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Artículo original

Nutritional and antioxidant properties of novel cookies enriched with oyster mushroom (*Pleurotus ostreatus*) flour

Propiedades nutricionales y antioxidantes de nuevas galletas enriquecidas con harina de setas *Pleurotus ostreatus*

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ABSTRACT

The use of *Pleurotus* mushrooms in the formulation of industrialized foods is not fully exploited. This study was conducted to investigate the effects of wheat flour (WF) substitution with 10 % of oyster mushroom (*Pleurotus ostreatus*) flour (POF) on the nutritional and antioxidant properties of cookies. Proximal composition, phenolic content, and DPPH radical scavenging activity were determined in flours and cookies. Results showed that the contents of crude protein, ash and fiber of supplemented cookies were higher than that of control cookies (P< 0,05). Furthermore, fortification of *P. ostreatus* flour improved the functional quality of cookies by significantly enhancing the phenolic contents (736 ± 42,07 in POF *vs.* 232,1 ± 15,50 μg tannic acid equivalents/g in WF cookies) and DPPH antioxidant activities (58,34 ± 1,65 in POF *vs.* 9,61 ± 2,07 % in WF cookies) (P< 0,05).

Thus, enriched cookies could provide the consumers a novel cereal-based product with health-promoting benefits.

Keywords: antioxidant activity; cookies; fortification; *Pleurotus ostreatus*; proximal composition.

RESUMEN

El uso de setas *Pleurotus* en la formulación de alimentos industrializados no ha sido del todo explotado. Este estudio se realizó para investigar los efectos de la sustitución de un 10% de la harina de trigo (WF) con harina de *Pleurotus ostreatus* en las propiedades nutricionales y antioxidantes de las galletas. Los resultados evidenciaron que el contenido de proteína bruta, ceniza y fibra de las galletas suplementadas fue mayor que el de las galletas control (P< 0,05). Además, el enriquecimiento con la harina de *P. ostreatus* mejoró la calidad funcional de las galletas al elevar significativamente el contenido fenólico (736±42,07 en POF *vs.* 232,1±15,50 µg equivalentes de ácido tánico/g en las galletas con WF) y la actividad antioxidante (58,34±1,65 en POF *vs.* 9,61±2,07 % en las galletas control) (P< 0,05). En general, las galletas enriquecidas podrían proporcionar al consumidor un nuevo producto basado en cereales con beneficios para la salud.

Palabras clave: actividad antioxidante; composición proximal; fortificación; galletas; *Pleurotus ostreatus.*

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Introduction

Edible and medicinal mushrooms are now the emerging and most important microbial agri-food chain, showing a good impact on food production for direct human consumption and as endless source of novel bioactive compounds with functional and medicinal properties, which are different from those found in foods of plant and animal origin.[1]

Pleurotus spp. is the second most important mushroom of culinary value worldwide. *Pleurotus* species have been recognized as a high nutritional value-food containing biologically active molecules with therapeutic effects.[2] Particularly, *Pleurotus ostreatus* is highlighted by its therapeutic properties such as anti-inflammatory, antimicrobial, antiviral, antitumor, antioxidant, antimutagenic, hypotensive, cardioprotective, antidiabetic, and immunomodulatory, among others.[3,4] These effects are attributed to the presence of biologically active compounds such as polysaccharides, peptides, proteins, glycoproteins, starch, polyphenols, nucleotides, triterpenoids, lectins, lipids, and other complex compounds.[5] Particularly, the antioxidant properties of *P. ostreatus* have been well documented in both mycelium and fruiting bodies at different maturation stages.[6,7]

Pleurotus mushrooms have become a staple food because of the variety in processing and consumption. In previous studies, some *Pleurotus* species were investigated as food additives: (I) the addition of *P. sajor-caju* to selected wheat- and rice-based products [8]; (II) the incorporation of oyster mushroom into biscuits in the range 5-30% [9]; (III) the production of Fettuccine pasta with partial replacement of wheat flour with *P. ostreatus* flour at 10% and 20% [10]; and (IV) sponge cakes supplemented with hot air or freeze dried *P. sajor-caju* powder at 5%, 7%, 10% and 12%. [11] However, the use of *Pleurotus* spp. in the formulation of industrialized food is not fully exploited.

To the best of our knowledge, there are no standard products developed with *Pleurotus* mushroom in the form of functional cookies with improved nutritional value, mycochemical profile and antioxidant properties. In this context, the purpose of this research was to evaluate the impact of partial replacement (10 %) of wheat flour (WF) with *Pleurotus ostreatus* mushroom flour in the elaboration of functional cookies. The hypothesis proposed was that the partial replacement of WF with POF improves its nutritional contribution in total protein, dietary fiber and mineral contents, as well as, in the antioxidant capacity of cookies enriched with this natural ingredient. This study intends to contribute to fortifying traditional types of cookies that lack nutraceutical compounds and antioxidant activity, thus providing reliable insight into fortified cookies functionality. Hence, obtaining these cookies represents a high potential in the development of a healthy functional food with benefits for the consumers. The research also promotes the renewable local bioresouces for a sustainable agri-food chain in the framework of Sustainable Development Goals (SDGs).

Materials and methods

Chemicals and Samples

Tannic acid, Folin-Ciocalteu's reagent, hydrochloric acid, sodium acetate trihydrate, glacial acetic acid, sulfuric acid, sodium carbonate (anhydrous), acetone, ethanol and sodium hydroxide were purchased from Merck. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

All the baking ingredients in customary quality, such as high grade Wheat Flour (WF), fat, white sugar, salt and yeast were supplied by the "Empresa Provincial de la Industria Alimentaria" (EPIA, Santiago de Cuba, Cuba). All samples in the original packaging were stored at ambient temperature until further processing.

Pleurotus ostreatus CCEBI-3024 is deposited at the Culture Collection of the Center for Studies on Industrial Biotechnology (CEBI, Santiago de Cuba, Cuba). The strain was maintained on slants with solid medium of potato dextrose agar (PDA) incubated at 5 °C. *Pleurotus* sp. cultivation was performed by solid-state fermentation of mushroom spawn on pasteurized coffee pulp used as substrate in plastic bags of 2 kg (30-40 cm).[12] Fruit bodies were harvested, sliced into small pieces and dried at 45 °C for 24 h. Dried material was milled and the resulting powder was preserved away from light and humidity in plastic bags for further use.

Formulation and Production of Fortified Cookies with *Pleurotus ostreatus* Flour

The production of cookies was performed at the specialized bakery "El Palacio de Santa Rita" (EPIA, Santiago de Cuba, Cuba). Standard cookie dough (control) was elaborated according to a traditional method. Wheat flour cookies (control) and POF-enriched cookies were prepared based on the formulations presented in table 1. The amount of POF to be incorporated in fortified cookies was established at the level of 10% because of according to sensorial analysis results, the 4-10% mushrooms powder in bakery products showed the best sensory properties.[13] For both formulations, shortening was creamed, blended with sugar in a mixer and then WF and POF were sieved into the above dough and mixed again until homogenous appearance was reached. The dough was rolled and cut into circular shapes and baked at 180°C for 12 min in a convection oven. After then, cookies were cooled at room temperature for 30 min and stored in hermetic bags, at ambient temperature and protected from the light until analysis. Figure 1 shows representative photographs of POF making and its utilization in the preparation of doughs and cookies.

Table 1 - Formulation of cookies with 10% of *Pleurotus ostreatus* flour (POF) and wheat flour (WF) as control cookies (g/100 g of formulation).

Ingredients	**Formulations**	
	WF	**POF**
Whole wheat flour (WWF)	72,2	62,2
Pleurotus ostreatus flour (POF)	-	10
Proportion of flours´ ingredients	**100% WF**	**90:10 (WF:POF)**
Water	19,6	19,6
Sugar	1,4	1,4
Salt	1,6	1,6
Fat	5,1	5,1
Yeast	0,1	0,1

Proximate and Energy Analysis of Flours and Cookies

Proximate composition of individual flours and cookies were performed in triplicate following the Association of Official Analytical Chemists standard protocols.(14) Chemical composition analyses were carried out for investigating moisture, ash, fat, crude protein and fiber contents in the samples. Total protein was determined by the Kjeldahl procedure (AOAC 976,05, N × 6,25); fat by Soxhlet extraction (AOAC 920,39); crude fiber by gravimetric method (AOAC 962.09); ash by muffle furnace dry ashing at 550 °C for 24 h (AOAC 942,05), and total carbohydrate by difference, subtracting 100 from the sum of the other components. Energy content of the flours and cookies was estimated by the equation:

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g total carbohydrate}) + 9 \times (\text{g fat}) \qquad \text{(n 1)}$$

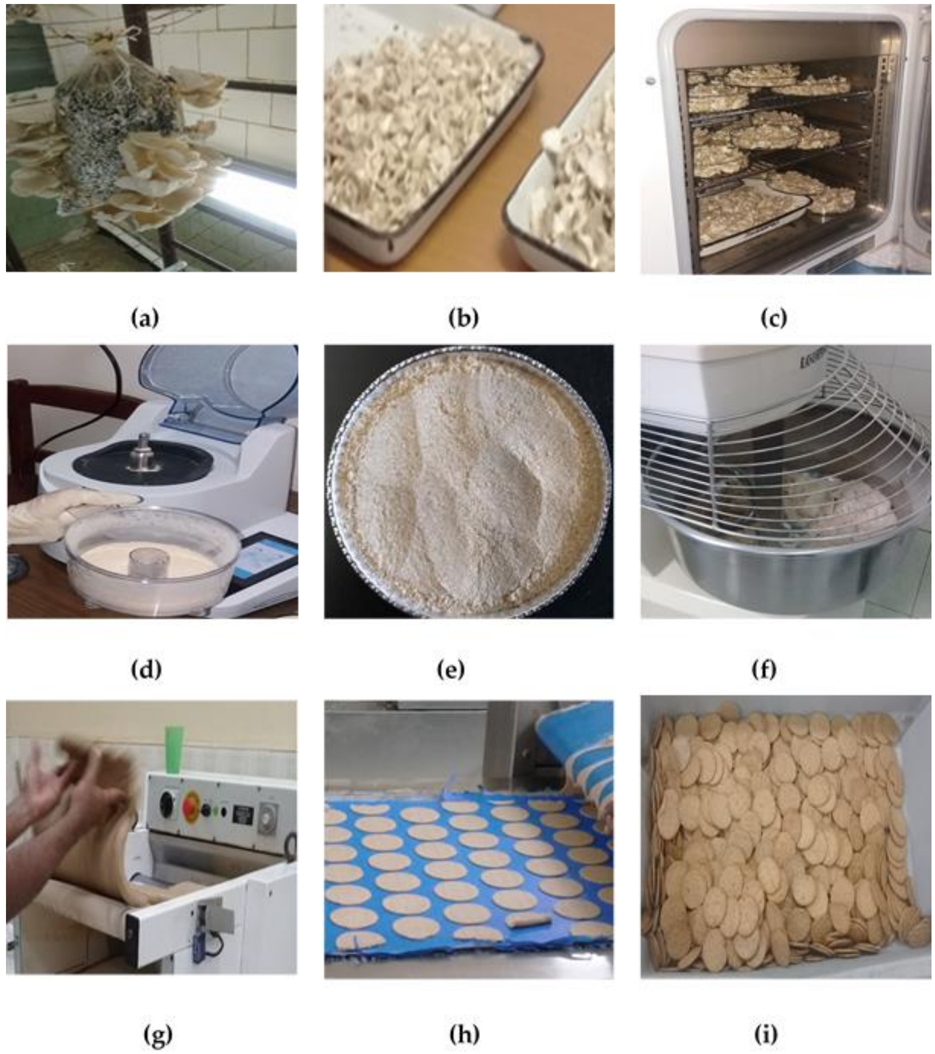

Fig. 1 - Representative photographs of POF making and its utilization in the preparation of doughs and cookies. (a) cultivation of *P. ostreatus* mushroom by solid-state fermentation; (b) slicing; (c) drying; (d) milling; (e) mushroom flour –POF; (f) homogenization; (g) dough rolling; (h) conformation of cookies for baking; and (i) resulting cookies

Determination of Total Phenolics and Antioxidant Activity in Flours and Cookies

One gram of flours and cookies were extracted by occasional shaking with 70:29.5:0.5 mixture of acetone-water-acetic acid (10 mL) at 37 °C for 60 min in the dark. Supernatant was obtained by centrifugation at 4,000 rpm for 30 min at 4 °C. The extraction was repeated by adding 5 mL of the solvent mixture and the supernatants were mixed and collected in individual vials and stored at 4 °C until use.

Total phenolic compounds (TPC) of extracts were determined according to the procedure reported by Slinkard y Singlenton with the Folin-Ciocalteu's phenol reagent.[15] Absorbance was measured at 765 nm in an spectrophotometer (VIS-723G spectrophotometer, Beijing Rayleigh Analytical Instrument Corporation, China), The results were expressed as μg of tannic acid equivalents per gram of sample, μg (TAE)/g.

The DPPH scavenging ability of extracts prepared from flours and cookies was determined by the procedure reported by Cheung et al.[16] using spectrophotometry. Extracts (1 mL) were mixed with 0.5 mL of 0.1 mM DPPH ethanolic solution. Then, the mixture was shaken vigorously and incubated at 25°C for 1 h in the dark. The absorbance of the sample was measured at 520 nm (VIS-723G spectrophotometer, Beijing Rayleigh Analytical Instrument Corporation) and the scavenging ability against DPPH radicals was calculated as a percentage of DPPH discoloration using the equation:

$$\% \, RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \qquad \text{(Equation 2)}$$

where

RSA means Radical Scavenging Activity,

A_S is the absorbance in the solution when the sample extract has been added, and A_{DPPH} is the absorbance of DPPH solution.

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) of triplicate determinations. The Student´s *t* test was used to determine the significance of differences between formulations. Differences were considered significant at P < 0,05. All statistical analyses were performed with SPSS version 22.0 (SPSS Inc, Chicago, Il, USA) software package for Windows.

Results and discussion

The development of new fortified flour products can have a demanding task capable of influencing metabolism and other health-related conditions. One of the categories of functional additions are components with antioxidative properties, which can reduce the level of oxidative stress in cells.[17]

Most of the time, cookies and biscuits are prepared using refined WF, but composite flour is healthier because it improves the nutritional value of bakery products when blended with other types of flour.[18] Thus, fortified cookies with POF offers the possibility of introducing substances including antioxidant molecules with beneficial properties for health through the diet.

Analysis of WF and POF Flours

The information about the proximate composition and energy is of great interest for biofunctional ingredients to be used in the formulation of health foods and nutraceuticals. Information about the complete characterization of flours will allow not only to establish possible combinations between them with the aim to enhance the nutritional profile of the bakery products, but also to be used in other types of products, such as beverages, soups, sauces, or food adjuvants.[19]

Natural *Pleurotus ostreatus* mushroom flour without preservatives, artificial colors, dyes or any additives were produced within the scope of this study by dehydration followed by grinding. The chemical composition of WF and POF is presented in Table 2.

Table 2 - Proximal composition, energy value, phenolic content, and antioxidant activity of WWF and *P. ostreatus* flours used in cookies´ formulation.

Parameter	WWF	POF
Moisture (%)	9,76 ± 0,03	8,36 ± 0,29 *
Total protein (%)	9,67 ± 0,10	27,4 ± 0,18 *
Carbohydrates (%)	75,15 ± 1,42	45,13 ± 0,49 *
Total lipids (%)	2,07 ± 0,41	4,04 ± 0,39 *
Crude fiber (%)	1,99 ± 0,20	7,50 ± 1,65 *
Ash (%)	1,36 ± 0,02	7,57 ± 0,17*
Energy value (kcal/100 g)	358 ± 23	326 ± 32
Total polyphenols (µg TAE/g)	250,3 ± 11,07	489,3 ± 97,42 *
Antioxidant activity (%, DPPH assay)	4,89 ± 0,07	49,43 ± 2,61*

(*) indicate significant differences of means for each parameter, according to the Student´s *t* test (P < 0,05, n = 3).

WF and POF used for cookies production differed significantly (P < 0,05) in the contents of basic proximate components. Our results showed that POF was rich in crude protein, crude fiber and ash, but lower in carbohydrate than those of WF. It is noteworthy that POF had approximately 2,8-fold higher levels of protein and 3,8-fold higher levels of fiber than those of the WF. The higher ash content in POF implied that they contained relatively higher mineral content with values 5,6-fold higher than WF. Lipids were also higher in POF with respect to WF (1, 9-fold). Moisture content of POF compared favorably with WF (P < 0,05), thus indicating a positive impact in terms of conservation. Even if there was variability on the chemical composition values POF with those obtained by other researchers, its proximate biochemical analysis is in agreement with the composition informed in previous reports. For oyster mushroom *Pleurotus ostreatus* ranges between 17-30,4 % for crude protein, 37-85 % for carbohydrates, 5,3-24% for total dietary fiber, 1,6-5,0% for fat and 5,9-9,8 % for ash have been reported depending on the strains, method of cultivation, substrates and growth conditions, etcétera.[20,21]

In addition to the high nutritional value, POF possess a significant polyphenols content, about two times higher than WF (P < 0,05). The content of phenolics is commonly determined by the Folin-Ciocalteu reaction. However, the comparison of our results with data regarding total phenolic content in literature is difficult because different extraction solvents and substances for calibration are used and data are variously expressed in quercetin (Q), catechin (C), gallic (GA), or other compound equivalents (E). Moreover, different methods have been used for measuring antioxidant activity based on diverse chemistry principles.[22] The TPC of POF tested in the present work was comparatively higher than the values reported in five wild culinary-medicinal species of *Pleurotus* collected from Northwest India (67,6 to 169.2 μg/g).[23] In addition, POF exerted a prominent scavenging activity against DPPH radical, about 10 times higher than control.

Functionality of Fortified Cookies

Level of enrichment of the cookies, i.e., functional characteristics of fortified cookies was determined based on an increase of nutrients and myco/phytochemicals content and antioxidant activity. Significant improvement of conventional cookies functionality through the incorporation of POF was confirmed. The results obtained for proximal composition of the cookies prepared from different blends of wheat and POF are depicted in table 3. Collectively, the differences in nutritional composition of cookies samples in this study are related to the original chemical compositions of wheat and POF, NF and AF, along with their relative inclusion level in the recipe. The moisture content of the composite POF cookies (3,16 %) was statistically higher than WF cookies (2,65 %), but within the recommended range of 0-10% for storage of biscuits. [24] POF fortified cookies had approximately 2-fold higher levels of protein, 1.9-fold higher levels of ashes, and 1,4-fold higher content of fiber than those of the WF cookies (P < 0,05). When POF was incorporated into cookies, a reduction of carbohydrates was observed coincidental with the increase in protein content. Moreover, it is widely known that WF cookies, commonly available in the market, lack good quality protein because of their deficiency in lysine. For this reason, the production of cookies enriched with POF can increase not only the protein content, but also improves the amino acid balance of the final product, due to the contribution of lysine and other essential amino acids by these biofunctional flours.[22] The results of the lipid content and energy values showed no significant differences.

Table 3 - Proximal composition, energy value, phenolic content, and antioxidant activity of traditional WWF cookies and those enriched with 10% of *Pleurotus ostreatus* flour.

Parameter	Traditional cookies	POF-10%
Moisture (%)	2,65 ± 0,15	3,16 ± 0,15 *
Total protein (%)	7,07 ± 0,08	14,12 ± 0,36 *
Carbohydrates (%)	83,03 ± 1,24	72,73 ± 0,48 *
Total lipids (%)	4,10 ± 0,36	5,01 ± 0,54
Crude fiber (%)	2,17 ± 0,53	3,11 ± 0,12 *
Ash (%)	0,98 ± 0,03	1,87 ± 0,05 *
Energy value (kcal/100 g)	397 ± 37	392 ± 30
Total polyphenols (µg TAE/g)	232,1 ± 15,50	736 ± 42,07 *
Antioxidant activity (%, DPPH assay)	9,61 ± 2,07	58,34 ± 1,65 *

(*) indicate significant differences of means for each parameter, according to the Student´s *t* test (P < 0,05, n = 3).

The incorporation of POF into WF significantly improved the total polyphenol content and antioxidant capacity in the DPPH radical-scavenging activity of composite cookies compared to control. In addition, phenolics retain their antioxidant activity after the baking process, which has potential health benefits for consumers, although some authors informed that baking could reduce the polyphenol content in the obtained cookies.[25] The antioxidant activity of the compound structure was reported to be dependent on the number of included active group (OH) and the position of the active groups.[22]

Therefore, determination of phenolic profile of cookie samples and correlation analysis between phenolic compounds and antioxidant activities are worthwhile to be studied further.

It is worth noting that POF is a complex matrix and the antioxidant activity may reflect the cumulative interactions (synergy or antagonism) among its constituents in addition to phenolics/flavonoids. For instance, this biochemical array contains antioxidant vitamins and minerals, as well as, dietary fiber - mainly β-glucans, polysaccharides, and polysaccharide- protein complexes.(2-6)

Taking together, our results indicated that supplementation of WF with POF in fortified cookies not only reinforces the nutritional quality, but also contributes to their stabilization against oxidative damage. Overall, enriched cookies have more functional components and effective antioxidant capacity than wheat cookies. Their supplementation could provide the consumers a novel product with health-promoting benefits.

Conclusions

In the light of the present data, it can be concluded that partial replacement (10%) of WF by POF in fortified cookies is an effective tool to offer nutritious, antioxidant-rich and healthy nutraceuticals/functional foods alternatives to consumers. Further studies are needed to evaluate the storability and sensorial acceptance of novel cookies by potential consumers. Additionally, *in vivo* intervention studies in animal models and humans will be advisable to verify the protective role of fortified cookies in modulating oxidative-stress related conditions.

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