Desorption isotherms and thermodynamics properties of anchovy in natura and enzymatic modified paste

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The thermodynamic properties of anchovy fillets and enzymatic modified pastes in two hydrolysis degrees (3% HD and 14% HD), at 50, 60 and 70 °C were evaluated. The GAB model was used to calculate the values of the monolayer moisture content and the thermodynamic properties of the samples. The enzymatic modification led to the increases of the superficial area and differential enthalpies, and decrease of the differential entropies in relation the samples in natura. The enthalpy–entropy compensation showed that the process was controlled by the enthalpy, it was only spontaneous for the samples in natura. Pore size decreased with enzymatic modification, and all samples were in the limit of region between micropores and mesopores (<2 nm) for moisture content of 15%, and mesopores (from 2 to 50 nm) to moisture content above 15%.

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1. Introduction

Anchovy (Engraulis anchoita) is a small pelagic fish found in the southwestern Atlantic Ocean (Brazil, Uruguay and Argentina), which share a stock named “Bonaerense.” In Brazil, potential alternative uses for new products based on anchovy were evaluated based on prototypes and new products such as dehydrated soup and risotto (Pastous Madureira et al., 2009). Recent advances in technology and food engineering have demonstrated the benefits of using enzymes in processing, especially of protein foods, increasing the utilization of raw materials such as fish of low commercial value (as the anchovy). Enzymatic hydrolysis, carried out under mild and controlled conditions, ensures the maintenance of the nutritional quality of hydrolysates and set peptide profile (Kristinsson and Rasco, 2000).

In the study of the drying operation and storage conditions, it is necessary to know the relationship between the equilibrium moisture content (Xe) of the material and water activity (aw) and this is named as the moisture sorption isotherms (Togrul and Arslan, 2007). They are also efficient tools to determine thermodynamic interactions between water and food materials, such as proteins, that lead to the information to evaluate the processing operations, such as energy requirement for heat and mass transfer (Iglesias and Chirife, 1976; Fasina, 2006; Kaya and Kahyaoglu, 2007). Several equations have been proposed to correlate the equilibrium moisture content of agricultural and food products, as a function of the relative humidity of air and temperature of solid material (Babetto et al., 2011), and the GAB and BET models being considered as the most used in literature to describe the food sorption (Van den Berg and Bruin, 1981; Al-Muhtaseb et al., 2002, 2004).

The sorption phenomena can be analyzed in terms of thermodynamic functions, which can provide important information on both the energy requirements in the processes of dehydration, as well as the microstructure of foods (sorption surface area and pore size) and physical phenomena in surface of food, water properties and kinetic parameters of sorption (Rizvi and Benado, 1984; Fasina, 2006). Physical phenomena such as sorption are often evaluated based on the isokinetic theory or theory of enthalpy–entropy compensation. This indicates that compensation arises due to changes in solvent–solute interaction, and that there is a linear relationship between enthalpy and entropy (Madamba et al., 1996; Tunç and Duman, 2007). The knowledge of the number and size of pores in a protein matrix is of great importance because it determines the total area of sorption. The speed and degree of hydration of food materials are mainly, determined by the pore surface properties (Singh et al., 2006), so the evaluation of pore size distribution is also important in the practice of drying. A variety of methods try to explain the increase of the film through the association of the Kelvin equation and isotherm pattern or curve “r” to describe the film thickness in the pre-condensation in the wall pores (Lastosckie et al., 1993).

Thermodynamic data for fish are scarce in the literature, in this context, the aim of this study was to obtain the desorption isotherms of in natura anchovy fillets and enzymatic modified paste...
### Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a_w)</td>
<td>water activity (dimensionless)</td>
</tr>
<tr>
<td>(A_{\text{H}_{2}\text{O}})</td>
<td>area of a water molecule in Eq. (2) (m²)</td>
</tr>
<tr>
<td>(c_c)</td>
<td>parameter in Eq. (1) (dimensionless)</td>
</tr>
<tr>
<td>(\Delta h_d)</td>
<td>differential enthapy of sorption in Eq. (3) (kJ/mol K)</td>
</tr>
<tr>
<td>(\Delta G_0)</td>
<td>free energy in Eq. (5) (kJ/mol)</td>
</tr>
<tr>
<td>(k)</td>
<td>parameter in Eq. (1) (dimensionless)</td>
</tr>
<tr>
<td>(MRD)</td>
<td>mean relative deviation (%)</td>
</tr>
<tr>
<td>(n_i)</td>
<td>total number of isotherms in Eq. (6) (dimensionless)</td>
</tr>
<tr>
<td>(N_A)</td>
<td>Avogadro number in Eq. (2) (6.0 × 10²³ molecules/mol)</td>
</tr>
<tr>
<td>(P M_{\text{H}_{2}\text{O}})</td>
<td>molecular weight of water in Eq. (2) (kg/mol)</td>
</tr>
<tr>
<td>(q_d)</td>
<td>isorhetic heat of sorption in Eq. (3) (kJ/mol)</td>
</tr>
<tr>
<td>(Q_d)</td>
<td>total heat of sorption in Eq. (3), (kJ/mol)</td>
</tr>
<tr>
<td>(r_c)</td>
<td>critical radius in Eq. (7) (m)</td>
</tr>
<tr>
<td>(R)</td>
<td>universal gas constant in Eq. (3), (kJ/mol K)</td>
</tr>
<tr>
<td>(R^2)</td>
<td>coefficient of determination (dimensionless)</td>
</tr>
<tr>
<td>(S_0)</td>
<td>surface area of sorption in Eq. (2) (m²/g)</td>
</tr>
<tr>
<td>(\Delta S_d)</td>
<td>differential entropy of sorption in Eq. (4) (J/mol K)</td>
</tr>
<tr>
<td>(T)</td>
<td>absolute temperature (K)</td>
</tr>
<tr>
<td>(t)</td>
<td>layer thickness in Eq. (8) (nm)</td>
</tr>
<tr>
<td>(T_F)</td>
<td>isokinetic temperature in Eq. (5) (K)</td>
</tr>
<tr>
<td>(T_{\text{hm}})</td>
<td>harmonic mean temperature in Eq. (6) (K)</td>
</tr>
<tr>
<td>(V_{\text{in}})</td>
<td>molar volume of adsorbate in the liquid state in Eq. (7) (m³/mol)</td>
</tr>
<tr>
<td>(X)</td>
<td>average moisture content of the sample (kgw/kgds)</td>
</tr>
<tr>
<td>(X_e)</td>
<td>equilibrium moisture content (kgw/kgds)</td>
</tr>
<tr>
<td>(X_m)</td>
<td>monolayer moisture content (kgw/kgds)</td>
</tr>
</tbody>
</table>

Greek symbol

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sigma)</td>
<td>surface tension in Eq. (7) (N/m)</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>latent heat of vaporization of water in Eq. (3) (kJ/mol)</td>
</tr>
</tbody>
</table>

in two different hydrolysis degree (3%HD and 14%HD), at different temperatures. Thermodynamic functions of enthalpy and entropy differentials, and the distribution and pore size of the samples by applying the Kelvin and Halsey equations were determined. The theory of enthalpy–entropy compensation was applied.

### 2. Material and methods

#### 2.1. Material

Anchovy (E. anchoita) was used in the form of in natura fillets and enzymatic modified samples. The enzyme used in the process of enzymatic modification was Neutrase® 0.8 L (Novozymes), which is an endopeptidase produced by Bacillus amyloliquefaciens.

#### 2.2. Methods

##### 2.2.1. Preparation of anchovy fillet

The anchovy was captured at sea by the “South Atlantic Oceanographic ship,” using mid-water trawling on the continental shelf of southern Brazil. The anchovy was stored on board with controlled temperature (±1 °C) for a time necessary to reach constant weight, which was measured every two days on an electronic balance accurate to 0.001 g (Kern, 430-21, Haubstadt, Germany). The equilibrium condition was achieved when the variation of water activity from 0.055 to 0.89 according to Perry (1984) for the sorption isotherms experiments 3 g of wet samples were used. The in natura anchovy fillets, containing moisture around 0.78 kg/kg, were cut in rectangular shapes of approximately (1.2 × 1.0 × 0.4) cm of size, and the enzymatic modified paste with moisture content of 0.80 kg/kg. The samples were placed on a support within each glass jar, ensuring that there was no contact of the sample with the acid solution. The glass jars were placed in an incubator (Marconi, B.O.D M.A 415/S, Piracicaba Brazil) with controlled temperature (±1 °C) for a time necessary to reach constant weight, which was measured every two days on an electronic balance accurate to 0.001g (Kern, 430-21, Haubstadt, Germany). The equilibrium condition was achieved when the difference between three successive measurements was 0.001 g. The time to achieve the equilibrium of the samples was in range from 14 to 16 days. After this time, the moisture content analysis was carried out according to AOAC (2000).

##### 2.2.2. Enzymatic modification

The anchovy fillets were hydrolyzed by Neutrase® in a stainless steel reactor, with agitation, connected to a thermostatic bath (Quimis, Q-304-264, São Paulo, Brazil) for obtaining the enzymatic modified pastes of low hydrolysis degree (3%HD) and high hydrolysis degree (14%HD). The reaction conditions for 3%HD were 0.01% enzyme:substrate ratio (E/S) and hydrolysis time 30 min; and for 14% HD were 0.25% enzyme:substrate ratio (E/S) and hydrolysis time 120 min. In both hydrolysis, the pHmuscle and temperature were, respectively, 6.8 and 55 °C. Thermal inactivation was carried out at 90 °C for 10 min (Moraes et al., 2010).

The hydrolysis degree (HD) was measured according to the methodology proposed by Adler-Nissen (1979), through the reaction of TNBS (trinitrobenzene sulfonic acid) with leucine liberated during hydrolysis, in order to determine the number of peptide bonds cleaved. The hydrolysis degree was then calculated by the ratio of the milliequivalents of leucine liberated per gram of protein and the milliequivalents of leucine total per gram of protein.

The number of leucine total adopted was 8.6 milliequivalents per gram of protein as suggested by Nielsen et al. (2001) for fish.

#### 2.2.3. Sorption isotherms

The desorption isotherms were determined at 50, 60, and 70 °C according to Moraes et al. (2008). The temperature range from 50 to 70 °C was chosen because this is range frequently used for fish drying. The static gravimetric method was used to determine the equilibrium moisture content of in natura anchovy fillets and enzymatic modified paste, with low (3%HD) and high (14%HD) hydrolysis degrees.

The isotherms experiments were carried out in eleven glass jars (height of 7 cm and diameter of 6 cm), sealed, ensuring a constant atmosphere. Inside each glass jar there was sulfuric acid solutions in eleven concentrations (range from 0.20 to 0.70 kg/kg), ensuring the variation of water activity from 0.055 to 0.89 according to Perry (1984). For the sorption isotherms experiments 3 g of wet samples were used. The in natura anchovy fillets, containing moisture around 0.78 kg/kg, were cut in rectangular shapes of approximately (1.2 × 1.0 × 0.4) cm of size, and the enzymatic modified paste with moisture content of 0.80 kg/kg. The samples were placed on a support within each glass jar, ensuring that there was no contact of the sample with the acid solution. The glass jars were placed in an incubator (Marconi, B.O.D M.A 415/S, Piracicaba Brazil) with controlled temperature (±1 °C) for a time necessary to reach constant weight, which was measured every two days on an electronic balance accurate to 0.001 g (Kern, 430-21, Haubstadt, Germany). The equilibrium condition was achieved when the difference between three successive measurements was 0.001 g. The time to achieve the equilibrium of the samples was in range from 14 to 16 days. After this time, the moisture content analysis was carried out according to AOAC (2000).

### 2.3. Data analysis

#### 2.3.1. Isotherm models

The experimental data of equilibrium moisture content (\(X_e\)) as a function of water activity (\(a_w\)) were fitted by the GAB model (Eq. (1)), which have a theoretical basis (Timmermann et al., 2001).

\[
X_e = \frac{X_m C_c k a_w}{(1 - k a_w)(1 - C_c k a_w)} \quad (1)
\]

The isotherms parameters were obtained by non-linear regression with the experimental data of equilibrium isotherms of the samples (in natura fillets, 3%HD and 14%HD) through the software.
Statistica 6.0 (Statsoft Inc., Tulsa, USA). To evaluate the fit of the model, the coefficient of determination ($R^2$) and mean relative deviation (MRD) were used, according to Thys et al. (2010).

### 2.3.2. Thermodynamic properties

The surface area of sorption plays an important role in determining the water bond of a particulate material, and is determined from the monolayer moisture values $X_m$, as shown in Eq. (2) (Oliveira et al., 2009). The monolayer moisture used to calculate the area was obtained from the GAB model,

$$S_0 = X_m \frac{1}{FMH_{1,0}} N_R A_{H_{1,0}} = 3.5 \times 10^3 X$$

The isosteric heat of sorption ($q_{st}$) or differential sorption enthalpy ($\Delta h_d$) is defined as the difference between the total heat of sorption ($Q_{st}$) and the latent heat of vaporization of water ($\Delta h_v$). This can be determined using Eq. (3) that is derived from the Clausius–Clapeyron equation (Rizvi and Benado, 1984).

$$\frac{\partial \ln(q_{st})}{\partial(1/T)}_{X_m} = -\frac{Q_{st} - \Delta h_v}{R} = -\frac{Q_{st}}{R} = -\frac{\Delta h_d}{R}$$

$$= \left[ \frac{\Delta h_d}{R} \right]$$

The isosteric heat of sorption is a differential molar quantity derived from the temperature dependence of the isotherm, and its application requires the measurement of isosteric sorptions in two or more temperatures. The change in molar differential entropy of desorption can be calculated by the Gibbs–Helmholtz equation. The free energy changes resulting from water sorption can be calculated by the Gibbs–Helmholtz equation (Rizvi and Benado, 1984). The effective pore size ($r_p$) can be calculated by adding the critical radius ($r_c$), where capillary condensation and evaporation occur, and multilayer thickness ($t$).

### 3. Results and discussion

#### 3.1. Sorption isotherm

The adsorption isotherm data for in natura and enzymatic modified samples (3%HD and 14%HD), at three different temperatures (50, 60 and 70 °C), are shown in Fig. 1. The desorption isotherms show sigmoidal shape type II, typical of foods isotherms according to the classification of Brunauer et al. (1940). An increase in moisture equilibrium with the modified enzyme and the increase in the hydrolsy degree can be seen in Fig. 1(a–c). Enzymatic hydrolysis of proteins, mainly of fish, is accompanied by three distinct effects: a decrease in molecular weight, an increase in the number of ionicizable groups, and lead to the exposure of hydrophobic groups. These changes alter the functional and biological properties of native protein. The presence of polar groups such as –COOH and –NH₂, which increase with the hydrolysis, has a substantial effect on the amount of adsorbed water, and consequently in the moisture sorption isotherms. Cândido and Sgarbiere (2003) studying hydrolysates obtained from Nile tilapia, also observed a slight increase in equilibrium moisture content with increasing degree of hydrolysis, to the same relative humidity.

Table 1 shows the estimated parameters obtained by nonlinear regression of the relationship between $X$ and $a_{aw}$, (Eq. (1)). The GAB model showed good fit to the experimental results in the different samples (MRD < 10% and $R^2 > 0.99$). The GAB equation has been recommended as a fundamental equation for the characterization of water sorption in food materials, because it works in a wide range of $a_{aw}$ (from 0.1 to 0.9) and has a theoretical base (kinetic model based on multilayer and condensate film). According to Timmerman et al. (2001), the GAB parameters are representative, when considering the hypothesis that the water sorption monolayer of proteins can be thought of in terms of fixing a water molecule to each polar group of the side chains of amino acids in proteins.

The monolayer moisture content is the minimum content covering hydrophilic sites on the surface of the material. In Table 1, the moisture monolayer ($X_{m0}$) decreased with increasing temperature, same as $C_{v_i}$, which is related to the energetic interactions between molecules of the monolayer in a given characteristic sorption site of the product. The decrease in moisture content of the monolayer with increasing temperature may be due to a reduction in the total number of active sites for water bonding as a result of physical and/or chemical changes induced by temperature (Iglesias and Chirife, 1976). The enzymatic modification caused an increase...
Many food science studies give preferential or almost exclusive attention to the monolayer value. However, the values of the energy parameters should not be ignored because they are results of the simultaneous process of regression, which influences the shape of the sigmoidal isotherms, since $C_d$ determine the form more or less pronounced as “knee”, in low water activity values. Furthermore, the parameter $k$ (GAB model) determines the profile of the isotherm at high values of $a_w$, which regulate the peak after a “plateau” in a middle range of water activity. The highest values of $k$ determine a more pronounced rise, which can be seen in Table 1, being close to the unit. With the increase of hydrolysis degree (14%HD) there was also a small decrease in the value of $k$, which may indicate a less structured state of the adsorbate in the multilayer. Already the parameter $C_d$ decreased with increase temperature, and increased with enzymatic modification, due to changes in protein structure.

The monolayer values found in this study are close to values reported in literature for fish: 0.061 kg/kg for salmon at 37 °C (Iglesias and Chirife, 1976); 0.058 kg/kg on average for fishmeal at 25 °C (Timmerman et al., 2001); 0.073 kg/kg for the protein fraction of myosin isolated dorsal fish (Labio rohita) at 45 °C (Das and Das, 2002); 0.052 kg/kg for sardines at 50 °C (Hadrich et al., 2008).

### 3.2. Thermodynamic properties

The values of desorption specific surface area of the in natura fillets, 3%HD and 14%HD samples (Table 2) were calculated according to Eq. (2), with the monolayer moisture values obtained by the GAB model to the equilibrium temperatures at 50, 60 and 70 °C. Table 2 showed that the total specific surface area for hydrophilic bonds was decreased with increase of the temperature and increased with enzymatic modification.

The sample 14%HD showed an increase in surface area of 36% compared to in natura fillet at 70 °C. This behavior has been described as a change in the number of active sites due to physical and chemical changes induced both by temperature and enzymatic modification. The increase in temperature decreases the number of active sites, and increasing the hydrolysis degree increases the number of active sites (Kristinsson and Rasco, 2000). The values found in this study are similar to those reported in literature for food at different temperatures, such as textured soy protein 162–206 m²/g (Cassini et al., 2006), garlic 196–315 m²/g and apple 374–588 m²/g (Moraes et al., 2008).

The values of enthalpy and entropy differentials of water desorption, for each moisture content, were estimated by Eq. (4), for the data calculated from the GAB model. The isosteric heat of sorption or enthalpy differential is the amount of energy above the heat of vaporization of water associated with the sorption process, this parameter is used as an indicator of water adsorbed by the solid particles. Whereas the differential entropy is proportional to the number of sorption sites available at a specific energy (McMinn et al., 2007).

An exponential relationship, widely used by researchers was obtained to adequately describe the dependence of the total heat of sorption ($Q_{st}$) and moisture content for different samples (Fasina, 2006). Eqs. ((9)–(11)) represent this relationship.

$$ Q_{st} = 45.23 + (59.42 \exp(-X_r/0.064)) \quad R^2 = 0.99 \quad (9) $$

$$ Q_{st} = 44.62 + (164.92 \exp(-X_r/0.046)) \quad R^2 = 0.99 \quad (10) $$

$$ Q_{st} = 43.60 + (214.87 \exp(-X_r/0.054)) \quad R^2 = 0.98 \quad (11) $$

Fig. 2 shows the total heat of desorption ($Q_{st}$) in function of the moisture content, and the models constructed by Eqs. ((9)–(11)). The energy for desorption increases with the decrease of the...
equilibrium moisture content because of the intensity of water bonding with the adsorbent material. There is the initial occupation of highly active polar sites to form the monolayer following by the progressive filling of the less accessible sites (with lower energies of bonding) and multilayer formation. So, the differential enthalpy decreased with the increase of moisture content, which tends to become asymptotic around 20% (dry basis, db). The enzymatic modification led to an increase in differential enthalpy, and consequently the total heat of desorption with moisture content decreased under 12.5% (db). This increase can be explained by the change in conformation of the biopolymer, which caused an increase in polar groups, making them more active. The differential enthalpy in a moisture content of 7.5% (db), for samples with 3% HD compared to \textit{in natura} fillet increased 72%, and for 14% HD there was an increase of 170%, which lead to an increase in energy costs of drying.

The results found in this study for \textit{in natura} fillets was similar to that reported by Hadrich et al. (2008) while studying the desorption of sardine fillets, where the total heat of sorption, at 5% (db) moisture content was approximately 70 kJ/mol. Ariahu et al. (2006) observed a variation of enthalpy of desorption, at 5% (db) moisture content of approximately 25 kJ/mol ($Q_{st}$=68 kJ/mol) for tropical freshwater crayfish flour. Das and Das (2002) reported a differential enthalpy change of 5 kJ/mol ($Q_{st}$=49 kJ/mol), at 7.5% (db) moisture content, for the fraction of myosin extracted from fish. Vivanco and Taboada (1998) observed a differential enthalpy change of 17 kJ/mol ($Q_{st}$=60 kJ/mol) during the adsorption process in fishmeal. Oliveira et al. (2009) found a maximum variation in enthalpy differential of approximately 100 kJ/mol ($Q_{st}$=143 kJ/mol) in \textit{Spirulina platensis} higher than the maximum found in this study. Singh et al. (2006) found in raw goat meat an enthalpy difference of 10,000 kJ/kg (180 kJ/mol), in moisture content of 10% (db).

The relationship between variation of the differential entropy of desorption ($\Delta S_d$) and the moisture content of the \textit{in natura} fillets, 3%HD and 14%HD are shown in Fig. 3. The differential entropy decreased with increase of hydrolysis degree at equilibrium moisture content under 20% (db). In the full extent of moisture content studied these values were negative. Rizvi and Benado (1984) attributes this to more polar groups that bond more strongly with water. This is indicative of thermodynamic compensation between the differential heat and differential entropy of anchovy, both \textit{in natura} and enzymatic modified forms. Similar

### Table 1

Estimated parameters, $R^2$ and DMR values of the models for the desorption isotherms of anchovy \textit{in natura} fillets, and hydrolysis degrees of 3%HD and 14%HD.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Temperature (°C)</th>
<th>3% Hydrolysis degree (HD)</th>
<th>14% hydrolysis degree (HD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td><strong>GAB model (Eq. (1))</strong> Parameters*</td>
<td></td>
<td>X_m (kg/kg dry solid)</td>
<td>0.074 ± 0.001d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_o</td>
<td>15.1 ± 3.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>k</td>
<td>0.085 ± 0.01d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2$</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRD (%)</td>
<td>4.82</td>
</tr>
</tbody>
</table>

* Mean value ± standard deviation (n = 3). Different letters in same line show significance difference ($p < 0.05$).

### Table 2

Specific surface area values of desorption for anchovy samples calculated by the GAB model at different temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Specific surface area (m²/g)</th>
<th>\textit{In natura} Fillets</th>
<th>33HD**</th>
<th>14HD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>263.2 ± 2.7³³a</td>
<td>267.0 ± 2.8³³a</td>
<td>330.1 ± 2.9³³a</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>232.5 ± 5.3³³b</td>
<td>237.4 ± 2.5³³b</td>
<td>299.0 ± 2.8³³b</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>205.6 ± 4.7³³c</td>
<td>212.7 ± 4.8³³c</td>
<td>279.7 ± 4.8³³c</td>
<td></td>
</tr>
</tbody>
</table>

* Mean value ± standard deviation (n = 3). Different small letters in same line show a significance difference ($p < 0.05$); different capital letters in same column show a significance difference ($p < 0.05$).

** HD: hydrolysis degree.

### Fig. 2

Total heat of sorption for \textit{in natura} fillet and enzymatic modified samples (hydrolysis degrees: 3%HD and 14%HD).

### Fig. 3

Differential entropy vs equilibrium moisture content for \textit{in natura} fillet and enzymatic modified samples (hydrolysis degrees: 3%HD and 14%HD).
The theory of enthalpy–entropy compensation or isokinetic theory has been widely applied to investigate physical and chemical phenomena (Madamba et al., 1996). Fig. 4 shows the linear relationship between 

\[
\Delta H_g, S_d C_0 / \Delta G_s C_1 = b \]

\( \Delta H_g \) (free energy), obtained from a linear regression (Eq. (6)) and Halsey (Eq. (8)), respectively. Table 3 shows the average size of pores for in natura fillets, 3%HD and 14%HD at different equilibrium moisture content and temperature.

The pore size increased with increment moisture content and temperature for all samples, however, the pore size decreased with enzymatic modification. At the temperatures and equilibrium moisture evaluated the pore sizes for in natura fillets, 3%HD and 14%HD ranged from 0.84 to 12.6 nm, 0.66 to 8.96 nm, and 0.55 to 8.21 nm, respectively. According to the classification defined by IUPAC (International Union of Pure & Applied Chemistry) (Miyata et al., 2003), all samples are in the limit of the region between micropores (<2 nm) for equilibrium moisture of approximately 15% (db), and mesopores (from 2 to 50 nm) to equilibrium moisture content above 15%. The pores generally increase with increment moisture content, and also with increase of temperature. A similar trend was found by Singh et al. (2001, 2006) for smoked chicken sausage and raw goat meat, respectively. However, the pore size for these products at 50 °C were lower than in the present study, and being classified as micropores. Rosa et al. (2010) while studying desorption of garlic and apple at 60 °C, found a pore size range from 0.5 to 30 nm. Moraes et al. (2008) evaluating the desorption of chitosan at 60 °C observed a variation in pore size from 0.5 to 6.8 nm in moisture contents range from 0.018 to 0.486 kg/kg\(_{\text{dry solid}}\).

The diffusion mechanisms depend on the properties within the local structure of the porous matrix, i.e., the force of interaction of molecules, in this case water, with the pore walls, as well as the relative proportion between the size of molecules and pores (Armatas, 2006). Enzymatic hydrolysis by Neutrase, which is an endoprotease, cleaves the peptide bonds within the protein profiles were also observed in some foods such as apple and garlic (Moraes et al., 2008).

Enzymatic hydrolysis by Neutrase, which is an endoprotease, cleaves the peptide bonds within the protein (Armatas, 2006). Evaluating starch products, McMinn et al. (2007) found a pore size range from 0.5 to 30 nm. Moraes et al. (2008) evaluating the desorption of chitosan at 60 °C observed a variation in pore size from 0.5 to 6.8 nm in moisture contents range from 0.018 to 0.486 kg/kg\(_{\text{dry solid}}\).

Table 3

Average pore size for anchovy samples at different temperatures and equilibrium moisture content.

<table>
<thead>
<tr>
<th>Sample in natura</th>
<th>3%H</th>
<th>14%H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>X (kg/kg(_{\text{dry solid}}))</td>
<td>0.05</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>0.10</td>
<td>1.44 ± 0.04</td>
<td>1.67 ± 0.08</td>
</tr>
<tr>
<td>0.15</td>
<td>2.17 ± 0.09</td>
<td>2.50 ± 0.09</td>
</tr>
<tr>
<td>0.20</td>
<td>2.91 ± 0.06</td>
<td>3.31 ± 0.11</td>
</tr>
<tr>
<td>0.25</td>
<td>3.65 ± 0.06</td>
<td>4.22 ± 0.12</td>
</tr>
<tr>
<td>0.30</td>
<td>4.38 ± 0.11</td>
<td>5.05 ± 0.20</td>
</tr>
<tr>
<td>0.35</td>
<td>5.13 ± 0.11</td>
<td>5.94 ± 0.26</td>
</tr>
<tr>
<td>0.40</td>
<td>5.91 ± 0.12</td>
<td>6.85 ± 0.32</td>
</tr>
<tr>
<td>0.45</td>
<td>6.67 ± 0.16</td>
<td>7.77 ± 0.39</td>
</tr>
<tr>
<td>0.50</td>
<td>7.46 ± 0.17</td>
<td>8.74 ± 0.46</td>
</tr>
</tbody>
</table>

* Mean value ± standard deviation (n = 3). Different letters in same line show significance difference (p < 0.05).

** HD: hydrolysis degree.
molecule, causing it to lose its conformation, producing relatively large peptides, which may explain the reduction in pore size of the protein matrix (Table 3) with increment in the hydrolysis degree and the consequent increase in surface area in relation to in natura fillets (Table 2).

4. Conclusion

The most extensive enzymatic modification (14%HD) caused increase in monolayer moisture and energy parameters in relation to in natura fillets, in the three different temperatures. Consequently, the surface area decreased with increment temperature and increased with enzymatic modification (range from 263 to 330 m²/g). The enthalpy and entropy differences show a strong relationship with the moisture content and the enzymatic modification. The compensation theory can be applied to the behavior of moisture desorption of the samples, indicating that the process was controlled by the enthalpy, and the process being spontaneous for the fillets samples. The pore size increased with increase of moisture content and temperature, but decreased with enzymatic modification. The pore sizes ranged from 0.84 to 1.26 nm for fillets, from 0.66 to 8.96 nm for 3%HD and from 0.55 to 8.2 nm for 14%HD.

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References


