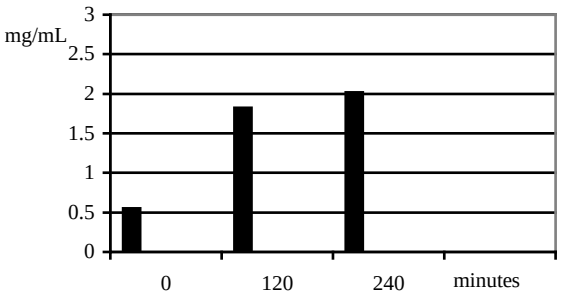

Figure 4. Dynamics of RG in intestinal digestion

4. Conclusions

The refractive index had an ascending trend as the intestinal digestion increased.

There is a high positive correlation between RG and RI (r = 0.7934), suggesting that RI may be used as a simple and rapid method for estimating the released glucose during *in vitro* intestinal digestion.

Acknowledgements

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