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ORIGINAL RESEARCH PAPER

THE INFLUENCE OF DIFFERENT SWEETENERS ON *IN VITRO* STARCH DIGESTION IN BISCUITS WITH WHEAT FLOUR AND WHOLE BARLEY FLOUR

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Abstract: Digestion of starch affects the glycemic index and it is important to know the percentage of starch digestion (degradation) in the body. The aim of this study was to investigate *In vitro* starch digestion of biscuits produced from wheat and barley flour in different ratios (100:0, 70:30, 50:50, 30:70, 0:100) with the addition of various sweeteners (sucrose, glucose solution and a mixture of sucrose and a glucose solution). *In vitro* starch digestion was evaluated after 0, 60, 120 and 180 min. It has been established that digestion of starch increases over time of hydrolysis. The results indicated that all types of biscuits produced from wheat and barley flour (in different ratio) had the slowest *In vitro* digestion when sucrose was used as a sweetener. The most rapid *In vitro* digestion was observed when a glucose solution was used as a sweetener, except in biscuits produced from 100% barley flour where the most rapid digestion was noticed for biscuits with a mixture of sucrose and glucose solution.

Keywords: *barley, biscuits, in vitro digestion, sweeteners*

INTRODUCTION

Cereal grains are consumed as mainly ingredient in a wide range of food products, which are of great economic importance and have many industrial applications, especially in the areas of food processing and marketing. The importance of starch in food depends on its technological characteristics, giving it various textural properties for formulating different recipes and developing wide range of food products. Starch is considered to be the most important ingredient of cereals in terms of adhesion, gelatinization, retrogradation, and other attributes of functionality that affect the quality of the product [1]. Starch is an integral part of many foods and its interactions with other ingredients, especially with water and lipids, are of great interest to the food industry and people's diet. Starch may be different in the form and functionality depending on its origin. Today, it is of great importance to explain the interaction of starch with other food ingredients [2]. Starch is the second most common cellulose biopolymer. It is synthesized from plants, stored in organs such as seeds in a form of different granules, and then used as a source of energy during germination and growth [3].

Starch is a carbohydrate, a polysaccharide composed of sub-units of α-D-glucopyranose with a general formula $(C_6H_{10}O_5)_n$. It is composed of two components: linear amylose and branched amylopectin. The ratio of amylose to amylopectin depends on the origin of the starch. In linear amylose, the glucose units are coupled via the α-(1,4)-glycosidic linkage, and the amylopectin is consisted of long syndicates of α-(1,4)-D-glucopyranosyl units by delineation of the α-(1,6) the links through which amylopectin form a branched structure [1].

One of the functional properties of starch is its digestion, which is particularly interesting from people's diet point of view. In a normal diet, 70-80% of the daily intake of carbohydrates is associated with starch. The starch is a polysaccharide that can be digested under the action of enzymes present in the human body. In order to be absorbed through intestinal epithelium, starch should be hydrolyzed to its core units, glucose molecules, which are the primary source of energy in humans. Depolymerization of the starch is performed with the help of amylase, which interrupts the α-1,4-glycosidic bonds, and with the help of amyloglucosidase, which interrupts α-1,6-glycosidic bonds. The rate and time of amylolytic hydrolysis of starch granules depends on the origin of the starch, while in the processed starch the effectiveness of the enzymes action is influenced by gelatinization or retrogradation of the starch [4]. The degradation or hydrolysis of starch begins in the mouth with the help of α-amylase dissolved in the saliva. It is considered that due to the short time of retention of food in the oral cavity, α-amylase from the saliva does not significantly affects the starch dissolution. The same enzyme is found in the pancreas juice, which digests the starch in the upper part of the digestive tract. Pancreatic amylase, as well as salivary amylase, are 1,4-glycosidases, which only hydrolyze, 1,4 glycosidic bonds. In order to be fully hydrolyzed the starch, enzymes that hydrolyze 1,6 glycosidic linkages such as pullulanase are required. The digestion is complete when the starch is fully amorphous, accessible to the enzymes, in small granules [2].

From a digestion point of view, there are three groups of starch: Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS).

RDS is a starch fraction that is rapidly adopted by the body, and therefore causes a rapid increase in a blood glucose levels. This starch is digested by enzymes in 20 minutes. SDS is a fraction of starch that slowly, but completely digests in the digestive system. Possible positive effects of SDS on human health includes stabilization of the glucose metabolism, controlling diabetes, and reaching the feel of satiety. This starch fraction is digested from 20 minutes to 1 hour. RS is a starch fraction that can't be digested in the small intestine, but can be fermented in the large intestine by the colonic bacteria. This fraction is not digested under the action of amylolytic enzymes, i.e it does not digested after 120 minutes incubation with enzymes [5 – 7]. Digestion of starch is an object of many investigations. In most cases it is performed *In vitro*, with different enzymes acting on the starch.

The barley grain belongs to the family *Poaceae*, the genus *Triticeae* and *Hordeum* [8]. In low-moisture recipes, such as for biscuits and pastes, the water level is generally too low and the competition for that water too high for significant gelatinisation to occur. Gelatinisation during baking plays a significant role in the formation of the product structure and the changes that subsequently occur as the product is cooled and stored. Biscuits are popular baking products that contain a large amount of fat and sugar [9 – 11]. They are stable foods and have advantages such as long shelf life and good eating quality [12]. The flour used for the production of biscuits is mostly white wheat flour [13 – 15].

The aim of this study was to determine the percentage of *In vitro* starch digestion from biscuits produced with wheat plain white flour and whole barley flour in different ratios (100:0, 70:30, 50:50, 30:70, 0:100) with the addition of various sweeteners (sucrose, glucose solution and a mixture of sucrose and glucose solution).

MATERIALS AND METHODS

Materials

Wheat plain white flour (Mill Popovo, Bulgaria) and barley flour (Ekosem Bulgaria OOD) were used in this research. The rest of the raw materials: sucrose (Agrana Bulgaria); glucose solution - D(+)- glucose monohidrat (Himiteks OOD Dimitrovgrad, Bulgaria); butter (Verea, Bulgaria); sodium bicarbonate ($NaHCO_3$, Radikom, Bulgaria); sodium chloride (NaCl, Global Food) were obtained from a local shops.

Methods

Production of biscuits

Biscuits made of plain white flour and barley flour in ratios 100:0, 70:30, 50:50, 30:70, 0:100, were prepared in the laboratory at the University of Ruse "Angel Kanchev" – branch Razgrad (Bulgaria) in accordance with the AACC Method 10-50D [16]. All ingredients were mixed using an electronic mixer with a flat beater. First sugar, salt, sodium bicarbonate and butter were mixed at low speed for 3 min (scraped down after each minute), then distilled water and glucose solution were added and mixed at low speed for 1 min, scraped down and mixed for additional 1 min at medium speed. Finally, the flour was added and mixed at low speed for 2 min, scraped down after each

30 s. After mixing, biscuits dough was stored in a refrigerator (up to 8 °C) for 30 min. Dough was flattened and rolled by a rolling pin to the desired thickness. At the end of the sample preparation process biscuits dough was cut with a round biscuit cutter and shaped biscuits were placed at the baking surface (cold baking pane covered with baking paper).
Fifteen types of biscuits were produced: basic control biscuits (100 % wheat flour), and biscuits in which wheat flour was partially or completely substituted with barley flour (30 %, 50 %, 70 % and 100 % barley flour), and with the use of different sweeteners (sucrose, glucose solution and mixture of sucrose and glucose solution). Biscuits were prepared in triplicate batches.

In vitro digestion of starch

In vitro method for digestion of starch was performed according to a method presented by Simonato *et al.* [17] with some changes. The method simulates the digestion of the starch in the human organism. The biscuit samples (1g) were weighted in the four separate test tubes (in order to determine the digestion of the starch during 0, 60, 120 and 180 min) and underwent 30 min of incubation with pepsin to simulate the gastric phase. Then, *p*H was adjusted with the addition of phosphate buffer (*p*H 7.2) and 4 mL of pancreatic amylase solution (10 $mg \cdot mL^{-1}$) were added in all tubes. The contents of the tubes were homogenized and tubes were introduced into a water bath with orbital movements at 37 °C. In the first test (0 min), 4 mL of absolute alcohol was added immediately. After 60, 120, and 180 min, all of the tubes were removed and the action of pancreatic amylase were stopped by the addition of 4 mL of absolute alcohol. The tube content was centrifuged at 5000 g for 10 min at 8 °C. The supernatant was diluted to 100 mL with 100 mM Na-acetate buffer (*p*H 4.5). Then, 100 µL aliquot were transferred into two test tubes. In both test tubes, 10 µL amyloglucosidase (AMG, 300 $U \cdot mL^{-1}$) were added and incubated in a water bath for 20 min at 50 °C. Then, 3 mL of glucose oxidase-peroxidase reagent (GOPOD) were added and the samples were incubated for 20 min at 50 °C before measuring absorbance at 517 nm. Distilled water was used as a control. Finally, the percentage of digested starch for a different period of time (0, 60, 120 and 180 min) was determined according to the Equation 1:

$$\%\ digestion\ of\ starch = \Delta E \times F \times 100/0.1 \times 1/1000 \times 100/W \times 162/180 \quad (1)$$

where:
ΔE - difference in absorbance of the sample and control,
F - absorbance conversion to µg (absorbance for 100 µg of glucose in GOPOD reaction which is calculated as F = 100 (µg glucose) / absorbance of GOPOD for 100 µg glucose,
100/0.1 - volume correction (0.1 mL taken from 100 mL),
1/1000 - conversion from micrograms to milligrams,
W - dry matter in the sample = mass of sample x dry matter content,
162/180 - a factor by which free glucose is detected as an hydroglucose found in starch.

RESULTS AND DISCUSSION

Starch is the main constituent of whole grain flour, and the rate of its enzymatic digestion is an important parameter to its nutritional property related to postprandial glycemic response and glucose homeostasis [18].
Figure 1 shows the *In vitro* digestion of the starch from biscuits produced of 100 % plain white wheat flour with different sweetener (sucrose, glucose solution and sucrose and glucose solution) and different time of hydrolysis (0 min, 60 min, 120 min and 180 min). It can be concluded that starch decomposition was increased with the time of hydrolysis. For a different period of time (60 min, 120 min and 180 min), the biscuits produced from 100 % wheat flour with a sucrose as a sweetener had the lowest rate of digestion (32.86 ± 0.10 % for 60 minutes to 50.16 ± 0.50 % for 180 minutes). The highest percentage of the starch digestion was determined in biscuits produced with a glucose solution as a sweetener (76.53 ± 0.30 % for 60 minutes to 98.40 ± 0.54 % for 180 minutes). In our previously analysis of *In vitro* digestion of biscuits produced with whole einkorn flour and white flour in different proportion we showed that by extending the digestion time, starch degradation increased [19].

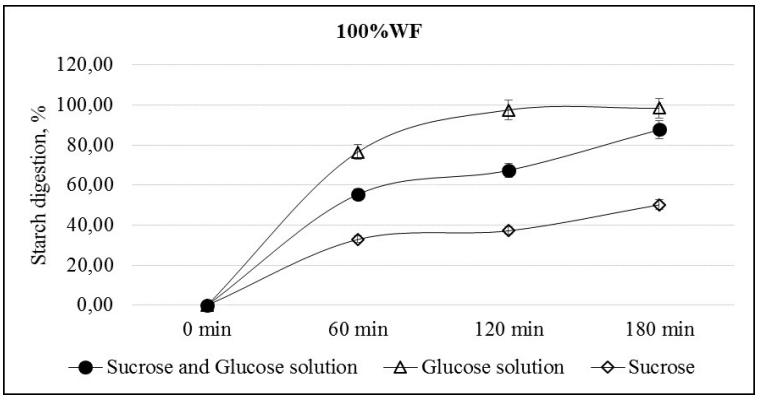

Figure 1. *In vitro digestion of starch in biscuits from 100 % wheat flour*

Figure 2 shows *In vitro* digestion of starch in biscuits produced from 70 % wheat flour and 30% barley flour with various sweeteners (sucrose, glucose solution and sucrose and glucose solution) for a different period of time (60 min, 120 min and 180 min). The highest percentage of starch digestion was also found in biscuits with glucose solution as a sweetener (92.64 ± 0.64 % over 60 minutes to 94.74 ± 0.31 % for 180 minutes). The lowest percentage of *In vitro* starch digestion had biscuits with sucrose as a sweetener (27.51 ± 0.24 % over 60 minutes to 60.35 ± 0.28 % in 180 minutes).

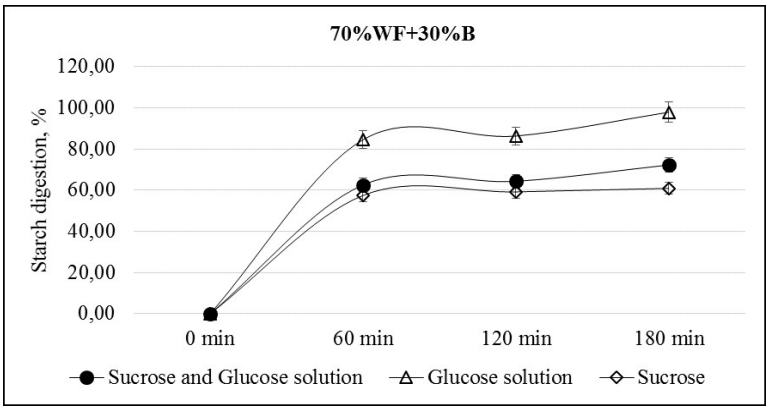

Figure 2. *In vitro digestion of starch in biscuits from 70 % wheat flour and 30 % barley flour*

The results of *In vitro* starch digestion in biscuits produced from 50 % wheat flour and 50 % barley flour with various sweeteners (sucrose, glucose solution and sucrose and glucose solution) for a different period of time (60 min, 120 min and 180 min) are presented in Figure 3. The highest percentage of starch digestion was found in biscuits with glucose solution as a sweetener (87.52 ± 0.16 % over 60 minutes to 96.35 ± 0.36 % for 180 minutes), and this lowest *In vitro* starch digestion had biscuits with sucrose as sweetener (57.45 ± 0.24 % over 60 minutes to 60.93 ± 0.28 % in 180 minutes).

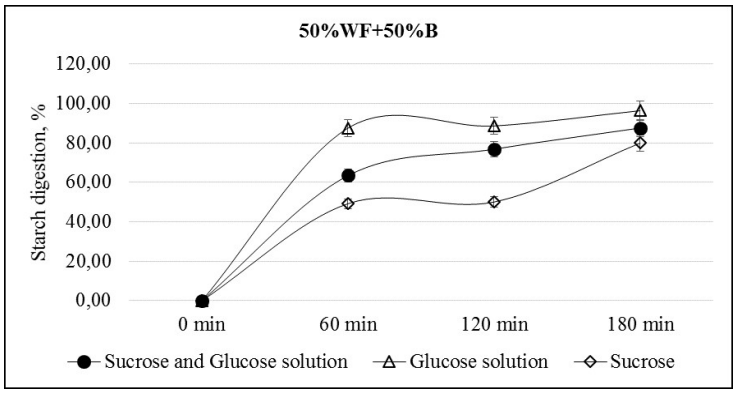

Figure 3. *In vitro digestion of starch in biscuits from 50 % wheat flour and 50 % barley flour*

The highest percentage of starch digestion among biscuits with 30 % wheat flour and 70 % barley flour was found in biscuits with glucose solution as a sweetener (70.57 ± 0.45 % over 60 minutes to 92.48 ± 0.51 % in 180 minutes). The slowest *In vitro* starch digestion had biscuits with sucrose as a sweetener, from 42.39 ± 0.32 % over 60 minutes to 66.23 ± 0.20 % in 180 minutes (Figure 4).

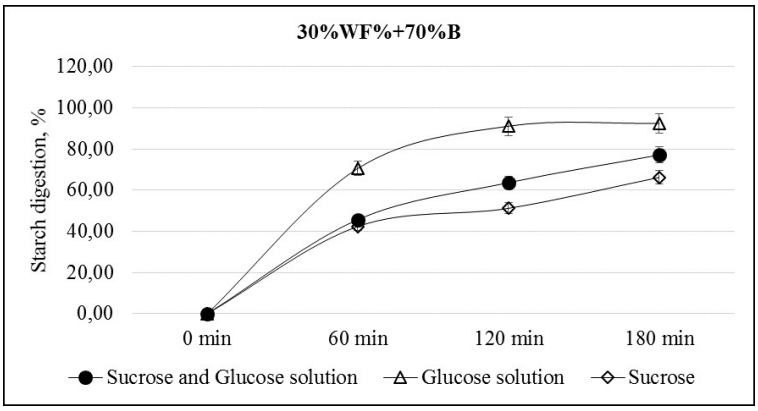

Figure 4. *In vitro digestion of starch in biscuits from 30 % wheat flour and 70 % barley flour*

The *In vitro* starch digestion in biscuits produced from 100 % barley flour with various sweeteners (sucrose, glucose solution and sucrose and glucose solution) for a different period of time (60 min, 120 min and 180 min) are presented in Figure 5. The highest percentage of starch digestion was found in biscuits with a mixture of sucrose and a glucose solution as a sweetener (44.45 ± 0.25 % over 60 minutes to 77.10 ± 0.64 % in 180 minutes). The lowest percentage of *In vitro* digestion of starch had biscuits with sucrose as a sweetener, from 24.21 ± 0.11 % for a period of 60 minutes to 51.12 ± 0.38 % in 180 minutes. According to Zhang *et. al.* [18] the higher amount of β-glucans in the biscuits can cause higher viscosity and reduce the starch-digestion rate.

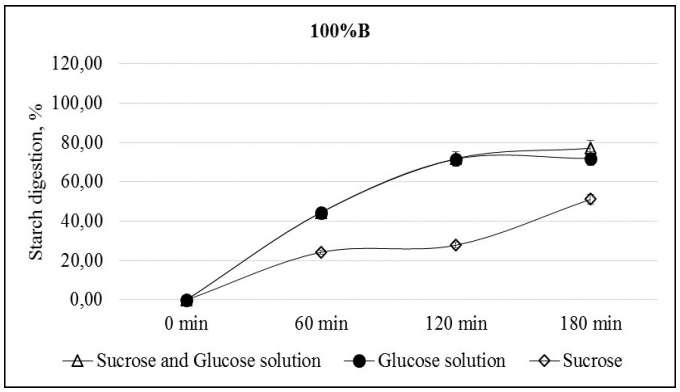

Figure5. *In vitro digestion of starch in biscuits from 100% barley flour*

Figures 1 to 5 present results for *In vitro* digestion of the starch contained in biscuits from wheat flour and barley flour in different ratios (100:0, 70:30, 50:50, 30:70, 0:100), with various sweeteners (sucrose, glucose solution and sucrose mixture and glucose solution). The figures show that during the time of 60 minutes the smallest digestion of starch had biscuits produced from 100 % barley flour with sucrose as a sweetener (24.21 ± 0.11 %), while the most rapid starch digestion for the same time (60 min) was observed in the biscuits produced with mixture of wheat and barley flour (ratio 50:50) with a glucose solution as a sweetener (87.52 ± 0.16 %).

During the 120 min of the *In vitro* starch digestion, the slowest digestion was noticed for biscuits produced from 100 % barley flour with sucrose as a sweetener (27.80 ± 0.29 %) and most rapid digestion rate was found for biscuits produced from 100 % wheat flour with glucose solution (97.51 ± 0.41 %).
At 180 minutes, the slowest digestion of starch has been determined for biscuits produced from 100 % wheat flour with sucrose as a sweetener (50.16 ± 0.50 %) and the highest starch digestion was found for biscuits produced from 100 % wheat flour with a glucose solution as a sweetener (98.40 ± 0.54 %).
From the obtained results it can be concluded that, by reducing the quantity of wheat flour in biscuits with a glucose solution as a sweetener, the percentage of starch digestion during 180 minutes decreases (from 98.40 ± 0.54 % for biscuits with 100 % wheat flour to 92.48 ± 0.51 % for biscuits produced from 30 % wheat flour and 70 % barley flour).
When sucrose was used as a sweetener, the starch digestion was slow in all types of biscuits, while use of a glucose solution significantly increased digestion of starch contained in biscuits. An exception to this were the biscuits produced from 100 % barley flour where the most rapid digestion was observed for biscuits with a mixture of sucrose and glucose solution.
Villemejane *et al.* [20] examined the *In vitro* digestion of starch from biscuits that have been fortified with dietary fiber and protein. In their investigations, they confirmed that, with increasing the time of degradation (up to 180 min), the digestion of the starch also increases, which has been confirmed in our investigation. Shumoy *et al.* [21] analyzed *In vitro* starch digestion of starch in various granular porridges, where the percentage of hydrolyzed starch during 180 minutes ranged from 68 % to 98 %. This percentage of digested starch coincides with almost all types of biscuit examined by us. It can be concluded that biscuits produced with sucrose as a sweetener show lower rates of starch hydrolysis. Kosović, [22] investigated digestion of wheat-barley pasta, and concluded that by increasing the amount of barley flour, the *In vitro* digestion of starch decreases. This positive influence of incorporation of barley flour in recipe has been also confirmed in our investigation.

CONCLUSION

Based on the results of the research carried out it can be concluded that all types of biscuits produced from wheat flour and barley flour (in a different ratio) had the slow rate of *In vitro* digestion when sucrose is used as a sweetener, while the most rapid *In vitro* digestion was observed when a glucose solution was used, except for biscuits produced from 100 % barley flour, where the highest *In vitro* digestion was when a mixture of a sucrose and glucose solution was used as a sweetener.

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