

Lovastatin concentration in *Pleurotus ostreatus* as affected by acid and blanching treatments combined to drying and canning processes

Abstract

Aims: Lovastatin is a cholesterol-lowering drug produced by several filamentous fungi as a secondary metabolite. Its concentration in mushroom can be affected by many post-harvest processes. In this study, fruiting bodies of *Pleurotus ostreatus* grown on corn cobs were used to evaluate the effect of conservation techniques on their lovastatin concentration.

Methodology: acid and blanching treatments combined to different processes of fruiting bodies conservation like sun drying, oven drying and canning by autoclave cooking were tested to evaluate their effect on lovastatin concentration.

Results: Sun drying, oven drying at 60°C/70°C and canning using autoclave cooking at 100°C did not significantly affect lovastatin concentration. On the contrary, oven drying at 80°C caused the reduction of this compound up to 45.4% with respect to fresh product irrespective of the precedent use of citric acid or blanching treatments. Also, during canning, the use of autoclave heat treatments at temperatures 110 and 121°C for 15 minute caused a significant reduction of lovastatin concentration of about 52.2% and 48.9% respectively compared to the control. In this regards, it can be concluded that processes that use dry thermal treatments higher than 80°C and autoclave heat treatments higher than 100°C will contribute to the reduction of lovastatin, the cholesterol-lowering compound in *Pleurotus ostreatus*.

Keys words: *Pleurotus ostreatus*, Conservation, Lovastatin, citric acid treatment, sun-drying, oven-drying, autoclave cooking.

1. Introduction

Fungi are the fleshy fruiting bodies and spores of a fungus produced using soil or agro waste [1]. In recent years, their worldwide production and consumption have increased significantly. The Food and Agriculture Organization statistics (FAOSTAT) database shows that the global production of mushrooms and truffles has increased significantly from 7.5 million tons in 2009 to 11.8 million tons in 2019 [2]. In accordance with what mushrooms represent in human nutrition and health, they can be classified into four classes: edible, medicinal, poisonous and other mushrooms, whose properties are not yet well defined [3]. Some comestible mushrooms also have medicinal properties and thus belong to the edible and medicinal groups simultaneously [4]. *Pleurotus ostreatus* belongs to the latter class. *P. ostreatus* has been preferred all over the world for many years due to its characteristic taste, agreeable aroma, culinary goodness, nutritional content, medicinal value, high yield, short life cycle and relatively easy growing method [5]. Mushrooms have a high nutritive value. They are rich in protein, dietary fiber, vitamins, minerals and phenolic compounds. Additionally, they have a low fat content (composed mainly of unsaturated fatty acids) and a low energy

density [3; 4; 6; 7]. They are the unique non-animal source of vitamin D [4; 8; 7]. Their proteins are of high quality and have all essential amino acids required by humans [9]. Scientists from the University of Oxford [10] have asserted that the amino acid composition of mushroom proteins is comparable to that of animal proteins. The mycelia and fruiting bodies of *P. ostreatus* contain a wide range of bioactive compounds produced through the polyketide biosynthetic pathway. Statins, among these classes of fungal metabolites, have gained a great deal of attention due to their capacity to affect the *de novo* synthesis of endogenous cholesterol [11]. Lovastatin is the most widely prescribed drug for hypercholesterolemia treatment in the world. It was the first statin that was approved by the US Food and Drug Administration in 1987 [12]. Lovastatin ($C_{24}H_{36}O_5$), a fungal secondary metabolite, is a competitive inhibitor of the enzyme hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate during cholesterol biosynthesis. Inhibition of HMG-CoA reductase, during Cholesterol biosynthesis results in the accumulation of HMG-CoA which is metabolized into a simple compound and there are no lipophilic intermediates noted [13]. The predominant form of lovastatin in the fermentation broth is the open hydroxyl-acid form (mevinolinic acid). However, it is generally in the lactone form when given to patients as a drug. In vivo, the lactone form of the compound is being converted to the open hydroxyl-acid, which is the biologically active form of the statin [14]. The commercialization of fresh mushrooms, however, is challenging because it is a highly perishable food product that has tendency to lose its quality directly after being harvested. Mushrooms have some characteristics that facilitate their rapid spoilage, namely high water content (85-95%) of fresh mushrooms [15], neutral pH, high respiration rate, high level of enzymatic activity, presence of microflora, and absence of protective cuticular layer on the skin [16; 17]. Previous studies have indicated a series of factors that may impact post-harvest mushroom quality attributes [17], grouping them into both internal factors associated with the mushroom itself (water activity, respiration rate, and microbial activity) and external factors related to storage (storage temperature, relative humidity, and mechanical damage). A recent review of post-harvest preservation techniques used to maintain the quality and extend the shelf life of mushrooms was conducted by Zhang et al [17]. These authors classified such conservation techniques into three groups: thermal, physical and chemical [17]. Some of these preservation methods, however, modify the chemical composition of mushrooms and, therefore, affect their nutritional value, bioactive properties, and organoleptic characteristics.

From the above discussion on the health benefits of some bioactive compounds that are present in mushrooms and taking into account all the factors that can influence their quality, the necessity of applying preservation methods to effectively reduce quality deterioration, extend shelf life, and conserve the nutritional value of fresh mushrooms is evident. In literature many authors have worked on the impact of conservation techniques on nutritional properties of mushrooms, it can be noted that very few works have targeted the stability of bioactive compounds during processing. The objective of this study was to evaluate the changes induced by different preservation treatments including sun-drying, oven-drying and canning on the lovastatin concentration of *Pleurotus ostreatus*.

2. Methodology

2.1. Research design

In this study, three distinct treatments were compared, sun-drying, oven-drying and canning through autoclave cooking. The experiment was performed in a completely randomized design (CRD) with three replications for each treatment. Three different drying methods were used, namely drying without treatment, drying after citric acid treatment and drying after blanching treatment. This was applied for sun and oven drying (figure 1 and 2). Before treatment, the fresh mushroom samples were firstly sliced into about 5 mm thick slices using a stainless steel knife. For each replicate of the drying treatments, 500 g of oyster mushrooms were used and for the canning treatment, 120 g were used. In the natural drying method, the mushroom samples were spread on perforated trays and sun-dried. The samples were mixed at regular intervals to ensure uniform drying.

2.2. *Pleurotus ostreatus* production

2.2.1. Mushroom strains

A pure culture of *Pleurotus ostreatus* was isolated from pileus tissue with sterile clamps by cutting longitudinally and transferring to Petri dishes containing potato dextrose agar (PDA) before incubating for 14 days at 25°C [18].

2.2.2. Spawn production

For spawn production, corn grains were used as substrate. Spawn medium was prepared according to [19] with slight modification. Each spawn medium was washed and soaked overnight, after this, water was drained, and they were boiled for 15 minutes with fresh water, and allowed to cool for 20 minutes. One hundred grams of each medium were transferred in tissue culture bottles, autoclaved at 121°C for 60 minutes, and allowed to cool. The bottles were inoculated with *P. ostreatus* mycelia and incubated for 14 days in the dark at 25°C.

2.2.3. *Pleurotus ostreatus* growing

Corn cobs were used for cultivating *Pleurotus ostreatus*. All substrate supplements were mixed manually with water to reach approximately 90% moisture as suggested by [19]. The mixture (1500 g) was then packed in polypropylene sacks and the sacks were sterilized at 121 °C for 1 hour. After cooling the temperature to 25 °C, spawns were aseptically inoculated into the bags (100 g). The bags were then incubated in the dark in the mycelial growth room at a temperature of 25±1 °C, with a relative humidity of 60-75%. After the mycelium had colonized the substrates of the bags, they were withdrawn in the dark for fruiting. The bags were kept at 25±1 °C, 70-80% humidity. Watering was done daily with a tap water sprayer until fruiting bodies were completely mature. Developed mushrooms were harvested after around 3-4 days from the initial fructification.

2.3. Experimental treatment processes

2.3.1. Treatments

The treatments administered to mushrooms before drying and canning were as follows: (a) blanching [B] in hot water at 93°C for 3 min, (b) dipping for 10 min in a solution containing 0,5 g/L citric acid [CA]. Untreated mushrooms were used as a control.

2.3.1.1. Drying Processes

Two drying processes were used in this study: sun drying (figure 1) and oven hot air drying (figure 2). For oven drying, mushrooms were dried with hot air at 60, 70 and 80°C with an air flow rate of 1.5 m/s up to 85% moisture lost. The material loaded on the tray was weighed constantly. The air was electrically heated prior to entering the dryer. Following drying, all samples were grounded in an electric mill individually and sieved through an 80 mesh screen to obtain fine powders. The obtained powder was cooled, packed hygienically and stored in a hermetic container for later usage.

2.3.1.2. Canning process

After cleaning, mushrooms were blanched and heat treated in autoclave at different temperatures including 100, 110 and 121°C for 15 min and conditioned in glass cans. The canned mushrooms were subjected to lovastatin content analyses and a microbiological quality assessment directly after production. The cans microbial stability was evaluated over a period of 5 months. For this purpose, the following flora were analyzed: total aerobic mesophilic flora and total aerobic spore flora. Following the enumeration, a visual evaluation of the stability marked by the development or not of the microorganisms was also carried out every week for the different conditions up to five months (figure 3).

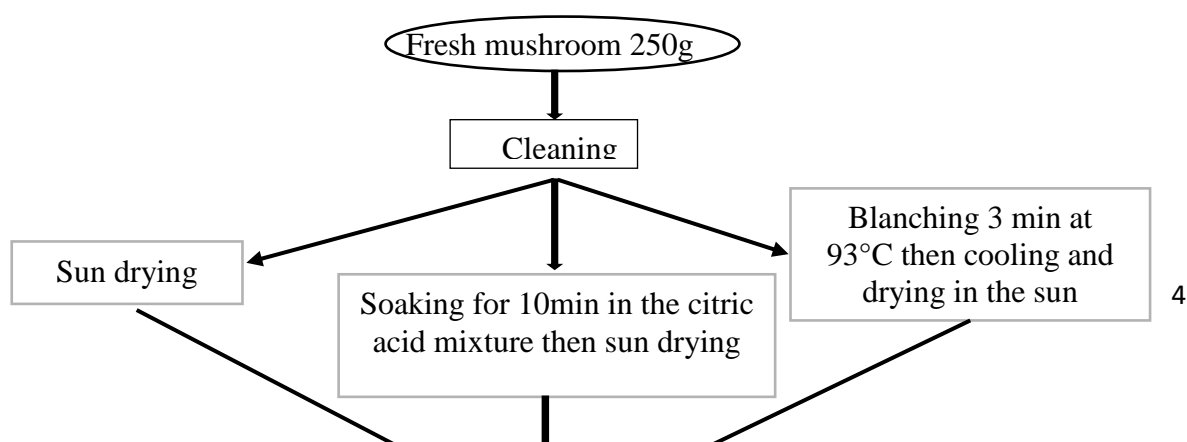


Figure 1: Protocol of sun drying processing

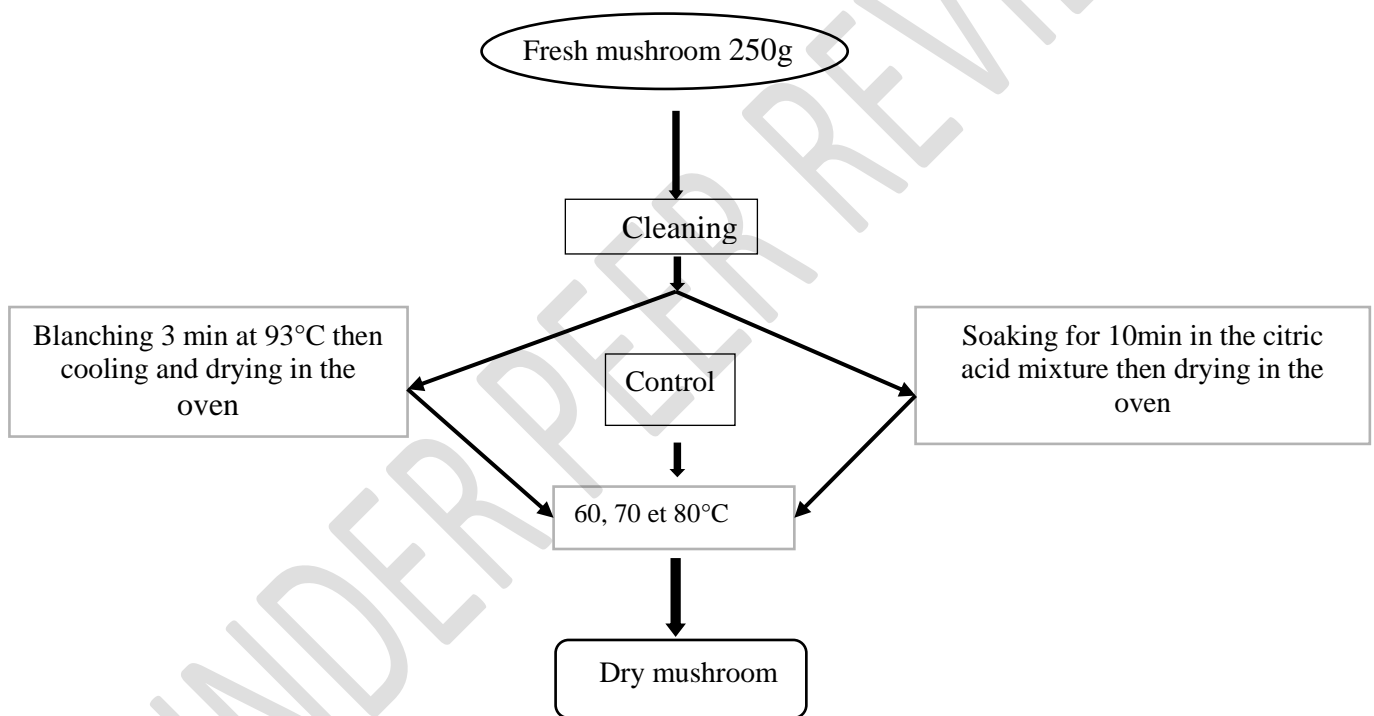


Figure 2: protocol of Oven drying processing

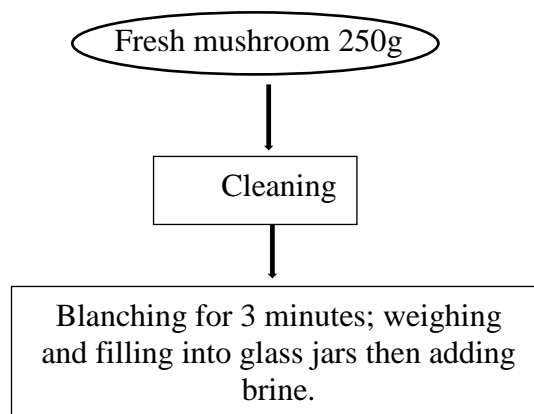


Figure 3: protocol of Canning processing

2.4. Method of data analysis

The data set was statistically analyzed for the mean difference using an LSD post hoc test with significant differences were considered for $P < 0.05$. Statistical analysis of the effect of different factors on lovastatin concentration was also performed using hypothesis decomposition by multivariate ANOVA analysis.

2.4.1. Preparation of lovastatin standard and standard curve

The beta hydroxyl acid form of lovastatin being the most produced by mushrooms, this was the form used for the lovastatin curve and was determined by UV/Vis spectrophotometer analysis at 238nm. Also, the beta hydroxyl acid is quite stable in solution [20]. Tablets of pharmaceutical-grade lovastatin (Lovastatin STADA®, lactone form) containing 40 mg of lovastatin per tablet was used to prepare the standards for the assays. The hydroxy acid form of lovastatin was prepared from tablets by hydrolysis as described by Yang and Hwang [21]. To obtain a stock solution of 20 mg/ml, lovastatin was dissolved in acetonitrile and other dilutions in acetonitrile were performed to have different concentrations which optical density (OD) was read by a UV/Vis spectrophotometer (Genesis 10S) at 238nm. A calibration curve was hence obtained. The absorbance of different solutions with concentrations ranging from 7 to 125 µg/ml was determined and a standard plot was realized to obtain a linear curve. The equation obtained was used to determine the lovastatin concentration present in mushroom extracts.

2.4.2. Extraction of lovastatin from *P. ostreatus*

This was carried out according to [22]. The test excluded the conversion step to the lactone form. Three grams of the mushroom powder were taken in a test tube and mixed with 50ml of ethyl acetate. Extraction of lovastatin was carried out by sonication for 45min, before filtration using Whatman No. 1 filter paper. The residual material was washed three times using ethyl acetate. The liquid phase was dried under a vacuum in a rotary evaporator at 45°C.

The dried residue was dissolved in 2ml acetonitrile and used to determine the lovastatin content by UV/Vis spectrometry. All the concentration was determined based on dry product basis.

3. Results and Discussion

The cultivation of mushrooms is a rather complex process that allows the production of a highly perishable product that need to be preserved. In fact, many mushrooms have become attractive because research has focused a lot on their health benefits, attributed to their bioactive compounds as reviewed in detail by different authors [23, 24, 25, 26, 27, 28, 29]. The chemical and nutritional attributes of mushrooms are interrelated not only to species but also to processing [30, 31, 32] and cooking [33, 34, 35]. Our experiment was focused on the stability of lovastatin after different transformations processes.

3.1. Variation of lovastatin concentration during solar drying combined to different treatments

Drying is a frequent and the most used preservation method used on mushrooms [36, 37]. In our study, different drying techniques were used and the products compared to fresh mushroom. Lowering the pH is generally used to convert most of the acidic form to the quantifiable lovastatin lactone form [38, 39, 40, 41]. However, most reports recognize that incomplete hydrolysis often occurs under these conditions, and that equilibrium between the lactone and acid forms is always present. For this reason, some studies have focused on the conversion of the lactone form of lovastatin to the acid form under alkaline conditions. In such cases, most lovastatin is present in the more stable hydroxy acid form [42, 43, 44, 45, 46, 47, 48, 49]. In our work, lovastatin was extracted in neutral medium and under these conditions lovastatin is present in both forms; the acidic form being predominant, which is in line with [22;50]. Our results show that the lovastatin concentration present in the fresh mushroom was 735.9 ± 7.2 $\mu\text{g/g}$ on dry weight basis and that solar drying without any additional treatment does not affect this concentration. In their work [50] also demonstrated that sun drying did not affect the concentration of statin. However, blanching and citric acid treatment before drying significantly affected this concentration. In fact, the acid treatment with citric acid followed by sun drying is considered as a combined treatments inducing a higher detection of lovastatin; 1471.7 ± 11.3 $\mu\text{g/g}$ of dry mushroom compared to 735.9 ± 7.2 $\mu\text{g/g}$ fresh mushroom (control). It is obvious that there is no creation of lovastatin after a treatment with respect to the fresh product. As mentioned previously, in fungi, lovastatin can be present in both its lactone and hydroxy acid form. This bioconversion is bidirectional and significantly affected by the prevailing pH conditions [51]. At low pH, most of the acidic form is converted to the quantifiable lovastatin in the lactone form, although equilibrium is still present [52]. Therefore, culture conditions, special pretreatments, or pH adjustments during analysis may affect the ratio of the two forms of lovastatin. In our case, the treatment in acidic medium favored the conversion of the acidic form to the lactone form, which was therefore dominant. In their work [53, 48] demonstrated that lovastatin in its lactone form, is poorly soluble in water and soluble in organic solvents, while the acidic β -hydroxy form is more soluble in water. Our extractions having been made in ethyl acetate, this would therefore

favor the extraction of the lactone form, predominant in the medium under these conditions. Blanching before sun drying produced and increased detection of lovastatin to concentrations of about $1085.2 \pm 54.1 \mu\text{g/g}$ (Figure 4).

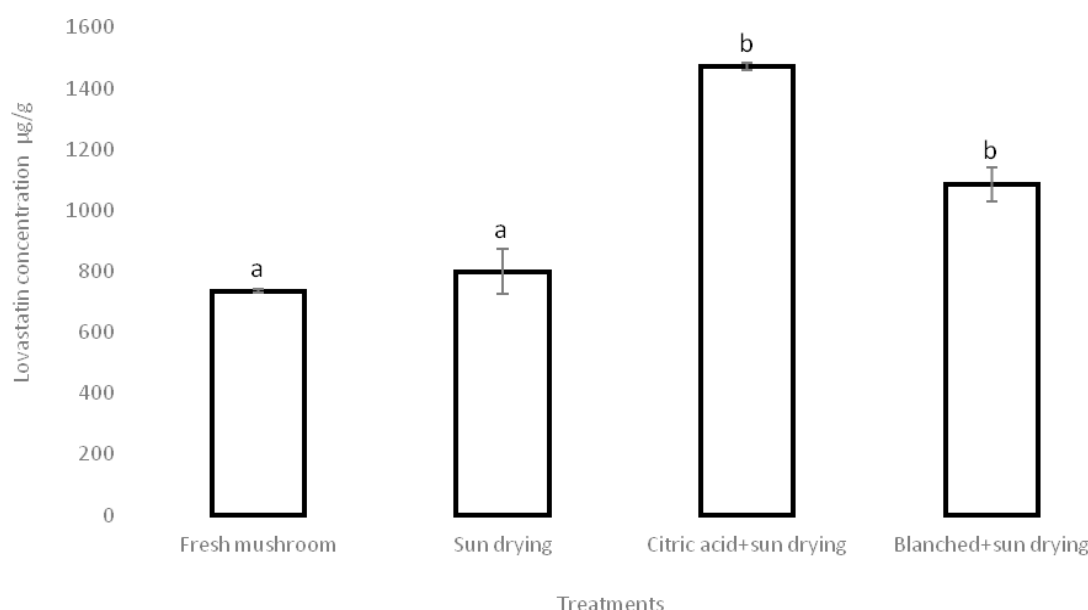


Figure 4: Variation of lovastatin concentration during solar drying according to treatments

3.2. Variation of lovastatin concentration during oven drying combined to different treatments

From figure 5 and 6 it appears that oven drying at 60 and 70°C without any treatment has no significant effect on the lovastatin concentration. However, citric acid treatment potentiated the detected concentration. In fact, acid treatment demonstrated to increase the detection of lovastatin to concentrations of $1216.7 \pm 191.6 \mu\text{g/g}$ and $1335.7 \pm 94.0 \mu\text{g/g}$ dry mushroom respectively at 60 and 70°C compared to $735.9 \pm 7.2 \mu\text{g/g}$ of the control. The increased in concentration could be related as indicated previously to compound availability during extraction or to the detection method. In fact, we also have to take into account the molar extinction coefficient values of the lactone and acid hydroxy forms of lovastatin. Indeed, [54] Seenivasan et al., 2015 in their work performed a regression analysis of the individual forms of lovastatin and the molar extinction coefficients (ϵ) of the lactone form and acid form of lovastatin obtained were $4.89 \times 10^4 \pm 21.8$ and $5.99 \times 10^4 \pm 36 \text{ M}^{-1}\text{cm}^{-1}$, respectively. Using the Beer Lambert equation, we can say that for equal optical density, a shift from total acid form to a total lactone form in a solution will increase the estimated concentration by about 1.22 times without any addition of lovastatin in the medium. Taking into consideration this, increase or reduction in concentrations lower or equal to 1.22 time that of the fresh product was not considered in this work as associated to the transformation process but linked to the spectrophotometer detection method and calculations. Contrary to the result observed during solar drying, blanching followed by oven drying at 60 and 70°C did not result in a change in lovastatin detection. This would reflect the fact that, at these temperatures, lovastatin is not degraded and the ratio of the two forms are not changed. Similar results were obtained by [55] when lovastatin was treated at 105°C for 4h. On the other hand, oven drying at 80°C

regardless of the treatments resulted in a significant decrease in lovastatin concentration in all conditions. However, in opposition with treatments at 60°C and 70°C, blanching pretreatment combined to oven drying at 80°C resulted in the greatest reduction of lovastatin concentration with regards of the control). This reduction is about 45.4% (401.8 ± 24.8 µg/g of dry mushroom compared to 735.9 ± 7.2 µg/g control (Figure 7). This would suggest that combining these two heat treatments, lovastatin is hydrolyzed to other by-products that would not absorb at the wavelength used in the work. As mentioned by [50] the treatment time effect plays a major role in lovastatin stability. They noted that the degradation of statins depended not only on the environmental conditions, but also on the duration of the stress exposure.

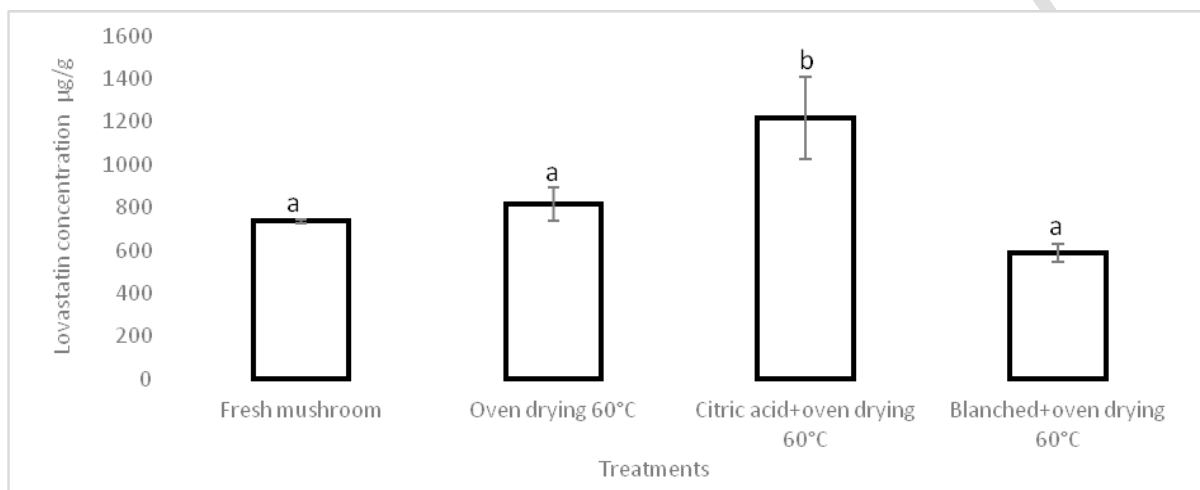


Figure 5: Variation of lovastatin concentration during oven drying at 60°C according to treatments

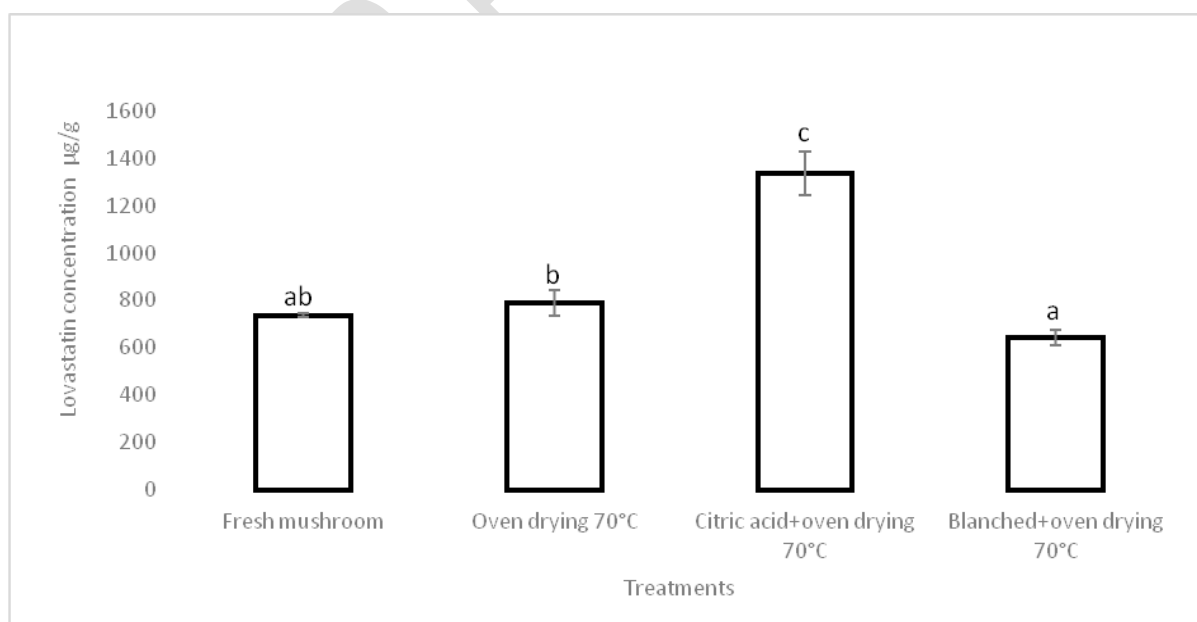


Figure 6: Variation of lovastatin concentration during oven drying at 70°C according to treatments

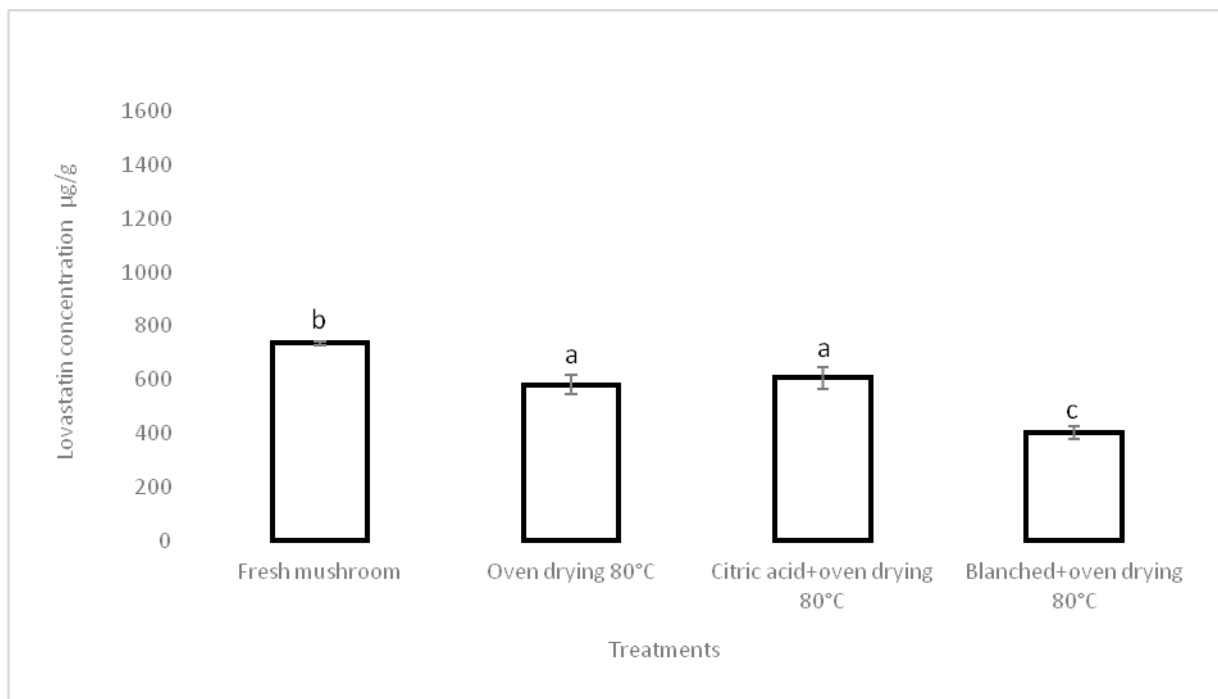


Figure 7: Variation of lovastatin concentration during oven drying at 80°C according to treatments

3.3. Variation of lovastatin concentration in canned mushroom as a function of processing temperature

Canning is a technique by which the mushrooms can be stored for longer periods up to a year at shelf condition. We assessed the effect of different appertization temperatures during canning on the lovastatin concentration. Appertization is intended as a thermal treatment of food already in its final container. In our work, autoclave was used for the thermal treatment. From figure 8, it appears that, blanching do not have a significant effect on lovastatin contain with comparison to the fresh control. This result was also observed after appertization at 100°C. According to [56], after neutral hydrolysis of statin the mostly and identified form of lovastatin present in the medium is the hydroxyl acid form which is the same observation with fresh mushroom. On the other hand, the appertization at 110 and 121°C significantly reduced this concentration by 52.2% and 48.9% respectively. This would suggest that at these temperatures lovastatin is hydrolyzed to other by-products that would not absorb at the wavelength used in the work.

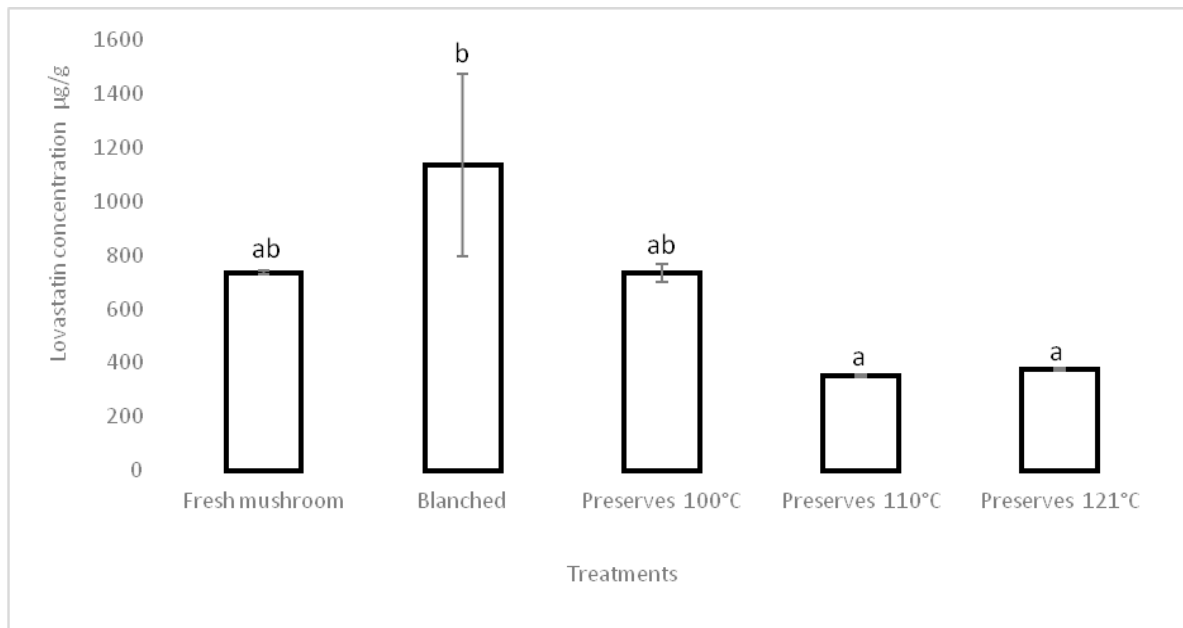


Figure 8: Variation of lovastatin concentration in canned mushroom as a function of processing temperature

3.4. Microbial analysis of preserves

Concerning the microbiological analysis carried out 1 month after the treatments, it appears that, except for the control which presented a microbial load of $6.6 \times 10^7 \pm 1.94$ CFU/ml in total aerobic mesophilic flora, no other condition presented the growth of both total aerobic mesophilic flora and total aerobic spore flora. The Image 1 and 2 below represent the visual aspect of the canned food at different periods of conservation. From this observation we can see that apart from the control which has not been appertized, no visual growth was observable in the different conditions after five months. This reflects the fact that pasteurization and appertization have resulted in deactivation of the microbial flora observed with the control.

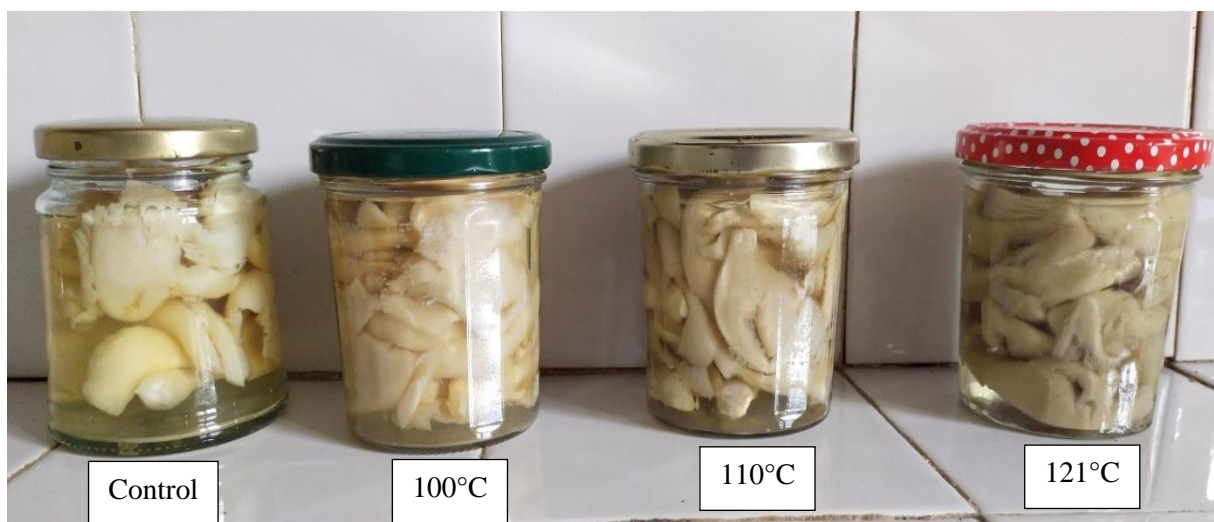


Image 1: Evolution of preserves after 1month of conservation

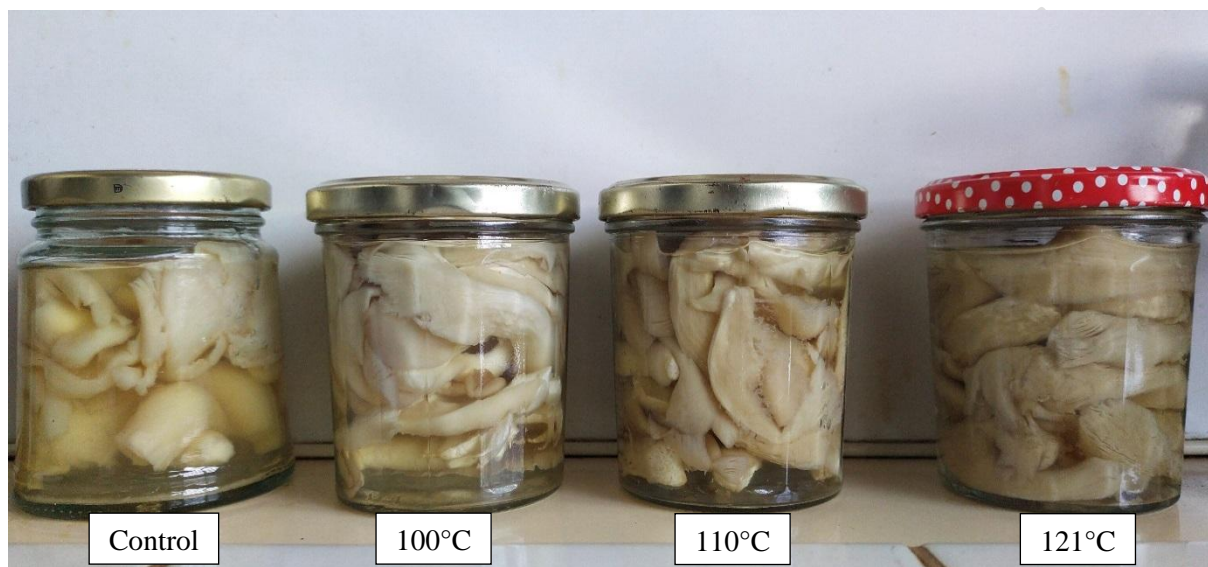


Image 2: Evolution of preserves after five months of conservation

4. Conclusion

One of the fear of the food industry is the loss of nutritional and health protection compound during food transformation and preservation. In this work, different processes for *Pleurotus ostreatus* storage were assessed with regard to lovastatin degradation. It was observed that application of citric acid and blanching treatment before sun drying or oven drying at 60 and 70°C did not caused lovastatin reduction. On the contrary, oven drying at 80°C caused the reduction of this compound irrespective of the type of the precedent acid or blanching treatments. Also, during canning, the use of autoclave cooking at temperatures higher than 100°C for 15 min caused a significant reduction of lovastatin concentration in comparison to the control, of about 52.2% and 48.9% depending of the pretreatment applied. We can conclude that, the treatment of mushroom at 80°C in dry oven, 110 and 121°C autoclave cooking during canning process decrease the lovastatin in mushroom.

ACKNOWLEDGEMENTS

We gratefully acknowledge the laboratory of biological control and applied microbiology of Research Institute for Development (IRAD) Yaoundé for the technical platform provided for the realization of part of this work.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author EYM contributed in designing the work, carried most of the farm production of mushroom, did most of the bench work and field work and data analysis. Authors GY and ADBB contributed in designing the work and farm production. Author ATN contributed in part of the bench work and statistical analysis. Author SLKS supervised the work, contributed in designing the work, interpreting and the statistical analysis of the data. All authors contributed in proof reading the work

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