Nutritional, antioxidant, and quality characteristics of novel cookies enriched with mushroom (Cordyceps militaris) flour

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ABSTRACT
This study was conducted to investigate the effects of incorporating varying proportions of nutraceutical-rich mushroom (Cordyceps militaris) flour (1%, 3% and 5%) on the physicochemical, antioxidant and sensory properties of cookies. Results showed that the contents of crude protein, ash and fiber of supplemented coo kies were higher than that of control cookies. Spread ratio and hardness decreased as the C. militaris flour incorporation level increased. The color values of cookies also varied with the increasing levels of C. militaris flour. Furthermore, fortification of C. militaris flour improved the functional quality of cookies by significantly enhancing the phenolic contents and antioxidant activities (P < 0.05). Sensory evaluation of cookies indicated that the addition of 3% C. militaris flour was more acceptable. Overall, enriched cookies have more functional components and effective antioxidant capacity than plain wheat cookies. Their supplementation could provide the consumers a novel cereal-based product with health-promoting benefits.

Características nutricionales, antioxidantes y de calidad de galletas nuevas enriquecidas con harina de hongos (Cordyceps militaris)

RESUMEN
Este estudio se llevó a cabo para investigar los efectos que conlleva la incorporación de proporciones variables (1%, 3% y 5%) de harina de hongos (Cordyceps militaris) rica en nutracéuticos en las propiedades físicas, antioxidantes y sensoriales de las galletas. Los resultados permitieron constatar que el contenido de proteína bruta, ceniza y fibra de las galletas suplementadas era mayor que el de las galletas de control. La razón de espaciamiento y la dureza disminuyeron al aumentar el nivel de incorporación de harina de C. militaris. Los valores de color de las galletas también variaron al incrementarse los niveles de harina de C. militaris. Además, el enriquecimiento con la harina de C. militaris mejoró la calidad funcional de las galletas al elevar significativamente el contenido fenólico y la actividad antioxidante (P < 0.05). La evaluación sensorial de las galletas mostró que la adición de 3% de harina de C. militaris fue la más aceptable. En general, las galletas enriquecidas tienen más componentes funcionales y una capacidad antioxidante más efectiva que las galletas de trigo normal. Su suplementación podría proporcionar al consumidor un nuevo producto basado en cereales con beneficios para la salud.

1. Introduction
Nowadays, with improvements in living standard and lifestyle, consumers no longer demand foods that only meet necessary nutrients, but desire foods with functional and nutraceutical properties (Bimbo et al., 2017; Irakli et al., 2019). Cookies are very popular sweet bakery products, widely consumed all over the world (Okpala et al., 2013). Cookies can be the right product for satisfying health-promoting dietary demands (Bhat et al., 2020). Thus, by application of bioactive components, production of novel cookies with improved nutritional value is a result of increasing interest.

Mushroom, including fruiting bodies and mycelia, have become attractive as functional foods and as a source of bioactive substances (Cui, 2015). In East Asia, Cordyceps militaris (L.) is an edible and medicinal mushroom, which has been used extensively as a crude drug and a folk tonic food (Shrestha et al., 2012). C. militaris is high in nutrients, such as proteins, vitamins, trace minerals, and so on (Dang et al., 2018). A number of other bioactive constituents isolated from C. militaris have been also reported such as cordycepin, polysaccharides, cordycepic acid, superoxide dismutase, and fibrinolytic enzyme (Kim et al., 2019; Tang et al., 2018; Zhang et al., 2019). C. militaris is well known for its health stimulating properties and medicinal effects, anti-inflammatory, antioxidant or anti-aging, antitumor and immunomodulatory effects (Reis et al., 2013). In view of its biological activities, C. militaris can be utilized as ingredients in nutraceuticals and functional foods for nutritional enhancement and fortification (Guo et al., 2020; Xiao et al., 2014). C. militaris-enriched food products fit into the health-conscious trend.

Utilization of C. militaris in cookies requires research attention because of growing demand for high nutritional bakery products. To the best of our knowledge, there is a paucity of information in literature on the usefulness of C. militaris as a functional supplement for cookies.
Therefore, the aim of this study was to compare physicochemical, antioxidant and sensory characteristics of cookies enriched with different levels of *C. militaris*, in order to assess its effectiveness as a functional addition of cookies.

2. Materials and methods

2.1. Raw materials

Mycelia of *C. militaris* were obtained from the edible fungus research institute of Jiangsu province (Jurong city, Jiangsu). All other ingredients (food grade) for making cookies were purchased from local market, and then stored at different conditions according to their individual requirements until further use.

2.2. Chemicals

All chemicals and reagents were of analytical grade and purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

2.3. Preparation of *C. militaris* flour

Mycelia of *C. militaris* were first dried at 50 ± 2°C for 12 h (DHG-9240A, Shanghai Yiheng Technical co., Ltd, China), and then milled using a food grinder (particles below 0.2 mm), and stored at −22°C for further analysis and cookies fortification.

2.4. Preparation of cookies

Wheat flour cookies (control) and *C. militaris* flour-enriched cookies were prepared based on the different formulations presented in Table 1. Control cookies were prepared without addition of *C. militaris* flour as blank sample. Cookies supplemented with *C. militaris* flour were prepared by replacing wheat flour with *C. militaris* flour at levels of 1%, 3% and 5% (CM-1, CM-3 and CM-5, respectively). For all formulations, shortening was creamed, blended with sugar and egg in a mixer (Model KVC3100, Kenwood, UK). And then wheat flour and *C. militaris* flour were sieved into the above dough and mixed again. Finally, water was added and thoroughly mixed to form homogenous dough. The dough was rolled and cut into round shape (12 cm, diameter of 5.5 cm) and baked at 180°C for 12 min. After then, cookies were cooled at room temperature for 30 min and packed into sealed polyethylene bags until analysis.

2.5. Proximate analysis

Proximate compositions were performed following the Association of Official Analytical Chemists AOAC (2006) standard protocols. Chemical composition analyses were carried out for investigating moisture, ash, fat, crude protein and fiber contents in the samples. Moisture content was determined by drying in a hot air oven (AOAC 934.01), ash by muffle furnace dry ashing (AOAC 942.05), protein by the Kjeldahl procedure (AOAC 976.05, N × 6.25), fat by Soxhlet extraction (AOAC 920.39), crude fiber (AOAC 962.09), and total carbohydrate by calculation (Bhat & Sridhar, 2008).

2.6. Physical and textural characteristics of cookies

2.6.1. Weight, diameter, thickness, spread ratio

Weight, diameter, thickness and spread ratio of control cookies and cookies fortified with mushroom flour were conducted by using the method reported by Sharma et al. (2016).

2.6.2. Color characteristics

Color characteristics of cookies were measured by using a CR-400 Minolta spectrophotometer (Tokyo, Japan). The instrument was calibrated before each analysis with white and black standard plate. L* (lightness, black = 0, white = 100), a* (redness > 0, greenness < 0), b* (yellowness > 0, blue < 0) were recorded on each sample. \( \Delta E = (\Delta L^2)^2 + (\Delta a^2)^2 + (\Delta b^2)^2)^{1/2} \). Each type of cookie sample was individually measured in triplicate.

2.6.3. Textural properties (hardness)

Texture of cookies was performed with a Texture Analyser (TA-XT2i, Stable Micro System Ltd, UK). A sharp cutting blade probe (HDP/BS) was selected to conduct the experiment with pre-test speed of 1.5 mm sec\(^{-1}\), test speed of 2.0 mm sec\(^{-1}\), post-test speed of 10.0 mm sec\(^{-1}\), distance 5.0 mm and trigger type auto (Biao et al., 2020). Each sample was conducted in hexplicate.

2.7. Extraction procedure

2.7.1. Buffer extracts (BE)

The powdered cookie samples (1 g) were extracted for 1 h with 20 mL of phosphate buffer (20 mM, pH 7.4). The extracts were separated by decantation and extraction procedure was repeated twice. The supernatants were combined and frozen in darkness at −20°C.

2.7.2. Extracts after digestion in vitro (DE)

An in vitro digestion procedure was performed based on the method of Gawlik-dziki et al. (2014) with some modifications, by mimicking subsequent digestion including gastric and intestinal steps. 15 mL stimulated salivary fluid (α-amylase, 200 U mL\(^{-1}\) in 1 L distilled water dissolved in 2.38 g Na\(_2\)HPO\(_4\), 0.19 g KH\(_2\)PO\(_4\), 8 g NaCl and 100 mg mucin) was homogenized into 5 g of cookies samples for 1 min to mimic buccal digestion. Subsequently, the digestion samples were incubated in the shaker at 37°C for 10 min. Then, samples were acidified to pH 1.2 using 5 M

| Table 1. Formulation of cookies with different levels of *Cordyceps militaris* flour (g 100 g\(^{-1}\), dry basis). |
| Ingredient | Control | CM-1 | CM-3 | CM-5 |
|------------|--------|------|------|------|
| Wheat flour | 100    | 99   | 97   | 95   |
| *C. militaris* flour | 0      | 1    | 3    | 5    |
| Sugar (g)  | 25     | 26   | 26   | 26   |
| Shortening | 60     | 60   | 60   | 60   |
| Water      | 10.5   | 10.5 | 10.5 | 10.5 |
| Egg        | 18     | 18   | 18   | 18   |

Note: Control, 100% of wheat flour only; control cookies: CM-1, formulation with 1% of *C. militaris* flour; CM-3, formulation with 3% of *C. militaris* flour; CM-5, formulation with 5% of *C. militaris* flour.
Note: Control, 100% de harina de trigo solamente, galletas de control; CM-1, formulación con 1% de harina de *C. militaris*; CM-3, formulación con 3% de harina de *C. militaris*; CM-5, formulación con 5% de harina de *C. militaris*.
HCl to stimulate gastric digestion, followed by adding 15 mL stimulated gastric fluid (pepsin, 300 U mL\(^{-1}\) in 0.03 M NaCl solution, pH 1.2). The samples were shaken for 60 min at 37\(^\circ\)C. Afterward, the pH of samples was adjusted to pH 7 with 0.1 M NaHCO\(_3\) solution. After that, 15 mL of mixture of bile acids and pancreatin (bile acids 0.3 g, pancreatin 0.05 g in 35 mL 0.1 M NaHCO\(_3\) solution) was added, and then placed in the shaker at 37\(^\circ\)C for 120 min. Thereafter, sample solutions were centrifuged (9,000 \(\times\) g for 15 min at 4\(^\circ\)C). Supernatants were collected and stored at −20\(^\circ\)C for further use.

2.8. Determination of total phenolic contents (TPC)

Total phenolic contents were determined for both BE and DE using Folin-Ciocalteu method as described by Xiao et al. (2014). TPC were calculated using gallic acid calibration curve (\(y = 0.1635x - 0.00019; R^2 = 0.9992\)). Results were expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight (mg GAE g\(^{-1}\) DW).

2.9. Evaluation of antioxidant activities

2.9.1. Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity was examined according to J. Liu et al. (2009) with slight modifications. Briefly, extracts (0.2 mL) were mixed with 3.8 mL DPPH solution (0.1 mM DPPH in methanol). The mixture was kept in dark at room temperature for 30 min. After incubation, the absorbance of the mixture was measured at 517 nm in a UV-Vis spectrophotometer (T6, Puxi, China). The DPPH radical scavenging activity was calculated as follows:

\[
\text{Scavenging activity (\%)} = \frac{1 - (A_1 - A_2)/A_0}{A_0} \times 100
\]

where A\(_0\) was the absorbance of the control (water instead of sample), and A\(_1\) is the absorbance of the sample, and A\(_2\) is the absorbance of the sample only (water instead of DPPH). Vitamin C was used as the positive control. The vitamin C calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were presented as micromograms of vitamin C equivalents (VCE) per gram of dry weight sample (\(\mu\)g VCE g\(^{-1}\) DW).

2.9.2. Assay of ABTS radical cation scavenging activity

The experiments were carried out by the method reported by Xiao et al. (2014) with minor modifications. The ABTS\(^+\) solution was produced by reacting ABTS stock solution (7 mM) with potassium persulphate solution (2.45 mM). The ABTS\(^-\) solution was stored in darkness for 16 h. The solution was then diluted (with ethanol) to an absorbance of 0.70 ± 0.05 at 734 nm. 0.2 mL of sample extracts was thoroughly mixed with 4 mL of the ABTS\(^+\) solution. Similar to other antioxidant activities, the results were compared with a calibration curve of vitamin C solution and expressed as vitamin C equivalents in mg g\(^{-1}\) DW.

2.9.3. Evaluation of ferric reducing power (FRAP)

FRAP of the test samples was carried out according to the method described by Wootton-Beard et al. (2011). FRAP of test samples was calculated as vitamin C equivalents in \(\mu\)g g\(^{-1}\) DW.

2.9.4. Assay of reducing power (RP)

The reducing powder of samples was examined based on the method of Xiao et al. (2014). A standard curve was plotted by different concentrations of vitamin C. The reducing powder was also expressed as \(\mu\)g VCE per g DW.

2.10. Sensory evaluation

Sensory evaluation was carried out for cookie samples enriched with different percentages of C. militaris flour by 30 trained panelists. Panelists were asked to evaluate color, odor, taste, texture and overall acceptability on a nine-point hedonic scale from extremely like (9) to dislike (1). In addition, plain water was provided to panelists for mouth rinsing in between every sample.

2.11. Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD) of triplicate determinations, except for texture (n = 6) and sensory evaluation (n = 30). One-way analysis of variance (ANOVA) and Duncan’s multiple range test were used to compare the means. Differences were considered significant at \(P < 0.05\). All statistical analyses were performed with SPSS version 22.0 (SPSS Inc, Chicago, II, USA) software package for windows.

3. Results and discussion

3.1. Physicochemical composition of raw materials

The chemical composition of wheat and C. militaris flour is presented in Table 2. The wheat and C. militaris flour used for cookies production differed significantly \((P < 0.05)\) in the contents of basic proximate components. Our results showed that the C. militaris flour used in this study was rich in crude protein, fat, ash and crude fiber, but lower in carbohydrate than those of wheat flour. It is noteworthy that the C. militaris flour had approximately 3.95-fold and 7.00-fold higher levels of protein and fiber than those of the wheat flour, respectively. Protein and fat contents of C. militaris flour was 33.44 and 2.33 g 100 g\(^{-1}\), which correlated well with the previously reported values of 30.23 and 4.58 g 100 g\(^{-1}\) of the mycelia of C. militaris (X. Liu et al., 2014). C. militaris as edible mushroom could be expected to be a good source of protein and fiber. The wheat flour showed low fiber content, which might be due to the removal of wheat bran during milling process (Oghbaei &

### Table 2. Proximate composition of wheat flour and Cordyceps militaris flour.

| Parameters                        | Wheat flour | Cordyceps militaris flour |
|-----------------------------------|-------------|--------------------------|
| Moisture (g 100 g\(^{-1}\))       | 13.74 ± 0.11a | 16.06 ± 0.59ab          |
| Crude protein (g 100 g\(^{-1}\))  | 8.46 ± 0.066a | 33.44 ± 1.58b          |
| Crude lipid (g 100 g\(^{-1}\))    | 0.71 ± 0.012a | 2.33 ± 0.21ab          |
| Ash (g 100 g\(^{-1}\))           | 0.35 ± 0.032a | 0.49 ± 0.19ab          |
| Crude fiber (g 100 g\(^{-1}\))   | 0.86 ± 0.026a | 6.02 ± 0.06ab          |
| Carbohydrates (g 100 g\(^{-1}\)) | 89.62 ± 0.017a | 53.31 ± 1.99ab         |
| Energy (kcal 100 g\(^{-1}\))     | 398.71 ± 0.20b | 368.00 ± 0.38ab        |

Note: The contents of total crude carbohydrates were calculated by difference. Means ± Standard Deviation followed by different letters are significantly different at \(P < 0.05\).

Nota: El contenido de carbohidratos brutos totales se calculó por diferencia. La media ± desviación estándar seguida de letras distintas es significativamente diferente en \(P < 0.05\).
Prakash, 2013). The higher ash content in C. militaris flour implied that it contained relatively higher mineral content (Cheng & Bhat, 2016). Based on these findings, it was found that C. militaris flour could serve as a good source of nutrients.

3.2. Proximate composition of the cookies produced from wheat and C. militaris flour blends

The results obtained for chemical composition of the cookies prepared from different blends of wheat and C. militaris flour are depicted in Table 3. No differences were observed on moisture content and fat content among the control cookies and mycelium-supplemented cookies (P > 0.05). It was found that a progressive increase in the protein, ash, fiber content of cookies as the supplement of C. militaris flour increased in the blend. The changes of the carbohydrates content showed a reverse trend. When 5% C. militaris flour was incorporated into cookies, protein content was increased up to 11.26 g 100 g⁻¹, which was coincidental with the reduction of carbohydrates to 59.83 g 100 g⁻¹. Similar results were also reported earlier wherein protein content of cookies with the addition of sclerotium flour of edible mushroom (Pleurotus tuberregium) was found to significantly higher than that of the control cookies (Kolawole et al., 2020). Moreover, cookies fortified with 5% of C. militaris flour had significantly (P < 0.05) the highest content of ash, where the lowest one was recorded for control samples without additions. Comparable results have been reported by Cheng and Bhat (2016) that the higher ash content of the supplemented cookies indicated that the cookies contained higher mineral content than the control wheat cookies. Collectively, these differences in nutritional composition of cookies samples are related to the original chemical compositions of wheat and C. militaris flour, along with their relative inclusion level in the recipe (Giuberti et al., 2018). The energy values (518.34–519.39 kcal 100 g⁻¹) did not show significant differences among cookies samples. The lowest energy value was recorded for cookies supplemented with 5% C. militaris flour. These findings indicated that supplementation of wheat flour with mushroom flour would therefore improve the nutritional profile of cookies.

3.3. Physical parameters of cookies

Results on the physical characteristics of cookie samples are presented in Table 4. Cookies containing C. militaris flour at all levels showed the similar diameter and thickness. Furthermore, spread ratio of cookies showed very slight decrease with increase in C. militaris flour levels. However, there was no significant difference with the control cookies (wheat flour) (P > 0.05). As also be shown in Table 4, the weight of mycelium-supplemented cookies varied from 16.33 to 16.57 g with the control cookies showing the greatest weight. This observation was consistent with the findings of Yusufu and Akhlige (2014) and Cheng and Bhat (2016), in which the control cookies were heavier than the composite cookies.

3.4. Color characteristics of cookies

Color is an important visual parameter of cookies. The data regarding color characteristics of cookies fortified with C. militaris flour at varying levels are given in Table 4. From Table 4. Physical and textural characteristics of cookies elaborated with wheat flour only and partial substitution of Cordyceps militaris flour.

**Table 3.** Nutritional composition of cookies elaborated with wheat flour only and partial substitution of Cordyceps militaris flour.

| Parameters                  | Control     | CM-1        | CM-3        | CM-5        |
|-----------------------------|-------------|-------------|-------------|-------------|
| Moisture (g 100 g⁻¹)        | 6.45 ± 0.39a| 6.48 ± 0.44a| 6.62 ± 0.15b| 6.70 ± 0.06c|
| Crude protein (g 100 g⁻¹)   | 8.65 ± 0.14a| 8.47 ± 0.40a| 9.53 ± 0.18b| 10.26 ± 0.12c|
| Crude lipid (g 100 g⁻¹)     | 26.34 ± 0.37a| 26.34 ± 0.02a| 26.40 ± 0.13a| 26.64 ± 0.30a|
| Ash (g 100 g⁻¹)             | 0.50 ± 0.02a| 0.51 ± 0.017| 0.55 ± 0.046| 0.62 ± 0.040|
| Crude fiber (g 100 g⁻¹)     | 2.57 ± 0.28a| 2.50 ± 0.035a| 2.62 ± 0.17b| 2.85 ± 0.032a|
| Carbohydrates (g 100 g⁻¹)   | 61.94 ± 0.43a| 62.17 ± 0.39a| 60.90 ± 0.33b| 59.83 ± 0.44a|
| Energy (kcal 100 g⁻¹)       | 519.39 ± 2.19a| 519.65 ± 0.13a| 519.35 ± 0.37a| 518.34 ± 1.32a|

**Note:** Control, CM-1, CM-3 and CM-5 represent the formulation containing 0, 1, 3 and 5% of C. militaris flour, respectively.

| Parameters                  | Control     | CM-1        | CM-3        | CM-5        |
|-----------------------------|-------------|-------------|-------------|-------------|
| **Weight (g)**              | 16.86 ± 0.12a| 16.57 ± 0.21a| 16.41 ± 0.26a| 16.33 ± 0.26a|
| **Diameter (mm)**           | 5.59 ± 0.10a| 5.50 ± 0.11a| 5.40 ± 0.16a| 5.37 ± 0.076a|
| **Thickness (mm)**          | 1.20 ± 0.095a| 1.33 ± 0.026a| 1.29 ± 0.095a| 1.30 ± 0.050a|
| **Spread ratio (mm)**       | 4.48 ± 0.31a| 4.34 ± 0.30a| 4.15 ± 0.076a| 4.12 ± 0.0058a|
| **Color**                   | L*          | 51.29 ± 1.46a| 48.86 ± 0.60b| 45.38 ± 2.94c| 41.39 ± 2.81c|
|                            | a*          | 6.09 ± 1.83a| 6.52 ± 0.24ab| 7.22 ± 0.27bc| 8.31 ± 0.50ab|
|                            | b*          | 26.51 ± 0.32a| 27.36 ± 0.26bc| 27.12 ± 0.32ab| 27.87 ± 0.36bc|
|                            | ΔE*         | 2.65 ± 0.47a| 6.08 ± 1.93a| 10.26 ± 2.74b|
| **Texture**                 | Hardness (N) | 3.75 ± 0.39a| 3.24 ± 0.17a| 3.21 ± 0.047ab| 3.17 ± 0.062ab|

**Note:** Control, CM-1, CM-3 and CM-5 represent the formulation containing 0, 1, 3 and 5% of C. militaris flour, respectively.

**Table 4.** Characteristics físicas y texturales de las galletas elaboradas con harina de trigo solamente y de las galletas con sustitución parcial con harina de Cordyceps militaris.

**Note:** Control, CM-1, CM-3 and CM-5 represent the formulation containing 0, 1, 3 and 5% of C. militaris flour, respectively.
the results, it was observed that *C. militaris* flour supplementation significantly (*P* < 0.05) decreased the brightness (L⁰) of the samples compared to the control. On the other hand, both the a* (redness) and b* (yellowness) increased with increasing *C. militaris* flour levels, indicating a browning of the cookies with the incorporation of *C. militaris* flour, which had a strong yellow color. However, there were no differences in a* and b* values between CM-1 and CM-3. The changes in the color of the cookies are associated not only to the color of the flour used, but also to Maillard reactions. Protein content was negatively correlated with color characteristics of cookies (Giuberti et al., 2018; Mancebo et al., 2015). Therefore, incorporating *C. militaris* flour in cookies could contribute to form a browner and more yellowish color due to the higher total protein content. In addition, total color difference (ΔE) increased significantly (*P* < 0.05) with the increase in *C. militaris* flour content. According to total color difference values, all *C. militaris* flour substituted cookies could be easily distinguished from the control.

### 3.5. Hardness of cookies

Hardness is another common index of cookies quality. As shown in Table 4, all the samples containing *C. militaris* flour had lower hardness (3.17–3.24 N), compared to the control sample (*P* < 0.05). In other words, the fortification of *C. militaris* flour caused cookies to soften. However, hardness of cookies slightly decreased with increasing level of *C. militaris* flour (*P* > 0.05). The decrease in hardness could be attributed to destruction of gluten network as result of the addition of *C. militaris* flour. These findings were in conformity with the previous studies (Gocmen et al., 2019; Sinthusamran et al., 2019) wherein incorporation of protein hydrolysate from cephalothorax of Pacific white shrimp and coffee silverskin resulted in the decrease in hardness of cookies.

### 3.6. Total phenolics of cookies enriched with *C. militaris* flour

The effect of addition of cookies with various levels of *C. militaris* flour on the phenolic contents (TPC) is illustrated in Figure 1. As expected, the supplementation of *C. militaris* flour positively affected the phenolic contents, but there was no significant difference between the control and CM-1. On analysis of buffer extracts (BE), by contrast with the control sample (Control), the TPC was significantly higher about by 82.14% and 139.29% in the cookies enriched with 3% and 5% of *C. militaris* flour (CM-3 and CM-5), respectively.

![Figure 1. Influence of *Cordyceps militaris* addition on the total phenolic contents in cookies.](image)

Figure 1. Influencia de la adición de harina de *Cordyceps militaris* en el contenido fenólico total de las galletas.

Note: BE, buffer extracts; DE, extracts after digestion in vitro; GAE, gallic acid equivalents; Control, plain wheat cookies; CM-1, CM-3, CM-5; cookies with 1%, 3%, 5% of *C. militaris* flour addition, respectively. Means with different small letters (buffer extracts) and capital letters (in vitro digestion extracts) indicate significant differences (*P* < 0.05) among the cookie samples.

Note: BE, extractos de tampón; DE, extractos después de la digestión in vitro; GAE, equivalentes de ácido gálico; Control, galletas de trigo normal; CM-1, CM-3, CM-5, galletas adicionadas con 1%, 3%, 5% de harina de *C. militaris*, respectivamente. Las medias con diferentes letras minúsculas (extractos de tampón) y mayúsculas (extractos de digestión in vitro) indican diferencias significativas (*P* < 0.05) entre las muestras de galletas.
after digestion (Wang et al., 2016; Wootton-Beard et al., 2011). As C. militaris flour is rich in phenolic compounds, thus which contribute to a good antioxidant capacity of cookies. Taking together, these results indicated that cookies supplemented with C. militaris flour might significantly contribute to the phenolics intake.

3.7. Antioxidant activities of cookies enriched with different levels of C. militaris flour

In this study, four common antioxidant activity assays with different approaches and mechanisms were explored to evaluate the antioxidant properties of cookie samples. Results are shown in Figure 2(a–d). The antioxidant activity of different cookie samples determined by DPPH radical scavenging effect is shown in Figure 2(a). All the cookies studied possessed the ability to scaveng DPPH radical. The DPPH radical scavenging activity of buffer extracts ranged from 261.47 to 1017.30 μg VCE per g DW, while it ranged from 397.33 to 1234.07 μg VCE per g DW for in vitro digestion extracts. According to the results, it was noted that the DPPH radical scavenging activity significantly increased with the increasing C. militaris flour supplement levels (P ≤ 0.05). The highest DPPH scavenging activity was determined for cookies with a 5% C. militaris flour addition (CM-5), while control cookie samples had the lowest values. DPPH radical scavenging activity of CM-5 was 1017.30 and 1234.07 μg VCE per g DW determined in BE and DE, respectively, which was about 3.89- and 3.11-fold, respectively, compared to the respective samples of the control. Furthermore, digestion significantly elevated the DPPH scavenging activity. The greater DPPH radical scavenging capacity of DE might be due to the release of the antioxidants through the in vitro digestion model.

Similar to DPPH radical scavenging activity, Figure 2(b) shows that the ABTS radical scavenging activity of both BE and DE significantly increased as the dosage of the C. militaris flour addition level increased (P < 0.05). The ABTS radical scavenging activity of buffer extracts were 2.64, 2.96, 3.98 and 5.10 mg VCE per g DW for cookies fortified with 0, 1, 3 and 5% of C. militaris flour, respectively. In terms of in vitro digestion extracts, the values of ABTS.

Figure 2. Influence of Cordyceps militaris addition on antioxidant activities of cookies.
radical scavenging activity were 13.18, 15.89, 23.09 and 31.43 mg VCE per g DW in those samples supplemented with 0, 1, 3 and 5% \textit{C. militaris} flour. The highest ABTS radical scavenging activity was obtained in cookies at substitution level of 5%, while the lowest scavenging was detected in control samples without additions. Additionally, the ABTS radical scavenging activity of \textit{in vitro} digestion extracts was found to be significantly higher than that of buffer extracts of all tested samples \((P < 0.05)\). It has been observed that the ABTS radical scavenging activity was closely related with the antioxidant activity of phenolic compounds. Digestion process might have liberated more reductants, which had quencher ability of ABTS radical, resulting in the higher ABTS scavenging activity of DE (Xiao et al., 2014).

As shown in Figure 2(c), it was found that ferric reducing antioxidant power (FRAP) of the cookies, regardless of extract methods, increased significantly with the enhancement in \textit{C. militaris} flour supplementation levels \((P \leq 0.05)\). The FRAP varied from 104.25 to 277.87 \(\mu\)g VCE per g DW, while it varied from 523.87 to 1091.19 \(\mu\)g VCE per g DW for \textit{in vitro} digestion extracts. As to buffer extracts, the FRAP of cookies fortified with 1, 3 and 5% of \textit{C. militaris} flour were about 1.22, 1.49, 2.67 times as high as that in no supplemented control, respectively. With regard to \textit{in vitro} digestion extracts, the FRAP of cookies enriched with 1, 3 and 5% of \textit{C. militaris} flour were about 1.10, 1.32, 2.08 times as high as that of the control, respectively. Therefore, it was obvious that partially substituted \textit{C. militaris} flour greatly enhanced the FRAP. Higher FRAP activity of DE might be due to the release of iron-chelated compounds \textit{in vitro} digestion.

According to the results presented in Figure 2(d), the same trend was observed for antioxidant activity assessed using the reducing power (RP) method. The RP of cookies extracts followed the order of CM-5 > CM-3 > CM-1 > Control. The highest RP was observed at 5% \textit{C. militaris} flour supplementation followed by those samples supplemented with 3% and 1% \textit{C. militaris} flour, while the lowest value was obtained in control samples. This implied that higher level of \textit{C. militaris} flour addition resulted in higher RP both in buffer extracts and \textit{in vitro} digestion extracts. The RP of buffer extracts of cookie samples increased by 42.47%, 135.21% and 333.26% at the substitution level of 1%, 3% and 5%, respectively, compared with control samples without additions. After \textit{in vitro} digestion, in contrast to the no supplemented control, the RP increased by 36.29%, 63.87% and 176.29% in those samples supplemented with 1%, 3% and 5% \textit{C. militaris} flour, respectively. The phenolics have been found to be significantly associated with the RP. Higher phenolic contents in the samples showed stronger RP.

Based on the above results, it can be concluded that higher supplementation levels tend to improve the antioxidant activities of cookies. All the results showed a similar tendency that was irrespective of the methods conducted in this study. This may be attributed to the fact that the addition of \textit{C. militaris} flour as sources of natural phenolic compounds remarkably increases the antioxidant activities of cookies. Moreover, similar to the changes of TPC, antioxidant activities of all samples were significantly increased after gastrointestinal digestion when in comparison with their respective buffer extracts. In the gastrointestinal digestion can promote the release the antioxidants to some extent. Therefore, cookies partially fortified with \textit{C. militaris} flour could be developed as a novel and functional food with more effective antioxidant properties. Determination of phenolic profile of cookie samples and correlation analysis between phenolic compounds and antioxidant activities are worthwhile to be studied further.

### 3.8. Sensory characteristics of cookies

The sensory properties of cookies fortified with \textit{C. militaris} flour at different levels were evaluated by using nine-point hedonic scales and the results obtained were illustrated as a radar plot (Figure 3). No statistically significant differences in scores for all sensory attributes between the control

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**Figure 3.** Radar plot of sensory evaluation of wheat cookies and mushroom flour-supplemented cookies.

**Figura 3.** Diagrama de radar de la evaluación sensorial de las galletas de trigo y las galletas con suplemento de harina de hongos.

Note: Control, cookies without \textit{C. militaris} flour; CM-1, CM-3 and CM-5, cookies fortified with \textit{C. militaris} flour at 1, 3 and 5% (flour substitution), respectively.

Nota: Control, galletas sin harina de \textit{C. militaris}; CM-1, CM-3 y CM-5, galletas fortificadas con harina de \textit{C. militaris} al 1, 3 y 5% (sustitución de la harina), respectivamente.
wheat cookies and cookies at 1% C. militaris flour content were noticeable ($P > 0.05$). It was noticed that the preference on the cookies in term of color reduced gradually with the increase in C. militaris flour inclusion levels. The increased substitution levels of C. militaris flour led to darker and browner appearance of the cookies. This was in accordance with the evaluated values measured by colorimeter. However, the color scores of the cookies fortified with C. militaris flour were acceptable to the panelists. Odor and taste scores of C. militaris flour supplemented cookies initially increased and then showed a decreasing trend with increase in C. militaris flour level. Cookies enriched with 3% C. militaris flour were rated for the highest scores for odor and taste, while cookies at substitution level of 5% had scored the least. This indicated that appropriate level of C. militaris flour incorporated into cookies had increased the acceptance for aroma and taste of cookies. C. militaris flour at incorporation levels of more than 5% had negatively impact on the organoleptic properties of cookies, which was attributed to the strong taste and odor of C. militaris flour (data not shown). All C. militaris flour supplemented cookies had higher texture likeness scores than that of the control cookies. Higher texture score might be due to the decrease in hardness and fracturability values of cookies, which was parallel to the results of the instrumental texture measurement. Furthermore, the cookies prepared by substituting 3% of wheat flour with C. militaris flour scored the maximum overall acceptability scores, as well as highest ratings in terms of odor, taste and texture. Therefore, 3% of C. militaris flour could be incorporated into the cookies to improve the sensory properties with higher acceptability.

4. Conclusions

In this study, novel functional cookies were developed by supplementing wheat flour with mushroom flour (C. militaris). In the light of the present data, it can be concluded that partial replacement of wheat flour with C. militaris flour enhanced the nutritional value of cookies. The developed cookies enriched with C. militaris flour generally had higher protein, ash, crude fiber compared to the control of wheat cookies. Another attractively benefits of C. militaris flour addition was the enhancement of phenolic contents and antioxidant activity of cookies. Furthermore, phenolic antioxidants were bioaccessible in vitro. Consequently, cookies supplemented with C. militaris flour had functional advantages over the conventional wheat-based cookies. The fortification of cookies with C. militaris flour is an effective tool to offer nutritious, antioxidant-rich and healthy alternative to consumers.

Disclosure statement

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