Glucose Release During *in Vitro* Digestion of Barley

Rodica Caprita, Adrian Caprita*, Iuliana Cretescu

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, 300645-Timisoara, Calea Aradului 119, Romania
"Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041, Timişoara, Romania

Abstract
The dynamics of released glucose (RG) and refractive index (RI) in the supernatant obtained during *in vitro* gastric and intestinal digestion of whole barley flour was studied. The *in vitro* digestion consisted of a two-step enzymatic procedure: (1) initial simulation of gastric digestion using pepsin and (2) simulation of small intestine digestion using multi-enzyme pancreatin. Experiments on 1 g barley flour showed an initial increase in RG concentration up to 0.379 mg / mL at one hour from the onset of gastric digestion, then a decrease in concentration, reaching the lowest value in 2 hours of gastric digestion (0.126 mg/mL). The concentration in reducing sugars increased throughout the intestinal digestion from 0.422 mg/mL up to 2.824 mg/mL, as a result of starch hydrolysis to dextrins, maltose and then to glucose. RI can give information on the amount of glucose released during digestion due to the high positive correlation found between RG and RI (r = 0.761).

Keywords: barley, released glucose, refractive index, digestion

1. Introduction

The chemical composition of cereal grains is characterized by the high content of carbohydrates [1,2]: available carbohydrates, mainly starch deposited in the endosperm (56–74%) and fiber, mainly located in the bran (2–13%) [3]. Cereal grains contain 66–76% carbohydrates. The major carbohydrate is starch (55–70%), followed by minor constituents, such as arabinoxylans (1.5–8%), β-glucans (0.5–7%), sugars (~3%), cellulose (~2.5%), and glucofructans (~1%) [4-6].
In cereal grains with high concentrations of non starch polysaccharides (NSP), water soluble arabinoxylans (AXs) and β-glucans are responsible for increased intestinal viscosity, and reduced starch, fat, and protein digestibility [7].
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" [8,9].
Cereal grains contain various amounts of NSP depending on the species and tissue type [2]: 1.14 g kg^{-1} in wheat kernel, 1.32 g kg^{-1} in rye and 1.67 g kg^{-1} in barley. 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}) [10-13]. The molecular weight of β-glucans is higher than that of AX, and both polymers contribute to the viscosity of the extract [14].
Exogenous carbohydrases are used when feed ingredients contain relatively high amounts of NSP [15-17]. Previous researches show that soluble NSP increased *in vivo* intestinal viscosity and slowed down the rate of diffusion of substrates, digestive enzymes, and final products of digestion, and therefore affect the digestion and utilization of nutrients [18-22].
Consumption of whole grain cereals results in a slow increase in blood glucose compared to refined flour consumption [23,24], although some results suggest otherwise [25]. The mechanism by

* Corresponding author: Adrian Caprita, Tel: +40-256-277298, adi.caprita@gmail.com

which whole grains could reduce the rapid increase in blood glucose is not yet clear [26]. The presence of soluble NSPs is thought to negate starch hydrolysis and glucose uptake by increased viscosity.

Digestibility of nutrients is very important when predicting the nutritional quality of feed. Digestibility is a measure of the biological availability of nutrients and is important in formulating a balanced ratio to maximize animal productivity. Only the part that is soluble or that becomes soluble by hydrolysis or other physic-chemical changes can be taken up in circulation and used by the animal as a building material or as a source of energy [27].

The purpose of the research was to investigate the dynamic of glucose released from whole barley flour during *in vitro* gastric and intestinal digestion.

2. Materials and methods

Samples of whole meal flour were digested *in vitro*, according to the method of Boisen et al. [28] with some modifications [29]. Two experiments were conducted. Experiment 1 (gastric digestion) was an enzymatic hydrolysis with a solution of pepsin at pH 2.0 and 37°C for 120 minutes. In Experiment 2, the first step was followed by the hydrolysis with the multi-enzyme pancreatin (intestinal digestion), at pH 6.8 and 37°C for 4 hours. Released glucose (RG) and refractive index (RI) were determined from the supernatant obtained after centrifugation. RG was determined using a Glucose Assay kit (GAGO-20, Sigma Chemical Company, St. Louis, USA) and a spectrophotometer Perkin Elmer UV/VIS-Lambda35. RI was measured with an Abbé refractometer Krüss DR301-95.

Statistical analysis

Average values, standard deviations, and variance coefficients were calculated. For continuous variables, the regression analysis was performed and the determination coefficients R^2 have been calculated. The results were statistically analyzed using the t-test. Significant differences were reported when $P \leq 0.05$.

3. Results and discussion

Values of RI determined in the experiments performed on barley samples subjected to *in vitro* gastric and intestinal digestion are shown in Figures 1 and 2. RI increased during pepsin digestion suggesting an increased glucose concentration in the supernatant. Pepsin digestion breaks down the cell walls and destroys the interactions between starch and protein. The release of physically trapped nutrients inside the cell wall allows a more efficient use of digestion.

Experiments showed an initial increase in RG from 1 g barley flour up to 0.379 mg/mL after 1 hour of gastric digestion, followed by a decrease (Figure 3). The lowest value was recorded after 2 hours of gastric digestion (0.126 mg/mL). Concentration of the RG increased during intestinal digestion from 0.422 mg/mL to 2.824 mg/mL (Figure 4) as a result of starch hydrolysis to dextrins, maltose and then glucose.

Determination of RI can provide information about the amount of glucose released during digestion, due to the high positive correlation (Figure 5) found between RG and RI (r = 0.761)

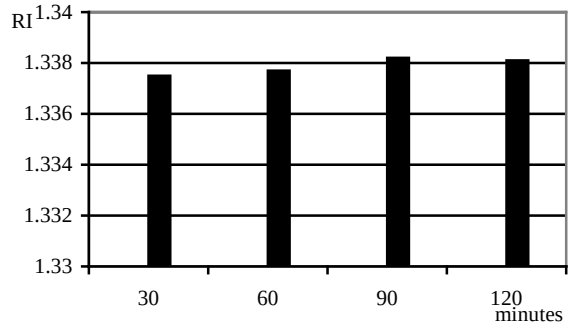

Figure 1. Dynamics of RI in gastric digestion

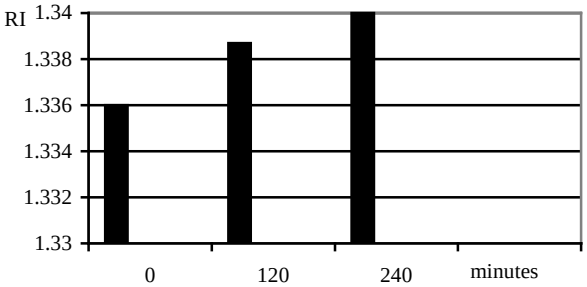

Figure 2. Dynamics of RI in intestinal digestion

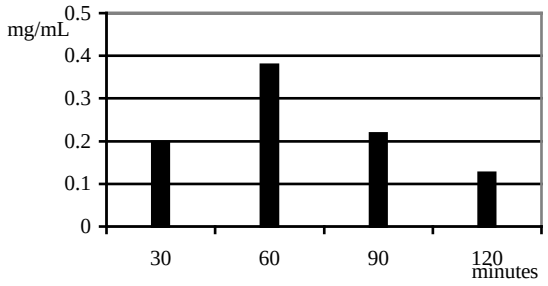

Figure 3. Dynamics of RG in gastric digestion

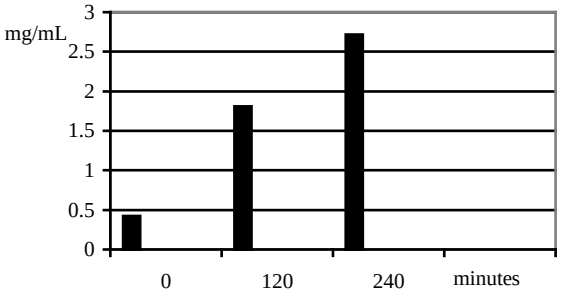

Figure 4. Dynamics of RG in intestinal digestion

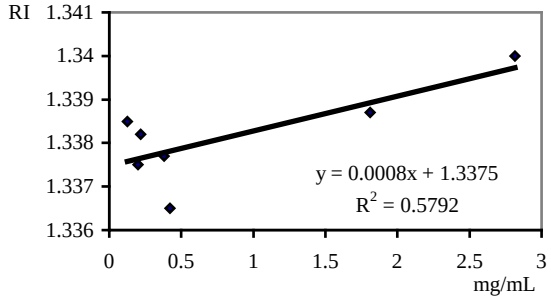

Figure 5. Correlation between RG and RI

4. Conclusions

RI has an ascending trend as intestinal digestion increases.

The correlation between RG and RI is elevated positive (r = 0.761), suggesting that RI can be used as a simple and rapid determination for estimating glucose released during digestion.

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