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RESEARCH

Effect of Household Cooking Treatments on the Stability of β-glucans, Ergosterol, and Phenolic Compounds in White-Button (*Agaricus bisporus*) and Shiitake (*Lentinula edodes*) Mushrooms

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Abstract
White-button (*Agaricus bisporus*) and shiitake (*Lentinula edodes*) mushrooms are widely consumed worldwide because of their organoleptic properties but also their bioactive compounds such as β-glucans, ergosterol, and phenolic compounds. Although these mushrooms can be eaten as raw food, they are usually subjected to household cooking treatments, so their effect on the stability of these molecules was evaluated in this work. The results showed that frying reduced β-glucan and ergosterol content but protected mushrooms from the loss of phenolic compounds that were mainly affected by boiling, being this procedure able to increase β-glucans and ergosterol concentrations. It can be concluded that culinary treatments had differential effects depending on the specific molecule, so they should be applied or avoided considering the target compound. Moreover, further research is encouraged to fully understand the mechanisms involved in the content variations and the impact on the biological activity of these mushrooms.

Keywords Household cooking · β-Glucans · Ergosterol · Phenolic compounds · *Agaricus bisporus* · *Lentiula edodes*

Abbreviations

UV	Ultraviolet
HPLC	High-performance liquid chromatography
GC-MS/MS	Gas chromatography coupled to tandem mass spectrometry
FID	Flame ionization detector

Introduction

Mushroom consumption has significantly increased in the last decades and, among the vast number of edible species (more than 2000 were identified), white-button (*Agaricus bisporus*) and shiitake (*Lentinula edodes*) mushrooms have attracted great interest constituting approximately the 50% of the total sum of marketed edible mushrooms worldwide (Cardwell et al., 2018; Li et al., 2021).

Their relevance is due not only to their organoleptic properties for culinary purposes, but also to their bioactive content that places them as a good source of functional molecules able to exert beneficial activities for human health (Cardwell et al., 2018; Wang, 2020). For instance, mushroom polysaccharides, and particularly β-glucans, have been widely studied in *A. bisporus* and *L. edodes*, being present in these species ranging between 9–13 and 20–33% (dry weight), respectively (Roncero-Ramos et al., 2017; Sari et al., 2017). These fungal polysaccharides are normally composed by a main linear glucose chain with (1 → 3)-β-linkages and (1 → 6)-β-branches and showed interesting properties, e.g., hypocholesterolemic, linked to their ability to increase intestinal viscosity, scavenge bile acids, and their use by colon microorganisms that can act as short chain fatty acids–producers (Khan et al., 2018; Palanisamy et al., 2014). Other properties attached to β-glucans include antioxidant effects, free radicals scavenging (Li et al., 2019), and antitumoral action, exerting a direct cytotoxic impact on cancer cells or stimulating immune system (Wang et al., 2014).

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Other bioactive component that can be obtained from these mushrooms is ergosterol (ergosta-5,7,22-trien-3β-ol), which is the main sterol that can be found in fungal membranes. *A. bisporus* habitually contained 5–9 mg/g and *L. edodes* 2–9 mg/g of ergosterol, depending on the strain, developmental stage, and cultivation conditions (Hammann et al., 2016; Morales et al., 2019; Sławińska et al., 2016). The structure of this lipid is similar to cholesterol, impairing its inclusion to micelles and exerting a hypocholesterolemic action affecting also to the expression of cholesterol homeostasis–related genes and inhibiting biosynthesis enzymes (Gil-Ramírez et al., 2018; He et al., 2019). Moreover, it can be transformed into vitamin D_2 (ergocalciferol) by ultraviolet irradiation (Morales et al., 2017) and other derivative, ergosterol peroxide has shown anti-inflammatory and antitumoral effects (Kobori et al., 2007; Tan et al., 2017).

In addition, phenolic compounds are molecules that play a key role in the secondary metabolism of mushrooms and as defense mechanism against pathogens, animals, and environmental stress. The total phenolic content of *A. bisporus* and *L. edodes* is very variable (≈ 1–25 mg/g), and it is directly influenced by the environment and the metabolic status of the mushroom since the mentioned stress promotes the synthesis and accumulation of these compounds (particularly in the external tissues) that show industrial and clinical significance because of the reported antioxidant and antimicrobial activities (Gąsecka et al., 2018; Morales et al., 2018a, b; Muszyńska et al., 2017).

Since mushrooms are rarely consumed as raw food, household culinary treatments should be studied in terms of their effects on the stability and integrity of these compounds and also as a factor that might increase or reduce the bioaccesibility and bioavailability of the active molecules. Cooking conditions such as temperature, medium (dry, water, oil), time, intensity, and pressure are crucial factors that must be taken in consideration (Ložnjak & Jakobsen, 2018; Morales et al., 2018a, b).

Thus, since there is a lack of these data in the scientific literature, the present work investigates the effect of different household culinary treatments (boiling, steam cooking, oven, grilling, frying, microwave, pressure cooking) that are usually applied to mushrooms, determining the variation in the content of β-glucans, ergosterol, and phenolic compounds in both *A. bisporus* and *L. edodes*.

Materials and Methods

Biological Material

Fresh *Agaricus bisporus* L. (Imbach) and *Lentinula edodes* S. (Berkeley) fruiting bodies were purchased in season from a local market in Madrid (Spain). Before treating them with the specific cooking methods, mushrooms were washed and sliced (0.5 cm × 5 mm).

Reagents

Solvents as methanol (HPLC grade), hexane (95%), and chloroform (HPLC grade) were purchased from LAB-SCAN (Gliwice, Polland) and absolute ethanol and sodium carbonate (Na_2CO_3) from Panreac (Barcelona, Spain). Potassium hydroxide (KOH), ascorbic acid, 2,6-Di-tert-butyl-*p*-cresol (BHT), bovine serum albumin (BSA), HCl (37%), Folin Ciocalteu's phenol reagent, hexadecane, ergosterol (95%), and gallic acid were purchased from Sigma-Aldrich Quimica (Madrid, Spain). All other reagents and solvents were used of analytical grade.

Household Cooking Treatments

Mushroom slices (30 g) were subjected to the different culinary treatments as described in Table 1. Boiling, oven, and frying methods followed the methodology described by Ložnjak and Jakobsen (2018). Other treatments such as steam cooking, grilling, microwave, and pressure cooking followed times and temperatures that are usually applied in household methods, following indications published by Soler-Rivas et al. (2009). All treatments were carried out in triplicate.

Once the cooking was finished, the treated mushrooms were freeze-dried, ground into fine powder, and stored at − 20 °C until further use.

Determination of β-glucan Content

Total β-glucan content was determined in the freeze-dried samples using a mushroom- and yeast-specific β-glucan determination kit (β-glucan Assay Kit Megazyme ®, Megazyme, Wicklow, Ireland) following the instructions of the user's manual, as described in Palanisamy et al. (2014). Sample absorbance was measured using a

Table 1 Household procedures details

Treatment	Time (min)	Temperature (°C)
Boiling	20	90 °C ± 2 °C
Steam cooking	20	100 °C ± 2 °C
Oven	10	200 °C ± 9 °C
Grilling	6	100 °C ± 2 °C
Frying (low)	20	120 °C ± 5 °C
Frying (high)	5	180 °C ± 5 °C
Microwave	10	800 MW
Pressure cooking	5	110 °C ± 5 °C

Genesys 10 UV spectrophotometer (Thermo Fischer Scientific, Madrid, Spain).

Determination of Ergosterol Content by GC-MS/MS-FID

Fungal sterols from freeze-dried samples were extracted following the procedure described by Gil-Ramírez et al. (2013). The unsaponified fractions that were obtained (6 mg/mL) were injected into an Agilent 19091S-433 capillary column (30 m × 0.25 mm ID and 0.25 µm phase thickness). The column was connected to a 7890A System gas chromatograph (Agilent Technologies, USA) including a G4513A autoinjector and a 5975C triple-axis mass spectrometer detector. The injector and detector conditions as well as the column temperature program were those described by Gil-Ramírez et al. (2013). Ergosterol was used as standard and hexadecane (10% v/v) as internal standard.

The GC-MS database identified the obtained peaks in concordance with previous studies (Gil-Ramírez et al., 2013; Jasinghe & Perera, 2005; Teichmann et al., 2007), being ergosterol (retention time = 12.6 min) the major detected sterol.

Determination of Total Phenolic Compounds Content

The total phenol content of freeze-dried samples (10 mg) was determined by the Folin-Ciocalteu methods according to the adapted procedure of Ramírez-Anguiano et al. (2007). Gallic acid was used as standard for quantification.

Statistical Analysis

Differences were evaluated at a 95% confidence level ($P \leq 0.05$) using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (and Student-*t* test for the results of raw mushrooms determinations). Correlation between different variables was evaluated by computing Pearson's correlation coefficient and determination coefficient. Statistical analysis was performed using GraphPad Prism version 9.3 (GraphPad Software, San Diego, CA, USA).

Results and Discussion

Effect of the Household Cooking Methods on the Weight Loss of *A. bisporus* and *L. edodes*

When fresh *A. bisporus* and *L. edodes* slices were subjected to the different culinary treatments, the weight changes were also recorded since these methods might provoke water losses and lixiviation of specific compounds, particularly polar molecules (Morales et al., 2018a, b).

For both mushrooms (Fig. 1), the most intense reduction was observed after oven or frying treatments that were the methods that utilized the highest temperatures (200 °C for oven, 120 and 180 °C for frying). Moreover, none of them was carried out in the presence of water (dry medium in the case of oven, oil medium in the case of frying). *A. bisporus* slices lost 92% of their initial weight after oven treatment, but *L. edodes* weight showed a softer decrease (55%). Frying affected *A. bisporus* with 78–79% losses and 62 and 66% reduction was registered in *L. edodes* at 120 and 180 °C, respectively. These differences between mushrooms might be due to the different moisture contents, structures, and water retention parameters of the species (Cardwell et al., 2022; Shi et al., 2022).

However, the less aggressive techniques were microwave and steam cooking in the case of *A. bisporus* (23 and 25% weight loss, respectively) and steam and pressure cooking and grilling in the case of *L. edodes*, with reductions lower than 10% and, in the case of steam cooking, below 1%. This specific method, widely used in many recipes with mushrooms as ingredients, takes advantage of the high temperature of the gas but without contact between the food ingredient and the liquid, as well as in the pressure cooking method. The results of this study are in concordance with other works that used these technologies and reported slighter weight losses than those obtained for other methods such as frying (Ilic et al., 2021; Kim et al., 2017).

Once the weight loss was calculated, the cooked samples were freeze-dried for further determinations.

β-Glucan and Ergosterol Content of *A. bisporus* and *L. edodes*

Prior to the analysis of household cooking effects on *A. bisporus* and *L. edodes* composition, β-glucan and ergosterol contents were measured in both mushrooms (Table 2). The results were similar to those published in previous works, showing that shiitake mushrooms contained a significantly higher β-glucan concentration than white-button mushrooms (32 and 6%, respectively) (Roncero-Ramos et al., 2017; Sari et al., 2017). However,

Table 2 Ergosterol (mg/g) and β-glucans (%) content in raw *A. bisporus* and *L. edodes* (dry weight)

	A. bisporus	*L. edodes*
β-Glucans (%)	5.96 ± 0.86b	31.92 ± 2.40a
Ergosterol (mg/g)	3.67 ± 1.24a	1.47 ± 0.14a

Different letters (a, b) denote significant differences ($P < 0.05$) between different mushrooms for the same compounds

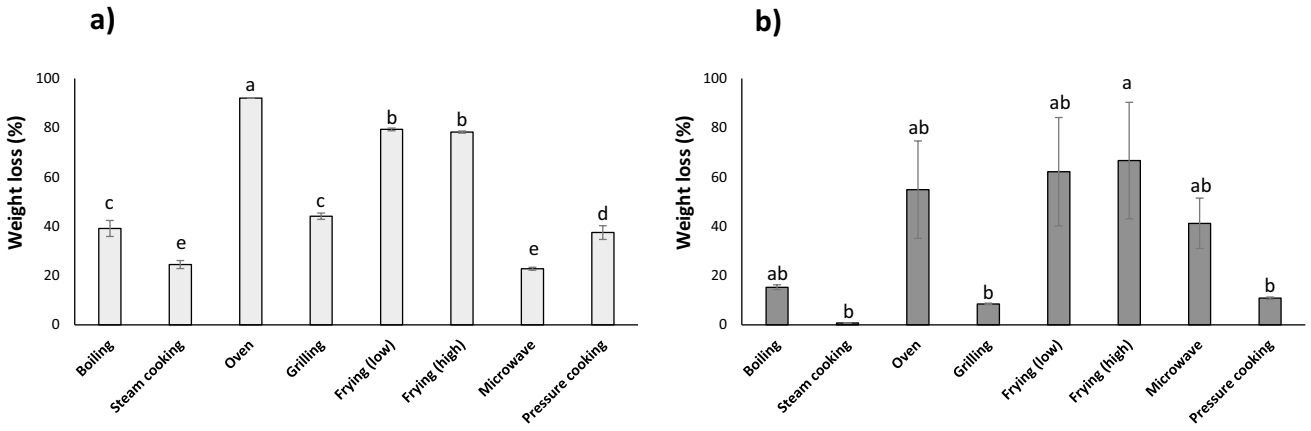

Fig. 1 Weight losses (%) after household cooking in *A. bisporus* (**a**) and *L. edodes* (**b**). Different letters (a–d) denote significant differences ($P < 0.05$) between different culinary treatments for the same mushroom

ergosterol amounts did not show significant differences between mushrooms and were slightly lower than those reported in the related literature (1.5 and 3.7 mg/g for *L. edodes* and *A. bisporus*, respectively). These variations in ergosterol content are usual and might be due to the specific cultivation conditions and developmental stage of the utilized mushrooms (Hammann et al., 2016; Morales et al., 2019; Sławińska et al., 2016).

Effect of the Household Cooking Methods on the β-glucan Content of *A. bisporus* and *L. edodes*

As commented before, *L. edodes* β-glucan content was higher in raw samples and these differences were also present after culinary treatments. However, a similar tendency was observed for both mushrooms (Fig. 2) since the only method that seemed to be detrimental for β-glucan stability was frying that reduced the concentration of these polysaccharides at the tested temperatures (120–180 °C). This effect can be linked to the reached temperatures; however, oven cooking utilized the highest one (200 °C) and no significant reduction was observed. This phenomenon might be provoked by the deep penetration of the oily medium into internal mushroom tissues, achieving a direct contact with these macromolecules. In contrast, dry heat methods such as oven cooking only affected the fungal skin and the first outer layers, but the inner tissues did not receive significant irradiation. This fact was previously observed for other fungal compounds such as eritadenine (Morales et al., 2018a, b). Although frying demonstrated to be harmful for the integrity of shiitake β-glucans, these polysaccharides were subjected to these practices by a specific study that enriched wheat noodles with them to act as a barrier to oil, reducing fat calorie content of instant-fried noodles and improving rheological properties of the pasta (Heo et al., 2013). However, in terms of β-glucan functionality, oil high temperature would compromise its beneficial properties in human health.

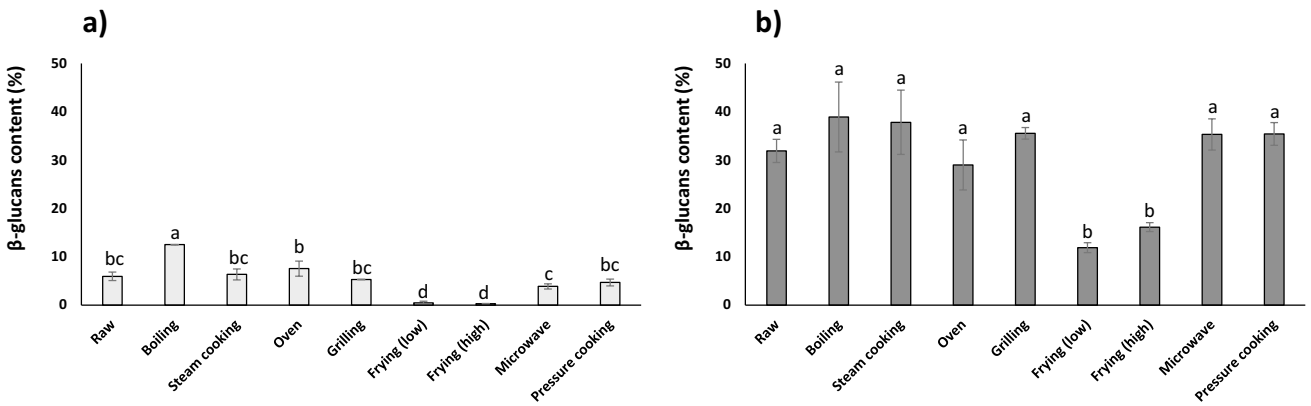

Fig. 2 β-Glucans (%) content before and after household cooking in *A. bisporus* (**a**) and *L. edodes* (**b**). Different letters (a–d) denote significant differences ($P < 0.05$) between different culinary treatments for the same mushroom

Furthermore, another relevant result must be highlighted for *A. bisporus* since boiling procedures increased the levels of β-glucans that were detected by the utilized quantification methods. The applied temperature (100 °C) showed to be not only innocuous but also beneficial, possibly contributing to break linkages and interactions within complex structures between macromolecules such as polysaccharides-proteins aggregates. This enhanced content was also observed in other studies using shiitake mushrooms, where the increment was correlated to the higher temperature up to 120 °C (Hwang et al., 2021). However, in the present case, the enrichment after boiling in shiitake was not statistically significant.

Effect of the Household Cooking Methods on the Ergosterol Content of *A. bisporus* and *L. edodes*

The results in ergosterol content variation after cooking processing (Fig. 3) were similar to those obtained for β-glucans, in the sense that boiling concentrated the amount of this molecule and, in this case, for both *A. bisporus* and *L. edodes*. The use of hot water seemed to be able to remove some polar substances from mushrooms, enriching the proportion of non-polar molecules such as ergosterol. Besides, this lipid showed heat stability as it was demonstrated in previous works that used high-temperature extractions (Morales et al., 2023; Taofiq et al., 2017).

Nevertheless, frying temperatures (120 and 180 °C) seemed to be excessive also for ergosterol, probably because of the deeper penetration phenomenon that was described in the previous section. Moreover, part of the ergosterol could be extracted from the fungal matrix and lost in the frying medium because of its lipophilic nature that leads to its complete solubilization in oil (Boarelli et al., 2020).

But frying was not the only detrimental procedure, since oven cooking provoked a significant ergosterol loss in *A. bisporus* that can be explained again because of the extremely high temperature (200 °C) degrading this compound. *L. edodes* ergosterol was affected by other techniques, such as grilling, microwave, and pressure cooking, indicating that a matrix effect was also occurring since the culinary methods led to different consequences depending on the species. This fact might be associated to the particular distribution of ergosterol in mushroom tissues and the specific morphological and structural features of white-button or shiitake mushrooms (Jasinghe & Perera, 2005; Jiang et al., 2010). Grilling and pressure cooking results were surprising since the utilized temperatures were not so high (100 and 110 °C, respectively) and the exposition time was even shorter than in boiling (6 and 5 min, respectively) but seemed to be enough to degrade ergosterol molecules. Besides, microwaving also lowered ergosterol amount, although a previous study applied microwave-assisted extractions to obtain ergosterol-enriched fractions with promising results. However, in the mentioned work, the mushrooms were freeze-dried prior to microwave extraction and, in the present procedure, fresh mushrooms with a significant water proportion in the inner tissues might allow more efficient heat transmission and more intense degradation of lipids (Heleno et al., 2016).

Effect of the Household Cooking Methods on the Total Phenolics Content of *A. bisporus*

A. bisporus phenolic compounds showed sensitiveness to high temperatures and the content was drastically reduced when water was used (Fig. 4). The losses because of leaching of water-soluble phenols were confirmed in microwaving and particularly in boiling, being this last method the one that recorded the lowest phenolic content. Significant decreases were also observed for oven, steam, and pressure cooking, but they were not so radical when compared

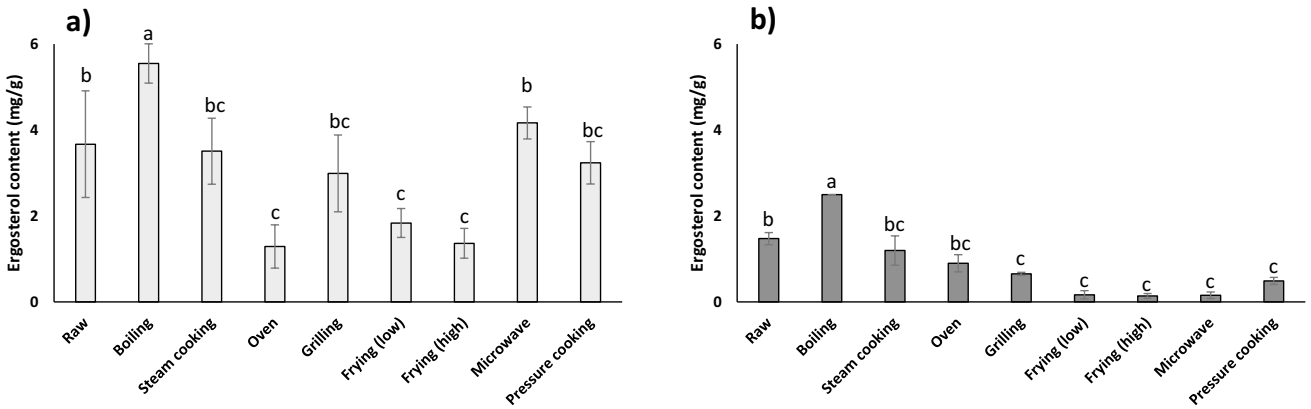

Fig. 3 Ergosterol (mg/g) content before and after household cooking in *A. bisporus* (**a**) and *L. edodes* (**b**). Different letters (a–c) denote significant differences ($P < 0.05$) between different culinary treatments for the same mushroom

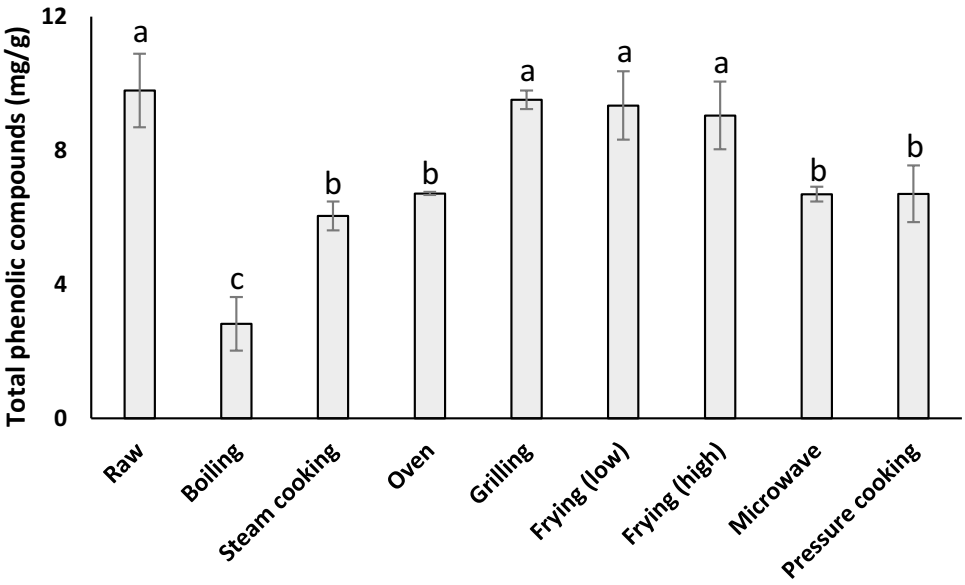

Fig. 4 Total phenolic compounds (mg/g) content before and after household cooking in *A. bisporus*. Different letters (a–c) denote significant differences ($P < 0.05$)

to boiling, since the mushrooms were not in direct contact with water. In this sense, the high oven temperature was the main fact that affected phenolics stability in this method and, for steam and pressure cooking, water losses seemed to be linked to total phenolic content decrease. The leaching out of water containing polar compounds was previously described for culinary methods applied to other hydrophilic molecules such as eritadenine (Morales et al., 2018a, b). However, when statistical analyses were performed in the current work, correlation between mass loss and phenolic compounds content was not significant. This analysis might be influenced by the results obtained from other methods such as grilling and frying that did not affect phenolic concentrations. These procedures did not utilize water, and the dry heat induced the formation of a Maillard crust in the skin that possibly hinder the lixiviation of the compounds that are solubilized in the constitutive water. This phenomenon was also previously observed for shiitake eritadenine (Morales et al., 2018a, b).

Moreover, it is known that culinary processing might lead to enzymatic oxidation of phenolic compounds by the action of polyphenol oxidases (PPOs). These enzymes are particularly active after breaking fungal cell walls caused by cooking techniques and strengthened by methods such as boiling. These reactions lead to the formation of dark-colored pigments that modify mushroom sensory attributes (Pathare et al., 2013). Therefore, PPO inactivation methods are being investigated, such as ultrasound-, high pressure–, pulsed light– or pulsed electric fields–processing (Pellicer et al., 2018; Tsikrika et al., 2022).

Conclusions

Household cooking treatments differentially affected the stability of specific mushroom compounds. High temperatures and deep oil penetration into inner fungal tissues reduced β-glucan and ergosterol content when *A. bisporus* and *L. edodes* slices were subjected to frying. However, the lack of water and the direct contact with the mushroom skin during frying time, as well as occurred in grilling, led to a Maillard crust that interestingly avoid the lixiviation of *A. bisporus* phenolic compounds that were drastically reduced in aqueous environments such as in microwaving and particularly boiling. In contrast, the boiling procedure led to an enrichment in *A. bisporus* β-glucans and in ergosterol in both mushrooms. This lipid was reduced not only by frying but also after oven cooking in the case of white-button mushrooms and after grilling, microwaving, and pressure cooking in shiitake.

The obtained results highlight the impact of cooking treatments on fungal bioactive compounds stability, increased the knowledge about culinary processing consequences, and encourage further investigation to fully elucidate the exact mechanisms that lead to increase or decrease the proportion of the relevant molecules and the concrete consequences regarding their biological activity.

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Author Contribution Diego Morales: investigation, methodology, formal analysis, writing of the original draft, figures; Monika Anna Bal: investigation, methodology; Sara Figueredo: investigation, methodology; Cristina Soler-Rivas: supervision, data curation, funding acquisition; Alejandro Ruiz-Rodríguez: supervision, validation, data curation, writing and reviewing.

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Data Availability Research data are not shared.

Declarations

Conflict of Interest The authors declare no competing interests.

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