Development and characterization of *Pleurotus ostreatus* mushroom- wheat bread

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Abstract

The *Pleurotus ostreatus* mushroom is a source of high nutritional value and bioactive compounds such as β -glucans, with immunomodulatory, hypoglycemic and prebiotic activity. The aim of this work was to develop a bread with *P. ostreatus* powder (POP) to increase its nutritional value and reduce its glycemic index.

Four formulations were proposed substituting 5, 10, 15 and 20% of wheat flour (w/w) for POP in the bread and their physical characteristics and nutritional were evaluated. In addition to determining the glycemic index and the *in vitro* digestion of starch in the proposed formulations. Results shown that with the addition of POP the specific volume of the bread decreased, and it became darker. The nutritional value of the bread was enriched, since the content on proteins, fiber and minerals increased. The results of *in vitro* simulated gastrointestinal digestion demonstrated that bread supplemented with POP showed more optimal starch digestibility with slower digestion rate, lower eGI and RDS contents, which could bring health benefits to humans. These findings suggest that the mushroom powder is an excellent alternative to produce bread with improved nutritional quality and decreased glycemic index.

Keywords: edible mushroom, glycemic index, *Pleurotus ostreatus*, wheat bread

1. Introduction

Bread is one of the most consumed food worldwide. Wheat flour (*Triticum aestivum*), water, yeast or other leavening agents and salt have been used as basic ingredients since ancient times. The product of the milling of the wheat grain is a white flour rich in starch and proteins of gluten. Unfortunately, many of the important nutrients such as protein, dietary fiber, B vitamins, and minerals are also removed if all parts of the grain are not preserved [1,2]. White bread is a food rich in starch and low in dietary fiber, is associated with a high glycemic index (GI) that promotes a rapid increase in blood glucose [3]. Eating high GI foods can affect human health, promoting the development of metabolic disorders and diseases, such as diabetes and cardiovascular disease [4]. Nowadays, adoption of a healthy lifestyle is increasing, and breads containing whole grains or other functional ingredients have positioned themselves on the market. Therefore, new ingredients are necessary to develop products that can compete with breads made with refined flours, with higher nutritional value and additional functional properties [2].

Recent research on edible mushrooms indicates that their bioactive compounds with nutraceutical and functional properties have multiple beneficial effects on human health, in addition to their high nutritional value and appreciated organoleptic qualities [5,6]. Likewise, consumption in their natural form in common dishes, edible mushrooms have been incorporated into food formulations to obtain new functional foods [7].

The edible mushroom *Pleurotus ostreatus* cultivated worldwide [8], known as oyster mushroom, is an important source of amino acids, proteins, unsaturated fatty acids, vitamins, fiber and has low energy [9]. *P. ostreatus* has been grown on lignocellulosic substrates, which are by-products of the food industry (e.g., grain

residues) and a relatively inexpensive material [10]. It receives a great interest from the scientific community due to its anti-inflammatory properties (e.g., curative and immunostimulatory effects) [11], prebiotic potential (e.g., stimulating the growth of some beneficial bacteria in the colon) [12] and hypoglycemic activity [13,14]. In addition it contains a large amount of ergothioneine and polyphenols with antioxidant activities [15].

The incorporation of edible mushrooms of the genus *Pleurotus* spp in foods in different forms with different purposes has been reported. In meat products, the incorporation of *P. ostreatus*, at an adequate level, has effect on the organoleptic characteristics of these products. This mushroom promotes the oxidation of lipids and proteins of the meat and generate compounds that improve the flavor; however, changes in color and texture also occur. The addition of mushrooms to these foods should be studied further to find the optimal amount [16]. In dairy products, there has been an increase in nutritional quality, especially changes in the content of protein and dietary fiber, in addition to providing functional activity [17,18]. There is research focused on fortifying starch-rich cereal products that have a favorable effect on lowering the glycemic index and increasing dietary fiber.

[19, 20].

According to the Mexican Standard NMX-V-022-1972, *aguamiel* is the "juice obtained by previously scraping the central cavity of the maguey pulquero". This agave sap is a sweet liquid, hence its name in Spanish - "agua" which means water and "miel" which means honey. By easily undergoing natural fermentation, it becomes a non-distilled alcoholic beverage called *pulque*, which is a drink consumed in Mexico since prehispanic times [21,22]. *Aguamiel* is rich in sugars such as sucrose, fructose and glucose, as well as composed of sugars such as glucose,

sucrose and fructose, fructans, gums, proteins, minerals, vitamins, amino acids, saponins, phenolic compounds and fructo-oligosaccharides (FOS) known for their prebiotic effects [23-25]. Due to these characteristics, in this work it was used as an ingredient in bread formulations. *Aguamiel* may become a novel alternative to sucrose commonly used in confectionery to promote yeast fermentation and increase the specific volume of wheat bread. [26].

The present research aimed to enrich wheat bread with powder of edible mushroom *P. ostreatus* (POP) to increase the nutritional value of the final bread and reduce its glycemic index.

2. Experimental Section

2.1 POP preparation

The POP was prepared and provided by the Plant Breeding Department and the Food Technology Department of the Autonomous Agrarian University Antonio Narro (UAAAN), located in Saltillo, Mexico. *P. ostreatus* mushroom were grown in sorghum and wheat straw in an incubator (ShelLbab), at 28 °C and in dark conditions. Ripe mushrooms were manually harvested after 40 days, dried in an electric oven at 60 °C for 48 h and grounded (mill Pulvex mini 100). The resulting powder was filtered through a 45- mesh (350 µm) sieve to obtain the *Pleurotus ostreatus* powder (POP) (sieve Tyler RO-TAP RX-29).

2.2 Bread formulations

The control bread (CB) was formulated with the following ingredients: wheat flour (160 g), yeast (4 g), whole milk (75 ml), aguamiel (10 ml), canola oil (12 ml), salt (3 g) and water (35 ml). 5, 10, 15 and 20% of wheat flour (w/w) was replaced with POP

and these bread with *Pleurotus ostreatus* (BPO) were named as BPO₅, BPO₁₀, BPO₁₅, BPO₂₀, respectively. The ingredients were thoroughly mixed with the help of an electric mixer (Black & Decker MX900). The dough was fermented for 1 h at room temperature (25 °C). The dough was weighed and divided into equal portions, then, it was placed in baking pans, fermented again for 30 min and finally baked for 22 min at 150 °C, in an electric convection oven (San Son, HCX PLUS 3). Once baked, the breads were allowed to cool for 1 h at room temperature (25 °C) and stored in polyethylene bags until further analysis. All breads were prepared at least in triplicate.

2.3 Chemical composition of POP and breads

Moisture, crude fat, crude protein, crude fiber and ash content were determined by AOAC official method [27]. A conversion factor of 4.38 was used to convert % N to % crude protein in the POP (presence of non-protein nitrogen in mushrooms) sample and for bread the conversion factor was 6.25 [28,29]. Carbohydrate contents were calculated by difference method (100 – (protein + lipids content + ash content + moisture content)) and energy was determined by Equation 1 [30]. All these analyses were performed in triplicate.

$$\text{Energy (kcal)} = 4 \times (g \text{ protein} + g \text{ carbohydrate}) + (9 \times g \text{ lipid})$$

(1)

2.4 Physical properties of bread

2.4.1 Specific volume

Specific volume of the bread was measured by the seed displacement method according to the AACC 10-05.01 method [31]. The bread volume and specific volume was evaluated using Equation 2 and 3, as following:

$$Volume (cm^3) = \pi \times radius\ of\ the\ cylinder\ (cm)^2 \times displacement\ distance\ (cm) \quad (2)$$

$$Specific\ volume\ (cm^3\ g^{-1}) = \frac{volume\ (cm^3)}{weight\ of\ unit\ bread\ (g)} \quad (3)$$

2.4.2 Color

The bread crumb color was measured according to CIE-L*a*b color space representation using a digital colorimeter (Colorimeter 3nH NR20XE).

2.5 Measurement of total starch content

For the determination of total starch bread, the Total Starch Assay Kit (AA/AMG) from Megazyme (K-TSTA, Ireland) was used. Bread sample (100 mg) was washed with an aqueous ethanol solution (80 % v/v, 5 mL) and incubated at 80 °C for 5 min. Subsequently, another 5 ml of 80 % v/v aqueous ethanol solution was added, and the samples were centrifuged (EBA 20, Hettich, Germany) at 1800 x g for 10 min. The supernatant was discarded, and 10 ml of 80 % v/v aqueous ethanol solution was added to the pellet and centrifuged at the same conditions before. Then, 2 ml of KOH 2 mol L⁻¹ was added to the resulting solution and stirred on cold water (4 °C) for 20 min. This process facilitated to resistant starch hydrolyzes. Sample was neutralized with 8 ml 1.2 mol L⁻¹ sodium acetate buffer solution at pH 3.8. The enzymes thermostable α-amylase (3000 U mL⁻¹ on Ceralpha reagent at pH 6.5 and 40 °C or 1600 U mL⁻¹ on Ceralpha reagent at pH 5.0 and 40 °C, Megazyme, Ireland) and of amyloglucosidase (3300 U mL⁻¹ on soluble starch or 200 U mL⁻¹ on p-

nitrophenyl β -maltoside at pH 4.5 and 40 °C, Megazyme, Ireland) were added and incubated at 50 °C for 30 min. The total volume was adjusted to 100 mL with distilled water and centrifuged at the same conditions before. Afterwards, a resulting solution sample (36.5 μ L) was added to glucose oxidase-peroxidase (GOPOD) solution (333.5 μ L), distilled water (666.5 μ L) and incubated at 50 °C for 20 min. The absorbance was measured at wavelength 510 nm (Synergy HT, Vermont, USA) and the percentage of D-glucose in the initial samples (100 mg) was determined. The total starch was performed by multiplying the percentage of D-glucose by the conversion factor (162/180), which represents anhydrous D-glucose and free D-glucose molecular weight, respectively. The starch available values were normalized with the percentage of total starch hydrolyzed.

2.6 Gastrointestinal digestion

2.6.1 Static *in vitro* simulation of gastrointestinal food digestion

To evaluate starch hydrolysis of all the bread formulations (CB, BPO₅, BPO₁₀, BPO₁₅, BPO₂₀) an static *in vitro* simulation of gastrointestinal food digestion was carried out following the standardized protocol INFOGEST [32] to mimic mouth, stomach, and small intestine conditions. Figure 1 summarizes the process of starch hydrolysis. Simulated digestion fluids (i.e., Simulated Salivary Fluid (SSF: KCl 15.1 mmol L⁻¹, KH₂PO₄ 3.7 mmol L⁻¹, NaHCO₃ 13.6 mmol L⁻¹, MgCl₂(H₂O)₆ 0.15 mmol L⁻¹, (NH₄)₂CO₃ 0.06 mmol L⁻¹, HCl 1.1 mmol L⁻¹); Simulated Gastric Fluid (SGF: KCl 6.9 mmol L⁻¹, KH₂PO₄ 0.9 mmol L⁻¹, NaHCO₃ 25 mmol L⁻¹, NaCl 47.2 mmol L⁻¹, MgCl₂(H₂O)₆ 0.1 mmol L⁻¹, (NH₄)₂CO₃ 0.5 mmol L⁻¹, HCl 15.6 mmol L⁻¹); Simulated Intestinal Fluid (SIF: KCl 6.8 mmol L⁻¹, KH₂PO₄ 0.8 mmol L⁻¹, NaHCO₃ 85 mmol L⁻¹, NaCl 38.4 mmol L⁻¹, MgCl₂(H₂O)₆ 0.33 mmol L⁻¹, HCl 8.4 mmol L⁻¹)) were prepared

for a volume of 400 mL with purified water (1.25 x concentration). Milled bread samples (100 mg) were dispersing in distilled water (5 ml) to ensure the full rehydration of samples. Before the *in vitro* digestion procedure, bread samples and simulated digestion fluids were inserted in a shaking water bath (B.BRAUN BIOTECH model CERTOMAT WR, Melsungen, Germany) with horizontal agitation of 120 rpm at temperature of 37 °C.

Initially, samples were exposed to the simulation of the oral phase by adding SSF, $\text{CaCl}_2(\text{H}_2\text{O})$ (to achieve the concentration of 1.5 mmol L^{-1}), α -amylase (Sigma-Aldrich A1031, CAS 9000-90-2) (to obtain 75 U mL^{-1} activity), and milli-Q water (necessary volume to dilute the SSF stock solution). The samples were incubated at 37 °C for 2 min. An aliquot of the samples were collected from the liquid medium in the end of oral phase (2 min) and preserved under frozen conditions for later analysis. The passage through to stomach was mimicked by adding SGF, porcine pepsin solution (Sigma-Aldrich P7012; CAS 9001-75-6) (to achieve an activity of 2000 U mL^{-1} in the final mixture), $\text{CaCl}_2(\text{H}_2\text{O}_2)$ (to obtain 0.15 mmol L^{-1} in the fluid). The pH value was adjusted to 3.0 with $1 \text{ mmol L}^{-1} \text{ HCl}$, and the gastric phase volume was complete with milli-Q water. The sample was incubated at 37 °C for 120 min. During the gastric phase, An aliquot of the samples was collected every 30 min and preserved under frozen conditions for later analysis. The intestinal phase was simulated by adding of SIF, $\text{CaCl}_2(\text{H}_2\text{O}_2)$ (to obtain 0.6 mmol L^{-1} in the fluid), pancreatin (Sigma-Aldrich P7545; CAS 8049-47-6) (to achieve the activity of 100 U mL^{-1} in the final mixture) and bile salts (Sigma-Aldrich B8631; CAS 8008-63-7) in a concentration of 80.0 mg mL^{-1} (to obtain the concentration of 10 mmol L^{-1} in the final mixture). Pancreatin and bile solutions were both prepared in SIF. The pH value was adjusted to 7.0 using $1 \text{ mmol L}^{-1} \text{ NaOH}$, when necessary. The final intestinal phase was adjusted with milli-

Q water and incubated under the same conditions as before for 120 min. An aliquot of the samples was collected every 30 min and preserved under frozen conditions for later analysis.

At the end of the intestinal phase, the enzymatic activity was stopped by adding 1 mmol/mL of enzyme inhibitor pefabloc[®] SC. All samples were tested at least in triplicate.

2.6.2 Measurements of starch fractions

To determine the total hydrolyzed starch (HS) during the digestion the collected samples were firstly centrifuged at 5000 x *g* for 10 min (MIKRO 120, Hettich, Germany). The supernatant was incubated with glucose GOPOD reagent from Megazyme (K-TSTA, Ireland) at 50 °C for 20. The absorbance was measured at wavelength 510 nm (Synergy HT, Vermont, USA). The percentage of D-glucose was converted to percentage of starch with the factor of 0.9 (which represents the anhydrous D-glucose divided by free D-glucose molecular weight). Obtained values of starch available were normalized to percentage of hydrolyzed starch.

Using the data obtained in the *in vitro* digestion, starch fractions corresponding to rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were determined by Eqs. [33]. Considering RDS as starch hydrolyzed during the oral phase (2 min), SDS as the maximum amount of starch hydrolyzed during gastric—intestinal phase and RS as starch that was not hydrolyzed. The following equations were applied:

$$\% RDS = \% HS_2 \quad (4)$$

$$\% SDS = \% HS_{150} - \% HS_2 \quad (5)$$

$$\% RS = TS - \% HS \quad (6)$$

where HS_2 represents the percentage of total starch hydrolyzed in the oral phase after 2 min, HS_{150} is the percentage of total starch hydrolyzed after 150 min in intestinal phase, HS is the percentage of total starch hydrolyzed until the end of the digestion.

2.6.3 Determination of estimated glycemic index

The glucose concentration determined during the *in vitro* digestion process was normalized to the percentage of total starch hydrolyzed. Then, the area under the curve (AUC) of the total hydrolyzed starch during the *in vitro* digestion process (0-240 min) was calculated using the GraphPad Prism 8 software. The AUC was used to provide the hydrolysis index (HI) according to Equation 7 [34]:

$$HI = \frac{AUC_{sample}}{AUC_{ref}} \times 100 \quad (7)$$

where AUC_{sample} corresponds to the AUC of the bread formulations and the AUC_{ref} corresponds to the AUC of white bread.

The estimated glycemic index (eGI) was determined as reported by Goñi et al., (1997), using the following Equation 8:

$$eGI = 39.71 + (0.549 \times HI) \quad (8)$$

2.7 Statistical analysis

All experiments were carried out at least in triplicate and results were reported as the average $\pm$ standard deviation (SD). The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test with a significance level of 5 %, using INFOSTAT software version 2018 for Windows (Córdoba, Argentina).

3. Results and discussion

3.1 Nutritional Composition of POP

Table 1 shows the results obtained from the evaluation of the chemical components and the energy value of the POP. The ash (9.9 ± 0.2 g / 100 g) and crude fat (1.9 ± 0.1 g / 100 g) content were similar as reported by other authors [35, 36]. *P. ostreatus* has also been described also as a source of minerals such as phosphorous, potassium and magnesium [37]. The crude protein content was 22.3 ± 1.4 g / 100 g, being the second greatest component found in the POP. The main component found in POP was carbohydrates (60.3 ± 0.7 g / 100 g). Other authors have also reported that carbohydrates are the main macronutrient present in the *P. ostreatus* [38]. The result of the crude fiber content was 5.5 ± 0.4 g / 100 g. The nutritional content of the POP and other edible mushrooms is mainly affected by the substrates used for their cultivation. Edible mushrooms can be cultivated on various substrates, such as lignocellulosic residues, since they degrade this type of material and use them efficiently to grow [39]. In the present investigation the POP grew in wheat and sorghum straw. Wheat straw is the material commonly used for this purpose, and it can provide nutrients necessary for the fruiting of the mushrooms [40].

The energy value determined for the POP was 347.5 ± 2.1 kcal / 100 g. Due to its low calorie content and nutritional contribution, mushrooms have be classified as healthy foods [41].

3.2 Nutritional Composition of *Pleurotus ostreatus* wheat bread

The nutritional composition of the breads formulated in the present work is shown in Table 2. The POP did not generate statistically significant changes in the moisture content of the breads (27.89 to 31.98 %) ($p \leq 0.05$).

The BPO₂₀ bread obtained a protein content of 1.55 ± 0.12 g / 100g, with the highest concentration of POP (20%), which represented that the POP increase a 40.9% the protein content compared to the CB with 1.10 ± 0.15 g / 100g of crude protein. There were no significant differences ($p \leq 0.05$) between the formulations with POP. This registered behavior may be due to the fact that by reducing the amount of wheat flour in the formulation, its protein contribution is also reduced. Despite this, the POP managed to increase and maintain this increase registered in protein levels, despite the decrease in wheat flour. POP contains proteins of high biological value since it provides all the essential amino acids, while the wheat flour is lacking in lysine and threonine [41, 42]. The POP on bread formulations promote an enhance of ash content t from 2.48 ± 0.09 g / 100 g in CB to 3.77 ± 0.21 g /100 g in BPO₂₀. The increase in the ash content in the bread can be attributed to the fact that the POP is a source of minerals as phosphorus, potassium, iron, copper and zinc [37]. There was no significant change ($p \leq 0.05$) in the total carbohydrate content due to the addition of POP, although these were the majority component. It should be considered that unlike wheat flour that contains up to 75 g /100 g starch [43] the polysaccharides of POP are fibers like β -glucans [44]. Regarding the crude fiber content, it increased as the concentration of POP in breads significantly increased ($p \leq 0.05$). POP could also increase the dietary fiber content in wheat bread, by containing in the cell walls β -glucans, which are soluble dietary fiber with benefits for

human health [45]. This is important, because white wheat bread has a very low fiber content due to the nature of refined wheat flour, which has separated fractions rich in dietary fiber (the germ and the bran of the grain) [46].

Regarding the crude fat content of the breads, its content increased from 4.50 ± 0.94 g / 100g in CB to 6.68 ± 1.13 g / 100 g in BPO₂₀. Mushroom includes monounsaturated and polyunsaturated fatty acids, which consume is related to a lowering risk of cardiovascular disease [47].

The energy value determined for the breads ranged from 289 to 319 kcal / 100g, without statistically significant differences ($p \leq 0.05$).

Fortifying wheat flour with POP can improve the nutritional quality of bakery products.

3.3 Bread physical properties

Table 3 shows the physical properties of the breads. The progressive addition of POP in the bread caused a significant increase ($p \leq 0.05$) in the weight of the bread unit going from 38.78 ± 0.34 g in CB to 41.74 ± 0.06 g in BPO₂₀ and an evident decrease in the volume of the bread (Fig 2.) The effect of the addition of POP on the physical properties of the bread has been significant, even at a lower concentration (4%) than that used in the present investigation [48]. These physical changes may be due to the fact that the substitution of wheat flour with POP (gluten-free) affected the formation of a firm gluten network that would retain the CO₂ produced during the fermentation of the dough with yeast, preventing an increase in volume [49]. In the present study, *aguamiel* was used as a source of sucrose, since it is known that sugar can activate yeast and increase the production of CO₂ [50]. More studies are needed to know the effect of *aguamiel* in bakery products.

There were also significant changes ($p \leq 0.05$) in the color parameters of the crumb of breads enriched with POP (Fig 2). L^* value corresponding to luminosity, decreased from 64.64 ± 3.45 in CB to 36.53 ± 1.90 in BPO₂₀. Mushrooms powder are slightly darker than refined wheat flour, which may explain the browning effect [51].

There was also a significant increase ($p \leq 0.05$) in the parameters a^* and b^* , which represent the red and yellow pigments, respectively. The appearance of red and yellow pigments is also associated with the Maillard reaction and caramelization of sugars during the baking of bread dough [52]. These changes may be due to chemical reactions that occur during baking, between the phenolic compounds, in the sugars and proteins contained in the mushrooms, thus altering the final color of the product [53].

3.4 *In vitro* digestion of starch and determination of nutritionally important starch fractions

The standardized INFOGEST protocol was used to study the *in vitro* digestibility of wheat bread starch with different amounts of POP. The starch digestion curves for bread enriched with POP are shown in Figure 3. In general, all bread samples showed a similar digestion curve in which the total starch hydrolysis rate increased markedly at 150 min, during intestinal digestion, achieving equilibrium in this phase. The starch digestion has been described to begin in the oral phase, during chewing, due to the action of the salivary α -amylase enzyme [54]. After the bread samples passed from the oral to the gastric phase, the hydrolyzed starch remained similar, with no statistically significant differences between both phases ($p > 0.05$). Conditions during the gastric phase were simulated to human physiological conditions, gastric conditions were similar to those described by Simões et al (2020), they were

characterized by high ionic strength (values around 100 mmol L⁻¹) and acidity (a pH value of 3) and these conditions inhibit the action of α -amylase and the hydrolysis of the starch was interrupted. In human digestion, the change in pH is gradual and enzymatic work continues, so that starch digestion continues even during the gastric phase [54], in the protocol carried out for the simulation of digestion, the change in pH was immediate, so the digestion of the starch was interrupted during this phase [56]. Results from the intestinal phase (Figure 3), demonstrated that the maximum starch hydrolyze was obtained at 150 min. These behavior can be attributed to starch availability to be hydrolyzed by α -amylase pancreatic enzymes when its function is favored by raising the pH to 7.0 [57-58]. Nevertheless, a significant decrease in total starch hydrolysis rate was observed with increased amount of POP, indicating that the addition of POP could reduce starch digestibility of bread.

In order to predict the nutritionally important fractions of starch of bread, the total starch content (TS) and the results obtained during *in vitro* digestion process were used to determine the total starch hydrolyzed (HS), rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) and estimated glycemic index (eGI).

The addition of POP in the wheat bread formulation decreased the TS from 99.33 % in CB to 66.53 % in BPO₂₀. Wheat flour is rich in starch [43], while POP has other types of carbohydrates. By reducing the wheat flour in the formulation and adding POP, the amount of starch present in the bread is decreased. A lower starchy bread can be included in a diet where carbohydrates are controlled. The consumption of low carbohydrate products is an appropriate approach to lose weight, with an impact on health, since obesity is associated with various chronic diseases such as cardiovascular disease, cancer, diabetes [59]. HS decreased significantly during *in*

vitro digestion ($p \leq 0.05$), presenting the lowest HS value of 35.24% in bread BPO₂₀, compared to the CB which was 41.73%.

The results on bread starch content (Table 5) revealed that the addition of POP in bread promoted a decrease in the content of RDS ($p \leq 0.05$). In this study, the bread formulations presented less than 3% RDS, this can be explained by the inhibition of the action of α -amylase by the acidic pH when passing from the oral to the gastric phase in the *in vitro* digestion protocol INFOGEST. These facts were accompanied by an increase in SDS values ($p \leq 0.05$).

These changes have been reported when low-starch and high-dietary fiber ingredients, as POP, are added to wheat bread [60]. The digestion of carbohydrates is compromised by different factors. As mentioned above, fortifying bread with POP increased the content of crude fiber, protein and fat (Table 2) and these components could interfere with the digestion of starch. A decrease in HS has been recorded during *in vitro* digestion of pasta enriched with 15% mushroom powder (porcini and shitake mushroom powder) [43]. Also, another reason why POP could decrease starch digestion, as observed in the RSD and SDS behavior of bread, could be due to the fact that POP contains a large amount of dietary fiber that could change the properties of the starch system, such as viscosity and degree of swelling. The dietary fiber in POP was also able to surround the starch granules, causing their swelling and gelatinization to be suppressed, and reducing the starch's susceptibility to digestive enzymes [4, 44].

The RS is a fraction of starch that can resist digestion and remains intact throughout the gastrointestinal tract, it can be fermented by bacteria in the colon and is associated with lower levels of blood glucose and plasma cholesterol [61]. In this study, the RS progressively decrease from 57.60 in CB to 31.29% as the

concentration of POP increase in the bread formulations. The highest percentage of RS is desired, due to its physiological effects in the gut (similar to fibers), which is also described as beneficial to health in diet-related non-communicable diseases. In this study, when replacing wheat flour rich in starch with another ingredient that was not a source of starch, such as POP, the amount of RS starch was also decreased. The lower the total starch concentration and lower amylose content, the lower the RS percentage. Studies have related, the amount of amylose present in starch is related to lower percentages of RS [62].

Bread formulated with wheat flour, used as a control (CB), obtained the eGI of 100. The addition of POP in the bread formulations promoted the reduction of eGI ($p \leq 0.05$), where the addition of 20% of POP in the bread BPO₂₀ obtained a decrease of more than 25% in the eGI of 72.21 in comparison with the CB. The reduction of eGI in bread formulations can be attributed to the fact that POP was a source of components, such soluble dietary fiber, as β -glucans. Dietary fibers have been shown to reduce GI when added to food products. Soluble fibers absorb water in low water conditions, limiting gelatinization of starch. Therefore, the accessibility of pancreatic amylase enzymes to act on partially gelatinized starch is reduced [19, 63]. A diet rich in low-glycemic foods has been associated with several health benefits, for instance, lower insulin demand, blood glucose control, and lower blood lipid levels, which could prevent or treat of chronic diseases (e.g., diabetes, heart disease and some types of cancer) [64]. *In vivo* studies are required to determine the hypoglycemic effect of the addition of POP in food formulations. There were no significant differences between the BPO₁₅ and BPO₂₀ formulation, so it can be used from 15% POP to achieve a significant decrease in eGI.

The results of *in vitro* simulated gastrointestinal digestion demonstrated that bread supplemented with POP showed more optimal starch digestibility with slower digestion rate, lower eGI and RDS contents, which could bring health benefits to humans. Sensory studies are needed to determine that formulations with high concentrations of POP do not affect the acceptability of the product.

4 Concluding remarks

The incorporation of POP improved the nutritional composition of bread formulations. The chemical characterization demonstrated that the addition from 5% of POP in the bread caused an increase in the content of protein, fiber, and minerals (ash). Replacing high-starch wheat flour with edible mushrooms reduced the amount of TS in the formulations. The *in vitro* digestion results indicated an improvement SDS starch fraction in the POP formulated bread. From the addition of 15% of POP, the greatest nutritional increases and reduction of eGI were presented. Therefore, the POP could be used as an alternative food ingredient to improve nutritional quality and reduce the glycemic index of bread. However, it is important that the incorporation of mushrooms does not negatively affect the physical and sensory quality of the bread, which is why additional studies on these parameters are needed. Is a trend to found sources of sugars with health benefits, such as *aguamiel* which was used in this work as an alternative to sucrose in bread formulations. However, in this study, the effects of *aguamiel* in bakery products was not the objective, which is why more research is needed on its properties and effects in these products.

Author contributions

Study concept and design: R.B., M.C. and C.L. Method development, sample preparation, sample analysis and interpretation of data: C.L., C.N., L.S.S and A.L. Preparation of the manuscript: C.L., R.B., M.C. and C.N. Study supervision and resources: R.B., M.C., R.M.R and J.T.

Conflict of interest statement

The authors declare “no conflict of interest” exists in this reported work.

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Table 1. Chemical composition and the energetic value of *Pleurotus ostreatus* powder (POP).

Chemical composition	g/100 g
Moisture (%)	5.6 ± 0.2
Ash	9.9 ± 0.2
Crude fat	1.9 ± 0.1
Crude protein	22.3 ± 1.4
Carbohydrates	60.3 ± 0.7
Crude fiber	5.5 ± 0.4
Energetic value (kcal/100 g)	347.5 ± 2.1

Values presented correspond to mean and standard deviations on a dry basis.

Table 2. Moisture, ash, crude fat, crude protein, total carbohydrates (TC), crude fiber (CF) and energetic value (EV) of bread formulations.

Sample	Moisture (%)	Ash (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	CF (g/100 g)	TC (g/100 g)	EV (kcal/100 g)
CB	27.89 ±	2.48 ±	4.50 ±	1.10 ±	0.06 ±	64.00 ±	289.18 ±
	1.37 ^a	0.09 ^a	0.94 ^a	0.15 ^a	0.02 ^a	2.31 ^a	1.18 ^a
BPO₅	24.52 ±	2.89 ±	6.11 ±	1.46 ±	0.21 ±	64.74 ±	290.17 ±
	2.14 ^a	0.12 ^{ab}	1.27 ^{ab}	0.06 ^b	0.01 ^b	2.90 ^a	2.40 ^a
BPO₁₀	28.46 ±	3.28 ±	6.83 ±	1.43 ±	0.51 ±	60.18 ±	299.09 ±
	3.28 ^a	0.09 ^{bc}	0.55 ^{ab}	0.03 ^b	0.07 ^{bc}	5.24 ^a	3.56 ^a
BPO₁₅	31.98 ±	3.79 ±	7.14 ±	1.48 ±	0.73 ±	54.99 ±	300.84 ±
	4.95 ^a	0.34 ^d	0.12 ^b	0.16 ^b	0.02 ^c	4.90 ^a	2.89 ^a
BPO₂₀	29.07 ±	3.77 ±	6.68 ±	1.55 ±	0.76 ±	58.19 ±	319.77 ±
	±1.45 ^a	0.21 ^{cd}	1.13 ^{ab}	0.12 ^b	0.04 ^c	1.90 ^a	5.72 ^a

Values presented correspond to mean and standard deviations on a dry basis.

^{a,b,c,d} Different superscript letters indicate a statistical difference in mean values of the same column ($p \leq 0.05$). CB: control bread, 0% POP; BPO₅: bread with 5% of POP; BPO₁₀: bread with 10% of POP; BPO₁₅: bread with 15% of POP; BPO₂₀: bread with 20% of POP.

Table 3. Specific volume and value of L^* , a^* and b^* of bread formulated with different proportion ranging from 0 to 20 % of *Pleurotus ostreatus* powder (POP).

Sample	Specific Volume (cm ³ g ⁻¹)	Color		
		L^*	a^*	b^*
CB	5.06 ± 0.04 ^a	64.64 ± 3.45 ^a	5.78 ± 0.90 ^c	19.32 ± 2.28 ^b
BPO ₅	4.14 ± 0.14 ^b	53.89 ± 4.80 ^b	11.13 ± 11.53 ^b	26.25 ± 2.25 ^a
BPO ₁₀	3.48 ± 0.15 ^c	47.29 ± 1.23 ^{bc}	12.92 ± 1.11 ^{ab}	26.20 ± 1.13 ^a
BPO ₁₅	3.33 ± 0.15 ^c	41.90 ± 1.76 ^{cd}	13.31 ± 0.70 ^{ab}	24.77 ± 1.10 ^a
BPO ₂₀	2.64 ± 0.29 ^d	36.53 ± 1.90 ^d	14.36 ± 0.09 ^a	24.47 ± 0.26 ^a

^{a,b,c,d} Different superscript letters indicate a statistical difference in mean values of the same column ($p \leq 0.05$).

CB: control bread, 0% POP; BPO₅: bread with 5% of POP; BPO₁₀: bread with 10% of POP; BPO₁₅: bread with 15% of POP; BPO₂₀: bread with 20% of POP.

Table 4. Total starch (TS), total starch hydrolyzed (HS), rapidly digested starch (RDS), slowly digested starch (SDS), resistant starch (RS) and estimated glycemic index (eGI) of breads.

Sample	TS	RDS	SDS	RS	HS	eGI
	(%)	(%)	(%)	(%)	(%)	
CB	99.33 ± 9.06 ^b	1.92 ± 0.32 ^b	39.36 ± 1.21 ^c	57.60 ± 0.23 ^d	41.73 ± 0.85 ^c	100.00 ± 1.30 ^c
BPO₅	97.65 ± 6.17 ^b	2.66 ± 0.42 ^d	37.29 ± 0.85 ^{bc}	57.24 ± 1.46 ^d	40.41 ± 0.70 ^c	85.00 ± 0.64 ^b
BPO₁₀	91.89 ± 8.12 ^b	2.31 ± 0.33 ^c	37.00 ± 2.52 ^b	52.61 ± 1.91 ^{cd}	39.28 ± 2.62 ^{bc}	83.33 ± 3.47 ^b
BPO₁₅	75.03 ± 2.88 ^a	0.46 ± 0.36 ^a	42.62 ± 2.81 ^a	38.13 ± 0.43 ^b	36.90 ± 2.87 ^{ab}	74.67 ± 1.63 ^a
BPO₂₀	66.53 ± 1.13 ^a	0.48 ± 0.67 ^a	42.26 ± 2.27 ^a	31.29 ± 0.73 ^a	35.24 ± 2.31 ^a	72.21 ± 1.78 ^a

Values presented correspond to mean and standard deviations on a dry basis.

^{a,b,c,d} Different superscript letters indicate a statistical difference in mean values of the same column ($p \leq 0.05$).

CB: control bread, 0% POP; BPO₅: bread with 5% of POP; BPO₁₀: bread with 10% of POP; BPO₁₅: bread with 15% of POP; BPO₂₀: bread with 20% of POP

Figure 1. Diagram of the static *in vitro* digestion procedure. Adapted from Minekus et al., 2014.

Figure 2. Images of cross section of bread enriched with *Pleurotus ostreatus* powder (POP). CB: control bread, 0% POP; BPO₅: bread with 5% of POP; BPO₁₀: bread with 10% of POP; BPO₁₅: bread with 15% of POP; BPO₂₀: bread with 20% of POP.

Figure 3. Hydrolyzed starch (%) during *in vitro* starch digestion of bread enriched with *Pleurotus ostreatus* powder (POP). CB: control bread, 0% POP; BPO₅: bread with 5% of POP; BPO₁₀: bread with 10% of POP; BPO₁₅: bread with 15% of POP; BPO₂₀: bread with 20% of POP.

Error bars indicate the mean values ± standard deviation.

a) Oral stage; b) gastric phase; c) intestinal phase.

Graphical Abstract

In this work, new wheat bread formulations were developed by replacing wheat flour with different concentrations of an edible mushroom, *Pleurotus ostreatus* powder (PPO), with the aim of increasing its nutritional value and reducing its glycemic index. These findings suggest that the mushroom powder could be used as an alternative food ingredient to improve nutritional quality and reduce the glycemic index of bread.