The effect of enzymatically treated ripe banana flour on the sensory quality and glycemic response of banana-wheat flour composite muffins

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Abstract Banana are the most consumed fruit worldwide, due to their good flavour and nutritional characteristics; however, when the banana is very or over ripe, the acceptability by the consumer decreases, and in many cases the fruit must be discarded. An alternative to consume these fruits and revalue these discards is their use as a food ingredient. The presence of bioactive compounds gives added value to this type of ingredients; therefore, using methods, such as enzymatic treatment, that increase their presence is of great interest. In this work a commercial pectinase (Viscozyme L) was applied in a flour produced from whole overripe banana; then, the treated flour was used to elaborate a baked product. The aim of this work was to evaluate the effect of the incorporation of an enzymatic treated overripe banana (*Musa cavendishii*) flour in the sensory evaluation of muffins and, to establish if the consumption of this food produce an effect on glycaemic response against a control food. The enzyme application produces an increment of 52% of antioxidant activity with a value of 12,791.6 $\mu\text{molTE}/100\text{ g}$, and a presence of 4.5% RS instead 3.5% in non-treated flour. The sensory evaluation study was conducted with 4 products, using an untrained panel; selecting a muffin with 50% of wheat flour replaced with the banana treated one. This one contains

9.49% of dietary fibre. The glycaemic response study was conducted with 20 healthy volunteers, using as control a 100% wheat flour product, non-observing significant differences between both products. This work contributes to the knowledge about the potential use of a food discard as an ingredient of a food of massive consumption.

Keywords Banana flour · Viscozyme L catalysts · Glycaemic response · Healthy food

Introduction

One of the most important crops worldwide after rice, wheat and maize, are bananas (*Musa spp.*: *Cavendishii* banana, which may be eaten raw when is ripe or can be eaten cooked when is green). This fruit is constituted mainly by carbohydrates, proteins, lipid (rich in polyunsaturated fatty acids), vitamins and minerals such as potassium, magnesium, calcium and sodium, both in its pulp and peel. Also, carotenoids, phenolic compounds and flavonoids, and amine compounds as dopamine are present in them (Pereira and Maraschin 2015; Tsamo et al. 2015). The production of the *Cavendishii* fruit is focuses on countries like Ecuador and India, being mostly exported for its consumption as dessert. United States and the European Community import about 27% each of the *Cavendishii* bananas traded worldwide, while, Latin-American countries import over 600,000 tons of them per year, being the Argentinean and Chilean the higher consumers (ODEPA 2019; DNAyB 2019).

The use of banana flour in food elaboration has been reported by different authors, observing good acceptability results. Flours from banana (pulp or peel) have been used in the preparation of cookies, noodles, bread, among other

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products (Ramli et al. 2009; Choo and Aziz 2010; Osorio-Díaz et al. 2014), observing a high presence of dietary fibre, a higher content of resistant starch (RS) than control foods, and a low glycemic index. Also, banana are used as ingredient in bars or drinks as energizers; as animal feed; or to produce concentrates rich in fibre. The green banana flour has also been used as an alternative for the preparation of pasta for gluten-free diets (Zandonadi et al. 2012). Flour prepared from *Musa cavendishii* banana (pulp and peel) has higher total phenolic content than those prepared from other type of banana (Fatemeh et al. 2012). As it is observed, not only the banana pulp has been used as food ingredient; but also, the peel, which is usually discarded. The use of agro-industrial discards as food ingredients rich in bioactive compounds is a way to added value to these wastes and to decrease the cost (economic and environmental) associated to their disposition (Caballero and Soto 2019).

Overripe (natural ripening) *cavendishii* banana, which contain a lots of brown and black spots on the peel and pulp, is generally discarded; but, a flour produced with this overripe fruit (pulp and peel) has a high amount of dietary fibre (18.2%), and an antioxidant capacity over 8000 ORAC units (Soto-Maldonado et al. 2018). The presence of those bioactive compounds, suggest that this raw material can be used as a healthy food ingredient. Muffins made with partial replacement of wheat flour with overripe banana flour are sensory well accepted. Regarding its healthy potential, when evaluating the glycaemic response of this food (muffin with 50% banana flour), no significant differences were observed compared to the result observed with white bread. This similarity may be due to the high content of available carbohydrates in both products (Soto-Maldonado et al. 2018), making it necessary to try to increase the presence of bioactive compounds available in banana flour. An alternative is the use of enzymatic catalysts, which due to their hydrolytic activities degrade the cell wall structure of the vegetable matrixes, releasing compounds such as oils (Soto et al. 2007), polysaccharides (Zhu et al. 2014) or phenolic compounds with antioxidant activity (Laroze et al. 2010; Soto-Maldonado and Zúñiga-Hansen 2017; Zhou et al. 2017). In addition, the presence of resistant starch, and some fructooligosaccharides can be increased using commercial enzymes (Zhang and Jin 2011; Vega-Paulino and Zúñiga-Hansen 2012).

Given the above, it is expected that when applying an enzymatic treatment on a flour made from overripe banana (peel and pulp), an increase in the presence of bioactive compounds will occur. Then, the purpose of this work was to evaluate the effect of the incorporation of an enzymatic treated overripe banana (*Musa cavendishii*) flour—in the sensory evaluation of a baked food; and stablish whether this food has any effect on the carbohydrate metabolism of the population (Glycaemic response).

Material and methodology

Enzymatically treated banana flour (ETBF)

Ripe bananas (stage 7 according to the Von Loesecke scale) were obtained in the local market. Subsequently, bananas were maintained under environmental conditions until many brown and big spots—in peel and pulp—were observed. The product obtained corresponds to overripe banana, which has similar characteristics to that product that is commercially discarded due to its overripening. Then, the overripe banana was washed in a citric acid solution, sliced (whole banana: pulp and peel), frozed at -20°C , lyophilized and grounded. Afterwards, the flour was treated enzymatically using Viscozyme L 5% w/w, at 70°C , using water at pH 6.5 (Free pH), for 1 h. The enzymatic process was stopped by an increase in temperature to 100°C . The mixture was lyophilized (-53°C , 5 mTorr) and milled again, using a ultra-centrifugal mill (Restch ZM 200, Restch GMBH, Germany) with a 0.5 mm sieve in order to obtain a particle size ≤ 0.5 mm.

Resistant starch (RS) and total dietary fibre (TDF) determination

RS and TDF content were determined according to Soto-Maldonado et al. (2018). Briefly, for RS determination, a resistant starch kit (K-RSTAR) of Megazyme was used, where 100 mg of the samples were incubated with pancreatic amylase and amyloglucosidase for 16 h at 37°C to produce glucose from digestible starch (non-resistant starch). The RS was recovered by centrifugation and was washed with ethanol. Then, RS was solubilized using an alkali solution and hydrolysed to glucose using a concentrated amyloglucosidase solution. Glucose was measured using an enzymatic kit determination. For TDF, the Megazyme K-TDFR kit was used, which consider a sequential removal of starch, and protein. Then the TDF was determined by gravimetric method after the precipitation of soluble fibre and filtration (TDF is the solid residue of filtration). The presence of residual protein—determined by Kjeldahl method— and residual ashes—determined by calcination at 500°C —in the solid was determined to elucidate the real content of TDF.

Phenolic content and antioxidant capacity determination

The total phenolic content (TPC) and the antioxidant capacity of ETBF were determined according to Soto-Maldonado et al. (2018). Briefly, for TPC method $3.75\ \mu\text{L}$ of distilled water, 0.5 mL of Folin–Ciocalteu reagent

(diluted twice), 0.5 mL of sample, and 0.25 of 10% sodium carbonate were mixed. The absorbance was recorded at 765 nm after 1 h at room temperature. Gallic acid was used as standard. In the case of antioxidant activity by ORAC method, 20 μ L of extract (obtained previously) or phosphate buffer sample (blank) and 200 μ L of fluorescein (1.5 mM) were added to each well in a 96-well opaque plate. The plate was incubated at 37 °C for 10 min. Then, 75 μ L APPH (79.7 mM) were added to each well. Fluorescence was recorded every 1 min, for 1 h at 37 °C, using excitation and emission wavelengths of 485 and 538 nm respectively. The antioxidant capacity was calculated as Trolox equivalent (TE), using the relative area under de curve (AUC) of the sample curves compared to a calibration curve, using Trolox as standard.

Muffin production with overripe banana flour

Four muffin prototypes were prepared using the following recipe: 22 g flour, 0.6 g stevia (sweetener), 6 mL canola oil, 5 g egg, 15 mL semi-skimmed milk, 0.7 g baking powder, 0.01 g salt. In the case of the flour, wheat flour was replaced by ETBF in 10, 30, 50 and 70% ratios. The mass was baked at 180 °C in a stove (Rational AG; Landsberg am Lech, Germany) for 25 min in individual portions in order to obtain a final product of 50 g. The samples were cooled to room temperature and individually packed until the assays.

Sensory evaluation

The sensory evaluation was done with 12 untrained subjects (aged 18–47). The test was done with volunteers after completing medical records and meet the inclusion criteria. An informed consent letter was signed by each participant. The sensory evaluation of the samples was performed asking to the panelists to evaluate for odour, texture, colour, and flavour and general acceptability using a 1–7 points hedonic scale (1, dislike extremely; 2, dislike very much; 3, dislike slightly; 4, neither like or dislike; 5, like slightly; 6, like very much; 7 like extremely). The results were collated and analyzed statistically. In the case of texture, the untrained panelists were asked to evaluate as a single concept that encompassed the multiparameter attributes of this sensory property (considering hardness, softness, chewiness, among others). One of the products containing banana flour was selected to continue the tests.

Determination of glycaemic response (GR)

The glycaemic response of the selected prototype was determined. A total of 20 volunteers (Body mass index—BMI of 22 ± 2 , average age 23 years) were recruited.

Each participant completed a medical record and signed the informed consent form. Glycaemia was measured using an electronic device (Accu-chek Active®, Roche Laboratory; Indianapolis, USA), which measured the blood glucose levels in capillary blood. The first analysis was performed after fasting. Subsequently, an amount of food equivalent to 50 g of available carbohydrates (CHO) was ingested by the subjects. The glycaemia was determined at 15, 30, 60, 120 and 180 min after ingestion of the sample. Both tests (Muffin with WF—only made with wheat flour; Muffin with ETBF—made with 50% of overripe whole banana flour treated enzymatically) were performed on non-consecutive days.

The composition of the elaborated muffins (selected and a control muffin) were determined using traditional methodology according to Soto-Maldonado et al. (2018).

Statistical analysis

For sensorial evaluation, the values were analysed using analysis of variance (ANOVA) with the Tukey test ($\alpha = 0.05$), to determine if there were significant differences in the Mean of the scores assigned to the samples. Results are expressed as mean $\pm$ standard deviation. Student's *t* test was used to compare GR between products. Statistical analysis Graphpad Prism 6. A *P* value ≤ 0.05 was considered significant.

Results and discussion

Effect of the enzymatic treatment on overripe whole banana flour

The overripe banana flour was treated with Viscozyme L in order to improve the presence of some compounds with biological activity. In this aspect, with the treatment it was possible to increase the presence of phenolic compounds from 250 to 510 mg gallic acid/100 g flour, the antioxidant activity of the sample from 8386.7 to 12,791.6 μ mol_{TE}/100 g_{flour}, and the presence of RS from 3.47 to 4.50 g/100 g. In the case of phenolic compounds, their presence was duplicated, potentially by the hydrolysis of macromolecules that release them; however, antioxidant activity only increases by 52%. This fact may indicate that the treatment releases compounds with a low antioxidant capacity (lower than those normally available in banana) or the enzymatic process produces the release of other molecules such as sugars and proteins, which can interfere in the determination of the content of total phenolic compounds by the Folin–Ciocalteu method, overestimating its value (Jakobek 2015). Beyond the above, the content of phenolic compounds is within the values reported for

banana (Soto-Maldonado et al. 2018). The antioxidant activity value observed for ETBF in this work [derived from that reported by Soto-Maldonado et al. (2018)] suggest that the enzymatic treatment generates a modification in the matrix plant, releasing these compounds, achieving values that even exceed some varieties of raspberries (Wang and Lin 2000), apple, kiwi, among other fruits (Wang et al. 1996)]. Also, it is possible to observe an increase in the RS presence, enhanced by the enzymatic treatment and the subsequent dehydration process to generate the flour. Viscozyme L is a multi-enzyme complex, which has different carbohydrases, including arabanase, cellulase, beta-glucanase, hemicellulase and xylanase activities. Viscozyme L is able to improve the availability of starch because of degrading the non-starch polysaccharides that are often bound to starch in plant matter. In addition, other enzymatic activities have been reported for Viscozyme L; for example, fructosyltransferase, which makes it possible to generate compounds such as short-chain oligosaccharides (Vega-Paulino and Zúniga-Hansen 2012). This last fact suggests that Viscozyme L also can include other glucosyltransferase enzymatic activities promoting the generation of RS in banana flour.

Muffin elaboration and sensory evaluation

Four muffins were made using the ETBF instead of wheat flour. The Fig. 1 and Table 1 shows the results of the sensory evaluation of muffins prototypes and a control muffin, done with 12 untrained volunteers. As it is possible to observe, flavour, colour and general appearance are the attributes more affected by the presence of ETBF. In the case of flavor, two factors can generate this behavior, a high sensation of natural astringency due to the presence of

banana peel in the flour (Soto-Maldonado et al. 2018), or that the enzymatic process causes the release of tannins from this same peel (Wu et al. 2014), increasing the astringency of the final product. On the other hand, an important improvement in the texture is observed when 50% of ETBF is used, probably due to the hydrolytic effect on the macromolecules, especially on the dietary fibre (pectin) (Sila et al. 2008). In relation to the other parameters evaluated, the colour acceptability was decreased with the increase of ETBF in the muffin, probably because the Maillard reaction (non-enzymatic glycosylation of proteins), which can occur during ETBF production (Carvalho and Conti-Silva 2017); however, in terms of general acceptability, there are no significant differences ($P > 0.05$) between the products made with 10, 30 and 50% of enzymatically treated banana flour.

In accordance with the foregoing, and with the aim that the product contains the largest possible number of bioactive compounds provided by the ETBF but is sensorially accepted, the muffin with a 50% of ETBF instead of wheat flour was selected. This muffin has scores, which are close to 5, which means that each attribute was classified as “like slightly”.

With this muffin, the study of its effect on the glycaemic response on a population of healthy individuals was carried out.

Muffin selected characterization and glycaemic response

The muffin made with 50% ETBF was characterized about its colour and instrumental texture parameters as hardness, gumminess, chewiness, and cohesiveness (Table 2). In addition, to establish the portion size of muffin required to

Fig. 1 Sensory evaluation (average media) of muffins elaborated overripe whole banana flour treated enzymatically

Table 1 Evaluation of sensory parameters of muffins made with overripe whole banana flour enzymatically treated. Muffin preselection (12 untrained volunteers)

	Control	Muffin A	Muffin B	Muffin C	Muffin D
Colour	6.42 ± 1.07	5.91 ± 0.90 ^a	5.83 ± 1.33 ^a	5.25 ± 1.05 ^{a,b}	4.63 ± 1.29 ^b
Odour	5.58 ± 1.13	5.54 ± 1.19 ^a	5.25 ± 1.13 ^a	5.41 ± 1.44 ^a	5.29 ± 1.35 ^a
Flavour	5.21 ± 1.08	4.67 ± 1.07 ^a	5.04 ± 1.17 ^{a,b}	5.63 ± 1.15 ^b	4.54 ± 1.69 ^a
Mouth texture	5.08 ± 1.13	5.04 ± 0.90 ^a	5.20 ± 1.46 ^a	6.29 ± 0.86 ^b	5.67 ± 0.88 ^{a,b}
General appearance	6.27 ± 0.92	5.72 ± 1.10 ^a	5.54 ± 1.03 ^a	5.63 ± 1.12 ^a	4.81 ± 1.40 ^a

Control: 0% replacement; Muffin A: 10% replacement; Muffin B: 30% replacement; Muffin C: 50% replacement; Muffin D: 70% replacement

The same letter in the row means there is no significant difference between muffins elaborated with ETBF instead WF

Table 2 Physical characterization of selected muffin elaborated with 50% of ETBF (enzymatically treated banana flour)

Parameter	Value
<i>Colour</i>	
L*	37.26 ± 1.77
a*	3.75 ± 0.66
b*	4.47 ± 0.91
Hardness (N)	87.46 ± 2.23
Gumminess (N)	32.28 ± 5.39
Chewiness (N mm)	5.81 ± 0.65
Cohesiveness	0.38 ± 0.07

do the glycaemia response test, the proximal composition of the selected muffin and the control muffin was determined (Table 3).

The selected muffin has a brownish colour, as well as occur with the muffin elaborated with non-enzymatically treated banana flour (Soto-Maldonado et al. 2018). The muffin possesses a good luminosity (L parameter), a similar red component (a*) to the non-treated muffin, but a lower yellow component (b*). However, the colour

Table 3 Proximate composition of muffin made with overripe whole banana flour treated enzymatically (Muffin with ETBF, 50% of banana flour and 50% wheat flour) and control muffin (Muffin with WF, 100% wheat flour)

	Muffin with ETBF (%)	Muffin with WF (%)
Moisture	27.17	31.05
Ashes	2.93	2.24
Lipids	15.16	13.96
Proteins	6.20	6.76
Total dietary fibre	9.48	3.86
Resistant starch	0.98	0.67
Carbohydrates (per difference)	39.06	42.13

acceptability of this muffin also is good (a value over 5), probably because this kind of colour (brownish) is associated with integral and healthy food, or on the other hand, to chocolate, which is a very appreciated food (Soto-Maldonado et al. 2018). The texture parameters of hardness and cohesiveness of the muffin made with ETBF shows non-differences with those prepared with non-hydrolyzed banana flour (Soto-Maldonado et al. 2018); but, gumminess and chewiness are higher in this work, maybe because the greater presence of dietary fibre in the final product, which is probably due to the effect of the enzymatic process which, through the hydrolysis of macromolecules, generates an increase in the presence of oligosaccharides, and of resistant starch, which are known as dietary fibre.

The proximal characterization of the selected muffin and a muffin made with 100% wheat flour (WF)—as standard for the study of the effect of carbohydrate metabolism generated by its intake—is showed in Table 3. The high presence of dietary fibre stands out in the product made with banana flour – 2.45 times the value observed in the control muffin. In addition, in both products, a low content of RS and a high content of available carbohydrates, are observed. The latter could negatively affect the glycaemic response of consumers.

Twenty volunteers older than 18 years (average of 23 years), of both sexes (4 male and 16 female), with a median of BMI (body mass index) value of 22 ± 2 were evaluated. The assay was random cross, and the volunteers consumed the products with 1 week apart. The food portion was according to obtain 50 g of available carbohydrates. As shown in Fig. 2, the fasting glycaemia was similar between the muffin with WF and the muffin with ETBF, with a median value of 89.6 mg/dL. After the consumption of both products, the glycaemia increased until reaching its maximum value at 30 min with no significant differences ($P > 0.05$) and a value of 123.3 mg/dL. Both curves are similar without appreciating differences in the results obtained with the product that contains 9.48% of fibre (muffin with ETBF) and the other one. This behavior can be associated with different personal factors,

Fig. 2 Glycaemic response to the consumption of a muffin made with enzymatically treated overripe Whole Banana Flour (Muffin with WF), as a partial replacement for wheat flour (50% of

replacement, muffin with ETBF). The result is compared to those of a standard muffin made only with wheat flour

as well as the composition and processing of the evaluated foods, such as the response to insulin, protein content, processing techniques, variety, particle size, fat, acidity, storage and harvest time of the food used (Soto-Maldonado et al. 2018). Also, the glycaemic response may also be affected by the portion of available carbohydrate ingested, as reported by Ray and Singhania (2014).

Conclusion

The overripe banana is a raw material that is currently discarded, but that given its nutritional potential could be used as a food ingredient. To consider this ingredient as healthy, in addition to being organoleptically acceptable to the consumer, it is expected to contain different potential bioactive compounds to positively affect consumer health. In the case of overripe banana flour, an enzymatic treatment was used in order to increase the availability of these bioactive compounds.

Viscozyme L commercial enzyme is able to produce a slight increase in the presence of available active compounds in overripe banana flour, including antioxidant compounds and resistant starch, which are recognized for their health benefit. The incorporation of this treated flour (ETBF) in muffins is technologically and sensorially feasible, so its consumption would promote the intake of dietary fibre.

On the other hand, although the processed food (a muffin) has a high content of available carbohydrates, potentially the presence of the bioactive compounds generates no differences in the glycemic response between this food and a control food. Then, the use of this type of ingredient could favor the development of new food products and improve the use of raw materials that are usually discarded.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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