Kelly de Moraes and Luiz Antonio de Almeida Pinto*

Drying Kinetics, Biochemical and Functional Properties of Products in Convective Drying of Anchovy (*Engraulis anchoita*) Fillets

Abstract: The aim of this work was to study the convective drying of anchovy (*Engraulis anchoita*) fillets and to evaluate the final product characteristics through its biochemical and functional properties. The drying temperatures were of 50, 60 and 70°C, and the fillet samples were dried with the skins down (with air flow one or the two sides) and skins up (with air flow one side). The drying experimental data were analyzed by Henderson–Pabis model, which showed a good fit ($R^2 > 0.99$ and REQM < 0.05). The moisture effective diffusivity values ranged from 4.1×10^{-10} to 8.6×10^{-10} $m^2\ s^{-1}$ with the skin down and 2.2×10^{-10} to 5.5×10^{-10} $m^2\ s^{-1}$ with the skin up, and the activation energy values were 32.2 and 38.4 kJ mol^{-1}, respectively. The product characteristics were significantly affected ($p < 0.05$) by drying operation conditions. The lower change was in drying at 60°C with air flow for two sides of the samples and skin up. In this condition, the product showed solubility 22.3%; *in vitro* digestibility 87.4%; contents of available lysine and methionine 7.21 and 2.64 g 100 g^{-1}, respectively; TBA value 1.16 mg_{MDA} kg^{-1}; specific antioxidant activity was 1.91 mM_{DPPH} g^{-1} min^{-1}, and variation total color was 10.72.

Keywords: drying fish, antioxidant activity, diffusivity, available lysine, lipid oxidation

***Corresponding author: Luiz Antonio de Almeida Pinto,** School of Chemistry and Food, Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil, E-mail: dqmpinto@furg.br
Kelly de Moraes, Federal University of Rio Grande (FURG), Rio Grande do Sul, Brazil, E-mail: kellydemoraes@globo.com

1 Introduction

Fish and seafood are important in many countries. These products are great sources of nutrients such as proteins (15–27%), with all essential amino acids, and high content of lysine, an amino acid starter of the digestive system. Besides the quality protein, fish are a good source of many lipids and polyunsaturated fatty acids [1, 2]; however, having approximately 80% of water, they are highly perishable [3]. Anchovy (*Engraulis anchoita*) is a pelagic species of high biomass in South America coast (Argentine, Brazilian and Uruguay); however, their stocks still remain little explored, but with possibilities for a sustainable exploitation. Therefore, in Brazil, there is a tendency to develop alternative products based on anchovy, which would assign new markets and could be included in government social programs to fight hunger. Research to use a formula to meet the requirements is necessary, especially in relation to the processing of dehydrated products [4].

Drying has been used by several countries around the world to preserve food and biological materials, such as fish species and processing wastes, this is an important method of processing and preserving aquatic products [5]. The removal of moisture content during the drying process is greatly affected by the conditions of the drying air as well as the dimensional characteristics of the material and composition. The effect of the drying parameters on the removal of moisture content expressed by kinetic models have been studied for different species of fish [3, 6–9]. However, the drying conditions such as temperature, moisture content, thickness, composition and geometry of the material have a great influence on the food properties, such as flavor, color and nutritional composition. Therefore, this operation must be done carefully to avoid any loss in quality, improving the life of these products [5].

The nutritional value loss during food processing may result from the interactions of lipid oxidation products, many reacting with proteins, which are vital components of our diet and need to be specially protected in all processes related to the technological processes. The nutritional value of proteins in meat products is determined by the qualitative and quantitative composition of amino acids and through the susceptibility of proteins to hydrolysis by digestive enzymes. The digestibility and amino acid availability decreases as a result of crosslinking of protein–lipid complexes and reactions of the functional groups of amino acids with lipid oxidation products. This is especially true for the amino-sulfhydryl

and hydroxyl groups [10–12]. Nutritional losses of lysine and amino acid chains containing sulfur (methionine) are crucial, since they are exogenous amino acids and, at the same time, may limit the nutritional value of proteins in many products [12]. The proteins are susceptible to oxidation, and these can then be used to inhibit oxidative reactions, thereby protecting the oxidatively labile fatty acids. The ability of the protein to react with free radicals in food may also lead to the development of new antioxidant technologies. The oxidative stability of foods is related to the balance between antioxidant and pro-oxidant factors [10].

In this context, the objectives of this study were to analyze the convective drying in thin layer of anchovy (*E. anchoita*) fillets and to evaluate the dried product compared to *in natura* fillets, for their characteristics (solubility, *in vitro* digestibility, available lysine and methionine, lipid oxidation, antioxidant activity and color).

2 Materials and methods

2.1 Materials

In this study, anchovy (*E. anchoita*) was used in the form of fillets. All reagents used in analyses were of analytical grade.

The anchovy samples were captured by the South Atlantic Oceanographic Ship in southern Brazil. The same were stored on board using a ratio of one part of ice, one of sea water and two of anchovy (1:1:2–ratio by mass) for 24 h. The anchovy samples were then washed in chlorinated water (5 mg kg^{-1} residual chlorine free), eviscerated and filleted, and for the drying tests, fillets with initial thickness of approximately 4.0 ± 0.3 mm were selected. The selected fillets were washed again with a 0.3% NaCl solution and then with deionized water. The samples were then drained for 2 h under refrigeration between 4 and 7°C and then frozen at –18° C until the time of use.

2.2 Drying operation

The experimental runs were performed in batch tray dryer with parallel flow of the drying air. The drying air conditions were: temperatures of 50, 60 and 70°C, absolute humidity of 0.013 ± 0.002 kg kg^{-1} (dry basis) and air velocity of 2.5 m s^{-1}. The fillet samples were dried through experimental runs with air flow on one side of the samples with the skin facing upward (SU1s) and with skins down (SD1s) and experimental runs with air flow on both sides of the samples with the skin facing down (SD2s). The fillets thickness was 4.0 ± 0.2 mm, and areas of the trays were 0.0168 and 0.0256 m^2 for the air flow on one side and two sides, respectively, keeping the load at approximately 3.25 ± 0.15 kg m^{-2}. The samples were weighed at pre-established intervals on an electronic scale (Marte, model AS 2000C, Brazil), with a precision of ±0.01 g, until they reached the commercial moisture (≅0.10 kg kg^{-1}, dry basis). The relative humidity of air was measured using a thermohygrometer (Cole Parmer model 331-00, USA) with a precision of 0.1%. After drying experiments, all products were ground in a mill (model Willey no. 3, Philadelphia, USA) and sieved (80–100 Tyler) The samples were stored in plastic bags under protection from light, at room temperature and analyzed a day after drying. All experiments were performed in duplicate.

2.3 Drying kinetics

The semi-theoretical models of thin layer drying have been used to model the drying of various foods of plant and animal origins [3, 9, 13, 14]. The Henderson and Pabis model [eq. (1)] is chosen to estimate the effective diffusivity for having physical significance, since it is analogous to simplification of the analytical solution of the diffusive model for long drying times, where only the first term of the series is considered [15].

$$[(X - X_e)/(X_0 - X_e) = A \exp(-(Kt)) \quad [1]$$

where [(X–Xe)/(X0–Xe)] is the dimensionless free moisture content, A is adjustment parameter, K drying constants (min–1) and t is drying time (min).

The drying constant K is thus related to the moisture effective diffusivity (D_{eff}) in accordance with eq. (2) for air flow on one side and eq. (3) for air flow from both sides [15].

$$K = \frac{\pi^2 D_{eff}}{4L_m^2} \quad [2]$$

$$K = \frac{\pi^2 D_{eff}}{L_m^2} \quad [3]$$

Due to the shrinkage that occurs in the material during drying, the average thickness value (L_m) should be considered in eqs (2) and (3). This can be calculated from the values of the initial (L_0) and final (L_f) thicknesses of the sample. Because, according to Pinto and Tobinaga [7], when studying the drying of hake and

sardine, it has been determined that in the fish fillets the shrinkage occurred mainly in the thickness of the sample, and it showed a linear behavior in function of the moisture content.

The dependency of the diffusion coefficient in relation to temperature of the drying air can be described based on the Arrhenius relationship [eq. (4)] [15].

$$D_{eff} = D_0 \exp\left(\left(\frac{-E_a}{RT}\right)\right) \quad [4]$$

where D_0 is a pre-exponential factor of diffusivity at infinite temperature ($m^2\ s^{-1}$), E_a is the activation energy ($J\ mol^{-1}$), R is the universal gas constant (8.314 J $mol^{-1}\ K^{-1}$) and T is the absolute drying temperature (K).

2.4 Analytical methodology

2.4.1 Proximal composition

The percentage composition of *in natura* and dried anchovy fillets were determined by methods of AOAC [16] for moisture content (950–46), ash content (920–153) and protein content (928–08). The lipids content was quantified according to Bligh and Dyer [17].

2.4.2 Protein solubility

The protein solubility was determined according to Morr et al. [18] in deionized water. Readings were performed using a spectrophotometer (Quimis, model 108DRM, Brazil) at a wavelength of 660 nm. Calculations were based on standard curve of bovine serum albumin, with concentrations between 0.1 and 1.0 $mg_{BSA}\ mL^{-1}$. The solubility was determined through the relation between the content of soluble protein and the total protein in the sample (determined by micro-Kjeldahl [16]).

2.4.3 *In vitro* digestibility

For *in vitro* digestibility, an equivalent of 50 mg protein (dry basis) was weighed and subjected to hydrolysis with 10 mL of pepsin suspension (1.5 mg mL^{-1} in 0.1 N HCl) for 3 h in a thermostated water bath at 37°C. Later, the pH was adjusted to 7.0 (noting down the required volume). A volume of 10 mL of a pancreatin suspension (1.5 mg mL^{-1} in phosphate buffer pH 8.0) was added to the system for continuous hydrolysis for 24 h at 37°C with stirring. Ten milliliters of trichloroacetic acid 40% was added to the system and allowed to stand in a refrigerator at 4°C for about 2 h. The sample was then centrifuged at 3,560 × *g* (Fanem, model Baby I 206BL, Brazil) for 5 min and later filtered. The filtrate was adjusted to pH 7.0 (recording the volume required), and the sample was stored at 4°C until use in the quantification of *in vitro* digestibility, in order to determine available lysine and methionine. *In vitro* digestibility was calculated according to eq. (5):

$$\text{Vitro digestibility}(\%) = \frac{N_{sol} - N_{enz}}{N_{total}} \times 100 \quad [5]$$

where N_{sol}, soluble nitrogen after enzymatic digestibility, N_{enz}, total nitrogen of the digestibility's blank without the sample measured and N_{total}, total nitrogen of the sample measured by micro-Kjeldahl [16].

2.4.4 Available lysine and methionine

The quantification of the availability of lysine and methionine was determined in the adjusted pH filtrate after the *in vitro* digestibility.

The available lysine was determined by colorimetric reaction of lysine with OPA (*o*-phtaldialdehyde) according to Dinnella et al. [19] with minor modifications. A known amount of the filtrate (pH adjusted) was diluted in phosphate buffer pH 7.0 at room temperature. An aliquot of 50 µL of the diluted filtrate was mixed with 1 mL of the OPA reagent (*o*-phtaldialdehyde – a complete solution of 1 $mg_{OPA}\ mL^{-1}{}_{2\text{-mercaptoethanol}}$). The sample was stirred in an ultrasonic bath for 2 min at room temperature and was read at 340 nm on a spectrophotometer (Varian Cary, 100 UV–VIS, USA). Quantification was based on a standard curve of L-lysine in phosphate buffer pH 7.0 (0.01–0.2 mg mL^{-1}).

For available methionine, an aliquot of 1 mL sample was removed from the filtrate (pH adjusted) and diluted to 10 mL. From this diluted sample, 1.0 mL aliquot was removed and mixed with 0.5 mL of NaOH 5 N, 1.0 mL of 2.5% sodium nitroprusside and 1.0 mL of distilled water. After 5 min, 1.0 mL of 8 N hydrochloric acid was added. After 5 min, reading was performed at 510 nm in a spectrophotometer (Quimis, model Q-108DRM, Brazil). The concentration calculations were based on methionine standard curve (from 0.02 to 0.2 mg mL^{-1}). The total contents of lysine and methionine *in natura* fillets were determined by acid hydrolysis with hydrochloric acid concentrated and autoclaved at 121°C for 30 min. Analyses were performed in triplicate and results were expressed as g (lysine and/or methionine) per 100 g protein (dry basis).

2.4.5 Specific antioxidant activity

The specific antioxidant activity (SAA) was determined by the ability to sequester free radicals, represented by the 1,1-diphenyl 2-picrylhydrazyl (DPPH), according to the methods described by Foh et al. [20] and Vega-Galvez et al. [9], with little modifications. A sample (2 mL) of soluble protein, previously diluted and of known concentration (~0.65 $mg_{protein}$ mL^{-1}) was mixed with 2 mL of 0.15 mM DPPH (in 95% ethanol). The mixture was stirred vigorously and allowed to stand in the dark for 30 min. The absorbance of the solution was read at 517 nm (Quimis, model Q-108DRM, Brazil). Analyses were performed in triplicate.

The antioxidant activity was expressed as the specific amount of free radical DPPH sequestered by the protein in 30 min, according to eq. (6).

$$SAA = \frac{1 - \left(\frac{(Abs\_DPPH_{reaction} - Abs\_sample)}{(Abs\_DPPH_{blank})}\right)}{(m_{protein} \times t)} \quad [6]$$

where Abs_DPPH_{blank} is the absorbance value of 2 mL ethanol 95% mixed with 2 mL DPPH solution 0.15 mM; $Abs\_DPPH_{reaction}$ is the absorbance value of 2 mL protein solution mixed with 2 mL DPPH solution 0.15 mM; Abs_sample is the absorbance value of 2 mL protein solution mixed with 2 mL 95% ethanol; $m_{protein}$ is a known amount of protein in solution (g), and t is time (min) of the sample that allowed to stand, in this case 30 min. The SAA was expressed in mM_{DPPH} $g^{-1}_{protein\ sol}$ min^{-1}.

2.4.6 Lipid oxidation

The lipid oxidation of *in natura* and dried fillet was evaluated by the TBA (thiobarbituric acid) values. Lipids from samples were extracted cold according to Bligh and Dyer [17], using 50 g of fillet samples and 10 g of dry minced fillet. The TBA value was determined according to Vyncke [21]. The quantification of the TBA values was based on a standard curve obtained from the reaction of tetramethoxy propyl ranging from 1×10^{-9} to 7×10^{-9} mol $mL^{-}_{TBA(0.02\ M)}$. The results were expressed as mg of malondialdehyde (MDA) per kg of sample on dry basis.

2.4.7 Color

The color of the surface of the *in natura* and dried fillets was determined using a colorimeter (Minolta, model CR–400, Osaka, Japan). Color was expressed in a three-dimensional diagram of colors – CIE (L^*, a^*, b^*) the coordinates being: L^* luminosity from (0) black to (100) white, a^* hue varying from (–) green to (+) red and b^* saturation from (–) blue to (+) yellow. D65 was used as a calibration standard and as observer 10°. Analyses were performed in triplicate.

The results were expressed as ΔE [eq. (7)], which is an absolute number which indicates the difference in total color sensation, including brightness, hue and saturation.

$$\Delta E = \left[(a_i^* - a_0^*) + (b_i^* - b_0^*) + (L_i^* - L_0^*)\right]^{1/2} \quad [7]$$

where a_i^* b_i^* L_i^* are the coordinate values of the dehydrated product in each experiment, and a_0^* b_0^* L_0^* are the values -of the control sample (*in natura* fillets).

2.4.8 Statistical analysis

For the statistical analysis of drying kinetics and characteristics of *in natura* and dried samples of anchovy fillets, the software Statistica 6.0 for Windows (StatSoft, Inc., USA) was used.

The drying kinetics data were adjusted by nonlinear regressions. The evaluation of the quality of the adjustment of kinetic model was checked by the coefficient of determination (R^2) and by root mean square error (RMSE) [eq. (8)].

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(X_{ei} - X_{pi})^2} \quad [8]$$

where X_{ei} and X_{pi} are, respectively, the experimental moisture content and that predicted by the model (kg $kg^{-1}_{dried\ solid}$), and N is number of experimental points.

The results were evaluated using analysis of variance and differences between means by Tukey test (HSD) in a confidence range at level 95% ($p < 0.05$) [22].

3 Results and discussion

3.1 Kinetics drying

Figure 1 shows the drying rate curves of the anchovy fillets, in thin layer, at three temperatures (50, 60 and 70°C) for the arrangements of the fillet samples and the air flow. It can be seen that the drying rate decreased with decreasing temperature of the different arrangements of the fillet samples and the air flow. In the drying air flow from one side, the rate was lower with the skin

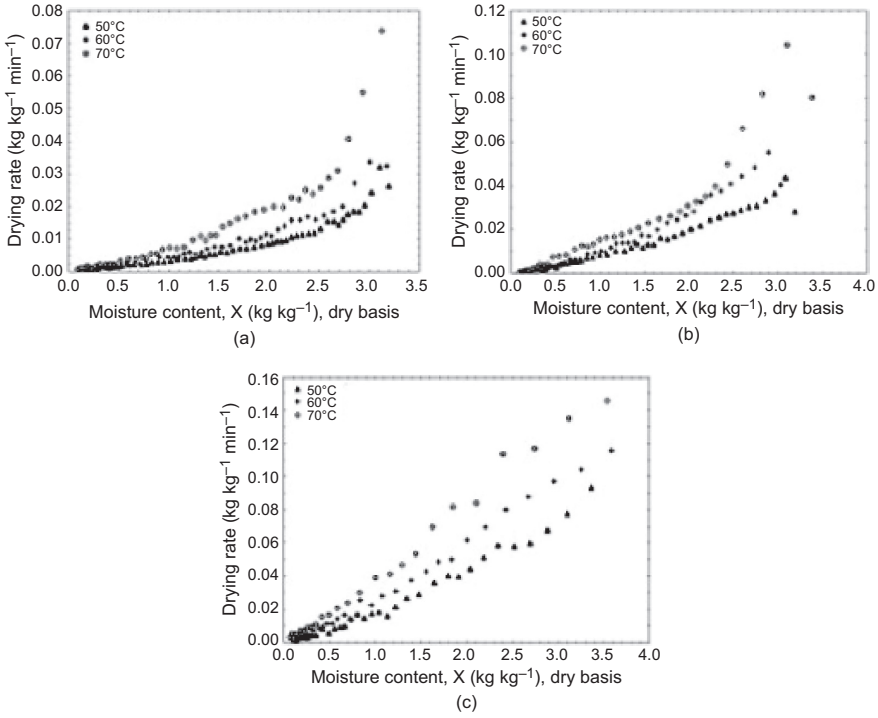

Figure 1 Curves of drying rate versus moisture content from anchovy fillets at air temperatures (▲) 50°C, (+) 60°C and (o) 70°C: (a) fillets dried with skin up with air flow along one side (SU1s); (b) fillets dried with skin down with air flow along one side (SD1s); (c) fillets dried with skin down with air flow along both sides (SD2s)

side up (SU1s) because of the barrier created by the skin for the moisture diffusion. The highest drying rates were observed with drying air flow on both sides (SD2s), due to the drying area being approximately double compared to the air flow on one side (SD1s).

The constant rate of drying, being one of the characteristics of foods with high protein content [15], was not observed in the experiments shown in Figure 1. All the drying experiments of the fillet samples occurred in the falling rate period, during which the internal molecular diffusion is the predominant mechanism of mass transfer. This behavior is typical of many biological materials as reported in the literature by Hadrich et al. [23] and Djendoubi et al. [3] in the drying of sardine fillets, Vega-Gálvez et al. [8] for residues of yellow lobster, Oliveira et al. [24] in the drying of microalgae and Kaya et al. [13] in the drying of kiwi.

Figure 2 shows the characteristic curves of dimensionless free moisture content versus time of drying, which were obtained from the data of the moisture content (X) of drying experiments and the equilibrium moisture content (X_e). Figure 1 shows the influence of air temperature and the arrangement of the fillet samples and air flow can be observed. The equilibrium moisture content values (X_e) were determined in drying experiments, for each condition studied until reaching constant weight of the samples. These values were 0.070, 0.039 and 0.024 kg kg^{-1} (dry basis), for temperatures of 50, 60 and 70°C, respectively. The moisture content values reached in experiments were found in a range from 0.074 to 0.115 kg kg^{-1}, dry basis (equivalent from 6.9 to 10.3%, wet basis). These values are within the range recommended for the production of aquatic products based snacks, which are 8–9%, wet basis [5].

The dimensionless free moisture content curves were adjusted by Henderson and Pabis model [eq. (1)] to describe the drying behavior of anchovy fillets. The results in the different drying conditions are shown in Table 1. In this table, it is observed that the drying constant (K) increased with temperature and varied with the disposition of the fillets and air flow, reaching the highest values in the samples dried on both sides.

For all experiments, the RMSE < 0.05 and the coefficient of determination (R^2) were above 99%, which represents a suitable adjustment of the model for the drying phenomena according to Denjadoubi et al. [3]. Konishi et al. [6], studying the drying of sausages based on fish paste, observed at 50°C, a drying constant of 0.22 h^{-1} (0.0037 min^{-1}), a value similar to that presented in this work, at the same temperature, for the fillets dried with skin side up (SU1s). The effective diffusivity values shown

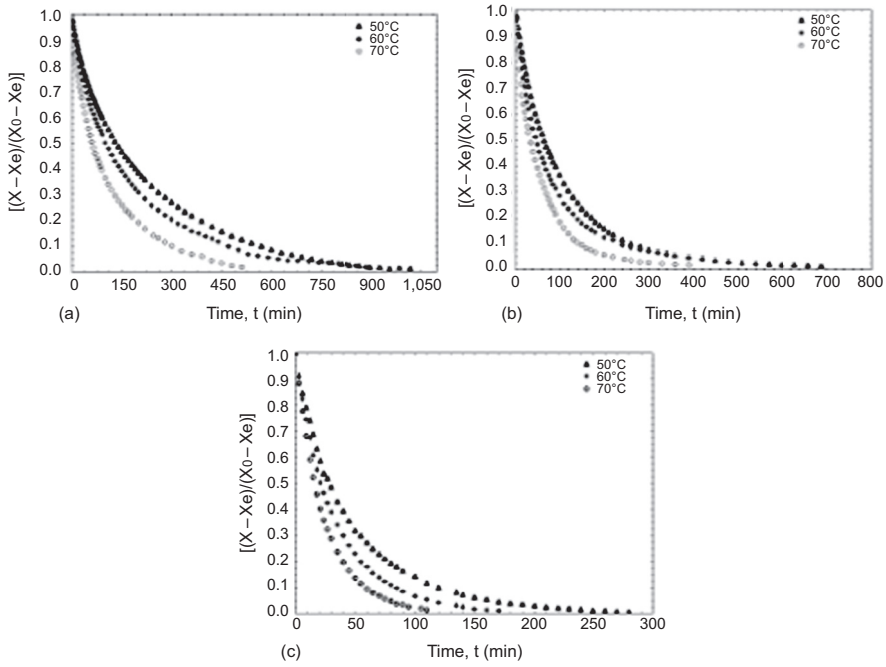

Figure 2 Curves of dimensionless free moisture content versus drying time from anchovy fillets at air temperatures (▲) 50°C, (+) 60°C and (o) 70°C: (a) fillets dried with skin up with air flow along one side (SU1s); (b) fillets dried with skin down with air flow along one side (SD1s); (c) fillets dried with skin down with air flow along both sides (SD2s)

Table 1 Drying constant (K) and the moisture effective diffusivity (D_{eff}) values of anchovy fillets drying for the arrangements of the fillet samples and air flow

Arrangements (fillets/air flow)	Temperature (°C)	K (min^{-1})	R^2	RMSE	Thickness ratio (L_f/L_0)	$D_{eff} \times 10^{10}$ ($m^2\ s^{-1}$)
SU1s	50	0.0043	0.995	0.021	0.38	2.24 ± 0.02
	60	0.0054	0.992	0.026	0.47	3.14 ± 0.03
	70	0.0087	0.990	0.027	0.52	3.47 ± 0.03
SD1s	50	0.0095	0.996	0.018	0.41	4.16 ± 0.04
	60	0.0120	0.988	0.031	0.50	5.95 ± 0.03
	70	0.0167	0.991	0.026	0.57	8.67 ± 0.04
SD2s	50	0.0214	0.997	0.014	0.51	3.31 ± 0.02
	60	0.0295	0.998	0.013	0.53	4.67 ± 0.03
	70	0.0412	0.999	0.009	0.59	7.05 ± 0.04

Notes: *Mean value ± standard error for two experiments. R^2: coefficient of determination; SU1s: fillets dried with skin upward with air flow along one side; SD1s: fillets dried with skin down with air flow along one side; SD2s: fillets dried with skin down with air flow along both sides.

in Table 1 was calculated using eqs (2) and (3) for air flow on one side and on both sides, respectively, which considers the drying values of the constant (K) and the average thickness (L_m) between the beginning and the end of drying experiments.

Table 1 shows that the values of the moisture effective diffusivity (D_{eff}) varied between 2.24×10^{-10} and 8.67×10^{-10} $m^2\ s^{-1}$, increasing with increasing temperature and varying according to the position of the skin in the tray. Drying of the samples on one side with the skin side up (SU1s) caused a decrease in D_{eff} values, compared with the samples with the skin facing down and dried on one (SD1s) and two sides (SD2s) at the same temperature, indicating that the skin presented resistance to moisture diffusion. D_{eff} values for dried fillets with skin facing down on both sides (SD2s) were somewhat lower than values of the samples of the fillets dried with skin down on one side (SD1s), probably due to lesser exposure to the drying air, although not as pronounced as when the skin was side up.

The moisture effective diffusivity values for anchovy fillets were within the range found in the literature for

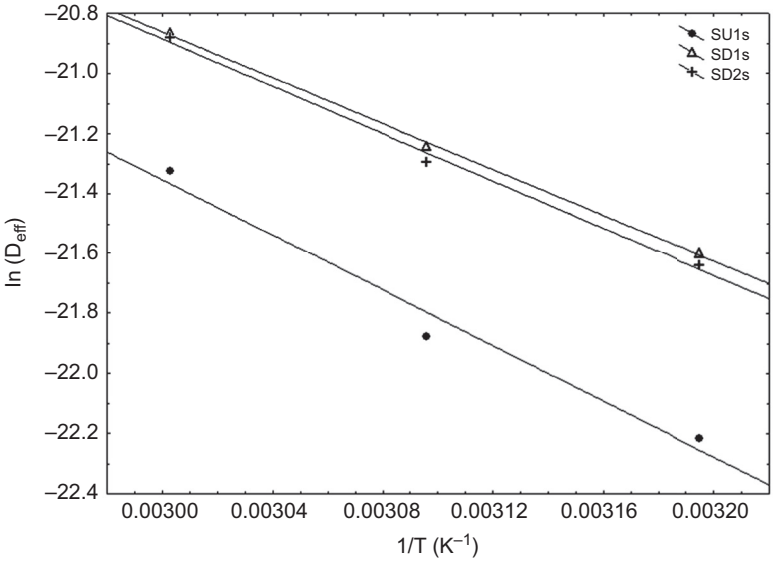

Figure 3 Effective diffusivity of moisture as function of air temperature of the fillet samples of dried anchovy. SU1s: fillets dried with skin upward with air flow along one side; SD1s: fillets dried with skin down with air flow along one side; SD2s: fillets dried with skin down with air flow along both sides

fish according to Panagiotou et al. [25] (from 1.30×10^{-11} to 1.89×10^{-9} m^2 s^{-1}). These authors reported D_{eff} values for dried cod at 60°C of 5.13×10^{-10} m^2 s^{-1}, similar to those found in this study at the same temperature with the skin side down. In a study on sardines drying, Djendoubi et al. [3] observed a variation in the D_{eff} values from 1.38×10^{-11} to 2.21×10^{-11} m^2 s^{-1}, varying the temperature between 40 and 80°C, these values were lower than those found in this work, caused by the fat content of the sardine, which, due to its hydrophobic character, causes a decrease in the rate of drying.

The activation energy (E_a) was calculated by the Arrhenius relationship [eq. (4)] using the effective diffusivity values for each temperature (Table 1), as shown in Figure 3. The E_a values were found to be 38.4 kJ mol^{-1} for samples of dried fillets with skin side up (SU1s), 31.7 and 32.7 kJ mol^{-1} for fillet samples with the skin side down and dried on one side (SD1s) and both sides (SD2s), respectively. These values were similar to fillets of different fish species, reported in the literature, which are approximately 20–30 kJ mol^{-1} [6–9].

3.2 Raw materials and product characterization

The proximate composition of the samples of *in natura* fillets (wet basis) were of 78.7 ± 1.2% moisture content, 17.1 ± 0.8% protein content, 1.3 ± 0.2% ash content and 2.2 ± 0.2% lipids content. The centesimal composition of the fillet had a higher moisture content compared to fresh anchovy fillets from Argentina, according to data reported by Czerner et al. [1], which consequently decreases the content (wet basis) of the other components. The protein and lipid contents (dry basis) were lower than those of anchovy from Argentina by approximately 12% and 30%, respectively. According to data presented by Pastous-Madureira et al. [4], for the centesimal composition of the anchovy from three different countries (Argentina, Uruguay and Brazil), there is a difference in these data due to the location and time of year of capture. According to the season, the moisture content can vary from 69.5 to 79.5%, protein from 15.8 to 19.2%, lipid from 1.68 to 9.43% and ash from 1.1 to 3.1%.

Table 2 shows the results of the characterization of the dried product in the different drying conditions and comparing them with the *in natura* fillets. It can be seen from this table that there was a significant difference ($p < 0.05$) between the dried products and *in natura* fillets with regard to all the above characteristics, except for the color perception range, which is a direct measure of comparison with the default (in this case *in natura* fillet).

The protein solubility, a functional technical characteristic measure of the protein [26, 27], decreased during the drying process reaching a minimum of 16% solubility in the sample 50°C_SU1s. This may be due to the exposure time of proteins to the heat. Myofibrillar proteins at temperatures above 65°C in the presence of water can

Table 2 Characterization of the *in natura* and dried products of anchovy fillets subjected to different drying conditions

Sample	Solubility (%)	*in vitro* digestibility (%)	Available lysine (g 100 $g^{-1}_{protein}$)	Available methionine (g 100 $g^{-1}_{protein}$)	TBA value (mg_{MDA} kg^{-1}_{sample}) (dry basis)	SSA (μMDPPH $g^{-1}_{protein}$ min^{-1})	ΔE
In natura	29.50 ± 0.32^{a}	91.53 ± 0.45^{a}	8.571 ± 0.019^{a}	2.671 ± 0.030^{a}	0.622 ± 0.054^{i}	2.127 ± 0.010^{a}	–
50°C_SU1s	16.07 ± 0.19^{f}	81.25 ± 0.23^{d}	6.772 ± 0.051^{d}	1.455 ± 0.006^{h}	3.894 ± 0.035^{a}	1.202 ± 0.006^{h}	11.89 ± 0.16^{c}
60°C_SU1s	19.35 ± 0.31^{de}	80.32 ± 0.13^{d}	5.769 ± 0.021^{e}	2.365 ± 0.006^{d}	1.593 ± 0.019^{f}	1.304 ± 0.005^{g}	13.00 ± 0.04^{b}
70°C_SU1s	21.98 ± 0.23^{c}	79.04 ± 0.18^{e}	5.500 ± 0.025^{f}	1.954 ± 0.017^{f}	2.408 ± 0.019^{d}	1.281 ± 0.004^{g}	14.31 ± 0.06^{a}
50°C_SD1s	22.08 ± 0.14^{c}	83.97 ± 0.15^{c}	6.924 ± 0.014^{c}	1.857 ± 0.011^{g}	3.099 ± 0.048^{b}	1.342 ± 0.005^{f}	10.02 ± 0.04^{g}
50°C_SD1s	26.47 ± 0.26^{b}	84.29 ± 0.22^{c}	6.723 ± 0.027^{d}	2.536 ± 0.021^{c}	1.437 ± 0.020^{fg}	1.651 ± 0.004^{c}	11.12 ± 0.01^{e}
70°C_SD1s	19.01 ± 0.12^{e}	81.29 ± 0.16^{d}	5.893 ± 0.019^{e}	2.207 ± 0.013^{e}	2.774 ± 0.020^{c}	1.557 ± 0.009^{d}	12.47 ± 0.17^{d}
50°C_SD2s	20.21 ± 0.13^{d}	86.23 ± 0.30^{b}	7.271 ± 0.028^{b}	1.968 ± 0.016^{f}	2.017 ± 0.042^{e}	1.387 ± 0.004^{e}	10.92 ± 0.19^{ef}
60°C_SD2s	22.28 ± 0.15^{c}	86.37 ± 0.18^{b}	7.215 ± 0.015^{b}	2.594 ± 0.024^{b}	1.161 ± 0.022^{h}	1.913 ± 0.010^{b}	10.72 ± 0.09^{ef}
70°C_SD2s	22.89 ± 0.26^{c}	84.45 ± 0.18^{c}	6.824 ± 0.017^{cd}	2.344 ± 0.020^{d}	1.348 ± 0.028^{g}	1.925 ± 0.006^{b}	10.45 ± 0.08^{fg}

Notes: Mean value ± standard error (experiments in duplicate and analysis in triplicate); SAA: specific antioxidant activity; TBA: thiobarbituric acid; ΔE: difference in total color sensation. Total lysine and total methionine were of 8.94 and 2.85 g 100 $g^{-1}_{protein}$, respectively; Different letters in the same column show significant difference ($p < 0.05$); SU1s: fillets dried with skin up on one side; SD1s: fillets dried with skin down on one side; SD2s: fillets dried with skin facing down on both sides.

undergo denaturation, contract, lose the ability to retain water and form aggregates, thereby decreasing the solubility. The sample in which occurred less change was the 60°C_SU1s with a 10.3% reduction in solubility in relation to *in natura* fillets. The dried samples, which were more exposed to temperature (60°C_SD2s and 70°C_SD2s) had on average 22.5% of solubility. Murueta et al. [28], studying different drying methods of different species of fish, observed a reduction in the solubility at 65°C (12 h of drying) in relation to the *in natura* samples, which in most cases had a higher solubility under this drying condition, than the freeze-dried samples. Candido and Sgarbiere [26] found a maximum solubility of myofibrillar proteins for protein concentrates of tilapia to be 18%.

The *in vitro* digestibility of *in natura* fillet (Table 2) was ~91.5%, which is expected for fish, however drying decreased the fillets digestibility significantly ($p < 0.05$g). The smallest change occurred after drying at 50 and 60°C with air flow on both sides (50°C_SD2s and 60°C_SD2s). The average decrease was 5.6%, with values of ~86.3%. The higher decreases occurred in the drying with skin up, reaching a loss of up to 12.7%. According to Fellows et al. [29], the protein digestibility does not substantially change with the drying, however, biological value can be reduced, like for example the reduction in lysine, which is thermosensitive. Abdul-Hamid et al. [30] who studied the hydrolysates drying of tilapia also showed a decrease *in vitro* digestibility of ~10% in comparison to the control sample (without addition of enzyme), with the rise in temperature during spray drying, this being ~77%. The loss of *in vitro* digestibility, measured by soluble nitrogen after digestion, did not show any great changes. However, the essential amino acids, namely available lysine and methionine after digestion, showed greater reductions. Processing operations increased oxidative stress by the introduction of oxygen, removal of natural antioxidants, destruction of endogenous antioxidants and increased pro-oxidative factors, the last two being most associated with the heat treatment. Proteins are susceptible to oxidative reactions. These reactive compounds can be generated in several ways, one being by lipid oxidation [10, 31].

The available lysine and methionine after *in vitro* digestibility of *in natura* fillets (Table 2) showed values similar to the total amounts mentioned for anchovy (*E. anchoita*) by Pastous-Madureira et al. [4], which were 8.96 and 2.88 g 100 $g^{-1}_{protein}$ for lysine and methionine, respectively. Wang et al. [2] reported quantities of 7.53 g 100 $g^{-1}_{protein}$ lysine and methionine 3.65 g 100 $g^{-1}_{protein}$ to anchovy Asian (*Engraulis japonicus*).

After drying, there was a significant reduction namely 36% ($p < 0.05$) of available lysine in the condition 70°C_SU1s. The smallest losses occurred after drying of the two sides at 50 and 60°C, with values above 7.2 g 100 $g^{-1}_{protein}$, being of high-quality protein. Moreover, a negative correlation of temperature increase with the amount of available lysine was observed. Abdul-Hamid et al. [30], studying the drying of tilapia in spray dryer, found a reduction of about 40% of the total content of lysine with the increase in outlet temperature of the spray dryer of 76–90°C. Girón-Calle et al. [11] showed that the loss of available lysine in foods in the form of N-2-propenal of lysine was due to its relation with the

decomposition products of lipids (peroxides of polyunsaturated fatty acids).

The available methionine was influenced by the drying conditions significantly ($p < 0.05$), and decreases are shown in Table 2. The highest loss in relation to the *in natura* sample, occurred in the treatment at 50°C_SU1s, resulting in a 50% reduction in the amount of this amino acid. The lowest loss occurred after the condition of 60°C_SD2s namely 4%. The drying conditions that satisfy the recommendation of the FAO/WHO/UNU ([32]), 2.5 g 100 $g^{-1}_{proteina}$ (for children aged 2–5 years), were 60°C_SD1s and 60°C_SD2s. Apparently there is a relationship between the methionine loss and drying time and temperature, because long times and higher temperatures lead to greater methionine loss. Methionine is important in sequestration of free radicals in biological systems and can be oxidized before other amino acids and fatty acids, thus assuming its antioxidant power [33], it is critical that it be preserved. The peroxidation of polyunsaturated lipids in food during processing and/or storage, also leads to loss of available lysine [34].

The drying operation had an important effect on the TBA values of anchovy fillets, and these values were significantly increased ($p < 0.05$), as shown in Table 2. The fillets dried under 50°C_SU1s condition presented higher amounts of secondary products of oxidation, reaching ~3.9 mg_{MDA} $kg^{-1}_{sample,}$ dry basis. The conditions that had values close to or below 2 mg_{MDA} kg^{-1} (dry basis) were 60°C (SU1s, SD1s and SD2s) and at 50 and 70°C (SD2s), and the condition of 60°C_SD2s showed the lowest value of TBA ($p < 0.05$).

TBA values are commonly used to measure oxidative rancidity, since they represent the values of secondary oxidation products, however, these do not necessarily show the quality level of freshness of the product. Peroxidative decomposition of lipids in foods containing polyunsaturated fatty acids has detrimental effects on nutritional, toxicological and functional foods properties. Among the products of lipid peroxidation, MDAs have been most extensively studied. This aldehyde can serve as an index of peroxidative decomposition of these lipids which readily react with functional groups present in proteins, nucleic acids and phospholipids, especially amino groups. There is no established amount of TBA, defining the occurrence of lipid oxidation and/or indicating that from that amount this the fish cannot be consumed. However, Connell [35] noted that rates of TBA around 2 mg_{MDA} kg^{-1} (dry basis) are usually recommended as a limit beyond which the fish usually develops unpleasant flavor and odor (rancidity). Meanwhile, Al Kahtani et al. [36] report that meat products can be considered in good condition in relation to oxidative change, if they present less than 3 mg_{MDA} kg^{-1} (dry basis). The TBA value *in natura* fillet (Table 2) was approximately 0.62 mg_{MDA} kg^{-1}_{sample}, dry basis (≅ 0.2 mg_{MDA} $kg^{-1}_{sample,}$ wet basis), indicating good preservation state.

The SAA of *in natura* fillets, as measured by the reduction of DPPH, was $mM_{DPPHseq}$ $g_{protein\ sol}$ min^{-1}, as shown in Table 2. With the drying, a positive correlation between drying time and the reduced SAA could be observed. The SAA in dried samples was significantly reduced ($p < 0.05$), resulting in losses of up to 43.5% (50°C_SU1s), compared to *in natura*. A better preservation of antioxidant activity occurred under the drying conditions 60°C_SD2s and 70°C_SD2s, not presenting significant difference between each other ($p > 0.05$), with a reduction of ~10%. Vega-Gálvez et al. [9] studying the drying of squid in thin layer, at different temperatures, evaluated the quality of the dried product, and found a smaller decrease in antioxidant activity, measured by DPPH reduction compared to 60°C. Under these conditions, 1.77 $mg_{protein}$ was necessary to reduce 50% of DPPH radical (IC_{50}), but at 70°C, 15.79 $mg_{protein}$ was necessary. In this study, under 60°C_SU2s condition, it would take 4.8 $mg_{ptna\ sol.}$, this result is lower than that found by the quoted authors. However, this study only evaluated the fraction of soluble protein and not a protein extract, which could limit the action of some polar amino acids and/or their location in the protein structure, as reported by Elias et al. [37]. Furthermore, this value is an estimate, since the calibration curve to determine IC50 was not employed (due to a likely nonlinear relationship). The ability of proteins to simply sequester free radicals is not conclusive evidence that they are antioxidants. The fact is that proteins can interact with free radicals and reactive oxygen species, suggesting that they may protect lipids from oxidation if they are oxidized preferentially to unsaturated fatty acids. The preferential oxidation of proteins can occur if the available amino acids that are more oxidatively labile than unsaturated fatty acids, or the physical location of the reactive sites of the protein are close to the sites of free radical or reactive oxygen species generated where the protein could rapidly sequester the free radical before the migration of the radical to the lipid. However, many studies do not address the relative kinetics of lipid and protein oxidation, and thus, it is not clear whether the protein protects the lipid or the lipid causes oxidation of the protein [10].

The *in natura* fillet color represented by the coordinates L^*, a^* and b^* was 49.49 ± 0.32, 3.59 ± 0.36 and 9.40 ± 0.35, respectively. Czerner et al. [1] observed color parameters L^*,

a* and b* for anchovy from Argentina (*E. anchoita*) of 34.66, 16.68 and 8.98, respectively, while the sample of this study showed higher brightness, that is lighter and less red. According to the same authors, the parameter a* is related to the content of myoglobin, depending on concentration and its derivatives. Wang et al. [2] observed values L*, a* and b* of 38.2, 8.9 and 3.2, respectively, in *E. japonicus*, being less luminous, more reddish and less yellow, than that of this study. Evaluating pâté produced on the Mediterranean based anchovy (*Engraulis encrasicholus*), Kilinc [38] observed initial L*, a* and b* values of 36.8, 4.67 and 13.0, respectively, and therefore, the sample in this study has greater luminosity, and the parameters a* and b* are closer to the Mediterranean species than the species from Japan. Variations of the coordinate values in drying experiments were observed, which can be represented by the total change in color or change in color sensation (ΔE) in relation to the *in natura* fillet. It can be seen in Table 2 that the greatest variation in the color perception ($p < 0.05$) was under the 70°C_SU1s condition, and lower variations occurred in drying on both sides, with no significant difference between the three temperatures ($p > 0.05$), with the ΔE average values of 10.7. Under the 60°C_SU2s condition, the coordinates L*, a* and b* values were 44.73, 2.89 and 18.97, respectively, being less luminous, less red and more yellowish than the *in natura* fillets.

4 Conclusions

The Henderson and Pabis model showed a better fit to the experimental data ($R^2 > 0.99$ and RMSE < 0.05), and the moisture effective diffusivity (D_{ef}) values found, at temperatures of 50, 60 and 70°C, of the samples dried from both sides and on one side with skin side facing down were approximately 3.31×10^{-10}, 4.67×10^{-10} and 7.05×10^{-10} $m^2\ s^{-1}$, respectively. However, the effective moisture diffusivity for drying of samples with skin side up showed lower values of 2.24×10^{-10}, 3.14×10^{-10} and 5.47×10^{-10} $m^2\ s^{-1}$ at 50, 60 and 70°C, respectively, indicating that the skin showed a resistance to moisture diffusion. The activation energy values for drying were of 32.2 and 38.4 kJ mol^{-1} for the fillets skin down and skin up, respectively.

In relation to the muscle *in natura*, the quality of the product decreased with drying. The smallest change was caused in the drying condition of 60°C in fillet samples dried on both sides (60°C_SD2s). In this condition, the solubility was 22.3%, *in vitro* digestibility was 87.4%; the contents of available lysine and methionine were 7.21 and 2.64 g 100 $g^{-1}_{protein}$, respectively; TBA value was 1.16 $mg_{MDA}\ kg^{-1}_{sample}$ (dry basis); SAA was 1.91 $mM_{DPPH}\ g^{-1}_{protein\ sol}\ min^{-1}$, and the variation in color sensation (ΔE) was 10.72. The samples obtained from dried anchovy fillets in thin layer with air flow from both sides at 60°C ensured a final product with good characteristics, especially regarding its available lysine content. This product was within the recommendation for fish meal for human consumption, according to FAO recommendations, and can be a great source of protein to be added in the future in the development of food products.

Acknowledgment: The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel) for the financial support.

References

1. Czerner M, Tomás MC, Yeannes MI. Ripening of salted anchovy (*Engraulis anchoita*); development of lipid oxidation, colour and other sensorial characteristics. J Food Sci Food Agric 2011;91:609–15.
2. Wang C, Xue C, Liu Y, Lou W, Chen X. Utilization of fish protein and oil from anchovy (*Engraulis japonicus*). Asian Fisheries Sci 1996;9:201–8.
3. Djendoubi N, Boudrhioua N, Bonazzi C, Kechaou N. Drying of sardine muscles: experimental and mathematical investigations. Food Bioproducts Process 2009;87:115–23.
4. Pastous-Madureira LS, Castello JP, Prentice-Hernández C, Queiroz MI, Espírito Santo ML, Ruiz WA, et al. Current and potential alternative food uses of the Argentine anchoita (*Engraulis anchoita*) in Argentina, Uruguay and Brazil. In: Hasan MR, Halwart M, editors. Fish as feed inputs for aquaculture: practices, sustainability and implications. FAO Fisheries and Aquaculture Technical Paper. Rome: FAO, 2009;518:269–87.
5. Wang Y, Zhang M, Mujumdar AS. Trends in processing technologies for dried aquatic products. Drying Technol 2011;29:382–94.
6. Konishi Y, Horiuchi J, Kobayashi M. Dynamic evaluation of the dehydration response curves of food characterized by a poultice-up process using a fish paste sausage. II A new tank model for a computer simulation. Drying Technol 2001;19:1271–85.
7. Pinto LA, Tobinaga S. Diffusive model with shrinkage in the thin-layer drying of fish muscles. Drying Technol 2006;24:509–16.
8. Vega-Gálvez A, Andrés A, Gonzales E, Notte-Cuello E, Chacana M, Lemus-Mondaca R. Mathematical modelling on the drying process of yellow squat lobster (*Cervimunida jhoni*) fishery waste for animal feed. Anim Feed Sci Technol 2009;151:268–79.

9. Vega-Gálvez A, Miranda M, Clavería R, Quispe I, Vergara J, Uribe E, et al. Effect of air temperature on drying kinetics and quality characteristics of osmo-treated jumbo squid (*Dosidicus gigas*). Food Sci Technol (LWT) 2011;44:16–23.
10. Elias RJ, Kellerby SS, Decker EA. Antioxidant activity of proteins and peptides. Crit Rev Food Sci Nutr 2008;48:430–41.
11. Girón-Calle J, Alaiz M, Millán F, Ruiz-Guitiérez V, Vioque E. Bound malondialdehyde in foods: bioavailability of the N-2-propenals of lysine. J Agric Food Chem 2002;50:6194–8.
12. Hęś M, Korczak J, Pyrcz J, Kowalski R. Influence of lipid stabilization on the retention of available lysine and methionine in stored raw polish sausage. Polish J Food Nutr Sci 2009;65:19–25.
13. Kaya A, Aydin O, Kolayli S. Effect of different drying conditions on the vitamin C (ascorbic acid) content of Hayward kiwifruits (*Actinidia deliciosa planch*). Food Bioproducts Process 2010;88:165–73.
14. Krokida MK, Founddoukidis E, Maroulis Z. Drying constant: literature data compilation for foodstuffs. J Food Eng 2004;61:321–30.
15. Chirife J. Fundamentals of the drying mechanism during air dehydration of foods. In: Mujumdar A, editor. Advances in drying. Washington/New York/London: Hemisphere Publishing Corporation (v.3), 1983:73–102.
16. A.O.A.C. Official methods of analysis, 17th ed. Maryland: Association of Official Analytical Chemists, 2000.
17. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–17.
18. Morr CV, German B, Kinsella JE, Regenstein JM, Van-Buren JP, Kilara A, et al. Collaborative study to develop a standardized food protein solubility procedure. J Food Sci 1985;50:1715–18.
19. Dinnella C, Gargaro MT, Rossano R, Monteleone E. Spectrophotometric assay using *o*-phtaldialdehyde for the determination of transglutaminase activity on casein. Food Chem 2002;78:363–8.
20. Foh MB, Amadou I, Foh BM, Kamara MT, Xia W. Functionality and antioxidant properties of tilapia (*Orechromis niloticus*) as influenced the degree of hydrolysis. Int J Mol Sci 2010;11:1851–69.
21. Vyncke BW. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette Seifen Anstrichmittel 1970;72:1084–7.
22. Box GE, Hunter WG, Hunter JS. Statistics for experiments: an introduction to design, data analysis and model building. New York: John Wiley & Sons, 1978.
23. Hadrich B, Boudhrioua N, Kechaou N. Drying of Tunisian sardine (*Sardinella aurita*) experimental study and three-dimensional transfer modeling of drying kinetics. J Food Eng 2008;84:92–100.
24. Oliveira EG, Rosa GS, Moraes MA, Pinto LA. Characterization of thin layer drying of *Spirulina platensis* utilizing perpendicular air flow. Bioresour Technol 2009;100:1297–303.
25. Panagiotou NM, Krokida MK, Maroulis ZB, Saravacos GD. Moisture diffusivity: literature data compilation for foodstuffs. Int J Food Properties 2004;7:273–99.
26. Cândido LM, Sgarbiere VC. Enzymatic hydrolysis of Nile tilapia (*Oreochromus niloticus*) myofibrilar proteins: effects on nutritional and hydrophilic properties. J Sci Food Agric 2003;83:937–44.
27. Kristinsson HG, Rasco BA. Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 2000;40:43–81.
28. Murueta JH, Navarrete del Toro MA, Carreño FG. Concentrates of fish protein from bycatch species produces by various drying process. Food Chem 2007;100:705–11.
29. Fellows P. Food processing technology: principles and practice, 2nd ed. Cambridge, England: Woodhead Publishing, 2000:338.
30. Abdul-Hamid A, Bakar J, Bee GH. Nutritional quality of spray dried protein hydrolysate from Black tilapia (*Orechromis mossambicus*). Food Chem 2002;78:69–74.
31. Davies MJ. The oxidative environment and protein damage. Biochim Biophys Acta 2005;1703:93–109.
32. FAO/WHO/UNU. Energy and protein requirements. Available at: http://www.fao.org/DOCREP/003/AA040E/AA040E08.htm#ch7.3. Accessed: April de 2011.
33. Levine RL, Mosoni L, Berlett BS, Stadtman ER. Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci USA 1996;93:15036–40.
34. Nielsen HK, Löliger J, Hurrell RF. Reactions of proteins with oxidizing lipids. Analytical measurements of lipid oxidation and of amino acid losses in a whey protein-methyl linolenate model system. Br J Nutr 1985;53:61–73.
35. Connell JJ. Control of fish quality, 4th ed. Farnham, Surrey: Fishing News Books, 1995:159–60.
36. Al-Kahtani HA, Abu-Tarboush HM, Bajader AS, Atia M, Abou-Arab AA, El-Majoddidi MA. Chemical changes after irradiation and post irradiation storage in tilapia and Spanish mackerel. J Food Sci 1996;61:729–33.
37. Elias RJ, McClements DJ, Decker EA. Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase beta-lactoglobulin in oil-water emulsions. J Agric Food Chem 2005;53:10248–53.
38. Kilinc B. Microbiological, sensory and color changes of anchovy (*Engraulis encrasicholus*) patties during refrigerated storage. J Muscle Foods 2009;20:129–37.