Effects of pseudocereals, legumes and inulin addition on selected nutritional properties and glycemic index of whole grain wheat-based biscuits

LOVORKA VUJIĆ – DUBRAVKA VITALI ČEPO – BLAŽENKA ŠEBEČIĆ – IRENA VEDRINA DRAGOJEVIĆ

SUMMARY

Based on the nutritionists‘ opinion that diet may have a preventive and protective role in many diseases, the possibilities of development of biscuits with lower glycemic index (*GI*) as functional cereal-based products were examined in this work. Results showed that, by substitution of 30% of white wheat flour with selected pseudocereals (buckwheat or amaranth flour) and legumes (soya or carob flour), it was possible to achieve better macronutritive quality of biscuits. Incorporation of selected flours resulted in a decrease of in vitro starch digestibility in terms of significant decrease of rapidly available glucose (9.6–47.6%), and rapidly digestible starch (9.8–50.4%). A significant increase in the resistant starch content was achieved by the implementation of pseudocereals (43.5–56.1%). Control biscuits, biscuits with added saccharose, and biscuits with buckwheat flour and without inulin, had medium *GI* (58.7–66.9), while samples with amaranth, soya or carob flour had low *GI* (44.9–52.5). None of the investigated biscuits had high *GI*. A very good correlation between the results obtained in vitro and *GI* values were determined indicating that the formulation of biscuits with lower starch digestibility represented a good approach in lowering *GI* of biscuits, and therefore increasing its functional properties.

Keywords

in vitro starch digestibility; glycemic index; biscuits

Biscuits are a very popular snack food used worldwide across all population groups [1]. They usually contain a high proportion of lipids and saccharose to achieve more appealing flavour, which classifies them as foods whose regular consumption represents a risk factor for obesity, and thus an increased risk of developing type 2 diabetes, high cholesterol and coronary heart disease [2]. In the modern world dominated by highly processed and nutritionally poor foods, there are efforts to complement the diet with valuable nutrients by so called functional products. Taking into account trends in the food industry, as well as the recommendations of nutritionists who are increasingly pointing out at preventive and protective role of diet in many diseases, the efforts of food technologists are focused in recent years to preparing biscuits with functional properties. Generally, functional foods are challenging the food industry in technological as well as in economic terms since studies have shown that most consumers rarely waive taste for health and it is therefore important to develop functional food products with retained traditional organoleptic characteristics [3]. Hence, many studies have focused on the creation of biscuits with enhanced nutritional characteristics without disruption the biscuit quality, whereby a number of improvements are based on the replacement of certain amount of refined wheat flour with various raw materials of high nutritional value such as soya, buckwheat or amaranth flour [4–6].

Buckwheat is a rich source of proteins, fibres, antioxidants, minerals and starch that contains substantial amounts of resistant starch, which may contribute to lowering of glycemic index (*GI*) of food [5]. Moreover, in the recent study [7] a decrease in *GI* was reported for bread made from a mixture of buckwheat and wheat flours. This was due to binding of fatty acids, rutin and epicate-

Lovorka Vujić, Dubravka Vitali Čepo, Blaženka Šebečić, Irena Vedrina Dragojević, Department of Food Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia.

Correspondence author:
Lovorka Vujić, tel.: +385 1 4612690, e-mail: lvujic@pharma.hr

chin-dimethylhallate, contained in buckwheat, to wheat flour starch.

Amaranth is another pseudocereal with protein content higher than wheat, with well balanced amino acid composition. It is also a rich source of dietary fibre, lipids and minerals [8]. According to study of KIM et al. [9], amaranth grain could be helpful in reduction of elevated serum glucose levels and preventing diabetic complications.

Legumes (soya and carob) are low glycemic foods, being excellent sources of dietary fibre, variety of micronutrients and phytochemicals. Soya contains high quality proteins as well as isoflavones with well documented health benefits [10]. Carob has high content of carbohydrates, appreciable content of proteins, low content of lipids, and has antioxidant properties due to the high content of condensed tannins [11].

Regarding the growing epidemic of obesity and type 2 diabetes worldwide, more attention is paid to creating lower *GI* products, since numerous studies have demonstrated positive effects of low glycemic diet on health. Hence, MARAGONI and POLI [12] added specific proprietary fibre mix to the biscuit formulation thus significantly reducing *GI*. JENKINS et al. [13] concluded that highly viscous fibre blend (PGX; InovoBiologic, Calgary, Canada) incorporated to a starchy snack (biscuit) reduced the *GI* by 74% in healthy, and by 63% in diabetic consumers.

Starch can be found in various physical and chemical forms in foods, which affect the rate and the extent of digestion and, consequently, its nutritional and health properties. When the rate of starch digestion is decreased, postprandial glucose and insulin responses are reduced or delayed [14]. Diets rich in slowly digested carbohydrates may protect against chronic disorders and/or diseases such as central obesity, hyperlipidemia and cancer [15]. Various factors affect starch digestibility such as nature of starch, protein and lipid interactions, presence of antinutrients and enzyme inhibitors, as well as food processing conditions [16]. This suggests that, by careful selection of raw materials, these parameters can be altered and also, consequently, the *GI* of the final product. Additionally, glycemia can be further reduced by replacing saccharose with indigestible carbohydrates such as sugar alcohols [17].

The goal of this work was to examine the possibilities of improving the traditional biscuits formulation by introduction of novel raw materials, such as pseudocereals, legumes or inulin. The proposed modifications are aimed primarily to reduce *GI*, while maintaining or enhancing the nutritional quality and functional properties of the final product in terms of increasing the contents of dietary fibre, slowly digestible and resistant starch, as well as proteins. The present work represents an attempt to decrease the *GI* of this frequently consumed snack.

MATERIALS AND METHODS

Preparation of biscuits

Seven types of biscuits were prepared under laboratory conditions in Technological development department in Kraš, confectionary factory in Zagreb, Croatia, in three series, in order to ensure the reliability of the obtained data. For biscuits preparation, whole grain and white wheat flour (Farina, Granolio, Zagreb, Croatia) were used as basic flours. Buckwheat flour (Do-It, Barneveld, Netherland), amaranth flour (Do-It), full fat soya flour (Biovega, Zagreb, Croatia) and carob flour (Šafram, Zagreb, Croatia) as substitute flours, were incorporated into biscuit by replacing the equivalent amount of white wheat flour in the biscuit formulation. All flours were purchased at the local market in Croatia.

Control biscuits were prepared with 949 g whole grain wheat flour, 633 g white wheat flour, 274 g vegetable fat without trans fatty acids (K_3-Z, Zvijezda, Zagreb, Croatia), 259 g inulin (Beneo, Tienen, Belgium), 87 g skimmed milk powder (Lactoprot, Kaltenkirchen, Germany), 20 g ammonium bicarbonate, 20 g salt (Solana Pag, Pag, Croatia), 7 g vanilla flavour (Symrise, Holzminden, Germany), 249 g isomalt (Beneo) and 0.3 g sucralose (McNeil Nutritionals, Fort Washington, Pennsylvania, USA).

In four biscuit formulations, a definite amount (480 g) of white wheat flour was replaced by different above mentioned substitute flour; one formulation was prepared using powdered saccharose (249 g) instead of saccharose substitute (isomalt) and artificial sweetener (sucralose), and one was prepared without inulin. Composition of the experimental biscuits is presented in Tab. 1.

Other ingredients were added in the same amounts as in control biscuits. Water was added as needed depending on the type of flour used. Resulting dough was laminated on the machine roller up to 0.5 cm thickness and followed by hand shaping of biscuits using moulds 4 cm in diameter. Biscuits were baked at 175 °C for 17–19 min, depending on the raw material used and the amount of water added. After baking, biscuits were cooled and, before mincing, samples for *GI* determination were separated.

Tab. 1. Composition of the experimental biscuits.

Sample	Whole grain wheat flour	White wheat flour	Raw material	Inulin	Isomalt/Sucralose	Saccharose
	[g]					
Control sample	949	633	–	259	249/0.3	–
With saccharose	949	633	–	259	–	249
With buckwheat flour	949	153	480 (buckwheat flour)	259	249/0.3	–
With amaranth flour	949	153	480 (amaranth flour)	259	249/0.3	–
With carob flour	949	153	480 (carob flour)	259	249/0.3	–
With soya flour	949	153	480 (soya flour)	259	249/0.3	–
Without inulin	949	633	–	–	249/0.3	–

Chemical analysis of biscuits

Moisture content was determined according to AACC 44-15A method, ash content according to AACC 08-01, proteins according to AACC 46-12 [18] using multiplying the nitrogen content by factor 6.25, and total lipids according to AOAC 920.39C method [19]. Total dietary fibre (*TDF*) content was assessed by enzymatic–gravimetric AOAC 991.43 method as the sum of the soluble (*SDF*) and insoluble dietary fibre (*IDF*) fractions [19]. The protein, ash, lipid and moisture contents were subtracted from the total weight and the difference was considered as total carbohydrates. Available carbohydrates were calculated as a difference between total carbohydrates and the sum of total dietary fibre, isomalt and inulin.

In order to calculate the energy value of the investigated biscuits, Atwater general factor system was used [20]. Conversion factors that were used were 17 $kJ \cdot g^{-1}$ for proteins and available carbohydrates, 37 $kJ \cdot g^{-1}$ for lipids and 8 $kJ \cdot g^{-1}$ for dietary fibre. Energy values used for isomalt and inulin were 9 $kJ \cdot g^{-1}$ [21] and 6.3 $kJ \cdot g^{-1}$ [22], respectively.

Energy value (*EV*) in kilojoules was calculated according to the following equation:

$$EV = 17P + 37L + 17avCHO + 8F + 9IS + 6.3IN \quad (1)$$

where *P* is content of proteins, *L* is content of lipids, *avCHO* is content of available carbohydrates, *F* is content of fibre, *IS* is content of isomalt and *IN* is content of inulin (all in grams).

In vitro protein and starch digestibility

In vitro protein digestibility of the biscuits was determined by enzymatic method with pepsin (P7000, Sigma-Aldrich, Steinham, Germany) and pancreatin (P1750, Sigma-Aldrich) according to Kumagai et al. [23]. In vitro starch digestibility was determined according to the procedure of Englyst et al. [14]. The analysis was carried out under controlled enzymatic hydrolysis with invertase (I4504, Sigma-Aldrich), pancreatin (P7545, Sigma-Aldrich) and amyloglucosidase (A3042, Sigma-Aldrich) at 37 °C in capped tubes immersed in a shaker water bath, followed by measurement of released glucose by GOD-PAP (glucose oxidase/peroxidase) method [24] using a diagnostic kit (Dijagnostika, Sisak, Croatia). The value G_{20} (rapidly available glucose) was obtained by measuring the release of glucose after 20 min of enzymatic incubation, while G_{120} was estimated after further 100 min of incubation.

The total glucose value (*TG*) was obtained by treating the sample with heat-stable α-amylase (A3306, Sigma-Aldrich) in boiling water, followed by immediate cooling, treatment with potassium hydroxide (7 $mol \cdot l^{-1}$) at 0 °C, and followed by complete enzymatic hydrolysis with amyloglucosidase.

Free glucose (*FG*) was determined by treating the sample with invertase in acetate buffer at 100 °C (water bath) for 30 min. Simultaneous tests were run in the same manner with standard glucose and blank.

Rapidly digestible starch (*RDS*), slowly digestible starch (*SDS*), resistant starch (*RS*) and total starch (*TS*) were calculated according to following equations [14]:

$$RDS = (G_{20} - FG) \times 0.9 \quad (2)$$

$$SDS = (G_{120} - G_{20}) \times 0.9 \quad (3)$$

$$RS = (TG - G_{120}) \times 0.9 \quad (4)$$

$$TS = (TG - FG) \times 0.9 \quad (5)$$

Glycemic index determination

Ten healthy volunteers (five men and five women) aged from 21 to 43, with an average body mass index of (22.36 ± 1.12) $kg \cdot m^{-2}$, were included in *GI* testing. The volunteers were non-smokers and had no history of metabolic disease, as determined by a questionnaire prior to recruitment. All subjects gave written consent prior to inclusion in the study after being given a detailed description of the study protocol and had opportunity to ask questions. The participants were asked to maintain standard diet and to refrain from heavy physical activity as well as consumption of alcoholic and caffeinated drinks the day prior to the test. The study protocol was approved by the Ethical Committee of the Croatian Institute for Transfusion Medicine and Committee on Ethics of the Experimental Work of the Faculty of Pharmacy and Biochemistry in Zagreb, Croatia.

The protocol procedure was in agreement with the recommendations of FAO/WHO [25] and Brouns et al. [17]. Each participant tested seven different types of biscuits once in a random order, while the reference food was tested twice. Tests were performed during morning after a 12 h overnight fast, with a gap of at least two days between measurements. Each volunteer ate a biscuit portion containing 50 g of available carbohydrates within 10 min with 250 ml of tap water. Reference food (anhydrous glucose) was prepared by dissolving 50 g of pure glucose in 250 ml of water. Capillary finger-prick blood samples were taken from participants at 0, 15, 30, 45, 60, 90 and 120 min after the beginning of the test, or after reference food intake (time 0 min). The blood glucose concentration was measured using Contour automatic glucose meter (Bayer HealthCare, Leverkusen, Germany), whose accuracy was verified using high, low and normal test solutions (Bayer Consumer Care, Basel, Switzerland).

Statistical analysis

One-way ANOVA was used to investigate differences between three examined series of biscuits. Since no significant differences were observed ($p > 0.05$), all results were presented as means of series of biscuits ± standard deviation. Depending on the applied method, analyses were conducted in duplicates or triplicates within one series of biscuits. One-way ANOVA and post-hoc Bonferroni test were applied to study the differences between different biscuit formulations, the significance being determined at the level of $p < 0.05$.

For *GI* calculation, the incremental area under curve (*iAUC*) of blood glucose response, ignoring the area beneath the baseline, was calculated geometrically [25]. *GI* of biscuits was calculated as the ratio between mean *iAUC* for the biscuit tested and mean *iAUC* of the reference food (glucose solution). The *GI* results are expressed as mean ± standard error (*SEM*). Correlation coefficients (*r*) between *GI*, different starch and dietary fibre fractions as well as protein and lipid contents were also calculated.

All statistical analyses were performed using GraphPad Prism 3 software (GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

Macronutritive and nutritional compositions of investigated biscuits are shown in Tab. 2. It can be seen from presented data that the addition of flours of different origins to the control biscuits resulted in significant improvement of certain nutritive parameters.

Total protein and lipid contents were the highest in biscuits enriched with soya flour, as was expected, since soya flour is rich in these components. Although soybeans are high in lipids, which are traditionally an undesirable ingredient in human diet, majority of them are unsaturated fatty acids with beneficial health effects [10]. Total dietary fibre content was significantly increased in biscuits enriched with buckwheat, amaranth and soya flours, with the strongest effect achieved by the addition of carob flour (an increase of 96% compared to the control biscuits) solely as the result of high insoluble fibre content. Since all investigated biscuits contained more than 6 g of fibre per 100 g dry matter, they can be classified as food rich in fibre according to EU regulation 1924/2006 [26].

In vitro protein digestibility is an important factor when assessing the nutritional quality of a food product. The obtained results showed that addition of all substitute flours to the control biscuits decreased protein digestibility of the final product, which is consistent with literature data for products of similar type [27, 28]. The observed results may be explained by the fact that high levels of dietary fibre (biscuits with carob flour) had a negative effect on protein digestibility due to possible complex formation between the fibre and the proteins, as reported Bilgiçli et al. [29], or by high levels of anti-nutritive components such as trypsin inhibitor present in soybean [30] and tannins present in carob [11]. Buckwheat contains antinutrients such as the protease inhibitor and tannins [31].

Bearing in mind that the nature of starch and consequently its digestibility has a fundamental role in the rate of its absorption, an attempt to reduce starch digestibility and, consequently, glucose response in vivo was made by introducing different raw materials to biscuit formulation. As pre-

Tab. 2. Chemical and nutritional properties of biscuits.

Sample	Proteins [g]	Lipids [g]	Ash [g]	Total carbohydrates [g]	Total dietary fibre [g]	Insoluble dietary fibre [g]	Soluble dietary fibre [g]	In vitro protein digestibility [g]	Energy value [kJ]
Control sample	9.69 ± 0.13^{a}	12.36 ± 0.18^{a}	1.84 ± 0.01^{a}	76.11 ± 0.06^{a}	8.07 ± 0.40^{a}	4.38 ± 0.14^{a}	3.69 ± 0.27^{ae}	79.38 ± 2.43^{a}	1644
With saccharose	9.50 ± 0.06^{a}	12.39 ± 0.19^{a}	1.75 ± 0.04^{b}	76.36 ± 0.28^{ad}	7.53 ± 0.29^{b}	4.01 ± 0.10^{a}	3.52 ± 0.25^{a}	78.26 ± 3.78^{a}	1732
With buckwheat flour	10.24 ± 0.09^{b}	12.25 ± 0.17^{a}	2.06 ± 0.03^{c}	75.45 ± 0.23^{b}	9.81 ± 0.32^{c}	5.27 ± 0.32^{b}	4.54 ± 0.13^{b}	69.70 ± 2.33^{b}	1623
With amaranth flour	10.97 ± 0.19^{c}	13.54 ± 0.58^{b}	2.30 ± 0.06^{d}	73.19 ± 0.49^{c}	9.95 ± 0.16^{c}	5.89 ± 0.05^{c}	4.06 ± 0.17^{c}	72.50 ± 3.37^{bd}	1648
With carob flour	9.04 ± 0.08^{d}	11.96 ± 0.27^{a}	2.26 ± 0.01^{d}	76.74 ± 0.33^{d}	15.82 ± 0.56^{d}	12.86 ± 0.53^{d}	2.96 ± 0.27^{d}	51.97 ± 3.33^{c}	1564
With soya flour	16.66 ± 0.19^{b}	17.61 ± 0.61^{c}	2.80 ± 0.07^{e}	62.92 ± 0.75^{e}	11.31 ± 0.13^{e}	7.35 ± 0.04^{e}	3.96 ± 0.14^{ec}	70.00 ± 3.47^{b}	1711
Without inulin	10.44 ± 0.03^{e}	13.05 ± 0.28^{b}	1.81 ± 0.01^{ab}	74.70 ± 0.25^{f}	6.37 ± 0.41^{f}	4.80 ± 0.21^{f}	1.57 ± 0.20^{f}	75.57 ± 1.74^{ad}	1744

The results are reported as means of three investigated series of biscuits ± standard deviation, per 100 g on dry basis. Data in the same column marked with the same letter are the part of the same statistical group ($p > 0.05$).

sented in Tab. 3, significant differences were found regarding the contents of rapidly available glucose (*RAG*), *RDS*, *SDS* and *TS*.

It is noticeable that incorporation of substitute flours resulted in a decrease of *RAG* and *RDS* content in relation to control sample, and the strongest impact was achieved by addition of amaranth flour (24.9% and 26.6%, respectively), carob flour (35.9% and 50.4%, respectively) and soya flour (47.6% and 50.1%, respectively). Obtained data are consistent with the fact that legumes (soya and carob) have a higher amylose/amylopectin ratio in starch granules compared to cereals, and are therefore less susceptible to amylolysis. This is because amylose molecules contain more hydrogen bonds making them less accessible to digestive enzymes action than amylopectin molecules with a branchy structure [32]. Additionally, carob and soya contain high amounts of dietary fibre, which may affect starch digestibility. Furthermore, as previously noted, soya contains high protein and lipid amounts that have a protective effect against amylolytic activity, diminishing the level of enzymatic hydrolysis by reducing the available surface area through blocking the adsorption sites [33]. Also, carob is rich in tannins with antinutritive effect [11]. Although amaranth contains relatively high amounts of starch, small size of starch granules and low amylose content [8], i.e. factors that increase starch digestibility, amaranth flour supplementation of the control formulation resulted in its reduction. The obtained results can be explained by the relatively high protein, lipid and dietary fibre contents, as well as low level milling used for amaranth flour.

Statistically significant increase in *RAG* content in relation to the control sample was determined in samples with saccharose (17.4%) and without inulin (22.0%). In this concern it should be noted that inulin removal from the control formulation had a more significant influence on the above mentioned parameter than the addition of saccharose. Also, a significant increase in *RDS* values was observed only in biscuit without inulin (25.4%), partly as a result of a higher total starch content.

SDS is the starch fraction completely digested in the small intestine but, as opposed to *RAG*, at a lower rate. Modification of the control formulation resulted in significantly reduced *SDS* content in all samples enriched with substitute flours, partially due to the reduction of the total starch content. Indeed, the greatest impact on *SDS* decrease was found in biscuits supplemented with carob flour (19.8%) and with soya flour (25.0%), which can be explained by the significant reduction in *TS* content in those samples (37.9% and

Tab. 3. Content of different carbohydrates fractions in experimental biscuits.

Sample	Rapidly available glucose	Rapidly digestible starch	Slowly digestible starch	Resistant starch	Total starch
	[g·kg^{-1}]				
Control sample	289.20 ± 4.37 ^{a}	250.72 ± 4.07 ^{a}	157.46 ± 9.70 ^{a}	35.56 ± 8.04 ^{a}	443.74 ± 18.69 ^{a}
With saccharose	339.60 ± 3.99 ^{b}	251.74 ± 4.16 ^{a}	162.88 ± 10.26 ^{a}	34.73 ± 6.72 ^{ad}	449.34 ± 12.06 ^{a}
With buckwheat flour	261.38 ± 10.78 ^{c}	226.22 ± 9.69 ^{b}	132.24 ± 11.66 ^{bc}	55.51 ± 1.40 ^{b}	413.97 ± 4.02 ^{b}
With amaranth flour	217.32 ± 4.29 ^{d}	184.12 ± 3.80 ^{c}	137.81 ± 8.67 ^{bd}	51.02 ± 0.79 ^{b}	372.95 ± 4.57 ^{c}
With carob flour	185.47 ± 3.57 ^{e}	124.37 ± 3.72 ^{d}	126.28 ± 9.34 ^{bc}	25.10 ± 2.25 ^{c}	275.75 ± 12.14 ^{d}
With soya flour	151.68 ± 5.25 ^{f}	125.16 ± 4.63 ^{d}	118.08 ± 13.42 ^{c}	25.08 ± 5.54 ^{c}	268.32 ± 9.35 ^{d}
Without inulin	352.71 ± 9.27 ^{g}	314.25 ± 8.30 ^{e}	151.10 ± 10.63 ^{ad}	27.35 ± 6.86 ^{dc}	492.70 ± 11.02 ^{e}

The results are reported as means of three investigated series of biscuits ± standard deviation. Data in the same column marked with the same letter are the part of the same statistical group ($p > 0.05$); values are expressed per kilogram on dry basis.

39.5%, respectively). In biscuits supplemented with pseudocereals (buckwheat and amaranth flours), *SDS* decrease occurred partially due to the increase in *RS* content. This is consistent with the results of Ragaee et al. [34], who determined in vitro starch digestibility in bread with 30% of white wheat flour replaced with various whole-grain flours.

In comparison to other food rich in starch, including biscuits [35, 36], the investigated samples had lower *RAG* and *RDS* contents, and higher *SDS* and *RS* contents, as a result of several factors. Namely, the basis of formulation of the investigated biscuits was whole grain wheat flour, i.e. low grade milled flour, where starch granules are trapped within the cell wall, which slows down their degradation. Additionally, degradation of the starch granules by grinding increases the sensitivity to enzymatic degradation [37]. The same effect has extended milling since smaller particles have a larger surface area available for amylolytic activity [38]. Another fact that might contribute to this effect was the high dietary fibre content and baking under low moisture conditions, which reduce starch gelatinization resulting in partially intact starch granules that are less sensitive to the amylolytic enzymes [36].

Glycemic index of biscuits

The primary aim of this study was to examine the impact of introducing different raw materials on the postpradial glycemia of whole grain wheat-based biscuits. The obtained results show that the highest glucose response in volunteers was observed 30–45 min after consumption of the test food (Fig. 1).

The highest increase in blood glucose concentration was observed after consumption of biscuits

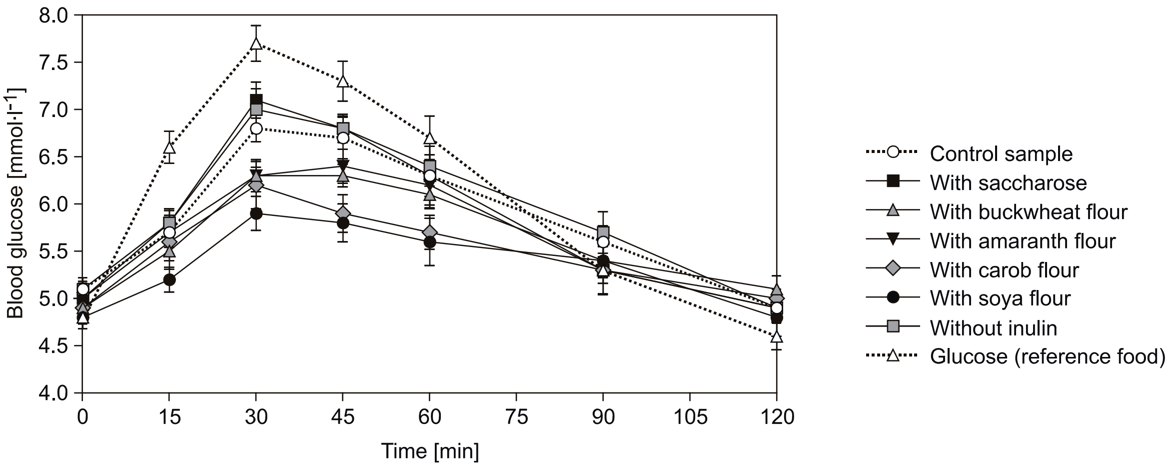

Fig. 1. Glycemic response to biscuits.

Values are expressed as mean ± standard error ($n = 10$).

with saccharose (after 30 min) although, compared to the reference sample, this difference was not statistically significant ($p > 0.05$). Consumption of biscuits with soya flour resulted in the lowest levels of blood glucose, which proved to be statistically significant compared to the sample with saccharose (after 30 min and 45 min), and statistically significant in comparison with the biscuit without inulin (after 45 min). Given that food can be classified according to its glycemic effect [39] as those that have a high (≥ 70), medium (56–69) and low *GI* (≤ 55) compared to the standard (glucose solution, *GI* = 100), the investigated samples were categorized in the following way: control biscuits, biscuits with saccharose, buckwheat flour and without inulin were medium *GI* products, while biscuits prepared with amaranth, soya or carob flours had low *GI*. None of the investigated biscuits had high *GI*. Although a wide range of *GI* values was observed for the investigated samples (Tab. 4), the differences were not statistically significant ($p > 0.05$) due to a large dissipation among the results. The obtained results indicate that by introducing pseudocereals (buckwheat and amaranth flour) and legumes (carob and soya flour) instead of white wheat flour, a decrease in blood glucose response can be achieved.

In order to establish the relationship between in vitro starch digestibility and in vivo glucose response after consumption of the investigated biscuits, *GI* values were correlated with the amount of starch fractions contained in 50 g of available carbohydrates, as the amount containing servings used to determine *GI* (Tab. 4). Although in vitro methods cannot completely imitate in vivo conditions, such as degree of gastric emptying or the influence of intestinal hormones, a very good correlation between the results obtained in vitro method and *GI* values was determined (Tab. 5).

Correlation between *GI* and *RAG* values proved to be the most significant ($p = 0.01$) among the tested starch fractions, which is consistent with literature [14]. Additionally, there was a strong negative correlation between the *SDS* and *GI* values ($p = 0.04$) indicating a reducing effect of *SDS* on in vivo glucose response. The obtained results suggest that creating low *RAG*/*RDS* and high *SDS* foods, is an effective approach for lowering *GI*. The analysis of the relationship between macronutrient content and *GI* values (Tab. 5) showed a significant negative correlation between the protein content ($p = 0.08$) and lipids ($p = 0.05$), which confirms the above mentioned allegations about the impact of these components on the digestibility of starch and, consequently, *GI*. These findings are in accordance with the results of Garsetti et al. [40] who investigated the relationship between *GI* of plain sweet biscuits and in vitro starch digestibility, and found similar negative correlation between *GI* and protein and lipid contents, as well as between *GI* and *SDS* content. Also, we established a very strong negative correlation between *GI* and *TDF* content ($r = –0.95$; $p = 0.00$) and its fractions (*SDF*, $r = –0.73$; $p = 0.07$ and *IDF*, $r = –0.87$; $p = 0.01$). Similar results were obtained by Wolever [41], who examined the relationship between dietary fibre content and *GI* of different foods and established a significant negative relationship with total fibre and its insoluble fraction ($p < 0.05$), but not with the soluble fibre content. Correlation with *SDF* observed in this study can be explained by the fact that the investigated samples were produced under strictly controlled conditions, had

Tab. 4. Portion size, rapidly available glucose, starch fractions and glycemic index of the examined biscuits.

Sample	Portion size	Rapidly available glucose	Rapidly digestible starch	Slowly digestible starch	Resistant starch	Total starch	Glycemic index
	[g]						
Control sample	105.0	30.4	26.3	16.5	3.7	46.6	60.1 ± 4.8 a
With saccharose	85.6	29.1	21.5	13.9	3.0	38.5	65.8 ± 5.6 a
With buckwheat flour	110.5	28.9	25.0	14.6	6.1	45.8	58.7 ± 6.2 a
With amaranth flour	116.7	25.4	21.5	16.1	6.0	43.5	52.5 ± 5.9 a
With carob flour	123.4	22.9	15.3	15.6	3.1	34.0	46.8 ± 6.0 a
With soy flour	160.2	24.3	20.1	18.9	4.0	43.0	44.9 ± 5.3 a
Without inulin	93.8	33.1	29.5	14.2	2.6	46.2	66.9 ± 4.6 a

Rapidly available glucose and starch fractions are expressed as grams per serving containing 50 g of available carbohydrates. Glycemic index is expressed as mean ± standard error ($n = 10$), data in the column marked with the same letter are the part of the same statistical group ($p > 0.05$);

Tab. 5. Correlations between rapidly available glucose, different starch fractions and macronutrient contents with glycemic index values.

	RAG	*RDS*	*SDS*	*RS*	*TS*	Proteins	Lipids	*SDF*	*IDF*	*TDF*
r	0.87	0.68	–0.78	–0.30	0.30	–0.70	–0.76	–0.73	–0.87	–0.95
p	0.01*	0.09**	0.04*	0.52	0.52	0.08**	0.05*	0.07**	0.01*	0.00*

* – correlation significant at level $p < 0.05$, ** – correlation significant at level $p < 0.1$.
RAG – rapidly available glucose, *RDS* – rapidly digestible starch, *SDS* – slowly digestible starch, *RS* – resistant starch, *TS* – total starch, *SDF* – soluble dietary fibre, *IDF* – insoluble dietary fibre, *TDF* – total dietary fibre.
RAG, starch fractions and macronutrient contents are expressed as grams per serving containing 50 g of available carbohydrates.

similar formulation and same processing conditions. Therefore, the sole impact of *SDF* could be noticed.

Jenkins et al. [42] examined the interaction between starch and proteins in whole grain wheat bread and found that the removal of gluten from wheat flour increased the amylolytic digestion in vitro and increased the in vivo glycemic response. This was explained by formation of a gluten-protein complex that interfered with the amylase action. Similar results were obtained by Berti et al. [43] who examined the same parameters in pasta and bread, with and without gluten. These authors also observed effects of the technological processing on in vitro starch digestibility. On the other hand, Packer et al. [44] explained the phenomenon solely by differences in the manufacturing process.

In our study, gluten was not removed but gluten-free flours were added at a rate of 30% instead of refined white wheat flour. In spite of this, no negative effects of dilution of gluten on the observed parameters were noticed. Moreover, the introduction of selected flours to control formulation resulted in a decrease of in vitro starch digestibility, as well as in vivo glucose response with the highest impact observed for biscuits enriched with soya flour (by 25.3% lower *GI* values compared to the control sample). These findings are consistent with the results of Blair et al. [45] suggesting that food based on soya has low or medium *GI* values, and is suitable for the regulation of blood glucose and insulin levels. Such statements were confirmed in the study of Sugiyama et al. [46], where the addition of grounded soybean into mixed meal foods resulted in a significant ($p < 0.001$) decrease of *GI* values.

In the present study, implementation of amaranth flour into the control formulation led to the reduction of *GI* by 12.6%, as opposed to findings of Chaturvedi et al. [47] who observed positive dose-dependent relationship between the amaranth flour added in the diet and *GI* values. Results were explained by physical properties of amaranth starch (lower viscosity, high amylopectin content, etc.). Taking into account that the strong causal relationships were established between *GI* values and macronutrient content in the examined samples (Tab. 5), as well as lower in vitro starch digestibility in biscuits enriched with amaranth, it could be concluded that quoted macronutrients and coarsely milled amaranth flour had a crucial impact on the glucose response in vivo.

The buckwheat flour incorporation into the biscuit formulation resulted in a slightly lower *GI* value (by 2.3%), which was not statistically significant. However, this effect suggests that the addition of buckwheat flour in larger quantities may result in lower *GI* values of the final product. This was previously indicated by Škrabanja et al. [48], who implemented 50% of buckwheat groats into wheat-based bread and achieved a significant reduction in postprandial glucose and insulin levels, compared with reference white wheat bread.

Inulin removal from the control biscuits resulted in an increase in *GI* (by 11.3%). The observed effect was stronger in comparison to *GI* of saccharose-containing sample, where the presence of saccharose instead of isomalt caused an increase in *GI* by 9.5%. This observation indicates a strong impact of inulin on the reduction of in vivo glucose response. It can be explained by the fact that inulin is highly hydrophilic and absorbs the water present, thus inhibiting starch gelatinization and digestion [49]. The obtained results are consistent with those of Brennan et al. [50], who added up to 10% of inulin in the wheat flour pasta recipe and lowered starch digestibility in proportion to the amount of the added inulin.

CONCLUSION

In the new millennium, the food industry is faced with the challenge of formulating novel products with optimal nutritional properties that may have beneficial influence on human health. The present study investigated the impact of an addition of selected raw materials on in vitro starch digestibility as well as on in vivo glucose response. Although in vitro methods cannot completely imitate conditions of digestion in the human body, a very good correlation between these two approaches was observed. It suggested that creation of biscuits with a low content of rapidly available glucose and rapidly digestible starch, and consequently with a high content of slowly digestible starch, represents an effective way to lowering the *GI* of this popular food. Therefore, in the era of increasing demands for products with health-enhancing effects, the obtained results could help the confectionery industry to offer new products as a part of a balanced diet, in particular for diabetics and persons with impaired glucose tolerance.

REFERENCES

1. Sudha, M. L. – Vetrimani, R. – Leelavathi, K.: Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chemistry, *100*, 2007, pp. 1365–1370.
2. Pareyt, B. – Talhaoui, F. – Kerckhofs, G. – Brijs, K. – Goesaert, H. – Wevers, M. – Delcour, J. A.: The role of sugar and fat in sugar-snap cookies: Structural and textural properties. Journal of Food Engineering, *90*, 2009, pp. 400–408.
3. Gambuś, H. – Gambuś, F. – Pastuszka, D. – Wrona, P. – Ziobro, R. – Sabat, R. – Mickowska, B. – Nowotna, A. – Sikora, M.: Quality of gluten-free suplemented cakes and biscuits. International Journal of Food Sciences and Nutrition, *60*, 2009, pp. 31–50.
4. Zaker, A. – Genitha, T. R. – Hashimi, S. I.: Effects of defatted soy flour incorporation on physical, sensorial and nutritional properties of biscuits. Journal of Food Processing and Technology, *3*, 2012, pp. 1–4.
5. Filipčev, B. – Šimurina, O. – Sakač, M. – Sedej, I. – Jovanov, P – Pestorić, M. – Bodroža-Solarov, M.: Feasibility of use of buckwheat flour as an ingredient in ginger nut biscuit formulation. Food Chemistry, *125*, 2011, pp. 164–170.
6. Hozová, B. – Buchtová, V. – Dodok, L. – Zemanovič, J.: Microbiological, nutritional and sensory aspects of stored amaranth biscuits and amaranth crackers. Nahrung, *41*, 1997, pp. 155–158.
7. Takahama, U. – Hirota, S.: Fatty acids, epicatechin-dimethylgallate, and rutin interact with buckwheat starch inhibiting its digestion by amylase: implications for the decrease in glycemic index by buckwheat flour. Journal of Agricultural and Food Chemistry, *58*, 2010, pp. 12431–12439.
8. Capriles, V. D. – Coelho, K. D. – Guerra-Matias, A. C. – Aręas, J. A. G.: Effects of processing methods on amaranth starch digestibility and predicted glycemic index. Journal of Food Science, *73*, 2008, pp 160–164.
9. Kim, H. K. – Kim, M. J. – Cho, H. Y. – Kim, E.-K. – Shin, D. H.: Antioxidative and anti-diabetic effects of amaranth (*Amaranthus esculantus*) in streptozocin-induced diabetic rats. Cell Biochemistry and Function, *24*, 2006, pp. 195–199.
10. Messina, M. J.: Legumes and soybeans: overview of their nutritional profiles and health effects. American Journal of Clinical Nutrition, *70*, 1999, pp. 439S–450S.
11. Avallone, R. – Plessi, M. – Baraldi, M. – Monzani, A.: Determination of chemical composition of carob (*Ceratonia siliqua*): protein, fat, carbohydrates, and tannins. Journal of Food Composition and Analysis, *10*, 1997, pp. 166–172.
12. Marangoni, F. – Poli, A.: The glycemic index of bread and biscuits is markedly reduced by the addition of a proprietary fiber mixture to the ingredients. Nutrition, Metabolism and Cardiovascular Diseases., *18*, 2008, pp. 602–605.
13. Jenkins, A. L. – Jenkins, D. J. A. – Wolever, T. M. S. – Rogovik, A. L. – Jovanovski, E. – Božikov, V. – Rahelić, D. – Vuksan, V.: Comparable postprandial glucose reductions with viscous fiber blend enriched biscuit in healthy subjects and patients with diabetes mellitus: acute randomized controlled clinical trial. Croatian Medical Journal, *49*, 2008, pp. 772–782.
14. Englyst, H. N. – Kingman, S. M. – Cummings, J. H.: Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition, *46*, 1992, pp. S33–S50.
15. Jenkins, D. J. A. – Kendall, C. W. C. – Augustin, L. S. A. – Franceschi, S. – Hamidi, M. – Marchie, A. – Jenkins, A. L. – Axelsen, M.: Glycemic index: overview of implications in health and disease. American Journal of Clinical Nutrition, *76*, 2002, pp. 266S–273S.
16. Goni, I. – Garcia-Alonso, A. – Saura-Calixto, F.: A starch hydrolysis procedure to estimate glycemic index. Nutrition Research, *17*, 1997, pp. 427–437.
17. Brouns, F. – Bjorck, I. – Frayn, K. N. – Gibbs, A. L. – Lang, V. – Slama, G. – Wolever, T. M. S.: Glycaemic index methodology. Nutrition Research Reviews, *18*, 2005, pp. 145–171.
18. Approved methods of the AACC. 10th ed. St. Paul : American Association of Cereal Chemists, 2000. 1200 pp. ISBN 1-891127-12-8.
19. Official methods of analysis. 17th ed. Washington : Association of Official Analytical Chemists, 2000. 2200 pp. ISBN 0-935584-67-6.
20. Food energy – methods of analysis and conversion factors. Food and Nutrition Paper 77. Rome : Food and Agriculture Organization, 2003. 87 pp. ISBN 92-5-105014-7.
21. Langkilde, A. M. – Andersson, H. – Schweizer, T. F. – Würsch, P.: Digestion and absorption of sorbitol, maltitol and isomalt from the small bowel. A study in ileostomy subjects. European Journal of Clinical Nutrition, *48*, 1994, pp. 768–775.
22. Roberfroid, M. B.: Caloric value of inulin and oligofructose. Journal of Nutrition, *129*, 1999, pp. 1436–1437.
23. Kumagai, T. – Kawamura, H. – Fuse, T. – Watanabe, T. – Saito, Y. – Masumura, T. – Watanabe, R. –

Kadowaki, M.: Production of rice protein by alkaline extraction improves its digestibility. Journal of Nutritional Science and Vitaminology, *52*, 2006, pp. 467–472.
24. Sacks, D. B.: Carbohydrates. In: Burtis, C. A. – Ashwood, E. R. (Ed.): Tietz textbook of clinical chemistry. 3rd Ed. Philadelphia : Saunders Company, 1999, p. 778. ISBN 0721-65610-2.
25. Carbohydrates in human nutrition. Food and Nutrition Paper 66. Rome : Food and Agriculture Organization, 1998. 140 pp. ISBN 92-5-104114-8.
26. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union, *L 404*, 2006, pp. 9–25.
27. Rayas-Duarte, P. – Mock, C. M. – Satterlee, D.: Quality of spaghetti containing buckwheat, amaranth, and lupin flours. Cereal Chemistry, *73*, 1996, pp. 381–387.
28. Rani, V. – Grewal, R. B. – Khetarpaul, N.: Sensory and nutritional evaluation of soy supplemented nutritious baked products. Journal of Dairying, Foods and Home Sciences, *27*, 2008, pp. 209–215.
29. Bilgiçli, N. – Ibanoğlu, Ş. – Herken, E. N.: Effect of dietary fibre addition on the selected nutritional properties of cookies. Journal of Food Engineering, *78*, 2007, pp. 86–89.
30. Gilani, G. S. – Xiao, C. W. – Cockell, K. A.: Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. British Journal of Nutrition, *108*, 2012, pp. S315 – S332.
31. Ikeda, K. – Sakaguchi, T. – Kusano, T. – Yasumoto, K.: Endogenous factors affecting protein digestibility in buckwheat. Cereal Chemistry, *68*, 1991, pp. 424–427.
32. Singh, J. – Dartois, A. – Kaur, L.: Starch digestibility in food matrix: a review. Trends in Food Science & Technology, *21*, 2010, pp. 168–180.
33. Oates, C. G.: Towards an understanding of starch granule structure and hydrolysis. Trends in Food Science & Technology, *8*, 1997, pp. 375–382.
32. Ragaee, S. – Guzar, I. – Dhull, N. – Seetharaman, K.: Effects of fiber addition on antioxidant capacity and nutritional quality of wheat bread. LWT–Food Science and Technology, *44*, 2011, pp. 2147–2153.
35. Bravo, L. – Englyst, H. E – Hudson, G. J.: Nutritional evaluation of carbohydrates in the Spanish diet: Non-starch polysaccharides and in vitro starch digestibility of breads and breakfast products. Food Research International, *31*, 1998, pp. 129–135.
36. Englyst, K. N. – Vinoy, S. – Englyst, H. N. – Lang, V.: Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. British Journal of Nutrition, *89*, 2003, pp. 329–339.
37. Lehmann, U. – Robin, F.: Slowly digestible starch – its structure and health implications: a review. Trends in Food Science and Technology, *18*, 2007, pp. 346–355.
38 Svihus, B. – Uhlen, A. K. – Harstad, O. M.: Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. Animal Feed Science and Technology, *122*, 2005, pp. 303–320.
39. Brand-Miller, J. – Foster-Powell, K.: Diets with a low glycemic index: from theory to practice. Nutrition Today, *34*, 1999, pp. 64–72.
40. Garsetti, M. – Vinoy, S. – Lang, V. – Holt, S. – Loyer, S. – Brand-Miller, J. C.: The glycemic and insulinemic index of plain sweet biscuits: relationships to *in vitro* starch digestibility. Journal of the American College of Nutrition, *24*, 2005, pp. 441–447.
41. Wolever, T. M. S.: Relationship between dietary fiber content and composition in foods and glycemic index. American Journal of Clinical Nutrition, *51*, 1990, pp. 72–75.
42. Jenkins, D. J. A. – Thorne, M. J. – Wolever, T. M. S. – Jenkins, A. L. – Venketschwer, R. – Thompson, L. U.: The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. American Journal of Clinical Nutrition, *45*, 1987, pp. 496–451.
43. Berti, C. – Riso, P. – Monti, L. D. – Porrini, M.: In vitro digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. European Journal of Nutrition, *43*, 2004, pp. 198–204.
44. Packer, S. C. – Dornhorstt, A. – Frost, G. S.: The glycaemic index of a range of gluten-free foods. Diabetic Medicine, *17*, 2000, pp. 657–660.
45. Blair, R. M. – Henley, E. C. – Tabor, A.: Soy foods have low glycemic and insulin response indices in normal weight subjects. Nutrition Journal [online]. Vol. 5, No. 35, 27 December 2006 [cit. 6 Juni 2013]. <http://www.nutritionj.com/content/5/1/35>. ISSN 1475–2891.
46. Sugiyama, M. – Tang, A. C. – Wakaki, Y. – Koyama, W.: Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. European Journal of Clinical Nutrition, *57*, 2003, pp. 743–752.
47. Chaturvedi, A. – Sarojini, G. – Nirmala, G. – Nirmalamma, N. – Satyanarayana, D.: Glycemic index of grain amaranth, wheat and rice in NIDDM subjects. Plant Foods for Human Nutrition, *50*, 1997, pp. 171–178.
48. Škrabanja, V. – Liljeberg Elmståhl, H. G. M. – Kreft, I. – Björck, I. M. E.: Nutritional properties of starch in buckwheat products: studies in vitro and in vivo. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 490–496.
49. Tudorica, C. M. – Kuri, V. – Brennan, C. S.: Nutritional and physicochemical characteristics of dietary fiber enriched pasta. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 347–356.
50. Brennan, C. S. – Kuri, V. – Tudorica, C. M.: Inulin-enriched pasta: effects on textural properties and starch degradation. Food Chemistry, *86*, 2004, pp. 189–193.

Received 16 July 2013; 1st revised 23 September 2013; accepted 30 October 2013; published online 5 May 2014.