



Postprandial Glycemic Response in Different Bakery Formulations with Adequate Palatability: Sex-related Effects

Journal:	<i>Nutrition and Food Science</i>
Manuscript ID	NFS-06-2021-0172.R1
Manuscript Type:	Original Article
Keywords:	glycemia, formulated food, nutritional values, sex differences

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Title:

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Abstract**Purpose**

Hyperglycemic diets are a critical risk factor for diabetes and other chronic diseases; therefore, food innovation is encouraged. In this sense, starchy foods with a better postprandial glycemic response (PGR) could contribute to disease prevention for consumers. This study aimed to evaluate the effect of three biscuit formulations on glycemic indicators -in general, and by sex- and their acceptability.

Methodology

We analyzed the nutritional composition of biscuits A, B and C (high, moderate, and low fiber content, respectively, among other differences) and the PGR, glycemic index (GI) and glycemic load (GL) after being consumed by 15 healthy adults. A sensory evaluation was also performed (n=54 subjects). An analysis of variance and generalized linear models were fitted to estimate the effect of formulations on glycemic indicators, including interactions by sex.

Findings

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4 For samples A, B and C (classic biscuit) the GI was 50, 54 and 51, respectively. The formulations did not show differences in the GI or GL. Lower
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6 values of PGR were observed in women ($p=0.01$) and inverse effects on glycemia in all formulations in women with respect to formulation C in men
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8 was found. Calories, fiber and monounsaturated fats showed inverse effects on the PGR, GI and GL. [Sample A had the highest value of acceptability](#)
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10 [\(86.1%\), considering all attributes assessed.](#)
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14 **Originality**

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17 A sex-related effect in glycemia was found, with all formulations triggered a better PGR in women. The nutritional profile of biscuits can be
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19 improved while maintaining palatability.
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23 **Keywords:** Glycemia, formulated foods, nutritional value, sex differences.
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26 ***Research paper***

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INTRODUCTION

The glycemic index (GI) plays a valuable role in food strategies to prevent chronic diseases. The recurrent intake of foods that induce postprandial hyperglycemia has been recognized as a risk factor for developing chronic metabolic diseases, such as obesity, type 2 diabetes mellitus and cardiovascular diseases (CVD) (Blaak *et al.*, 2012; Vinoy *et al.*, 2016).

The GI, a concept introduced by Jenkins *et al.* (1981), is a property of carbohydrates which is defined as the increased area under the curve (AUC) after a tested food, expressed as a percentage of the corresponding area following an equivalent carbohydrate load of reference food. The GI is also used to quantify the glycemic response (GR) to different types of carbohydrates, as key components to be considered in the nutritional formulation of processed food products. Complementary to the GI, the glycemic load (GL) aims to measure the glycemic impact of a regular food portion. This value is calculated by multiplying the GI value by the amount of carbohydrates contained in the food consumed.

Starchy foods have gained considerable interest among food industries, given that changes in their structure and composition may exert a positive impact on consumers' health by reducing the postprandial glycemic response (PGR) and insulin resistance (Nazare *et al.*, 2009). In addition, the technology and cooking methods applied to cereal products have a significant impact on the PGR. Food manufacturing, heat, moisture and pressure significantly modify the digestibility of starch in processed foods. In this sense, the combination of high moisture levels and high temperatures or high pressure leads to a high degree of starch gelatinization with an increase in their rate of digestion, which enhances the PGR (Vinoy *et al.*, 2016). These processing techniques also influence the concentration and availability of some nutrients and compounds, such as antioxidants, fibers and enzymes (Adedayo *et al.*, 2018).

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4 Biscuits are one of the most widely consumed starchy foods worldwide by all age groups. This type of processed food has a great amount of starch
5 and sugar with elevated GI. A high intake of these products increases the risk of developing metabolic diseases (Blaak *et al.*, 2012; Vinoy *et al.*,
6 2016), which have a different incidence between men and women (Woodward, 2019). In this sense, there is scarce published data about the
7 variability by sex of postprandial glycemic. In addition, industry shows a growing interest in improving the nutritional profiles of these products to
8 respond to the demand of consumers, who are increasingly interested in their health, and to policies avoiding the excess of unhealthy nutrients in
9 processed foods (Hoek *et al.*, 2017; Maganja *et al.*, 2019; Pieniak *et al.*, 2016). Therefore, it is encouraged to better characterize the products
10 developed, specifically in terms of carbohydrates and other macronutrients, to explore and understand the health-related effects of both a low
11 and a high GR in the general population and by sex. Increased availability of palatable snacks in the market, with an improved nutritional profile at
12 the same time, will constitute a relevant development for health promotion and prevention of pathologies with high morbidity and mortality rates
13 worldwide. This study aimed to evaluate the effect of three biscuit formulations on the PGR and their acceptability, in order to provide the required
14 evidence for processed products with a better nutritional profile.
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30 **MATERIALS AND METHODS**

31 **1. Study Design**

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35 The study was divided into 3 stages: a) nutritional composition analysis of the tested products, b) measurement of the postprandial glycemic
36 response after consumption, and c) acceptability evaluation of different formulations.
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2. Subjects

Healthy male and female individuals from Córdoba city (Argentina) were selected as they met the following criteria:

- For GR, GI and GL measurement: fifteen subjects over 18 years old, with breakfast habits and without food allergies, dietary behavior disorders, metabolic diseases, dietary restrictions or pregnancy, and fasting blood sugar levels <110 mg/dL, as recommended (WHO, 1998; Brouns *et al.*, 2005; Wolever, 2006).
- For sensory evaluation: fifty-four subjects over 18 years old were selected. The inclusion criteria were based on the participants' ages and the absence of food allergies.

All the participants signed an informed consent to be voluntarily included in the study, which was approved by the Ethical Committee of the Clinics National Hospital in Córdoba, Argentina, in accordance with the Declaration of Helsinki and later amendments, and the local legislation (Córdoba registration code: REPIS-3245).

2.1. Test Products

Two different biscuits were formulated on the pilot lines of a multinational food company in Córdoba, Argentina. Sample A (high fiber content) was a biscuit with 27-31% oat sweetened with sugar and high fructose corn syrup (HFCS); sample B (moderate fiber content), 8-12% oat sweetened with sugar only. These samples were compared with sample C, a classic dried sweet biscuit with low fiber content; some of its main ingredients were enriched wheat flour, sugar, high oleic sunflower oil, and glucose syrup.

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4 All samples had a grainy texture as well as a low-moisture and anti-caking mass. They were manufactured by rotary molding and a rapid cooking
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6 method was used.
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9 The variables considered in the analysis of the nutritional formulation were quantity and type of carbohydrates, fatty acids profile, and content of
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11 protein, fiber, and sodium. The proximal composition of biscuits was calculated from the nutritional information of the ingredients reported by the
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13 corresponding suppliers, according to act 69 of the National Commission on Food and the Argentine Food Code (Law 18284, Chapter V and XX)¹.
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16 **2.2. Postprandial Glycemic Response, GI and GL Determination**

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19 For glycemic measurements, 15 adult volunteers (9 males and 6 females), with a mean age of 30 (DE 6.72) years and a mean body mass index of
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21 22.38 (SD 1.54) kg/m², participated in the study. Each test was conducted in subjects with an 8 hour overnight fasting, the last meal being a light
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23 dinner without alcoholic beverages. In addition, they were asked not to perform any physical activity 2 days before the study. Participants
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25 consumed a portion of each test product containing 50 g of available carbohydrates, corresponding to 77.2, 70.5, and 69.9 g of samples A, B, and
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27 C, respectively, along with a glass of water. Capillary blood was collected using an ISO standard glucometer. One fasting blood sample was collected
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29 from each subject, and then at 15, 30, 45, 60, 90, and 120 minutes participants began to eat the test products. Each sample (white bread, sample
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31 biscuits A, B, and C) were measured one time in all persons with at least one week interval (maximum 14 days). The incremental area under the
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38 ¹ The methodology approved by AFC is as follows: Total caloric value: calculated by factors (Chap. V AFC); Carbohydrates by difference; Proteins: Kjeldhal,
39 (factor 6.25); Fats (total, saturated, monounsaturated, polyunsaturated, trans, w9 (AOAC Method 996.06); Cholesterol (Gas-Liquid Chromatography); Dietary
40 fiber (AOAC Method 985.29); Sodium (atomic absorption); Sugars (HPLC).
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4 curve (IAUC) of each sample biscuit was estimated, and then compared with the IAUC of the reference food (white bread) for GI determination. A
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6 correction factor (1.33) was applied to the resulting GI, adjusting the values to make results equivalent to GI with glucose as standard, as suggested
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8 by Wolever (2006). The GL was also calculated, based on a standard portion size of each product.
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11 **2.3. Sensory Evaluation**

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15 The sensory properties of the biscuits were determined by an internal tasting panel constituted by 54 evaluators, habitual consumers of these
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17 products and non-smokers (21 men and 33 women). Panelists received a serving of each biscuit formulation, with a glass of water at reasonable
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19 intervals for consumption. Subsequently, they were asked to evaluate the taste, color and texture of each biscuit sample through a 5-point hedonic
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21 scale (I like it a lot; I like it a little; I neither like nor dislike; I don't like it a little; I don't like it a lot), to then calculate the relative acceptability of
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23 new formulations with respect to a classic biscuit (sample C).

24 **3. Statistical Analysis**

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27 An analysis of variance (ANOVA) for repeated measurements was applied, followed by Bonferroni tests to evaluate differences in PGR at 15, 30,
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29 45, 60, 90 and 120 minutes after consumption between the biscuit formulations and the standard food as well.

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32 In addition, generalized linear models were fitted to estimate the effect of different biscuit formulations on six PGR measurements, as well as the
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34 effect on global glycemic indicators (IAUC, GI or GL). Gamma distribution was chosen for the responses, using identity as the link function. Age,
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36 body mass index (BMI) and interaction terms of different formulations by sex were included in the linear predictor.
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4 Multiple linear regression models were also fitted to identify the effect of specific nutrients that varied significantly among formulations on
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6 glycemic indicators. First, fiber and calories, and then fats, were evaluated as predictors. Finally, a log linear model for each indicator of acceptability
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8 was fitted to assess the sensory properties of the formulations. Stata 15 (Statacorp, 2016) and Infostat were used for data analysis, and $\alpha=0.05$
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10 was used as the significance level.
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13 RESULTS

14 15 16 Nutritional Composition

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19 Sample A presented the lowest content of available carbohydrates (CHO) per 100 g of product (64.7 g) and the highest fiber content (4.4 g), though
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21 it had the highest level of free sugars (22 g). In addition, sample B presented 70.9 g% of CHO and 2.7 g% fiber, while sample C had the highest
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23 content of CHO (71.5 g%) and the lowest one of fiber (1.9 g%). Regarding fat content, sample B had the lowest (13 g%) and sample A the highest
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25 percentage of that nutrient (16 g%); however, in both cases, unsaturated fatty acids made up most of it (about 88% of total fat). In addition,
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27 samples A and B doubled the amount of monounsaturated fatty acids in sample C, the biscuit with the largest amount of saturated fatty acids (46%
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29 of total fat). There were no differences in the amount of protein among samples (Table I).
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33 Postprandial Glycemic Response, GI and GL

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36 The three studied samples showed lower postprandial glycemic values than the reference food, mainly after 15 min following intake. Sample B
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38 seems to produce more stable levels of blood glucose in relation to the other samples, including the reference food. After consuming this
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4 formulation, from 15 to 90 min, participants presented average blood glucose values between 100 and 110 mg/dL, which led to the flattest curve
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6 observed in all formulations. On the other hand, samples A, C and white bread showed values of blood glucose peaking at 30 min of 120 mg/dL,
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8 115 mg/dL and 140 mg/dL, respectively (Fig. 1). The PGR values were different between the 3 samples and the white bread at 30 minutes ($p=0.001$).
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10 The GI and the GL (in brackets) obtained in the biscuit samples were 50 (8), 54 (9) and 51 (10) for samples A, B and C, respectively. The glycemic
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12 response patterns tend to be different by sex, with lower values of IAUC ($p=0.01$) observed in women, and more variable glycemic response to
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14 each formulation in this group with respect to men (Fig. 2).
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17 Results of the gamma generalized linear model analysis, which were fitted to compare the effect of the three different biscuit formulations on PGR,
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19 showed no significant effects when only principal elements –the three formulations– are included (data not shown). However, a differential sex-
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21 effect of formulations was found when interaction terms are included in the models. In female sex, the effect of three products A, B and C showed
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23 negative values on glycemia average with respect to the intake of formulation C (classic biscuit) in males at 30, 45, and 60 minutes of the intake.
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25 At 15 minutes, only the combination between females and product B presented a negative coefficient. Age and BMI had no effect on the PGR. The
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27 product B in female had a negative effect on the IAUC with respect to formulation C and to male. The different formulations did not show effects
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29 on the GI or GL. In addition, the BMI showed a positive effect on the GI and GL, while age showed an inverse effect (Table II).
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33 According to the results of the multiple linear regression models, calories have a significant effect on the decrease in blood glucose between 30
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35 and 90 minutes, and at 120 minutes the same effect was observed for fiber. Also, an inverse effect of dietary fiber on the GL was found.
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4 Monounsaturated fats showed an inverse effect on postprandial glycemia at 15, 30, and 60 minutes (consistent with the effect of calories), as well
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6 as on IAUC, GI and GL (data not shown).
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9 10 11 **Sensory Evaluation** 12

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14 From the three attributes assessed, only *color* showed significant differences between samples ($p=0.028$), with higher percentages of positive
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16 responses for sample B, followed by A. The “basic and common” appearance of sample C was the major reason for its low score in the respective
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18 attribute. When biscuit *texture* and *flavor* were assessed, there were no significant differences among samples ($p>0.05$) (Fig. 3).
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21 Considering the three attributes assessed (*flavor*, *color* and *texture*), global acceptability was higher than 80% for the three samples, with sample
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23 A having the highest value (86.1%). However, sample B was chosen as the favorite one by a higher percentage of judges (40%, followed by sample
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25 A and C with 32% and 28%, respectively).
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28 29 30 31 **DISCUSSION** 32

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34 Food intake is linked to the pathogenesis of chronic metabolic diseases, mainly when processed products, frequently poor in fiber and rich in
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36 refined starch and added sugar, predominate in the diet (Nagaraju *et al.*, 2020). Glycemic indicators constitute important parameters to be
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38 considered for developing new food products, given that elevated postprandial glycemia induces proinflammatory mediators, metabolic
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4 disturbances and oxidative stress, which trigger several pathological pathways (Wolever, 2008). In addition, stable levels of glycemia improve
5 metabolism and glucose tolerance (Roumen *et al.*, 2008). Thus, this study aimed to evaluate the impact on postprandial glycemic response of three
6 biscuits based on the same type of dough and equal elaboration processing but formulated with different additional ingredients, as healthier food
7 alternatives.
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13 The composition of the formulations was examined based on the nutritional profile of processed foods recommended by the Pan American Health
14 Organization (PAHO, 2016). Thus, sample B met five out of six nutritional criteria (not containing/absence of artificial sweeteners, and low content
15 of total fat, saturated fat, trans fat and sodium), and only the free sugar was above the suggested limit. Sample A met the criteria for artificial
16 sweeteners, saturated and trans fats, while sample C only did so for artificial sweeteners, total fat and sodium. Furthermore, this emphasizes that
17 biscuits A and B have 2.3 and 1.5 times more fiber than the control biscuit (sample C). The lipid profile achieved and the fiber content in sample A
18 and B are relevant characteristics, since reducing fat or increasing fiber in biscuit production is difficult because these nutrients perform important
19 functions associated with food sensory and rheological properties (Aboshora *et al.*, 2019). However, those formulations showed a global acceptance
20 of over 80%, higher than the values found by other authors (Cutullé *et al.*, 2012; Ayensu *et al.*, 2019; Skalkoset *et al.*, 2020).
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31 It is generally accepted that fats decrease PGR; however, there is a controversy about low-GI fat-enriched foods that can be deleterious for
32 dysmetabolic patients due to the risk of cardiovascular disease (Kaur *et al.*, 2020). Nevertheless, the high content of mono- and polyunsaturated
33 fatty acids, along with a low content of trans and saturated fatty acids of formulations A and B, constitute an advance for these bakery products
34 because of their healthier lipid profile and effects on the PGR. Consequently, although all samples had a GI below 55, which is considered low
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4 (Wolever, 2006), and a better PGR than white bread, the lowest GI of sample A (50) was in accordance with its higher content of monounsaturated
5 fatty acids and fiber, as they modulate the rate of carbohydrate digestion and absorption (Wolever *et al.*, 1991). Sample B presents a GI of 54,
6 although it showed a stable postprandial glycemic response curve, which could prevent abrupt increases in insulin concentration, thus reducing
7 the adverse effects of hyperinsulinemia (Vinoyet *et al.*, 2016; Wolever, 2006). In addition, a standard portion of samples A and B had a low GL (less
8 than 10), whereas the GL was higher in sample C, which is considered moderate according to the classification used (Wolever, 2006).
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11 Differences in the amount of fat and fiber in food may explain the differences in the PGR between the samples analyzed (Brounset *et al.*, 2005; Vinoyet
12 *et al.*, 2016; Wolever, 2006). Meynier *et al.* (2015) evaluated the influence of several nutrients in the PGR of 190 cereal products, pointing out that
13 fat and fiber significantly affect the glycemic response to these products, with a respective impact of 13.7% and 15.9%. In this sense, we found that
14 fiber and monounsaturated fats significantly controlled the GR, up to 90 minutes for fats and longer for fiber. The effect of calories observed in the
15 first measurements was consistent with the mentioned effects of monounsaturated fats.
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18 The biscuits in this study (samples A and B) are made with oat, which contains primarily soluble fiber in the form of *b*-glucan. The ability of *b*-glucan
19 to attenuate the PGR has been attributed to its viscous nature. Other authors have found significant decreases in postprandial blood glucose after
20 15 minutes with this type of fiber; however, the effect was achieved at higher doses (27.3 g) (Steinert *et al.*, 2016). In contrast, researchers who
21 used amounts similar to the current ones (4.8 g) or higher (9.6 g) found no effect (Meng *et al.*, 2017).
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24 Fiber was also inversely associated with the GL; however, none of these nutrients had an impact on the GI; thus, it is possible that the GL better
25 represents the overall quality of food. Consequently, to achieve substantial changes in the GI, it may be necessary to manipulate other aspects,
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4 such as the procedures to which the food is subjected or the physical state (microstructure) of starch (Adedayo *et al.*, 2018; Parada and Rozowski,
5 2008). Several studies demonstrated that a higher content of slow-digesting starch can be preserved by controlling process parameters such as
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7 heat, humidity and pressure, and that the GR effects are independent of the content of other macronutrients (Nazareet *et al.*, 2009; EFSA, 2011;
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9 Vinoyet *et al.*, 2016).
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13 Finally, a differential effect of formulations between sexes was found by this study, suggesting a dimorphism of the GR. González-Rodríguez *et al.*
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15 (2019) also found that the PGR in men was different from that in women, who showed a significantly different GR after consuming fat, with a
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17 “flattening” of the glycemc curve. Thus, the lipid profile of samples A and B would be responsible for the stronger PGR decrease found in women.
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19 Other studies found differences by sex in adolescents (Cooper *et al.*, 2017) and diabetic subjects (Dennis *et al.*, 2018). In consequence, this shows
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21 that an analysis by sex should be encouraged to develop specific food products.
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24 In conclusion, our results suggest that the nutritional profile of a highly consumed wheat-based product such as biscuits, can be improved – while
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26 maintaining palatability – by adding monounsaturated fat and soluble fiber (e.g., oat) in their formulations, and thus providing viable options to
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28 include biscuits as part of a healthy diet. The three formulations presented a better PGR profile than white bread, with further modifications being
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30 required to differentiate their effects on the PGR, such as in mechanic and cooking procedures, to generate a more remarkable option among
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32 them. Although more studies are necessary for food innovation to respond to specific nutritional requirements, some easily available alternatives
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34 have been established herein.
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Conflict of interest statement

The Company that participated in the development of the cookies did not participate in the data collection and analysis. The authors declare that they have no conflict of interest.

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4 **Figure captions**
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7 Figure 1. Postprandial glycemc response for three biscuit formulations and white bread.
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11 Figure 2. Glycemic indicators of three biscuit formulations, in women and men. a) IAUC by sex, b) IG by sex, c) GL by sex, d) IAUC by
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13 formulations and sex, e) IG by formulations and sex, f) GL by formulations and sex.
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17 Figure 3. Acceptability of three biscuit formulations assessed in terms of flavor, color and texture through a hedonic scale of 5 points.
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Test Foods	Sample A		Sample B		Sample C (control)	
GI (GL)	50 (8)		54 (9)		51 (10)	
Nutrient content	g/100 g	g/portion*	g/100g	g/portion*	g/100g	g/portion*
Energy (Kcal)	435.2	100.4	435.0	100.1	453.3	126.9
Carbohydrate	64.7	15.5	70.9	16.3	71.5	20.0
Sugar	22	5.3	20.3	4.7	19.0	5.3
Protein	8.1	2.0	8.5	2.0	8.6	2.4
Fat	16	3.8	13.0	3.0	14.7	4.1
Saturated Fats	1.8	0.4	1.3	0.3	6.8	1.9
Trans Fats	0.0	0.0	0.1	0.0	0.7	0.2
Monounsaturated Fats	11.6	2.8	10.4	2.4	6.0	1.7
Unsaturated Fats	2.5	0.6	1.1	0.3	0.9	0.2
Cholesterol (mg)	0.0	0.0	0.1	0.0	26.4	7.4
Fiber	4.4	1.1	2.7	0.6	1.9	0.5
Sodium (mg)	416.3	99.9	213.9	49.2	370.3	103.7

*Portion sizes: Sample A: 24g, Sample B: 23g, Sample C: 28g.

	Glc 15'		Glc 30'		Glc 45'		Glc 60'		Glc 90'		IAUC		GI		GL	
	Coef.	p-value	Coef.	p-value	Coef.	p-value	Coef.	p-value	Coef.	p-value	Coef.	p-value	Coef.	p-value	Coef.	p-value
Interactions: Sex x Products																
Male x Product A	4.21	0.53	3.78	0.61	0.10	0.99	1.04	0.89	3.51	0.60	-6.65	0.53	-1.35	0.91	-2.57	0.22
Male x Product B	-5.05	0.44	-8.85	0.21	-2.11	0.80	-2.86	0.71	7.34	0.28	-3.96	0.41	0.94	0.94	-1.80	0.40
Female x Product A	-12.01	0.11	-15.63	0.06	-25.42	0.01	-23.07	0.01	-18.56	0.01	-53.73	0.11	6.33	0.65	-1.09	0.66
Female x Product B	-15.87	0.04	-22.91	0.01	-25.53	0.01	-22.65	0.01	-12.26	0.10	-22.72	0.04	25.60	0.13	2.26	0.44
Female x Product C	-4.42	0.57	-19.17	0.02	-21.25	0.02	-19.44	0.02	-14.24	0.05	-46.27	0.57	11.47	0.47	1.60	0.58
BMI	-0.06	0.97	-0.62	0.68	0.19	0.90	-0.71	0.64	-1.65	0.24	0.69	0.61	5.11	0.04	0.81	0.05
Age	0.27	0.45	0.33	0.38	0.51	0.24	0.52	0.20	-0.09	0.77	6.59	0.17	-1.52	0.02	-0.27	0.02

Glc, glycemia; IAUC, glycemic incremental area under the curve; GI, glycemic index; GL glycemic load; BMI, body mass index.

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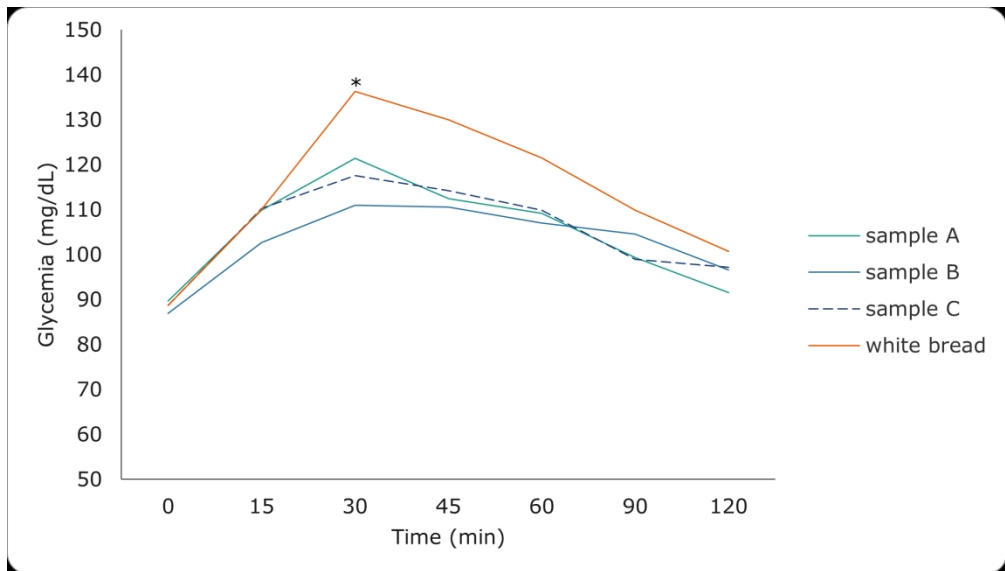


Figure 1. Postprandial glycemic response for three biscuit formulations and white bread

440x250mm (130 x 130 DPI)

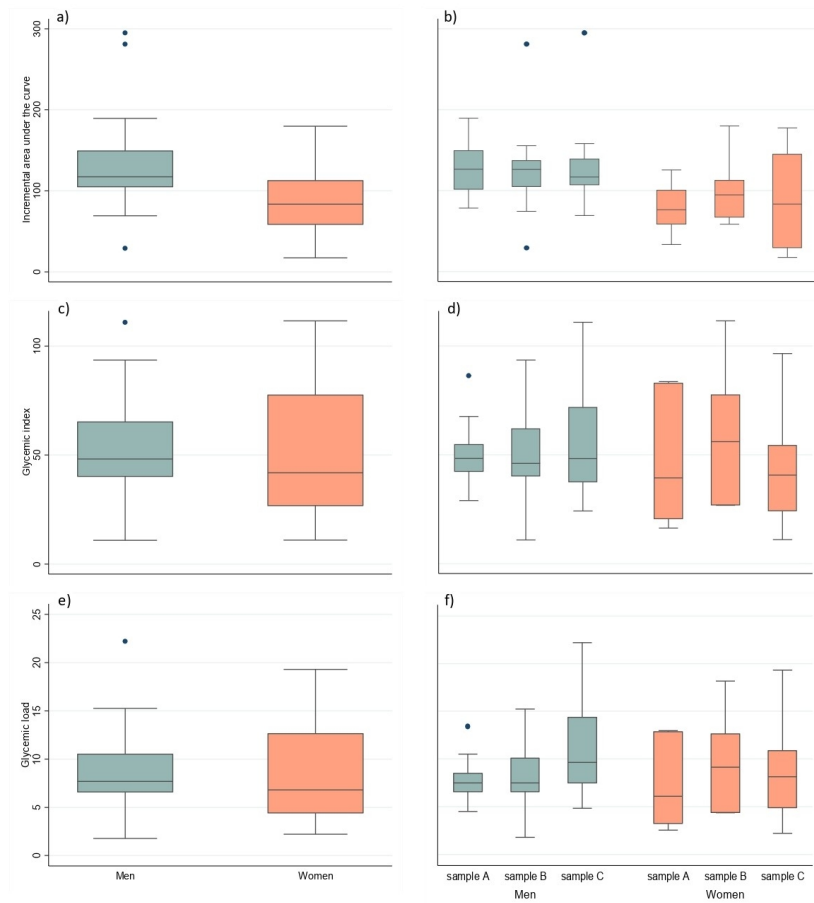


Figure 2. Glycemic indicators of three biscuit formulations, in women and men. a) IAUC by sex, b) IG by sex, c) GL by sex, d) IAUC by formulations and sex, e) IG by formulations and sex, f) GL by formulations and sex

239x222mm (150 x 150 DPI)

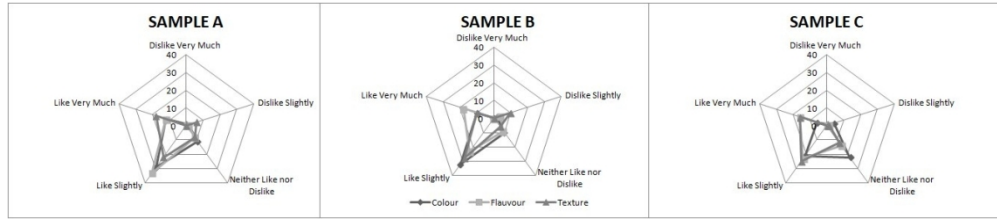


Figure 3. Acceptability of three biscuit formulations assessed in terms of flavor, color and texture through a hedonic scale of 5 points.

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