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EFFECT OF SWEETENERS AND NON-TRADITIONAL FLOURS ON SENSORY ACCEPTABILITY AND GLYCEMIC INDEX OF BISCUITS

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ABSTRACT

Nowadays, food manufacturers are trying to include alternative sweeteners in their product formulations because they are perceived by consumers as preferable to sugar, which plays an important role in the development of civilisation diseases such as diabetes and obesity. One possible indicator of the suitability of a food regarding the prevention and treatment of these diseases may be the glycemic index value, which in bakery products may also be influenced by the type of flour used. The aim of this study was to investigate the effect of different types of sweeteners (sugar, aspartame-acesulfame, Stevia) and non-traditional flours on the sensory acceptability and glycemic index of biscuits. Both quantitative and hedonic descriptors were assessed by panellists ($n = 14$). Using quantitative descriptive and hedonic analysis, it was found that the use of alternative flours was only appropriate up to 15% replacement of wheat flour. Biscuit samples sweetended by sugar performed above average in the overall evaluation. The results also showed that flaxseed flour was rated better than hemp flour, which in addition showed the least suitable combination with stevia sweetener. The use of flaxseed flour in combination with the sweetener aspartame-acesulfame appeared to be less acceptable. The glycemic index was determined using the GOPOD test kit and the results showed that none of the flax and hemp flour additions had a significant ($p > 0.05$) effect on the glycemic index value, nor did the sweeteners used.

Keywords: aspartame-acesulfame, steviol glycoside, sucrose, flax flour, hemp flour, sensory analysis

INTRODUCTION

Food manufacturers are using alternative natural and synthetic sweeteners in place of conventional sugar in their formulas to meet market demand. However, some synthetic sweeteners (e.g. aspartame, saccharin, sucralose) are becoming less popular among consumers due to health concerns, lower nutritional values and controversies about their safety. In contrast, natural sweeteners are perceived favourably by consumers due to their association with healthier lifestyles and higher nutritional values **(Castro-Muñoz *et al.*, 2022)**. The glycemic index (GI), which is a recognised valid and reproducible method of classifying carbohydrate foods, can be an important indicator of food suitability for overweight or diabetic individuals. Foods with a low GI are important for the prevention and treatment of diabetes, coronary heart disease and possibly obesity. Possible associations have been observed for some cancers. Given the high prevalence of diabetes worldwide, consumer awareness of the composition and glycemic index of foods is important. This information is available to consumers on food labels or may be available through food composition tables and in some cases in the form of dietary recommendations **(Augustin et al., 2015)**. The glycemic index value may be influenced by the fibre content or the type of flour used. The current trend of producing nutritionally healthier bakery products follows the addition of non-traditional flours to wheat flour **(Švec *et al.*, 2015)**. The interest in using certain plant species that can be used for flour production stems mainly from the search for and promotion of nutritional properties **(Dini *et al.*, 2012)**. An example is the use of hemp flour in bread **(Hofmanová *et al.*, 2014)** or biscuits **(Lukin & Bitiutskikh, 2017)** in order to increase the nutritional value of the food. Hemp seeds are rich in fibre, lipids, protein and a number of minerals **(Gumus *et al.*, 2023)**, which partially remain in the pomace after pressing the seeds. The addition of hemp seed meal significantly increases the protein and fibre content of foods **(Švec *et al.*, 2015)**. Flaxseed meal can also be used in a similar way **(Pankratov *et al.*, 2020)**. Currently, flaxseed meal is used as livestock feed, where it is a common source of protein. From a nutritional point of view, important components of flax meal are being investigated for bioactivity and potential benefits to human health **(Oomah, 2020)**. The use of proteins and other ingredients in the food industry is limited, due to the lack of pilot studies that could make the procedures applicable to industry **(Oomah, 2020)**. Enabling the use of by-products derived from hemp and flaxseed oil processing in industry could meet the ever-increasing need for sustainable protein, while reducing food waste **(Oomah, 2020; Setti *et al.*, 2020)**. This trend of using by-products in the food industry is also followed by this study focusing on the sensory acceptability of biscuits with the addition of flax and hemp meal obtained as by-product during oil pressing. In addition, this work investigates the addition of different sweeteners to biscuits and their effect on the glycemic index.

MATERIAL AND METHODS

Material

A total of 13 samples of biscuits with flax and hemp flour and various sweeteners were prepared. The flax and hemp flour were obtained as a by-product by pressing the seeds at the Institute of Hygiene and Technology of Food of Plant Origin, Veterinary University in Brno. The raw materials including sweeteners and seeds used for pressing were purchased from the local market. The hemp pomace, which was in the form of flakes, was ground to a finer consistency using a grinder (Bosch, Slovenia). The sweeteners aspartame-acesulfame and Stevia in tablet form were purchased from a local market. The commercial sweetener under the name 'aspartame-acesulfame' contained acidity regulators (sodium citrates and sodium bicarbonate), sweeteners (erythritol, aspartame (15 %) and acesulfame K (15 %)) and anti-caking agents (microcrystalline cellulose and magnesium stearate). The sweetener 'Stevia' contained steviol glycoside, sodium bicarbonate, tartaric acid and natural flavouring. The quantity of sweeteners used was according to the sweetness indicated on the packaging, so that the quantity of sweetener corresponded to 20 g of sugar indicated in the biscuit formula.

Sample preparation

Simple formula consisting of basic ingredients was used to prepare biscuits, in order to better identify the differences between the different modifications of the formula.-The composition of the different types of biscuits are shown in Table 1.

Table 1 Composition of the different types of biscuits

	Control	S_H 10	S_L 10	S_H 20	S_L 20	As_ H10	As_L 10	As_ H20	As_L 20	St_H 10	St_L 10	St_H 20	St_L 20
Butter	45 g	45 g	45 g	45 g	45 g	45 g	45 g	45 g	45 g	45 g	45 g	45 g	45 g
Wheat flour	65 g	55 g	55 g	45 g	45 g	55 g	55 g	45 g	45 g	55 g	55 g	45 g	45 g
Hemp flour	-	10 g	-	20 g	-	10 g	-	20 g	-	10 g	-	20 g	-
Linseed flour	-	-	10 g	-	20 g	-	10 g	-	20 g	-	10 g	-	20 g
Sucrose	20 g	20 g	20 g	20 g	20 g	-	-	-	-	-	-	-	-
Asp.-acesulfam	-	-	-	-	-	6 tbl.	6 tbl.	6 tbl.	6 tbl.	-	-	-	-
Stevia	-	-	-	-	-	-	-	-	-	5 tbl.	5 tbl.	5 tbl.	5 tbl.

Legend: Control - sucrose, wheat flour; St_H10 - stevia, 10g hemp flour; St_H20 - stevia, 20g hemp flour; St_L10 - stevia, 10g flax flour; St_L20 - stevia, 20g flax flour; As_H10 - aspartame-acesulfame, 10g hemp flour; As_H20 - aspartame-acesulfame, 20g hemp flour; As_L10 - aspartame-acesulfame, 10g linseed flour; As_L20 - aspartame-acesulfame, 20g linseed flour; S_H10 - sucrose, 10g hemp flour; S_H20 - sucrose, 20g hemp flour; S_L10 - sucrose, 10g linseed flour; S_L20 - sucrose, 20g linseed flour.

The ingredients were mixed and worked into a dough, which was wrapped in cling film and left to rest in the fridge for several hours. The dough was then rolled out to a thickness of approximately 2 mm and biscuits were cut out in the shape of a circle with a diameter of 4 cm (figure 1). Baking in an oven (UNOX Elena XFT 183, Italy) was carried out for 9 min at 170 °C.

control hemp flour biscuit flax flour biscuits

Figure 1 Control sample and samples with the addition of 30% g of hemp and flax flour (Authors photo)

Sensory analysis

Sensory analysis was conducted by trained panellists (*n* = 14) aged 26-58 years (9 women and 5 men). The panel consisted of academic staff and students of the University of veterinary sciences Brno. Each panellist was provided with 1 piece of biscuit labelled with a 3-digit numerical code (figure 2). In the sensory analysis, quantitative (color, foreign smell intensity, sweet taste intensity, foreign taste intensity, hardness) and hedonic (pleasantness of appearance, aroma, taste, texture and overall rating) descriptors were assessed using categorical ordinal scales with extreme values of 1 and 9. A value of 1 corresponded to the lowest intensity or pleasantness and a value of 9 to the highest intensity or pleasantness in a given descriptor. Still water was used as a neutralizer.

Figure 2 Set of experimental samples coded for sensory analysis (photo by authors)

The results of the sensory analyses were evaluated by principal component analysis using statistical software R version 3.3.3 (The R Foundation for Statistical Computing, Austria).

Determination of the glycemic index

Samples most acceptable to the consumer from a sensory point of view were used to determine the glycemic index. The glycemic index was determined for 6 samples and a control sample. The GOPOD test kit (Megazyme, Ireland) was used to analyse the selected samples for the determination of glucose content.
The methodology **(Pečová & Zielińska, 2022)** was applied to the samples to determine the values of predicted glycemic index in vitro. From the start of amylolysis, samples were taken at time intervals of 20, 30, 60, 90, 120 and 180 min. Absorbance was measured at 510 nm after 20 min incubation of the samples at 40 °C according to the manufacturer's instructions for the test kit used. Based on the glucose content determination, curves were constructed and the Area Under the Curve (AUC) of each sample was calculated. The areas of the samples were compared with the area of the control sample and the glycemic index was then calculated from these data according to the formula of **Goñi *et al.* (1997)**.
GI (%) = 39.71 + 0.549×HI

The GI results were statistically processed using the Kruskal-Wallis test (one-way ANOVA) at a significance level of p=0.05 (Unistat ltd., UK).

RESULTS AND DISCUSSION

Quantitative descriptive analysis

Using quantitative descriptive analysis, the results (figures 3 and 4) of the effect of the addition of non-traditional flours and different sweeteners on the descriptors of the biscuits were obtained. The graph of the principal component analysis results shows 76% of the total variability of the data evaluated.

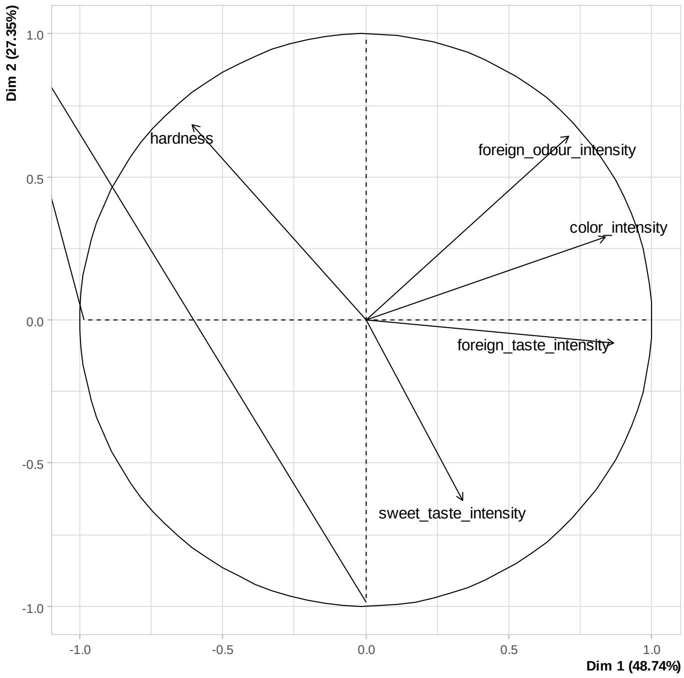


Figure 3 Results of quantitative descriptive analysis of sweetener and non-traditional flour biscuit samples – Map of variables

The sample map (figure 4) shows that the control sample was significantly separated from the other experimental samples. A statistically significant difference ($p < 0.05$) was confirmed between the control and the samples of biscuits containing Stevia and the addition of both flax and hemp flour, with the exception of the Stevia sample containing 10 g of flax flour. Conversely, no statistically significant difference was confirmed between the control and the samples containing sugar, except for the sample containing sugar and 20 g of hemp flour ($p < 0.05$). The sample map further shows that the samples containing aspartame-acesulfame were significantly different from the control ($p < 0.05$), except for the sample containing aspartame-acesulfame and 10 g of flax flour ($p > 0.05$).
In accordance with the original hypothesis, the control sample showed the lowest intensity of foreign smell, foreign taste (figure 3 and 4) and also the lowest intensity of color, which can also be seen in figure 5. The results further show that the samples with lower addition of flaxseed flour (10 g), namely S_L10, As_L10 and St_L10, had statistically significantly lower intensity of color. On the other hand, statistically significantly higher color intensity was observed in the biscuit samples with higher addition of alternative flour, especially in the case of hemp flour content (St_K10, S_K20, As_K20 and St_K20). The intensity of foreign smell (5.3 and 5.1) and foreign taste (6.5 and 5.9) were found to be statistically significantly higher ($p < 0.05$) in the samples of biscuits containing Stevia and hemp flour (St_K10 and St_K20). The values for the hardness descriptor were statistically significantly higher ($p < 0.05$) in the control sample, and in all experimental biscuit

samples containing sugar. On the contrary, the samples of biscuits containing Stevia and aspartame-acesulfame (As_L10, As_L20, St_L10, St_L20, and As_K10) were perceived as more brittle, suggesting that the sweetener used has an effect on the texture of the biscuits, which is also confirmed by **Pourmohammadi *et al.* (2017)** and **Savitha *et al.* (2008)**.

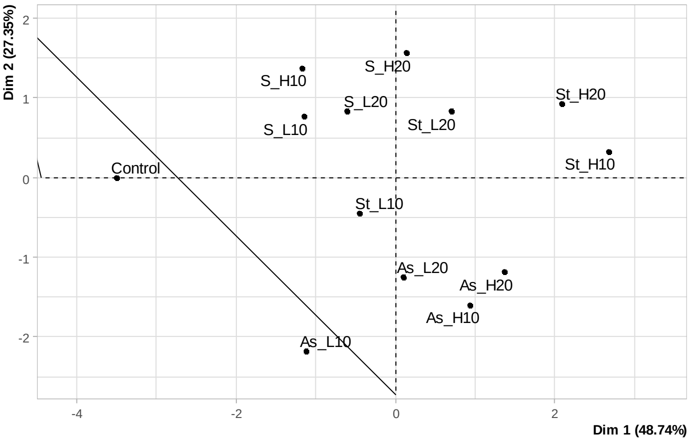
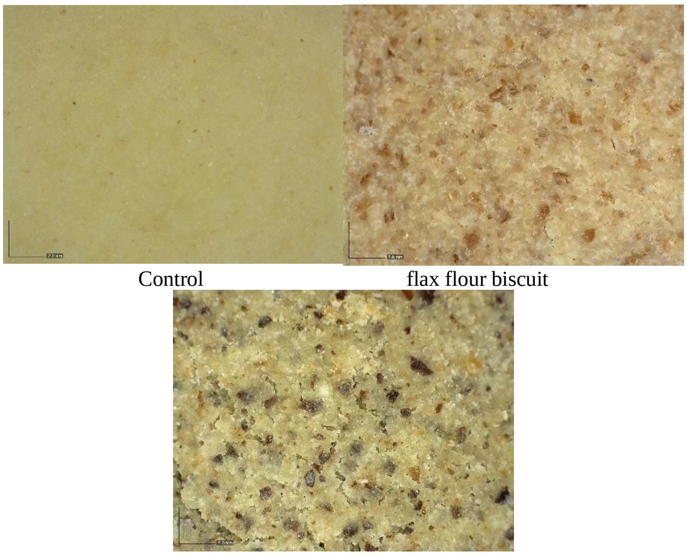


Figure 4 Results of quantitative descriptive analysis of sweetener and non-traditional flour biscuit samples – Map of samples

Legend: Control - sucrose, wheat flour; St_H10 - stevia, 10g hemp flour; St_H20 - stevia, 20g hemp flour; St_L10 - stevia, 10g flax flour; St_L20 - stevia, 20g flax flour; As_H10 - aspartame-acesulfame, 10g hemp flour; As_H20 - aspartame-acesulfame, 20g hemp flour; As_L10 - aspartame-acesulfame, 10g linseed flour; As_L20 - aspartame-acesulfame, 20g linseed flour; S_H10 - sucrose, 10g hemp flour; S_H20 - sucrose, 20g hemp flour; S_L10 - sucrose, 10g linseed flour; S_L20 - sucrose, 20g linseed flour

The effect of the raw materials used on the texture can be seen in more detail in figure 5, which shows images of the surface of the control sample, samples with the addition of flax and hemp flour at 30%. These images were taken with a Dino-Lite AM4115T digital USB microscope (AnMo Electronics, Taiwan). In the case of the 30% addition of hemp flour, signs of disturbed structure (cracks) are clearly visible, which negatively affects the integrity of the surface.



Control flax flour biscuit

hemp flour biscuit

Figure 5 Images of biscuit samples (photo by authors)

Mikulec *et al.* (2019), who used hemp flour in their work for bread production, reported that hemp flour affects the color of bread, thus confirming our results where hemp flour had a significant effect on the color intensity of the biscuits ($p < 0.05$). They further reported that the proportion of 30% and 50% hemp flour contributed to a reduction in the organoleptic rating of bread, therefore, for industrial production, the proportion of hemp flour should not exceed 30%. Bread with the addition of hemp flour was characterized by higher protein and polyphenol content **(Mikulec *et al.*, 2019)**. Among the experimental samples analyzed in our study, only those with a maximum content of 30% (20 g) of alternative flours were included.

Other authors have used flaxseed flour in bread to increase the nutritional value of wheat-rye bread. Thus, the addition of flax meal increased the protein, calcium and iron content of the bread. During the study, organoleptic properties were also investigated, through which it was found that the introduction of flaxseed flour into bread formulas was appropriate up to 15% of the total bread weight **(Safronova & Fatyanov, 2021)**.

Hedonic analysis

Using hedonic analysis, results (figure 5 and 6) on the effect of the addition of non-traditional flours and different sweeteners on descriptors related to the pleasantness of appearance, aroma, flavour, texture and overall biscuit evaluation were obtained. The graph of the principal component analysis results describes 89.8% of the total variability of the evaluated data.

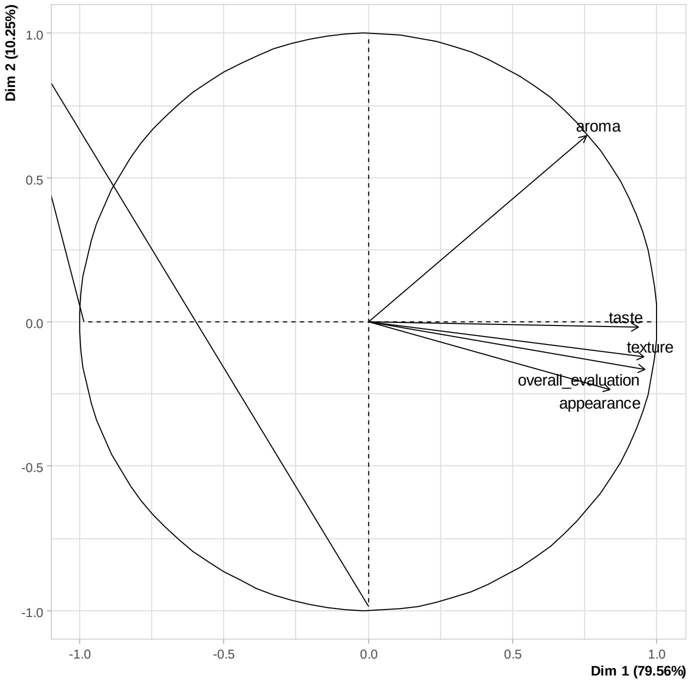


Figure 5 Results of hedonic analysis of biscuit samples containing sweetener and non-traditional flour – Map of variables

The sample map for hedonic analysis shows that the control is again significantly separated from the other experimental samples. The results of the hedonic analysis of the experimental biscuit samples showed that, apart from the control sample (overall score of 7.0), only samples S_H10 (6.1) and S_L20 (6.1) scored statistically significantly ($p < 0.05$) higher in the overall evaluation. Furthermore, samples S_L10 (5.5) and S_H20 (5.4) achieved above average values in the overall evaluation. The sugar-containing samples (S_L10, S_L20 and S_H10) together with the control were rated statistically significantly better ($p < 0.05$) in the texture pleasantness descriptor. On the other hand, the samples of biscuits containing Stevia St_K10, St_K20 and St_L10 ($p < 0.05$) were statistically significantly lower in the overall evaluation, while at the same time showing statistically significantly lower values in taste and texture pleasantness. The results show that the combination of steviol glycosides and hemp flour was not perceived positively by the evaluators.

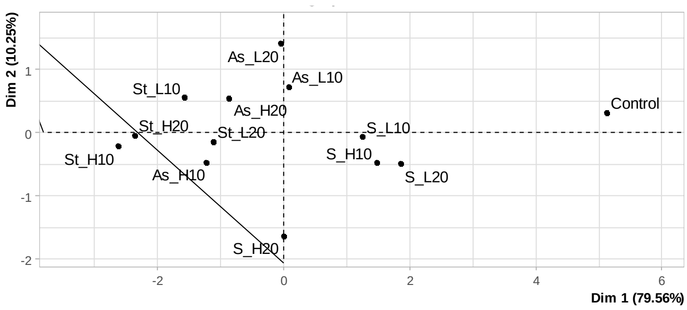


Figure 6 Results of hedonic analysis of biscuit samples containing sweetener and non-traditional flour – Map of samples

Legend: Control - sucrose, wheat flour; St_H10 - Stevia, 10g hemp flour; St_H20 - Stevia, 20g hemp flour; St_L10 - Stevia, 10g flaxseed flour; St_L20 - Stevia, 20g flaxseed flour; As_H10 - aspartame-acesulfame, 10g hemp flour; As_H20 - aspartame-acesulfame, 20g hemp flour; As_L10 - aspartame-acesulfame, 10g flaxseed flour; As_L20 - aspartame-acesulfame, 20g flaxseed flour; S_H10 - sucrose, 10g hemp flour; S_H20 - sucrose, 20g hemp flour; S_L10 - sucrose, 10g flaxseed flour; S_L20 - sucrose, 20g flaxseed flour

The biscuits containing flaxseed flour with the sweetener aspartame-acesulfame achieved slightly below average values in the overall evaluation (4.3-4.9). In terms of pleasantness of appearance, no statistically significant differences were described between the experimental samples, with values ranging from 4.7-5.9; only the control sample achieved a statistically significant ($p < 0.05$) higher rating for pleasantness of appearance (7.4).

The experimental samples with the addition of flaxseed flour and aspartame-acesulfame showed higher aroma acceptability (5.3 - 5.6), while the samples with the same sweetener and the addition of hemp flour showed lower aroma acceptability values (4.4 - 4.8), suggesting that the addition of alternative flours may also affect the aroma of the final product. As the samples of biscuits containing sugar as a sweetener were the best sensory evaluated, irrespective of the alternative flour used and its concentration, it seems preferable not to use alternative sweeteners to achieve a product formulation with the highest possible nutritional value, as they introduce a specific and less usual taste to the product for ordinary consumers without any diet restrictions regarding sugar and may lead to an unsatisfactory sensory quality.

Glycemic index

For the determination of the glycemic index, the most acceptable samples from the sensory evaluation were selected, as well as samples in which both types of flour and all types of sweeteners used were represented. The glycemic index is an indicator of the ability of a food to raise blood glucose levels(**Chlup *et al.*, 2019**). The results of this study showed that the addition of flaxseed and hemp flour did not have a significant effect on the GI of the biscuits ($p > 0.05$). **Lal *et al.* (2021)** in their study reported that various factors affect the digestibility of starch and hence the GI of foods. These can be internal factors such as amylose, lipids, proteins, phytic acid, fibre and resistant starch (RS) or external factors which include cooking, processing, retrogradation, soaking and sprouting. The change in GI can also be influenced by the food matrix **(Lal *et al.*, 2021)**. Our results showed that none of the flax and hemp flour additions had a significant ($p > 0.05$) effect on the GI value. Similarly, no statistically significant difference ($p > 0.05$) was found for the sweeteners used (sugar, aspartame-acesulfame, Stevia).
The literature reports an average glycemic index (GI) of 41% for chocolate-covered biscuits **(Chlup *et al.*, 2019)**. The GI of fibre-enriched biscuits with bread as the reference food was 58.9% **(Schuchardt *et al.*, 2016)**. Another source reported a GI value of 47% for biscuits containing 50% tempeh gembus flour **(Manullang *et al.*, 2020)**.
The graph (figure 7) shows the gradual release of glucose for each biscuit sample as a function of time. As expected, the rate of increase in blood sugar at 30, 60, 90 and 120 min was highest in the control sample.

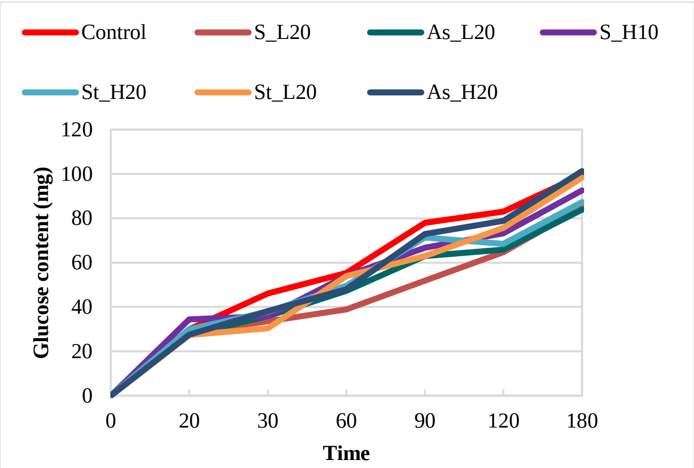


Figure 7 The release of glucose of biscuit samples in time

The graph also shows that the fastest increase in GI occurred in the first 20 minutes for all biscuit samples, with the sample containing sugar and 10 g of hemp flour showing a higher increase in glucose content compared to the other samples. The slowest increase in glucose was observed in the biscuit sample containing sucrose and 20 g of flaxseed flour, while the largest increase was observed in the control sample.

CONCLUSION

Flours derived from by-products represent a nutritionally valuable ingredient potentially useful in commercial bakery formulas. Flax and hemp flours were selected for this work because they are naturally gluten-free and also because they have the potential to contribute to addressing the ever-increasing need for sustainable protein while reducing food waste. The results of our work have shown that the use of alternative flours is only suitable from a sensory analysis perspective at lower concentrations and some combinations of flours and sweeteners used are not suitable. Of the flours mentioned, flaxseed flour at 15% appeared to be more suitable for the selected sweeteners used, while hemp flour at more than 15% and in combination with Stevia sweetener appeared to be the least suitable. Because of the possible strong specific taste, it is preferable not to combine alternative flours above 15 % with sweeteners other than sugar, as this could lead to a reduction in sensory acceptability and consumer rejection. Sweeteners in the form of aspartame-acesulfame or steviol-glycosides may be used commercially in the formulation of biscuits enriched with nutritionally valuable flour derived from by-products only in the case of the addition of non-traditional flour up to 15%, which does not reduce the acceptability particularly of taste and flavour below average values. The results also show that the addition of flax or hemp flour and the use of different sweeteners does not have a significant ($p > 0.05$) effect on the glycemic index value at any of the concentrations used.

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