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Original article

From Sun to Air Drying: Comparative Quality Evolution of Dried *Octopus vulgaris*

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Abstract

This study aimed to evaluate the effects of two drying methods on the physicochemical and microbiological quality of Octopus vulgaris. Samples were dried using sun drying (SD) at 28 ± 0.75 °C and convective air drying (CAD) at 30 ± 0.12 °C. Drying and rehydration kinetics were investigated. Physicochemical analyses included pH, water activity (a_w), color parameters, total volatile basic nitrogen (TVB-N), and trimethylamine (TMA), while microbiological quality was assessed through total viable count (TVC) and yeast and mold (YM) enumeration using standard methods. Quality attributes of the dried products were analyzed and compared using analysis of variance (ANOVA). CAD resulted in a significantly higher drying rate, whereas no significant differences were observed in rehydration behavior between the two drying methods. Drying led to the inhibition of yeasts and molds in both treatments, while lower total viable counts were observed in CAD-treated samples. TVB-N content increased after drying, from 5.1 ± 1.2 mg N/100 g in fresh samples to 6.0 ± 0.59 and 9.46 ± 1.81 mg N/100 g in CAD- and SD-treated samples, respectively. A similar trend was observed for TMA levels. Sun-dried octopus exhibited higher total color difference (ΔE) and browning index (BI) values, indicating more pronounced color changes and intensified non-enzymatic browning reactions. Overall, low-temperature convective air drying at 30 °C appears to be a suitable method for preserving the quality of dried octopus.

Keywords: Octopus Vulgaris, Sun Drying, Convective Drying, Kinetics, Quality

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INTRODUCTION

Octopus vulgaris or common octopus is a widely distributed benthic cephalopod occupying a large variety of habitats. This specie has long been considered abundant in the Mediterranean, Atlantic and Japanese waters; more recently its distribution has been extended to include the central Indian Ocean islands (Fadhlaoui-Zid et al., 2012).

Proteins are the most abundant macronutrient found in the common octopus (15.8% as reported by Zlatanos, Laskaridis, Feist, and Sagredos (2006). Analysis run by these researchers resulted in a high digestibility of these proteins that exhibited a quality similar to that of beef or fish (Zlatanos et al., 2006). Despite their low fat content, as other cephalopods, common octopus contain large amounts of polyunsaturated fatty acids specifically n-3 fatty acids and a rich minerals and vitamins composition (Zlatanos et al., 2006). However, high water and protein contents make *O. vulgaris* sensible to chemical and microbial degradation. In addition, octopus contains endogenous and bacterial enzymes which accelerate quality deterioration and proliferation of spoilage microorganisms (Atrea, Papavergou, Amvrosiadis, & Savvaidis, 2009).

In most developing countries sun or solar drying is the main conservation technique used to lengthen seafood shelf life. Dried fish may represent the main animal protein source in several coastal regions (Prasad, Gopakumar, & Seenayya, 1999). In several Mediterranean countries, dried octopus is a much-appreciated product which is added in local dishes such as soups and other cereal based meals. These products may also be a part of complex product formulations in food industry. Traditionally, in several Mediterranean, Asian and African regions, small-scale artisanal fisheries as well as local fishermen dry in the sun the product of their capture. Indeed, although sun drying subjects the product to the risk of insects infestation or microbial proliferation and lipid oxidation, this process does not require specific equipment, saves energy and appears to be convenient and economic(Qiu, Chen, & Lin, 2019). Currently, industrials are aware of consumer demand as well as export opportunities and seek for better controlled drying alternatives w. Convective air drying is still the most used method allowing a better microbial control and protection against weather disturbances although it is an energy-intensive treatment.

Drying food products aims mainly their long-term preservation and availability at ambient temperature with no further energy consumption. Recent syntheses compare drawbacks but also positive effects of different drying modes on various dried food products. Indeed, different drying modes will lead to different extent of organoleptic, biochemical and nutritional changes (Calín-Sánchez et al., 2020). Hence, sun drying, which is a long lasting process, often leads to biochemical degradation and then to the revaluation of the drying process, as described during shrimp sun drying at 35.7°C during 4 days(Hernández Becerra et al., 2014). In addition, longer drying periods at relatively low air flow temperature supports microbial proliferation. Indeed, a slow water activity decrease during drying allows degradation reactions to take place. Hernández Becerra et al. (2014) compared several quality parameters in sun dried salted Wolf Herring (*Chirocentrus dorab*) and Coastal Trevally (*Carangoides coeruleopinnatus*) and reported an important increase in total vitamin based nitrogen (TVB-N). These researchers directly related the observed lower quality to the longer drying period under sun drying mode. Similarly, in a study on convective drying of pre-salted squid, higher temperatures (up to 90°C), led to higher TVB-N levels highlighting an increase of free volatile nitrogen compounds and a poorer product quality(Vega-Gálvez et al., 2011).

Moreover, component denaturation and biochemical degradation extents are mainly related to drying temperature and drying rate. Gliguem, Hajji, Rekik, Allaf, and Bellagha (2021); Tajudin, Tasirin, Ang, Rosli, and Lim (2019) reported a better biochemical retention and organoleptic preservation while drying *Roselle calyx* at 40°C in comparison with dried product quality at 50 and 60°C. More specifically, high protein content products, such as seafood, encounter protein denaturation during drying. Gliguem et al. (2021) described the effects of temperature on blue crabmeat protein content and quality during drying under various modes and operating conditions. They reported that lower temperature encountered in the product and higher drying rate led to better protein preservation.

This research study aims to analyse and compare the effect of two drying methods, sun drying (SD) and convective air drying (CAD) on the drying kinetics and on the quality indicators such as total volatile based nitrogen (TVB-N), trimethylamine (TMA), color and microbial counts of common octopus.

MATERIALS and METHODS

Biological material

Octopuses (*Octopus vulgaris*) used in this study were caught off the coast of Bizerte (Tunisia). Undersized octopus with a weigh of 133.66g and a size of 30cm were used. Water content of fresh samples was determined according to Hajji et al., (2022) and was 89.10±0.177 g/100g wb (wet basis). To ensure all the analyses carried out in this study, 6 kg of octopuses was used.

Drying treatments

Drying methods

Two drying modes were used. In the first mode, *octopus* samples were directly placed in an open air for sun drying at 28 ± 0.75°C and 49 ±1.25% relative humidity (RH) during 4 to 5 days. A second sample was dried in a convective air drier well described by Hajji, Bellagha, and Allaf (2022) at 30°C with an air velocity of 2m/s, for almost a day and a half. Drying was stopped for both modes when water activity reached a 0.45 value.

Drying kinetics

Water content evolution throughout the drying period has been recorded for the two drying methods. The drying process was stopped when sample water activity reached 0.45 for stability reasons (Hajji et al., 2022). Final water content (X) calculation during the drying processes was made based on relation 1:

$$X = \frac{m_t - m_s}{m_s} * 100 \quad (1)$$

Where m_t is the mass sample in gram at time t and m_s is the dry matter content in gram of the product. X is thus expressed in g/100g db (dry basis).

Rehydration

For rehydration purposes, dried octopus samples (19.47± 3.74 g) were immersed in distillated water at 20°C. Changes in sample weight were measured every 6 min. Before weighing, the samples were removed from water and blotted with paper tissue to remove superficial water. Rehydration is stopped after 2 h. The rehydration rate (RR) was calculated as follows according to (Rekik et al., 2021) (Eq 2):

$$RR = \frac{Mass\ of\ rehydrated\ sample - Mass\ of\ dried\ sample}{Mass\ of\ dried\ sample} \quad (2)$$

Physico-chemical analysis

Water content, Water activity and pH

About 2 g of ground sample placed in a crystallizer was weighed using a precision balance (10^{-3}g) then placed in an oven at 102 ° C for 16 to 18 hours up to constant mass. The dried sample was weighed and water contents on a fresh and dry basis were calculated(Gliguem et al., 2021). Water activity of the ground octopus flesh was assessed by a pre-calibrated rotronic water activity meter (Model: Hygro-Lab C1, Decagon Devices, Inc. France) at 20°C.

pH analysis was run by mixing 10 g of crushed and homogenized octopus with 50 ml of distilled water in a beaker. A microprocessor pH meter (BT-500, Boeco, Hamburg, Germany) was used.

Total Vitamin-Based nitrogen TVB-N

As described in the Cobb et al. (1973) method, 100 g of fish were chopped and added to 50 ml of distilled water, until liquid slurry was obtained. The latter was mixed with 50 ml of trichloroacetic solution and filtered. One millimeter of the extract with 1.5 ml of distilled water was placed in the outer ring of the Conway cell while 1 ml of boric solution was added into the inner ring. After adding 1ml of saturated K_2CO_3 in the outer chamber, the cell was closed and incubated for 2 hours at 35 ° C. Then the volatile bases contained in this solution were titrated with HCl 0.02 N. TVB-N content was expressed as described in equation 3:

$$TVB - N = N_{HCl} * V_{HCl} * m_{NH_3} * 10^2 \quad (3)$$

Where N_{HCl} is hydrochloric acid normality (0.02N), V_{HCl} is the volume (ml) of hydrochloric acid used and m_{NH_3} is the molar mass of NH_3 in g/mole.

Trimethylamine TMA

The same liquid extract obtained for TVB-N determination was used with 1ml of distilled water and 0.5 ml of formalin and placed in the center of a Conway cell then gently mixed. 1 ml of saturated K_2CO_3 was introduced into the outer chamber which is then closed and incubated for 2 hours at 35 ° C. The inner ring solution was then titrated with 0.02N-HCI using a micro-burette until green color turns to pink (Ng, 1987). TMA content is expressed in mg of nitrogen per 100g of the sample as described in equation 4:

$$TMA = N_{HCl} * V_{HCl} * m_{TMA} * 10^2 \quad (4)$$

Where N_{HCl} is hydrochloric acid molarity (0.02N), V_{HCl} is volume (ml) of hydrochloric acid used and m_{TMA} is molar mass of TMA in g/mole.

Color

Color measurement was evaluated using a colorimeter (Konica Minolta CR-410, Tokyo, Japan) and measured in Hunter L, a, b scale. Color parameters L, a and b represent the lightness, the green to red scale and the yellow to blue scale, respectively. Total color difference (ΔE) and browning index (BI) were calculated using equations 5 and 6. In this work, ΔE has been assessed between the fresh samples (L_0, a_0 and b_0) and the SD and CAD treated octopus samples (L, a and b).

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (5)$$

The browning index (BI) allows evaluating the brown color evolution after drying.

$$\mathrm{BI} = \frac{100(\mathrm{x} - 0.31)}{0.172} \quad (6)$$

$$\text{where } \mathrm{x} = \frac{(\mathrm{a} + 1.75\mathrm{L})}{(5.645\mathrm{L} + \mathrm{a} - 0.3012\mathrm{b})}$$

Microbial analysis

Ten grams of each ground sample was suspended in 90 ml of sterile peptone water using a Stomacher 80 Bio-master. This suspension was diluted 10-fold in sterile peptone water then homogenized during 2 min. Appropriate dilutions were prepared according to the analysis to be performed.

Total viable count (TVC) is determined on Plate Count Agar (PCA) according to ISO 4833-1 (2013) standard. Total coliforms counts were conducted on Desoxycholate Agar medium after 24 h at 37°C (ISO 4832, 1991). According to ISO 6888 (1999), *Staphylococcus aureus* were counted on Baird Parker Agar added with sodium tellurite and egg yolk after 48h at 37°C. Sulfito-reducer bacteria were determined according to ISO 15213 (2003) standard on tryptose- sulfite – néomycine (TSN) medium after 24h at 37°C. Yeast and molds were enumerated on Sabouraud medium after 5 days at 25°C(ISO 7954, 1987).

All chemical, physical and microbial analyses were run in triplicate.

Statistical analysis

The analyses were carried out in triplicate and the values were presented as the mean ± standard deviation (SD). Analysis of variance was applied with source of variance being the drying modes (CAD and SD) in order to estimate the least significant differences (LSD) among the media of water content, pH, aw, color properties, TVB-N and TMA content and some microbial flora, at a confidence level of 95% ($p < 0.05$). Statistical treatment of results was executed using the analysis design procedure of Statgraphics Plus software for Windows (1994, version 4.1, Levallois-Perret, France).

RESULTS and DISCUSSION

Drying behavior

Octopus samples were dried using two modes: sun drying in open air at 28°C and convective air drying at 30°C. Monitoring the water content during drying, enabled setting up the drying kinetics curves (Figure 1).

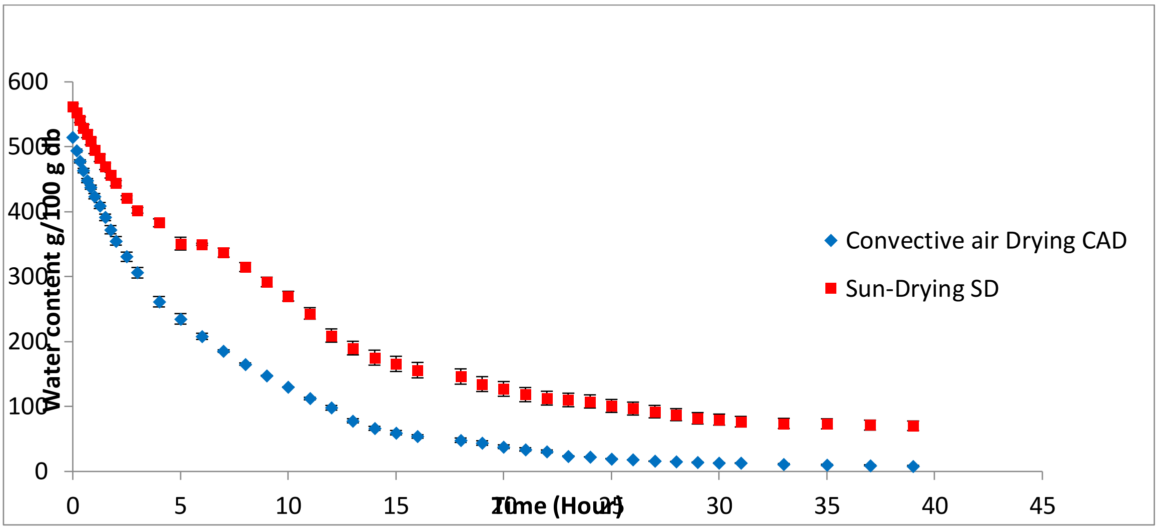

Figure 1. Water content evolution during drying in two different modes (CAD and SD).

Both curves showed similar behavior where the rate of water content decrease was high during a first drying phase then slowed down reaching equilibrium. This behavior is classically encountered when drying food products. Indeed, during drying, two simultaneous mechanisms take place in the product i.e. surface convective evaporation and water diffusion throughout the product (Hajji, Bellagha, & Allaf, 2020) . However, water transfer from the product surface to the drying air flow becomes less important and is overwhelmed by the internal mass flow resistance and the drying curves show a slowing down (exponential) shape. The diffusional phenomenon is a complex mechanism involving water in both liquid and vapor states which depends on temperature, pressure and water content (Hajji et al., 2020). As water content decreases during drying, more energy is required for water evaporation and diffusion through tissue capillaries, which results in a drying curve slowdown (Fig. 1).

Despite the similarity in behavior described in Figure 1, SD method clearly led to a slower drying treatment. Open air drying depends on weather conditions and recorded temperature during this study was about 20°C which was already a lower temperature than the convective drying one (30°C). This figure showed that throughout the drying process, octopus water content dried by the traditional method (in the open air) were higher than that of octopus treated in the dryer. Thus, after 39h drying, octopus samples exhibited a water content of 84.78 and 70.352 g/100g db, for SD and CAD, respectively. This subjected the SD samples to risks of contamination and proliferation of microorganisms and allowed setting up degradation reactions which often require higher water content. A water activity between 0.4 and 0.5 was targeted using these treatments. Octopus samples submitted to CAD reached this water activity after 39 hours while 168 hours were necessary for open air-dried samples.

Dried octopus samples quality

Drying treatments were stopped when the product water activity was about 0.45 (0.435 ± 0.029 for CAD and 0.463 ± 0.001for SD). The final dried octopus samples quality was assessed through several physico-chemical and microbial parameters.

Physico-chemical parameters

Table 1 presented values obtained for the various quality parameters analyzed.

Table 1. Physico-chemical parameters of sun dried and convective air-dried octopus samples.

Parameters		Before drying (fresh)	Convective air drying	Sun drying
Water content (g H_2O/**100g** wb)		$83.73^{a} \pm 0.3$	$15.00^{b} \pm 0.22$	$15.78^{b} \pm 1.64$
a_w		$0.860^{a} \pm 0.008$	$0.435^{b} \pm 0.029$	$0.463^{b} \pm 0.001$
TVB-N (mg N/100 g wb**)**		$5.1^{a} \pm 1.2$	$6\ ^{b}\pm0.59$	$9.46\ ^{c}\pm1.81$
TMA (mg N/100 g wb)		$4.21^{a} \pm 0.5$	$5.19^{b} \pm 0.15$	$6.68^{c} \pm0.59$
pH		$6.58^{a} \pm0.02$	$6.49^{b} \pm 0.01$	$6.38^{b} \pm 0.07$
Color properties	L	$66.60^{a} \pm 0.12$	$50.28^{b} \pm 0.8$	$44.04^{c}\pm1.83$
	a	$0.61^{a} \pm 0.22$	$6.46^{b} \pm 0.47$	$7.38^{c} \pm 0.04$
	b	$3.16^{a} \pm0.15$	$12.83^{b} \pm 0.72$	$11.46^{c} \pm0.35$
	ΔE	-	19.84^{a}	24.97^{b}
	BI	1.11^{a}	11.48^{b}	14.2^{c}

Data are recorded as the mean ± standard deviation. Values having the same lowercase letter (a,b, and c) for different Physico-chemical parameters are not significantly different at a confidence level of 95%.

Proximate octopus composition before drying showed a high water content (83.73 g/100g) which is higher than 80.4% found by Zlatanos et al. (2006). At the end of the drying treatments, water content reached 15 g/100g for CAD samples and 15.78 g/100g for SD octopus. These low water contents ensure product stability as long as the product is kept from potential humidity absorption thus as long as it is stored in waterproof packaging. Indeed, low moisture content with water activity below 0.6 ensures dried product quality and stability.

TVB-N and TMA may be used as quality parameters to evaluate the state of deterioration of the product for some treated seafood products. TVB-N may be related to spoilage bacteria and endogenous enzymes activities. Low TVB-N content under the threshold limit of 20mg N/100g (Lu, Luo, Zhou, Bao, & Feng, 2014) was observed in fresh octopus highlighting its quality. The TVB-N values recorded in this study for octopus dried under sun and convective air-drying modes are lower than the limits set by the standard (20 mgN/100g). Hence, considering the TMA values found for the dried samples which are also beneath the recommended standard (17%) (OFIMER, 2006), dried samples obtained by sun drying and by convective air treatment were well preserved. However, TVB-N and TMA values recorded in this work are lower for CAD treated octopus samples than sun dried ones, which suggested that CAD treatment leads to a better preservation. In their study on sun drying at 30°C during 5 days of *C. dorab* and *C. coeruleopinnatus,* Arulkumar, Balamurugan, Paramasivam, Rameshthangam, and Paramithiotis (2017) observed an increase in TVB-N content from an initial 22.8 and 16.2 mg/100 g to 35.9 and 33.13 mg/100 g respectively. While drying squid at different temperatures between 50 and 90°C, (Vega-Gálvez et al., 2011) observed an increase in the TVB-N level with drying temperature increase and explained this behavior by the release of volatile compounds during high temperature treatments.

Dried octopus pH has slightly dropped regardless of the drying mode. This observed pH drop may be explained by the release of COOH groups from amino acids following protein denaturation. In addition, as higher water contents were observed all along of the sun drying treatment (fig. 1), the slightly higher acidity in the sun-dried samples may be correlated to a higher anaerobic lactic flora proliferation.

Figure 2 shows dried octopus obtained by both treatments. The dried product, which has acquired a brown color, appears darker than the fresh octopus. These observations are supported by the values of the Hunter color parameters L, a and b. Indeed, L decreased after drying while the parameters a and b increased; Lower L values describes a darker product (**Table 1**). These observations agree with other described results as described in the work of (Vega-Gálvez et al., 2011) . where L values decreased from 78 to 65 for fresh and dry-rehydrated jumbo squid samples.

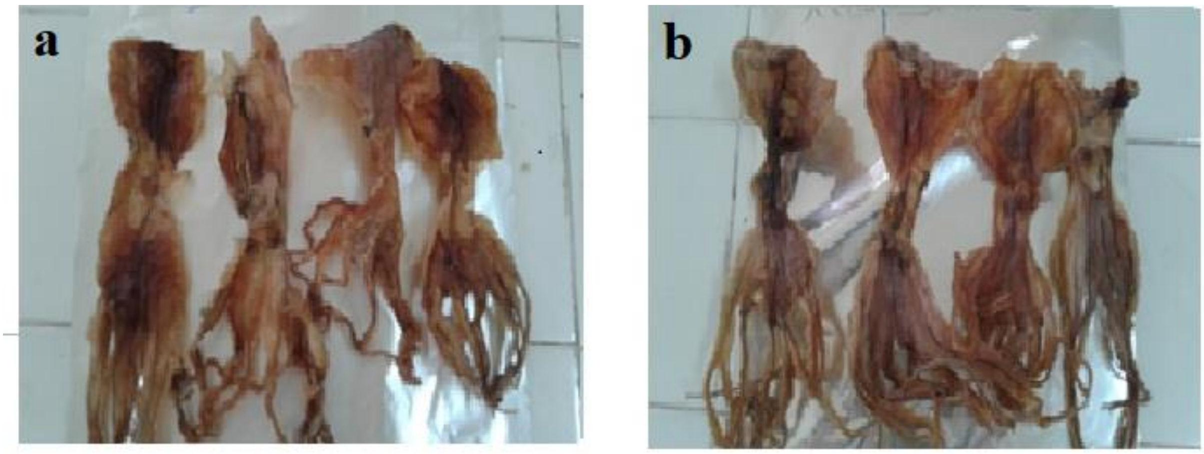

Figure 2. Dried octopus (*octopus vulgaris*): (a) convective air dried; (b) sun dried.

Color change is mainly due to non-enzymatic browning reactions (Maillard) which take place during drying. Amino acids react with sugar molecules under the action of increased temperature but also of lower drying rate which allows keeping the product under intermediate water content accelerating the Maillard reaction (Tamanna & Mahmood, 2015) . Indeed, SD octopus samples showed a L value lower than the CAD ones, thus a stronger brownish color illustrating that the magnitude of these non-enzymatic reactions were more important with the longer drying time required for SD treatment. ΔE value which represents color change between fresh and dried samples was lower for CAD treated octopus illustrating the higher impact on sample surface color after SD (**Table 1**). Browning extent during drying may also be evaluated trough the browning index. Indeed, the calculated BI increased from 1.11 for fresh octopus to 11.48 and 14.2 for CAD and SD treatments respectively, supporting the dried samples darkening but also the stronger browning impact during SD treatment. The difference in the BI increase between SD and CAD treated samples was supported by the appearance of the products (**Fig. 2**) and may be explained by the difference in browning reaction rates. As well described by Labuza (1975), non-enzymatic browning reactions are accelerated in intermediate water content products, which is the case of foods during drying. Thus, the magnitude of these reactions will be all the more important as the time required for the product to reach its final water content is long. In this study, SD samples keep having water content as high as 70.352 g/100g db while CAD octopus samples exhibit a water content of 8.478g/100g db (**Fig. 1**). Interestingly, observed data strongly support the fact that intermediate water content, which is maintained during a longer time in SD treatment, has a stronger effect on product darkness than higher temperature. However, this may also be related to the fact that used temperatures in this work are not that high (28°C, for SD and 30°Cfor CAD). Ideally then, drying treatments should be thought as short treatments reducing water content in order to reduce browning time.

Microbial quality

Microbial composition of fresh, convective air dried and sun dried octopus samples are summarized in **Table 2**.

Table 2. Microbial flora detected in convective air dried and sun-dried octopus.

Microorganism (log UFC/g)	Fresh octopus	Convective air dried	Sun dried
Yeast and Molds	3.79±1.89	0	0
Total Viable Count	4.11a±3.25	2.90b±2.08	4.30c±3.26
Total Coliform	3.40a±2.72	1.78b±1.04	2.78c±1.79

Data are recorded as the mean ± standard deviation. Values having the same lowercase letter (a, b, and c) for different microbial flora are not significantly different at a confidence level of 95%.

Initial counts were 3.79±1.89, 4.11±3.25 and 3.40±2.72 log cfu/g respectively for YM, TVC and TC while no FC, nor *Staphylococcus aureus* and sulfite reducer bacteria were detected. Drying through either method has inhibited YM flora and decreased the TC. Similar behavior has been reported in other studies(Arulkumar et al., 2017). This may be explained by the dried product composition and mainly by the water content reduction and the low water activity (Arulkumar et al., 2017). However, it is interesting enough to notice that the TVC in the SD sample has increased (4.30103±3.26 log UFC/g) while CAD samples show an important reduction (2.90±2.08 log UFC/g). Lower TVC was also registered after convective air drying than sun drying of Freshwater Barb, Punti (*Puntius sophore*) and of Gangetic Catfish, Gulsha (*Mystus cavasius*) (Arulkumar et al., 2017). The longer drying period required to dry products in open air often leads to microbial proliferation since high water content levels are maintained during longer periods and are associated to temperatures (20°C in this work) favorable to bacteria multiplication. In other words, low drying rate observed during SD maintained high water levels e.g. over 120g/100g db after 20h (**Fig 1**) promoting bacterial proliferation.

Rehydration

Dehydrated foods often need to be rehydrated before consumption. Water imbibition is expected to induce tissue swelling. Thus, rehydration is a complex process aimed at restoring the properties of the raw material when applicable and may be used as a tool to evaluate the extent of damages suffered by the heat-treated product (Vega-Gálvez et al., 2011).

Rehydration rate (RR) kinetic of octopus dried according to the two drying methods is illustrated in **Figure 3**. Rehydration curves show a high rate of water absorption during the first 15 minutes of imbibition followed by a lower slope which illustrates that rehydration is reaching rapidly equilibrium. Rehydration capacity of a food product is linked to the extent to which protein, as a water holding component, among others, may have been denatured during drying (Murali et al., 2021).

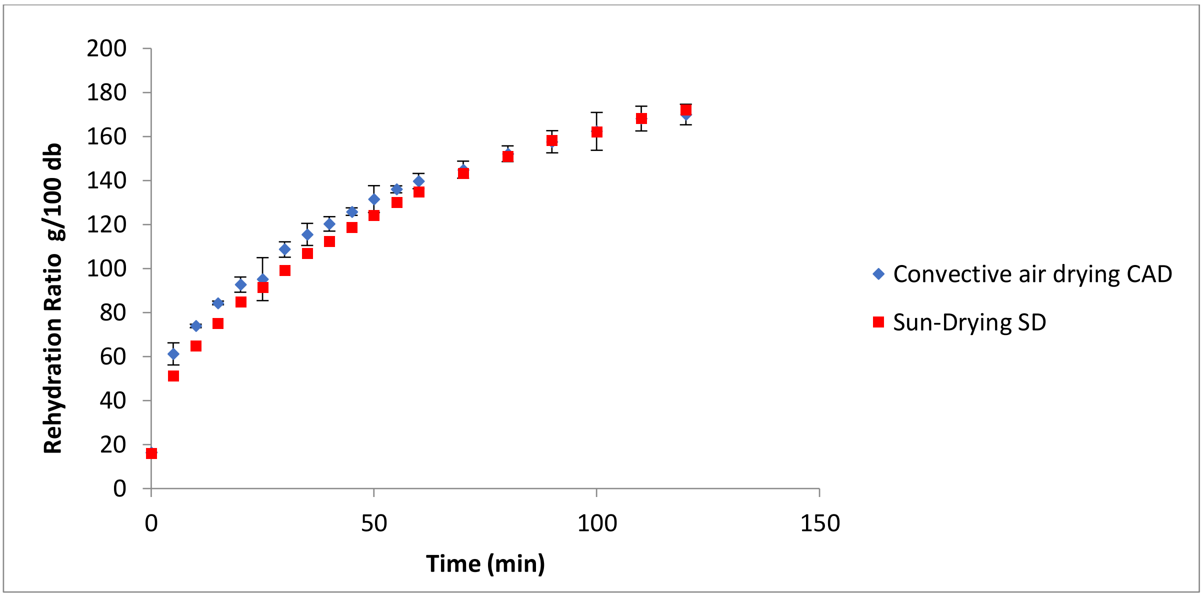

Figure 3. Rehydration Ratio of sun dried and convective air-dried octopus.

Drying modes i.e. SD at 20°C and CAD at 30°C, did not seem to influence significantly the rehydration capacity of the dried octopus samples which is clearly observed trough the curves that nearly coincide (figure 3). Vega-Gálvez et al. (2011) in a study on drying of jumbo squid (*Dosidicus gigas*) observed that rehydration ratio was related to drying temperature, the higher the temperature the lower the RR. However, drying air temperature essayed in their works ranged from 50 to 90°C and the largest RR decrease was noted starting at 70°C. Hydration recovery is directly related to the damages and depends on the structural changes in the tissues and cells undergone during drying. Irreversible changes such as capillaries obstruction due to soluble substances deposition, tissue shrinkage and collapse as well as cells disruption are enhanced by higher drying temperature preventing complete rehydration.

Indeed under moderate drying temperature and higher drying rate milder deformation in food matrixes should be observed (Doymaz, 2014).

In both cases, it should be noted that rehydrated octopuses no longer regain their initial water content.

CONCLUSIONS

Comparing sun dried and convective air-dried octopus in this study showed a faster drying kinetic for CAD treatment which reduced microbial flora, TVB-N and TMA contents in the dried product. In addition, color which is one of the main buying criteria of food products was better preserved after convective air drying at 30°C with a lower ΔE and browning index. BI value may be used to illustrate the extent of non-enzymatic reactions which may participate in lowering the nutritional value of a product.

Hence, convective air drying at low temperature, 30°C, may be considered as a quality preserving option for dried octopus. In addition, unpacked samples were considered in this work in order to better approach the usual practice of local small fisheries, however, packaging effect may be studied and a better preservation should be expected.

Conflict of interest

All authors declare no conflict of interest.

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Author Contributions

The theoretical framework of the study, the data collection and analysis process was carried out by Wafa HAJJI, the article was written by Wafa HAJJI. The revision of the article was jointly by both authors and the final version was approved together.

Responsible Artificial Intelligence Statement

No artificial intelligence support was received in any part of this study.

Ethics Approval

This study does not require ethics committee approval

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