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Biochemical quality of conserves of *Agaricus bisporus* mushroom seasoned with lentisk oil

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1. Introduction

A significant increase is known is in the worldwide need for healthy nutrition and eating, not only to guarantee a safety life for humans but also to treat chronic diseases and healthy warnings. Thus, growing consumer demand has focused on functional food products with ingredients that are able to exert a regulatory effect on the entire body or its specific systems and organs (Siddiqui *et al.*, 2022). Experts around the world recognize that many natural resources are restorative nutrient-rich foods (Satish *et al.*, 2021).

Various functional products are currently being considered by several edible mushroom species. Indeed, mushrooms are macro-fungi that have different fructifications within shape and ultra-structure. There are more than 14,000 species of mushrooms worldwide, with approximately 3,000 species considered edible (Chowdhury *et al.*, 2015). Nearly 1400 are rather toxic species while 700 others are of medicinal importance (Mowsurni *et al.*, 2013, Gaur and Rao, 2017). Moreover, Sharifi-Rad *et al.* (2020) reported that mushrooms have been used significantly in the pharmaceutical, nutraceutical, and cosmetic industries in recent decades.

There are several species of mushrooms, including *Agaricus bisporus*, *Pleurotus* spp., *Lentinus edodes* and *Volvariella* sp., which are among the most sought after and cultivated mushroom, through their health benefits and biochemical properties (Gupta *et al.*, 2022).

Flavonoids are phytochemical composites that occur in several plant and mushroom species (Chen *et al.*, 2023; Zhou *et al.*, 2024). They are categorized into various types according to their chemical assembly, unsaturation degree, and oxidation of carbon ring (Bondonno *et al.* 2019).

Flavonoids possess a number of medicinal benefits, including anticancer, anti-inflammatory, and antiviral properties (Zhao *et al.*, 2019; Elmsellem *et al.*, 2019; Ullah *et al.*, 2020; Haddou *et al.*, 2023; Cherriet *et al.*, 2023; Hasnat *et al.*, 2024). Many mushroom species equally *Agaricus bisporus* (Blumfield *et al.*, 2020) are actually confirmed to enclose significant flavonoid and antioxidant potential (Hassan *et al.*, 2021; Sevindik, 2024).

Moreover, *Agaricus bisporus*, also known as cultivated mushroom, is commonly used in the food industry and has great nutritional value. The presence of B and D vitamins, along with essential minerals like potassium and phosphorus, makes it a valuable source of vitamins (Smith *et al.*, 2023). Furthermore, *Agaricus bisporus* possesses intriguing physiological properties; including substantial antioxidant capabilities (Jones *et al.*, 2022) and potentially advantageous effects on metabolism control (Venturella *et al.*, 2021).

Nevertheless, *Agaricus bisporus* is highly perishable with known short shelf life of 1–2 days when commercialized under ambient temperature. This short is a great hindrance to its marketing as fresh produce. Making food canning for vegetables, mushrooms and many other foods in the industrial level was highly proposing in the use of food additives. However, some of these additives could have drawbacks and can pose a health human risk (Kane *et al.*, 2024)

Lentisk oil is obtained from the mastic tree (*Pistacia lentiscus*), an evergreen shrub mainly distributed in the Mediterranean areas. Many study proved many health benefits of lentisk oil (Mezni *et al.*, 2018, Ameur *et al.*, 2024).

This study was conducted to explore the effect of biological and natural lentisk oil on the properties of preserved mushrooms by comparing it with the effects generated by olive oil. The main objective is to evaluate how these oils, known for their nutritional benefits and organoleptic characteristics, influence the biological qualities of this mushroom. The study will focus on the analysis of the impact of the biochemical value of canned *A. bisporus*.

2. Methodology

2.1 Process of mushrooms conservation

Careful cleaning (**Figure 1a**) with sterile distilled water mixed with some drops of vinegar was carried out for a quantity of commercial mushrooms purchased from a food store. The mushrooms were picked on the day they were placed on the market. Once cleaned, the mushrooms were cut into small pieces with a sterile knife (**Figure 1b**).

A solution of 500 ml distilled water mixed with a few drops of lemon and white vinegar was prepared to boil the cut pieces of Paris mushroom (**Figure 1c**). The addition of lemon is essential to avoid oxidation of the product, thus preserving its texture and natural color. The mushroom pieces were then boiled for 2 to 3 minutes then directly placed in direct contact with glace water to ensure the cooling of mushroom pieces and stop cooking (**Figure 1d**). Then, the well-drained products were filled in small plastic boxes previously sterilized. The first lot marked "Control" or control includes the mushrooms no additive. For the other lots, two additives (olive oil and lentisk oil) were added with the following percentages: 0.5%, 1% and 2%. Olive oil used was purchased from local market and lentisk

oils from GDA (Agricol Developpement Group) of Oued Maaden (Beja Governorate- Tunisia). Five replicates or boxes of 10 g were prepared for each group. The abbreviations used for button mushroom conserves and oils percentages are summarized in Table 1.



Figure 1a. Cleaning button mushroom



Figure 1c. Boiling of button mushroom



Figure 1b.Cutting of button mushroom



Figure 1d. Cooling of button mushroom before conservation

Abbreviation	Oil type and added percentage
Control	Without any addition
L-0.5	0.5% Lentisk oil
L-1	1% Lentisk oil
L-2	2% Lentisk oil
O-0.5	0.5% Olive oil
0-1	1% olive oil
0-2	2% Olive oil

s percentages

2.2 Preparation of button mushroom conserves' extracts

Aqueous extracts were prepared by macerating 5g of conserved button mushroom in 20 ml of sterile water for 24 hours. The solvent was evaporated and the dry extracts were collected, weighed and then diluted in 50 ml of ethanol.

2.2.1 pH

The pH was measured for all tested samples in three replicates using a pH meter (PH920 Precision pH/ORP Meter). Measurements were effectuated in the first day of conserve preparation, in day 2 and 5 days after conservation for pH and all other measurements.

2.2.2 Antioxidant properties

The antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to Tohidi *et al.* (2017); Loukili *et al.* (2023) ; Elbouzidi *et al.* (2024). About 100 mL of sample extract was added to 2,500 mL DPPH methanolic solution (1024M). The mixtures were shaken vigorously and then placed in the dark for 30 min. The absorbance of the solutions was measured at 517 nm. All samples were analyzed in triplicate. Butylated hydroxytoluene (BHT) was used as positive control. The percentage inhibition of the DPPH radical was calculated according to the following equation:

%Inhibition = [Acontrol - Asample] /Acontrol * 100

Where, $A_{control}$ is the absorbance of the DPPH solution without sample solution and A_{sample} is the absorbance of the sample at 517 nm.

2.2.3 Total phenols contents

Total phenols were determined by the method described by Singleton and Rossi (1965). 150 μ l of each sample was mixed with 500 μ l of Folin Ciocalteu reagent (1:10 v/v distilled water) and 2 ml of aqueous Na2CO3 (2%). The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetry at 755 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25, 0.5 g/L solutions of Gallic acid in water. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.2.4 Flavonoid contents

The total flavonoid content of crude extract was determined by the Aluminium Chloride Colorimetric Method (Quettier-Deleu *et al.* 2000). 1 ml of each sample extract was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per mL of juice (mg RE/g).

2.2.5 Total tannins contents

The determination of the total tannins was performed with the Folin-Denis colorimetric reagent. In this; 50 μ l of each sample extract, 10 μ l of Folin-Denis reagent and 25 μ l of saturated Na₂CO₃ solution were added; the final volume was adjusted to 500 μ l with distilled water. After 90 minutes of incubation at room temperature, the absorbance was measured at 760 nm. Result was expressed in mg Catechin equivalent per g of dry weight (mg CE / g dry mass) (El Ouadi *et al.* 2017).

2.3 Statistical analysis

Statistical processing of data was performed using the SAS General Linear Models (GLM) procedure. An analysis of variance relating to the parameters studied was carried out. The most significant correlations between the latter are noted. The results obtained are presented as the mean of three replicates \pm standard deviation.

3. Results and Discussion

3.1 Antioxidant activity

The antioxidant activity (**Figure 2**) on the first day of making conserves (day 1) of the control is around 52% and on the second day a small increase was recorded as well for the day 5. This indicates that button mushroom conserve without any additives (Control) have a relatively high antioxidant activity (between 52 and 55%). The antioxidant activity of the product flavored with 0.5% lentisk oil is closely inferior to that of the control. On day 2 we noticed a fairly significant increase (from about 40 to 68% respectively) which proves an effect related to lentisk oil. The decrease in antioxidant activity on day 5 may be correlated with contamination and degradation of the product previously observed and quantified (data not shown).

Adding oils, improves the antioxidant activity of mushrooms. However, too high concentration can reduce this activity in the long term, especially with lentisk oil. Olive oil at 2% seems to be the best option to maintain high antioxidant activity. The differences observed are explained by the properties of the oils. Lentisk oil and olive oil form a protective layer around the mushroom, which helps retain moisture and slow down oxidation (Nejatian *et al.*, 2024). However, if too much is used, this protection can become less effective. The oils also react with some natural substances of the mushroom, which can make certain concentrations or types of oil more effective than others.



3.2 Total phenols contents

The addition of lentisk oil showed a dose-dependent effect on total polyphenol content (Figure 3). All tested conserves showed highest contents in day 2 with superior data than controls. Lentisk oil has more distinguishable effect on polyphenol content than olive oil. This may be explained by the fact that lentisk oil is rich in bioactive compounds that stimulate polyphenol production in the mushroom. Although olive oil also led to an increase in total polyphenols, the effect was less pronounced than for lentisk oil. This could be due to the difference in the composition of fatty acids and other phenolic compounds between the two oils, with lentisk oil potentially being more effective in inducing this response. For most samples, an increase in polyphenols was observed over the days, with notable peaks on day 5. This suggests a cumulative effect where polyphenols due to the amount of oil added. This accumulation may be related to a metabolic adaptation of the fungus to the oil-enriched environment, stimulating the production of phenolic compounds for antioxidant protection. The decrease in polyphenols on day 2 may be due to an adjustment in the amount of this compound between the different constituents of preserves.

The diagram clearly shows that the addition of lentisk oil has a significantly greater effect on the increase in total polyphenols than olive oil, with notable variations depending on the percentage added and the day of analysis. The cumulative effect observed at day 5, especially with the higher percentages, can be attributed to the progressive enrichment of bioactive compounds in the mushroom environment, stimulating the production of polyphenols for reasons of cellular protection or stress response.



Figure 3. Total polyphenol content in different samples of button mushrooms conserves

Mushroom pH varies with storage time (**Figure 4**). Adding oil makes mushrooms more acidic (lower pH), especially on day 2, which can help with their preservation. Higher concentrations of oil stabilize the pH, reducing variations. According to Food pH and mushroom conservation, a slightly acidic pH (below 7) is favorable for inhibiting microbial growth and extending the shelf life of mushrooms. Samples with pH values that decrease to more acidic levels on day 2 are in a safer storage zone. On the first day of analysis and just after canning, the pH is high because the mushrooms release basic compounds (such as certain minerals or proteins) into the storage medium, increasing the pH.

On Day 2, it can be assumed from a literature review that there has been a slight fermentation that can occur, generating acids such as lactic acid, which makes the mushrooms more acidic and lowers the pH (Jabłońska-Ryś *et al.*, 2019; Skrzypczak *et al.*, 2020). Also the drop in pH can be due to the chemical reactions that will take place at the level of the products: *A. bisporus* and the preservation liquid react with each other, which can also lower the pH by seeking a new balance (Guo *et al.*, 2024). Several researches have shown that the pH varies because of the chemical reactions between the fungi and the preservation liquid, and these variations are normal during conservation.

On the other hand, on Day 5, the pH values showed little increase because some acids present at the start degrade, and the system seeks to stabilize. With an intervention of the microbial flora previously revealed with the microbiological tests (data not shown).



Figure 4. pH values in different samples of button mushrooms conserves

3.3 Flavonoids

The initial flavonoid concentration (day 1) in control button mushroom was about 10 mg RE/g Extract dry mass (**Figure 5**). This value decreases in day 2 to the half. In the 5th day, flavonoids content increase reaching about 9 mg RE/g Extract dry mass. This fluctuation in flavonoids content in the control could indicate a natural stabilization of the conserve product between its entire constituents.

In day 1, flavonoid concentrations were highest by the two concentrations of lentisk oil, (1 to 2%) than control. However, when added at 0.5% lentisk oil decreases flavonoids content of conserve of button mushroom than the control. In day 2, a reduction in flavonoid levels is observed in all subgroups, with a more marked decrease with 0.5%. While, in day 5, button mushroom conserved and added by 2% of lentisk oil group shows a notable increase, reaching about 18 mg RE/g Extract dry mass, which is the highest amount among all group. Flavonoids increase in the L-2 group could indicate that the higher concentration of lentisk oil stimulates a significant antioxidant response in button mushrooms. While, its decrease observed on day 2 could be due to an adjustment phase of metabolisms between all constituents of mushroom conserve (Chiu *et al.*, 2000).



Figure 5. Flavonoids contents in different samples of button mushrooms conserve

Flavonoid levels also increased with olive oil concentration, with a similar trend to that observed in the lentisk oil groups. In the second a decrease was observed in all conserve groups of button mushroom flavored with olive oil with a content diminishing to approximately 5 mg RE/g Extract dry mass for O- 0.5% (conserve with olive oil added at 0.5%).

In the 5th day, flavonoid values increased in groups O-1 and O-2 conserves, reaching about 9 to 12 mg RE/g Extract dry mass.

Olive oil also appeared to increase flavonoid levels, but less strongly than lentisk oil. The moderate increase in groups O-1 and O-2 on day 5 could still be related to secondary metabolites secreted into the medium by the mix (mushroom and canning solution) coupled or not with those related to the presence of the microbial flora already recorded on day 5.

The increase in flavonoids in the oil groups (especially L-2) compared to the control can be attributed to the antioxidant properties of lentisk and olive oils. These oils are rich in phenolic compounds (Mezni *et al.*, 2018; Costa *et al.*, 2024), which can stimulate flavonoid production in mushrooms.

It is evident that lentisk and olive oils have a stimulating effect on flavonoid production in preserved button mushrooms, with a more pronounced response for high-concentration lentisk oil (L-2). The control shows a natural fluctuation, while the additives show a potential to increase the antioxidant response, which could be beneficial for health (Dula *et al.*, 2018; Ruskovska *et al.*, 2020; Bassolino *et al.*, 2022).

3.4 Tannin content

Control; L-0.5; L-1; L-2 and O-0.5 conserves treatments, were the only doses and treatments that promote a decrease in the amount of tannins in the button conserves from day 1 to 2 (Figure 6). On

the first day, the added oils (lentisk and olive oils) at 0.5% were recognized with high amounts of tannins compared to control mushrooms conserved without oil.

In the second day, 1% of lentisk oil ensures a lesser tannin content to conserved button mushrooms than the same concentration of olive oil added. While when the two oils were used with 2% in conserves, results demonstrated that olive oil conserves have the lowest tannin content.



Figure 6. Tannin contents in different samples of button mushrooms conserves

In high amounts, tannins are considered nutritionally invaluable because their abilities to precipitate proteins and to reduce the utilization of vitamins and minerals (Souza *et al.*, 2024). However, tannins have the capacity to restrain microorganism's growth in the media and serve as a natural resistance system against microbial infections (King-Thom *et al.*, 1998). Tannins have also been reported to produce other physiological effects such as immunity and hepatotoxicity. While, presence of tannins in foods could ameliorate their benefits by appropriate level and dose. Tannins doses found here were very acceptable when compared with many fruits, legumes and cereals ones. In fact it was demonstrated that edible *Quercus infectora*; *Kaki tipo* and *Pigen pea* contain 4.4 and 32% (Medugu *et al.*, 2012; Kumari and Jain, 2015; Sharma *et al.*, 2021). Moreover, it was reported that the daily intake of tannin varies from 1500–2500 mg in India and 1 g per day in USA (Sharma *et al.*, 2021).

Each Constituent of button mushroom conserves treatments could differentiate tannin composition separately but also difference could be attributed to interaction between them or due to packaging format, humidity and temperature conservation condition or also may be owing to direct or indirect secondary metabolite excretion (Castellanos-Reyes *et al.*, 2021; Feng *et al.*, 2023; Shonte *et al.*, 2024). Statistical analysis of all results relating to the assays for antioxidant activity, tannins, flavonoids and total phenols according to the type of treatment adopted (only for three doses of lentisk oil) for the conservation of the Paris mushroom (**Table 2**) show that the variation of these parameters is not significant and that all were considered by the SNK test being part of the same group this means that statistically there is no difference between the composition of the different canned products with the three tested doses of lentisk oil. This result confirms the biochemical quality of all canned foods and that each type guarantees the presence of biochemical virtues comparable to others.

 Parameter	р	SNK Groups	
 Antioxidant activity	0,149***	А	
 Tannins	0,181***	А	
 Flavonoids	0,320***	А	
 Total phenols	0,173***	А	

 Table 2. Analysis of the variance of antioxidant activity, tannins, flavonoids and total phenols according to the effect of button mushroom converse's treatments with three doses of lentisk oil (0.5, 1 and 2%)

***Mean difference is not statistically significant p>0.05

According to Vetter (2003) there are some negative consequences (most of all the changes in minerals) derived from the classical technology of conservation which diminish the nutritional value of the mushroom. However, conservation process experienced in this work promise button mushroom quality during tested period. Edible mushrooms are generally high in moisture, the main cause of their rapid perishing (Vetter, 2007); cooking and canning *A. bisporus* was demonstrated to exclude such alteration.

Lentisk oil is rich in phenols which are natural antioxidants with a total amount around 4260.57 mg/kg oil (Mezni *et al.*, 2018; Siano *et al.*, 2020). Besides, it is well known that mushrooms are rich sources for antioxidants like lectins, terpenoids, beta-glucans, ascorbic acid, tocopherols and carboxylic acids (Sharma and Vaidya, 2011). Therefore, making mushroom conserves added by lentisk oil was demonstrated here as a good quality antioxidant food. Results showed the highest quality of button conserves with lentisk oil than olive oil, mainly used and consumed in mushrooms and many other conserves. Antioxidants are the most widely used additives in the food industry to prevent or at least slowdown oxidation reactions in order to prevent the browning and rancidity of foods. In addition, for some commonly used artificial antioxidants such as BHA and BHT, several studies have approved their health risks (Kane *et al.*, 2024).

This paper presents the first work and process involving an edible mushroom associated with lentisk oil and confirms the resulting amount of antioxidants. This opens up prospects for the development of this additive to deal with rapid browning of mushrooms and possibly for other products.

Conclusion

This study is the first conservation and characterization test of button mushroom flavored with lentisk oil. Olive oil was used only to compare the results found after adding lentisk oil since olive oil is widely consumed. The study showed flavonoid richness of *A. bisporus* conserves when added with lentisk oil than those added with olive oil. Moreover, a highest antioxidant activity was found for canned mushroom with 1% lentisk oil added but also all other conserves showed significant antioxidant activity, demonstrating the nutritional value of canning button mushroom coupled to lentisk oil use. The high antioxidant outcome of lentisk oil could restrain industrial use of chemical conservators in food conservation and the canning process, besides its health benefits and pleasant taste.

This form of preservation of flavored mushrooms is a proven innovation that promotes a high nutritional value, but it requires an optimization of the packaging pattern which could improve the marketing prospects of such product.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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