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Antioxidant capacity of frozen *Pleurotus ostreatus* during convective drying

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ABSTRACT

The influence of drying conditions on antiradical power, total phenols, flavonoids, and surface color in frozen basidiocarps of *Pleurotus ostreatus* was studied. Drying was carried out in an experimental rig, air velocity of 2 m/s, 5% relative humidity, and at different temperatures (50 C, 60 C, 70 C). Total phenols were determined according to the Folin-Ciocalteau method, flavonoids by complex formation with $AlCl_3$, and antiradical power by 1,1-diphenyl-2-picrylhydrazyl (DPPH). Surface color was also measured. A decrease was observed in the content of flavonoids during the first 15 min of drying in all temperatures tested. The content of total phenols increased, but subsequent stabilization was noted as drying progressed. Higher temperatures of the drying air preserved the antiradical power. A drying air temperature of 60 C was suitable for variables studied, showing *P. ostreatus* adequate stability of total phenols, antiradical power, and color. This process allows the use of oyster mushrooms out of season.

Key words: Antiradical power, dehydration, flavonoids, *Pleurotus ostreatus*, polyphenols.

* E. Ohaco and A. De Michelis are members from CONICET.

Capacidad antioxidante de *Pleurotus ostreatus* congelado durante el secado por convección

RESUMEN

Se estudió la influencia de las condiciones de deshidratación sobre el poder antirradicalario, fenoles totales, flavonoides, y color superficial de basidiocarpos congelados de *Pleurotus ostreatus*. El secado se efectuó en equipo experimental, con una velocidad de aire de 2 m/s, 5% de humedad relativa, y distintas temperaturas (50 C, 60 C, 70 C). La determinación del contenido de fenoles totales se realizó de acuerdo al método de Folin-Ciocalteau, flavonoides por la formación de complejo con $AlCl_3$, y poder antirradicalario por el 1,1-difenil-2-picrilhidracilo (DPPH). También se evaluó el color superficial por colorimetría. Se observó un marcado descenso en el contenido de flavonoides durante los primeros 15 min de secado, en todas las temperaturas ensayadas. También se observó un aumento en el contenido de fenoles totales, así como su posterior estabilización a medida que transcurría el secado. A mayor temperatura del aire de secado más se preservó el poder antirradicalario. Una temperatura del aire de secado de 60 C fue adecuada para las variables estudiadas, mostrando *P. ostreatus* estabilidad adecuada de fenoles totales, poder antirradicalario, y color. Este proceso permite la utilización de *P. ostreatus* fuera de estación.

Palabras clave: Deshidratación, flavonoides, *Pleurotus ostreatus*, poder antirradicalario, polifenoles.

INTRODUCTION

The cultivation of edible mushrooms is a biotechnological industry in continuous expansion, gaining economic importance in many countries. Commonly known as oyster mushrooms, *Pleurotus* species are prized not only for their texture and flavor, but also for their functional properties[8]. Mushrooms are a source of proteins, fibers, carbohydrates, antioxidant vitamins and polyphenols, and essential minerals, but they are low in fat[3,9,16,18,19,20]. Numerous studies have reported that antioxidant activities of mushrooms are related to their polyphenolic content[2,3,10,12,29]. Polyphenols interfere with the initiation and progression of cancer[6,14]. They also act as antiageing[17], anti-inflammatory[4,25], and brain-protective factors[26], and protect against cardiovascular diseases[11,31]. *Pleurotus* mushrooms have the capacity to grow over a wide range of temperatures, using various organic materials rich in lignin and cellulose as substrates. In the Alto Valle de Río Negro and Neuquén regions, Argentina, oyster mushrooms are grown on poplar trunks. Basidiocarps are produced from March to May, and during October and November to a lesser extent. Oyster mushrooms should be preserved to be available all year round.

Mushrooms contain about 90% moisture and are highly perishable in nature. They

start deteriorating immediately after harvest, having a shelf life of 1-2 days only at ambient temperature[27]. Stem elongation, cap opening, discoloration, and textural changes are important factors lowering quality and affecting consumer acceptability. The application of suitable post-harvest techniques to extend shelf life and maintain mushroom quality plays an important role in marketing[8]. Short-term preservation methods, such as low-temperature storage, steeping preservation, irradiation, and chemical treatment, help to prolong shelf life up to three weeks. Long-term preservation methods, such as canning, pickling and drying, can make mushrooms available throughout the year at reasonable cost[15]. Drying reduces bulk, thus facilitating transportation, handling and storage. Hot air drying is a widely used processing practice for food products in developing countries. For drying mushrooms, a wide temperature range of 37.8-70.0 C, and a finishing temperature of up to 82.2 C have been reported, including several recommendations for the dehydration process in terms of product quality. Mudahar and Bains[21] dried mushrooms in two stages of development at 45 C and 60 C. Pruthi *et al.*[24] and Lal *et al*.[13] proposed a temperature of 60 C. Yapar *et al*.[30] reported that high moisture associated with low temperature caused mushroom browning derived from enzymatic activity, but the use of high temperature resulted in Maillard reaction. They proposed a drying temperature ranging from 60-70 C. However, more systematic work is needed to determine a safe drying temperature that ensure desirable mushroom quality and consumer acceptability. Food color is one of the most important marketing attributes because it causes acceptance or rejection by the consumer. It is used accordingly as a parameter of quality in the final product[23].

In order to extend the maximum period of drying raw material, which is limited as it is associated with the appearance of the fresh product, it is also important to evaluate frozen mushrooms during the dehydration process. In this study, we assessed the influence of drying air temperature on antioxidant properties of ethanol extracts from *P. ostreatus* previously frozen, as well as the surface color of basidiocarps.

MATERIALS AND METHODS

Raw material. Mushroom spawn was produced at the Laboratory from Centre for Research and Services for the Production of Edible and Medicinal Mushrooms, according to standard methods (strain code: A02). Basidiocarps of *Pleurotus ostreatus* (Jacq.) P. Kumm. were used in all experiments, which were cultivated on poplar trunk and harvested at the high valley of Río Negro and Neuquén, Argentina. *P. ostreatus* basidiocarps had an average water content of 92.7 ± 1.0% on a fresh weight basis, and they were kept frozen at -20 C until use.

Drying process. Experiments were carried out in a purpose-built pilot scale dryer, consisting basically of a closed system with forced air circulation and appropriate control of drying variables (**Fig. 1**)[23]. Different experiments were conducted based on drying temperature (50 C, 60 C, 70 C), keeping constant speed (2 m/s) and relative humidity (5%) of air. In order to assess the evolution of bioactive compounds according to the drying time, the drying tray was divided into six equal compartments, rectangles

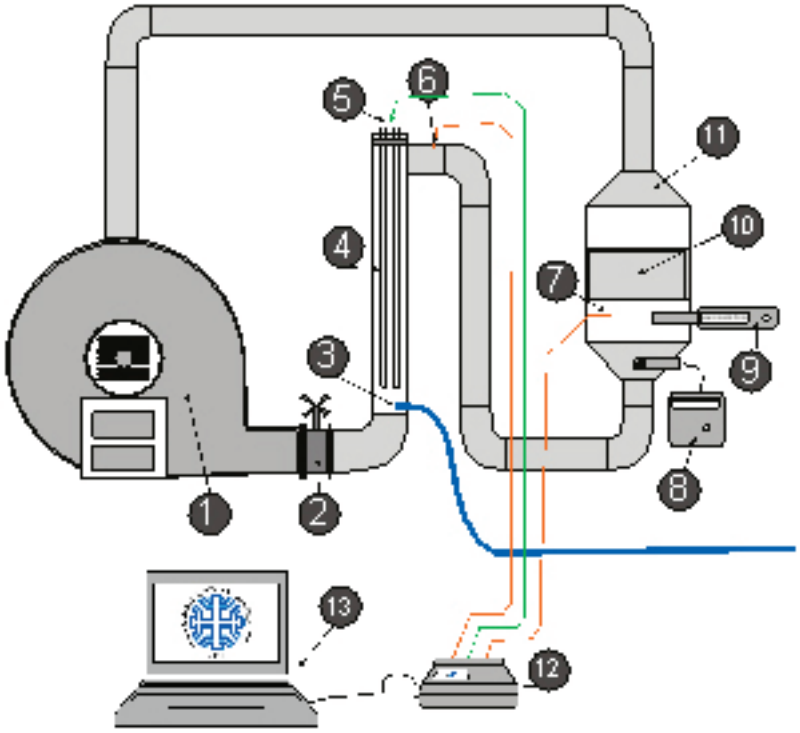

Fig. 1. Experimental drying equipment. 1: Fan. 2: Air velocity control. 3: Humidifying air circuit. 4: Electric heat exchanger. 5: Power electric heating regulator. 6: Air dry bulb temperature sensor. 7: Dry bulb temperature sensor in the drying chamber inlet. 8: Anemometer. 9: Hygrometer. 10: Device with sample tray holder. 11: Drying chamber. 12: Data logger. 13: Computer.

(2 x 4 cm) of fresh pre-weighed frozen oyster mushrooms were placed at each compartment and distributed in monolayers. As the drying process progressed, at certain time intervals, oyster mushrooms were placed in a different compartment. They were weighed and placed in sealed glass containers to avoid relative humidity effects. Containers were labeled and stored at -20 C in a freezer until use.

Measurement of weight loss and dry weight. The weight loss of partially dehydrated samples was obtained by discontinuous weighing using an analytical scale (Ohaus, Canada). The dry weight of each sample was determined by drying to constant weight in a forced air oven at 102 C[1].

Preparation of extracts of bioactive compounds. About 2 g of fresh or dry sample was weighed. The dry sample was rehydrated with 5 ml of distilled water for 30 min; then 10 ml of 70% ethanol was added and the sample was placed in a Dubnoff thermostatic bath at 37 C for 30 min. It was vacuum filtered, and two more extracts were obtained, each with 10 ml of the solvent. These volumes of three extracts were pooled and made up to 50 ml with distilled water. Each sample was prepared in duplicate and the extracts were kept frozen until analysis.

Determination of total phenols (TP). The total phenols concentration was measured according to a modified method from

Swain and Hillis[28]. Distilled water (1,500 µl) and 100 µl of 1N Folin-Ciocalteau reagent were added to one aliquot of 50 µl of the extract. The solution was stirred with a vortex for 3 min, and 300 µl of 20% Na_2CO_3 were added. It was then incubated for 30 min at 40 C. Absorbance readings were performed at 765 nm using a spectrophotometer (Metrolab 1700, Argentina), against an external standard of gallic acid (mg/l). Data were expressed as milligrams of equivalent gallic acid per 100 grams of fresh or dried mushrooms, on a dry weight basis (mg GAE/100 g).

Determination of flavonoids (Fv). Total flavonoids were determined by the method of complex formation with $AlCl_3$, against an external standard of catechin (mg/l). An aliquot of the extract was mixed with 300 µl of 5% $NaNO_2$. After 5 min, 300 µl of 10% $AlCl_3$ were added, and after 6 min 2 ml of 1N NaOH were also added. The solution was made up to 10 ml with distilled water. Absorbance readings were performed at 510 nm using a spectrophotometer (Metrolab 1700, Argentina). Data were expressed as milligrams of equivalent catechin per 100 grams of fresh or dried mushrooms, on a dry weight basis (mg CE/100 g)[32].

Antiradical power (AP). The antiradical power was analyzed using the 1,1-diphenyl-2-picrylhydrazyl stable radical (DPPH) in methanol[5]. The degree of discoloration of the solution was measured spectrophotometrically. A typical reaction consisted of adding to a glass cell containing 3 ml of methanol solution of DPPH at 60 µM concentration, different aliquots of extracts adjusted to consume between 30% to 70% of the radical. Absorbance decay was monitored for an hour, the first 30 min in cycles of 60 s and thereafter every 5 min. The absorbance value of the solution to steady state was estimated by the exponential fit of kinetic curves. The amount of antioxidant present in the matrix (mg tissue on a dry weight basis) required to decrease the initial concentration of DPPH by 50% was designated as EC_{50}, and for simplicity the antiradical power was defined as $1/EC_{50}$ (mg^{-1} of fresh or dry tissue, on a dry weight basis). This means that the higher the antiradical power, the more effective was the tissue as an antioxidant.

Surface color. The surface color of samples was determined using a Minolta CR400 photocolorimeter. CIELAB parameters were as follows: L* (lightness), a* (degree of red or green component), and b* (degree of yellow or blue component). The Croma (*C**) and tone (*hue*) parameters were calculated, Equation 1 and Equation 2, respectively.

(1) $C^* = (a^{*2} + b^{*2})^{1/2}$

(2) $hue = arc\ tg\left(\frac{b^*}{a^*}\right)$

where *C** is the degree of saturation of color and *hue* is the chromatic color.

Statistical Analysis. Data were subjected to simple analysis of variance ANOVA (Statistica 7.0), $\alpha = 0.05$, to determine significant differences between mean values of the content of different bioactive compounds according to the drying air temperature (50 C, 60 C, 70 C). Conclusions were confirmed by the Least Significant Difference Test (LSD).

RESULTS AND DISCUSSION

Table 1 shows the concentrations of bioactive compounds studied, total phenols (TP), flavonoids (Fv), and antiradical power (AP), which were analyzed in frozen

Table 1. Content of bioactive compounds in fresh frozen, and dehydrated (D) basidiocarps of *Pleurotus ostreatus* at different temperatures. GAE: Galic acid equivalent; CE: Catequin equivalent; db: Dry weight basis.

Sample	Total phenols* (mg GAE/100 g db)	Flavonoids* (mg CE/100 g db)	Antiradical power* (mg^{-1} db)
Fresh frozen	483 ± 22 [a]	841 ± 52 [a]	105 ± 6 [a]
D 50 C	665 ± 23 [b]	165 ± 6 [b]	98 ± 10 [ab]
D 60 C	965 ± 10 [c]	160 ± 23 [b]	76 ± 1 [b]
D 70 C	780 ± 30 [d]	162 ± 18 [b]	86 ± 3 [b]

* Equal letters in the same column indicate no significant differences ($p > 0.05$).

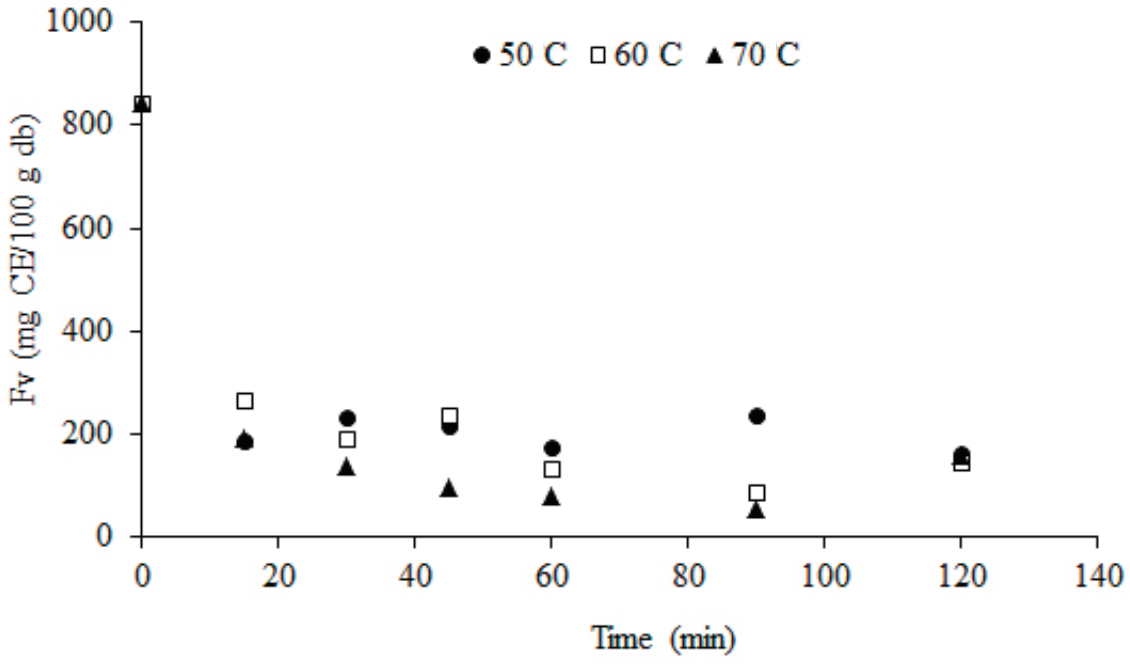

Fig. 2. Content of flavonoids (Fv) as a function of time and temperature of the drying air. CE: Catechin equivalent.

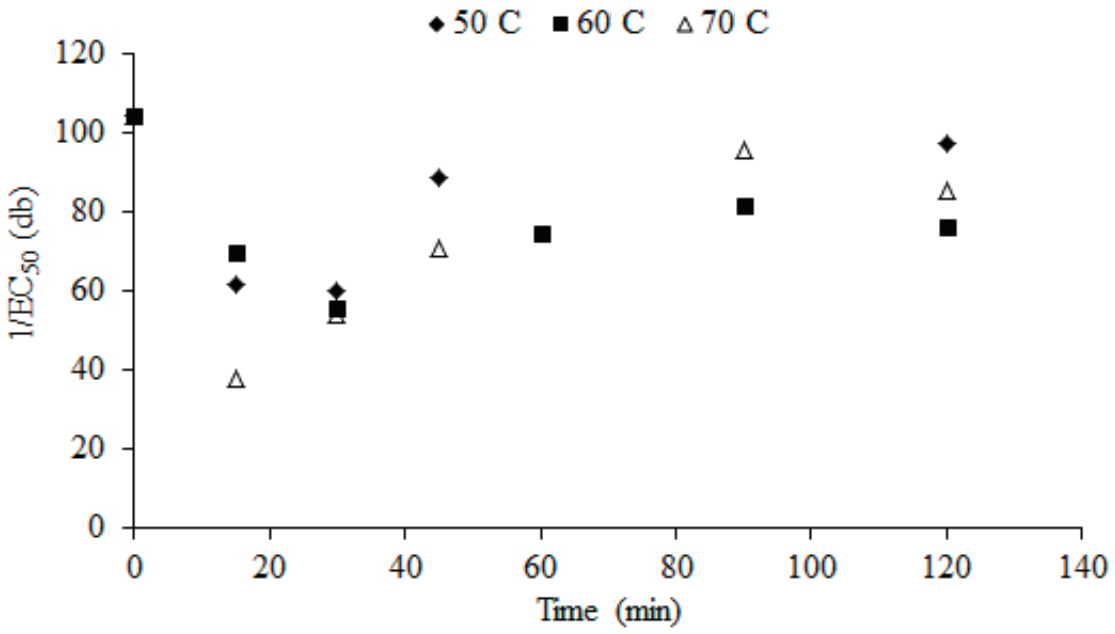

Fig. 3. Antiradical power ($1/EC_{50}$) as a function of time and temperature of the drying air.

and fresh oyster mushrooms after 2 h of drying at different temperatures (50 C, 60 C, 70 C). Increased total phenolic content was observed after heat treatment. Choi *et al.*[7] suggested that heat can cause changes in the extractability of phenols due to the rupture of cellular structure. Murakami *et al.*[22] showed that polyphenolic compounds are more stable in processed and cooked foods than in the original matrix, due to the greater antiradical activity of treated products. Flavonoids content decreased significantly (80%) after 2 h of drying. **Figure 2** shows a marked decrease of flavonoids content during the first 15 min of drying. This trend was observed at all temperatures studied, and continued over time.

The antiradical power decreased in all experiments as shown in **Figure 3**, but recovered the initial value after 2 h of drying. This recovery could be explained by the condensation of oxidation products from polyphenols, which determines the formation of procyanidins with greater aromatic structures and higher antioxidant capacity[7]. It should be noted that *in vitro* determinations of antioxidant capacity are only an approximation of what might happen in more complex situations (*i.e.*, *in vivo*), where a large number of biochemical reactions occur. Further effects are also determined not only by the number of antioxidant capacities of compounds, but also by various synergistic or inhibitory effects that occur between these components and the environment. In plant extracts, there is presumably a direct relation between total phenolic content and antioxidant capacity, because the phenolic or polar compounds cause higher antioxidant activity.

Figures 4-5 show the evolution of *C** and *hue* color parameters on the upper side

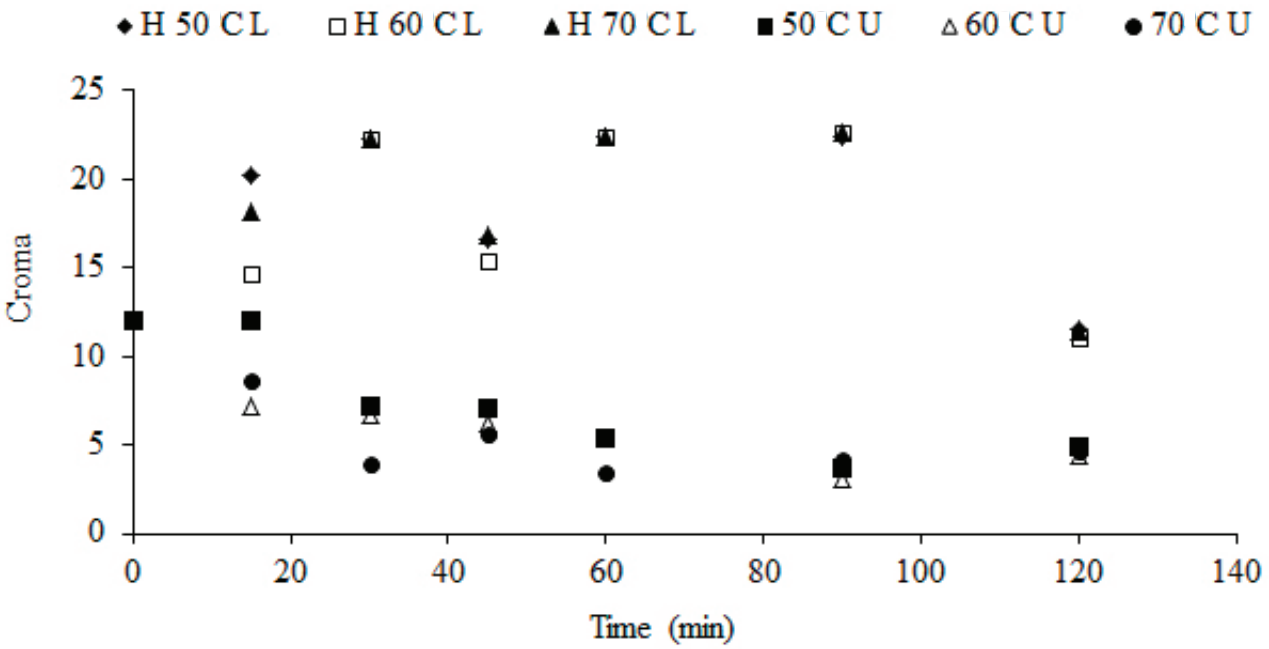

Fig. 4. The *Croma* parameter as a function of time and drying temperature. L: Lower side. U: Upper side.

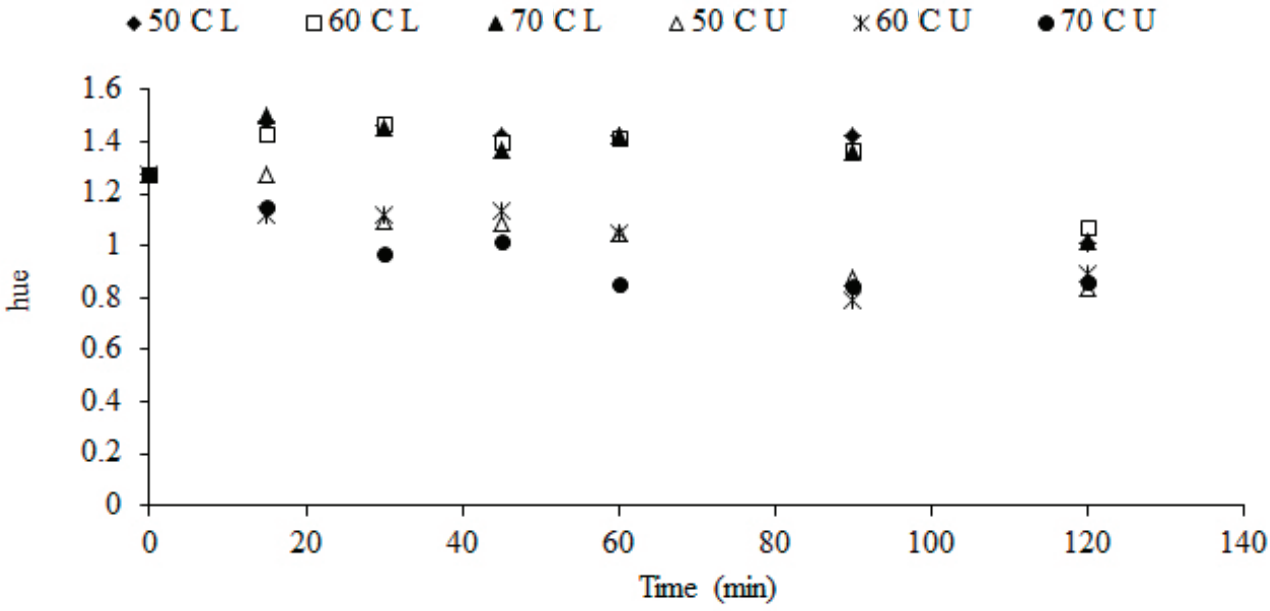

Fig. 5. The *hue* parameter as a function of time and drying temperature. L: Lower side; U: Upper side.

(U) and the lower side (L) of mushrooms dehydrated at 50 C, 60 C, and 70 C. Increased C^* was observed on the lower side as drying time progressed. This means that the color was intensified, probably by the appearance of products derived from the Maillard reaction due to exposure to high temperatures. Furthermore, decreased C^* was observed on the upper side and subsequent stabilization at 2 h of drying, indicating a decrease in color sharpness. The *hue* showed a decrease of 0.4 units after 2 h of drying on both sides of the product, and regardless of the temperature of drying air. This indicates a trend towards yellow orange hues in the dehydrated product.

It is well known that natural components (nutrients or non nutrients) may be significantly lost by heat treatment because most bioactive compounds are thermally unstable. In this study, the increase in total phenols compensated for flavonoids degradation. The antioxidant capacity of *P. ostreatus* remained stable during the drying process in the temperature range studied. A drying temperature of 60 C can be recommended as suitable, since a significant increase in total phenols was evident at this temperature. It is concluded that frozen *P. ostreatus* showed adequate stability during hot air drying, allowing increased use of the product out of season.

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