Water Activity Role on Dried *Agaricus Bisporus* L. Lipid Oxidation During Storage Time

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Abstract: The aim is to determine the influence of water activity on dried *Agaricus bisporus* lipid oxidation stored for 180 days at a temperature of 25°C. Water sorption isotherms were determined using desiccators methods. *Agaricus bisporus* samples were equilibrated on saturated salt solutions in a range of water activity from 0.1 to 0.753. The equilibrium moisture content was obtained when there was no appreciable change in sample weight. GAB model was tested to determine the monolayer of *Agaricus bisporus*, $m_0$, and there were monitored peroxide value, acidity value and refraction index as a function of *Agaricus bisporus* moisture (0.032 and 0.062 g water g$^{-1}$ *Agaricus bisporus*) during the period of storage and the influence of *Agaricus bisporus* moisture on lipid oxidation.

Keywords: *Agaricus bisporus*, sorption isotherm, water activity, lipid oxidation

1. Introduction

Removing water from food to obtain an optimum preservation is a process commonly used in the food industry. Numerous speciality studies have been performed to establish an optimum amount of water that does not significantly affect physical, chemical and biochemical changes during storage. Studies have shown that the stability and changes that take place in the food preservation process depend on the activity of water and not on its content in food [1].

Water activity is defined as the ratio of the vapor pressure of water in food (p) to the vapor pressure of pure water at the temperature [2, 3].

The relationship between these two parameters under defined conditions of temperature and equilibrium is represented graphically by an adsorption or desorption isotherm [4].

Water sorption isotherms have a sigmoidal shape and are available in literature several mathematical equations used to model the experimental data [5-9]:

\[
X_e = \frac{(X_m \cdot C_{BET} \cdot a_w) \cdot \left[1 - (n+1) \cdot a_w^n + n \cdot a_w^{n+1}\right]}{(1 - a_w) \cdot \left[1 + (C_{BET} - 1) \cdot a_w - C_{BET} \cdot a_w^{n+1}\right]}
\]

BET linear

\[
X = \frac{X_m C_{BET} a_w}{(1 - a_w) \left[1 - (C_{BET} - 1) a_w\right]}
\]

GAB

\[
X_e = \frac{X m \cdot C_{GAB} \cdot K_{GAB} \cdot a_w}{(1 - K_{GAB} \cdot a_w) \cdot (1 - K \cdot a_w + C_{GAB} \cdot K_{GAB} \cdot a_w)}
\]

Halsey

\[
a_w = \exp\left(-\frac{A}{X_e^B}\right)
\]

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On this isotherm there is a point called monolayer which is the optimum moisture content for maximum storage stability of the food [2, 9, 10]. Also at the monolayer moisture content, theoretically all polar groups have adsorbed one molecule of water vapour. It is at or just above this point where chemical compounds begin to dissolve, diffuse or to react in the aqueous phase [2, 3].

The monolayer of *Agaricus bisporus* can be determined using GAB equation:

\[
m = \frac{m_0 \cdot K_b \cdot C \cdot a_w}{(1-K_b \cdot a_w)(1-K_b \cdot a_w + K_b \cdot C \cdot a_w)}
\]

where:
- \(m\) is the equilibrium moisture content, g water g\(^{-1}\) *Agaricus bisporus*;
- \(m_0\) is the monolayer (the optimum moisture), g water g\(^{-1}\) *Agaricus bisporus*;
- \(a_w\) - water activity;
- \(K_b\) - correcting constant;
- \(C\) - Guggenheim constant.

2. Materials and methods

Fresh button mushrooms (*Agaricus bisporus*) were obtained from a local supermarket.

Reagents and methods

Chloroform, Ethanol, Glacial acetic acid, Lithium chloride, Magnesium chloride, Magnesium nitrate, Potassium carbonate, Potassium hydroxide, Potassium iodide, Sodium chloride, Sodium thiosulfate were purchased from Merck.

Sample preparation

The mushrooms were washed with tap water, dried and grounded.

Determination of the moisture content

The initial moisture content of mushroom was determined by using the moisture analyzer (Mx-50), based on the principle of thermogravimetric analysis.

Determination of the water sorption isotherm

Water sorption isotherms were determined for *Agaricus bisporus* samples whose moisture was below and above the monolayer value. The equilibrium moisture content, \(m\), can be determined using the method of static system (isopiestic method) and to calculate the monolayer, \(m_0\), were applied the GAB equation [3, 11-13].

Water sorption isotherm were determined by placing the *Agaricus bisporus* samples in desiccators containing saturated salt solutions, Table 1. Salt solutions should present a liquid layer above the crystals.
Table 1. Water Activity of Saturated Salt Solutions at 25°C

<table>
<thead>
<tr>
<th>Saturated salt solution</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>0.113</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.328</td>
</tr>
<tr>
<td>K₂CO₃</td>
<td>0.432</td>
</tr>
<tr>
<td>Mg(NO)₃</td>
<td>0.529</td>
</tr>
<tr>
<td>KI</td>
<td>0.689</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.753</td>
</tr>
</tbody>
</table>

The desiccators were stored for 3 weeks at a constant temperature of 25°C and the water activity of saturated salt solutions is varying from 0.1 to 0.753. Sample are dehydrated by drying for 24 h at 60°C in a vacuum oven. “Zero” water content can be obtained for dehydrated *Agaricus bisporus* kept over P₂O₅ in a desiccator for 7 days. The weighings of samples were done to the tenth of a milligram in a Precisa XB 120 A analytical balance, before and after placing samples in desiccators. Water sorption isotherm was obtained by modelling experimental data with Aur-Buildgab Model software. Due to the fact that monolayer, m₀, is an optimum moisture which confer a maximum stability during the period of storage of food, we modified the value of monolayer forming two types of samples:

- *Agaricus bisporus* with a value of monolayer of 0.032 g water g⁻¹ dry solids;
- *Agaricus bisporus* with a value of monolayer of 0.062 g water g⁻¹ dry solids;

The samples were packed in plastic pouches laminated with aluminium foil and stored at room temperature for 6 months. Daily we measured temperature resulting an average temperature of 25°C. Once a month oil were extracted in a Soxhlet apparatus using petroleum ether as a solvent, the length of extraction being 10 h [14]. To study the influence of water activity on lipids were determined the following indexes.

**Determination of the Peroxide index**

Peroxide value is defined as the milliequivalents of oxygen per kilogram of oil. It was determined according to the methodology provided by ISO 3960:2017 [15].

**Determination of the Acidity index**

Acid value is number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of fat. It was determined according to the methodology provided by ISO 660:2009 [16].

**Determination of the Refraction index**

The refractive index (RI) of an oil is defined as the ratio of the speed of light in air (technically, a vacuum) to the speed of light in the oil. Refractive index was determined using Abbe refractometer according to ISO 6320:2017 [17].

**3. Results and discussions**

By processing the experimental data obtained with the Aur-Buildgab-Model software program, was obtained the adsorption isotherm for *Agaricus bisporus*, Figure 1.
The value of monolayer of *Agaricus bisporus* using GAB equation was 0.051 g · g$^{-1}$ dry solid. Experimental values of peroxide, acidity and refraction indexes for *Agaricus bisporus* with moisture below monolayer and with moisture above monolayer is show in Table 2-3.

Table 2. Experimental values of peroxide, acidity and refraction indexes for *Agaricus bisporus* with moisture below monolayer.

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>Peroxide index, mEq O$_2$ Kg$^{-1}$ oil</th>
<th>Acidity index, mg KOH g$^{-1}$ oil</th>
<th>Refraction index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5</td>
<td>0.11</td>
<td>1.4621</td>
</tr>
<tr>
<td>30</td>
<td>3.1</td>
<td>0.12</td>
<td>1.4626</td>
</tr>
<tr>
<td>60</td>
<td>8.9</td>
<td>0.21</td>
<td>1.4631</td>
</tr>
<tr>
<td>90</td>
<td>12.1</td>
<td>0.29</td>
<td>1.4637</td>
</tr>
<tr>
<td>120</td>
<td>22.0</td>
<td>0.49</td>
<td>1.4642</td>
</tr>
<tr>
<td>150</td>
<td>30.6</td>
<td>0.69</td>
<td>1.4646</td>
</tr>
<tr>
<td>180</td>
<td>35.2</td>
<td>0.80</td>
<td>1.4650</td>
</tr>
</tbody>
</table>

Table 3. Experimental values of peroxide, acidity and refraction indexes for *Agaricus bisporus* with moisture above monolayer

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>Peroxide index, mEq O$_2$ Kg$^{-1}$ oil</th>
<th>Acidity index, mg KOH g$^{-1}$ oil</th>
<th>Refraction index</th>
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<tr>
<td>0</td>
<td>2.5</td>
<td>0.12</td>
<td>1.4621</td>
</tr>
<tr>
<td>30</td>
<td>2.9</td>
<td>0.15</td>
<td>1.4624</td>
</tr>
<tr>
<td>60</td>
<td>6.1</td>
<td>0.19</td>
<td>1.4628</td>
</tr>
<tr>
<td>90</td>
<td>9.5</td>
<td>0.31</td>
<td>1.4631</td>
</tr>
<tr>
<td>120</td>
<td>11.7</td>
<td>0.38</td>
<td>1.4633</td>
</tr>
<tr>
<td>150</td>
<td>25.7</td>
<td>0.57</td>
<td>1.4636</td>
</tr>
<tr>
<td>180</td>
<td>27.0</td>
<td>0.86</td>
<td>1.4638</td>
</tr>
</tbody>
</table>

The values of peroxides indexes obtained during the first 90 days of storage increased reaching to a value which ranged from 2.5 meq O$_2$ Kg$^{-1}$ oil to 12.1 meq O$_2$ Kg$^{-1}$ oil for *Agaricus bisporus* samples whose moisture was 0.032 g water g$^{-1}$ solids respectively 2.5 meq O$_2$ Kg$^{-1}$ oil to 11.7 meq O$_2$ Kg$^{-1}$ oil during the first 120 days of storage of *Agaricus bisporus* samples whose moisture was 0.062 g water g$^{-1}$.
solids. All samples had increased values of peroxides during the first 150 days of storage and started to decrease in the last 30 days. The decrease of the peroxides values at the end of storage may occur due to decomposition of hydroperoxides into secondary oxidation products.

Lipid oxidation follows two distinct phases. A lag phase first produces low and undetectable levels of oxidation products. This is followed by an exponential phase where the concentration of oxidation products increases dramatically [18]. The experimental values indicate lipid oxidation in two periods [19-21]. In the first 90 days of storage of oil extracted from samples with \( a_w = 0.032 \), we observed a slower growth in peroxide values (12.1 meq O\(_2\) Kg\(^{-1}\) oil). In the next period, up to 180 days, the rate of oxidation increases and peroxide values increase toward the value of 35.2 meq O\(_2\) Kg\(^{-1}\) oil.

In the first 120 days of storage of oil extracted from samples with \( a_w = 0.062 \) we observed a slower growth in peroxide values (11.7 meq O\(_2\) Kg\(^{-1}\) oil). In the next period, up to 180 days, the rate of oxidation increases and peroxide values increase toward the value of 27 meq O\(_2\) Kg\(^{-1}\) oil.

The main factor affecting lipid peroxidation reaction rates seemed to be the reaction initiation type (monomolecular or bimolecular). The monomolecular or bimolecular reaction can be responsible for initiating the lipid oxidaton chain of oil by decomposing peroxides.

During the first 90 and 120 days of storing oil (\( a_w = 0.032 \) and \( a_w = 0.062 \)), the low concentration of peroxides favors monomolecular initiation:

\[
P V^{1/2} = PV_0^{1/2} + \frac{1}{2} k_a t, \quad 0 \leq t < t_b
\]

and when it reaches the critical value of 12.0 meq O\(_2\) Kg\(^{-1}\) oil (the sudden increase of the peroxide index value), the reaction is controlled by the bimolecular mechanism:

\[
P V^{1/2} = PV_b \cdot e^{k_b (t - t_b)}, \quad t_b \leq t
\]

where:
- \( PV \)– the value of peroxide, meq O\(_2\) Kg\(^{-1}\) oil;
- \( PV_0 \)– the value of peroxide at the beginning of experiment (time \( t_0 \));
- \( PV_b \)– the value of peroxide at time \( t \), days;
- \( k_a, k_b \)– constants, (meq O\(_2\) Kg\(^{-1}\));
- \( t_b \)– the time needed to reach the critical point, days.

Based on the data presented in Tables 2 and 3, the highest acidity value is for \( Agaricus bisporus \) samples with a monolayer moisture value of 0.8 mg KOH g\(^{-1}\) of oil versus the moisture content over the monolayer of 0.69 mg KOH g\(^{-1}\) of oil. Changing the acidity of oils occurs in the formation of peroxides which decompose and interact with the formation of several oxidation products, including aldehydes, which are oxidized in acids [3; 21].

The variation of the refractive index of oil samples extracted from \( Agaricus bisporus \) with a monolayer value of 0.062 g water g\(^{-1}\) dry solids during storage is between 1.4621 and 1.4650 compared to that of oil samples extracted from \( Agaricus bisporus \) with a monolayer value of 0.032 g water g\(^{-1}\) dry solids whose values are between 1.4621-1.4638. This variation is due to the change in the degree of unsaturation of fatty acids due to oxidative damage to the oil during storage. Changes in the refractive index show that the rancidity process of oil is enhanced for samples of oil extracted from \( Agaricus bisporus \) whose monolayer value was 0.032 g of water g\(^{-1}\) dry solids. Is observed an increase almost constant of refraction index for \( Agaricus bisporus \) samples whose moisture was above monolayer. At the end of storage period the refraction index of \( Agaricus bisporus \) samples whose moisture was above monolayer was about 1.4638 compared to \( Agaricus bisporus \) samples whose moisture was below monolayer where the increase in the acidity index is more pronounced reaching a value of 1.4650. This variation is due to the change in the degree of unsaturation of fatty acids due to oxidative damage to the oil during storage.
Analyzing the experimental data we noticed that Agaricus bisporus samples with moisture above the monolayer value show an oxidation stability superior to Agaricus bisporus samples with moisture value below monolayer.

During the dehydration process of Agaricus bisporus samples, extraction of water from capillary and pores takes place and is replaced with atmospheric air. Also there is a direct contact between lipids and atmospheric oxygen thus increasing the possibility of initiation of oxidation reaction. For samples with moisture above the monolayer value, the direct contact between the lipids and the atmospheric oxygen is reduced due to the presence of water in the pores and capillaries thus reducing the possibility of initiation the oxidation reaction.

The increased moisture of Agaricus bisporus samples above the monolayer value increases the capillary water mobility, followed by a much higher dissolution of the existing chemical compounds, including polyphenols [22] which by their antioxidant activity contributes to slow down the oxidation process. Dissolved substances bind water molecules through physical bonds (van der Waals bonds).

For samples with moisture above the value of the monolayer, it is assumed that water is sufficient to bind all the substances found in Agaricus bisporus samples, leaving a small excess of free water. In the meantime, the lipid oxidation phenomenon continues with the formation of peroxides. Excess water has the role of blocking the reactivity of peroxides by stabilizing them due to the hydration process. At the same time, the process of hydration of the free radicals formed takes place. The free radical solvation process contributes to the reduction of the acidity index, refraction index and peroxide index [2, 3].

A low moisture below the monolayer value leads to a reduced dissolution of the chemical compounds and implicitly to a partial solubilisation of the free radicals. The occurrence of higher peroxides in oil samples with moisture below the monolayer contributes to the formation of new oxidation compounds followed by the reduction in the number of double bonds of unsaturated fatty acids with consequence in increasing in the acidity index, refraction index and peroxide index [2, 3].

4. Conclusions
Water activity and the monolayer value of food preserved by drying are two parameters of major importance in ensuring their quality and safety. Depending on their value in food, different physico-chemical processes take place with a direct effect on organoleptic qualities. Critical upper and lower water activity levels can be established by determining water sorption isotherm.

References


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