Abstract - Wheat germ represents approximately 3% of the grain and it contains 8-14% oil, which is a rich source of tocopherols (vitamin E) and polyunsaturated fatty acids, mainly linoleic acid. The present work shows the influence of temperature (27ºC and 45ºC) and storage time (maximum 35 days) of the wheat germ on the concentration of tocopherol in the oil. Their effect on other quality parameters was also investigated. Results indicated that oil oxidation and free fatty acid formation increased markedly with temperature and storage time. The initial sample contained 3134 µg/g total tocopherol, of which 67% was α-tocopherol and, in a lower proportions, β-tocopherol and γ-tocopherol (30.5% and 2.4%, respectively). In the temperature range studied, tocopherols decreased as a function of storage time following first-order kinetics. The rate constant k for β-tocopherol increased with temperature. The fatty acid composition was not affected by the storage conditions applied.

Keywords: Wheat germ; Storage; Oil; Tocopherols; Kinetics.

INTRODUCTION

Wheat germ represents 2-3% of the entire wheat grain and it contains between 8 and 14% of oil (Sonntag, 1979; Pomeranz, 1988). It is a by product of industrial wheat milling, being separated from the endosperm during this process, and used mainly as forage and as a source from which oil is extracted. It is a valuable product for its nutritious and pharmaceutical properties (Itoh, 1973; Barnes, 1983; Kahlon, 1989; Bramley et al., 2000; Zacchi et al., 2006; Eisenmenger and Dunford, 2008).

Wheat germ oil is well known for its beneficial health effects due to its high content of vitamin E and polyunsaturated fatty acids, mainly linoleic acid (omega 6, between 44 and 65%) and linolenic acid (omega 3, in a lower proportion, 4-11%) (Wang and Jonson, 2001; Megahad and El Kinawy, 2002). Tocopherols protect vegetable oils against oxidation-related deterioration and they perform an important biological activity as vitamin E. However, the high content of polyunsaturated fatty acids makes the oil highly prone to oxidation. Thus, it can undergo transformations that may affect both its nutritional and organoleptic qualities.

Gustone et al. (1994) reported that one gram of wheat germ oil contains approximately 2188 µg of total tocopherols, consisting of 53.9% α-tocopherol, 18.2% β-tocopherol, 22.5% γ-tocopherol and 5.4% of δ-tocopherol. Along with sunflower oil, wheat germ oil is one of the oils with the highest concentration of α-tocopherol (Gustone et al., 1994; Nolasco et al., 2004; Nolasco et al., 2006).

The mechanical treatment undergone by the wheat grain during the milling process produces cell rupture and oil diffusion (Apro et al., 2004). This,
together with the germ features mentioned above, makes the product highly prone to a rapid deterioration. The highest possible retention of tocopherols during processing and storage of the germ will contribute to prevent or decrease oxidation processes in the oil with a high percentage of polyunsaturated fatty acids (Wang and Jonson, 2001). Therefore, it is important to know the effects that different storage conditions may have on the oil quality and, specifically, on the tocopherol concentration.

There are few studies on the kinetics of tocopherols in cereal degradation during storage. Hidalgo et al. (2009) showed that the kinetics of tocols degradation in wholemeal and white flours from einkorn and bread wheat decreased during storage as a function of time and temperature, following first-order kinetics.

The main purpose of this work was to study the effect of temperature and storage time of the wheat germ on the oil tocopherol concentration and also analyze their influence on other quality parameters, such as free fatty acid content, peroxide rates and fatty acid composition.

**MATERIALS AND METHODS**

**Sample**

The sample consisted of 50 kg of wheat germ provided by Molinos Cañuelas SACIFIA (Argentina). The germ was stored until use in plastic recipients at 5°C. The moisture content was determined according to the AACC method 44-19 (1993) and the oil yield was extracted in a Soxhlet (n-hexane, 80°C for 8 h), following the IUPAC method 1.122 (1992). After oil extraction, the solvent was evaporated on a rotary evaporator (Buchi, Waterbath B-480) under vacuum at 50°C. The recovered oil was kept in a caramel-colored bottle and the residual hexane was eliminated under a nitrogen stream.

**Sample Processing**

Two hundred grams of wheat germ were placed on aluminum trays (covered with aluminum foil) and stored in a culture stove at 27°C (68% of relative humidity) for 35 days, extracting samples every 7 days for quality analysis. A similar treatment was carried out for storage at 45°C (25% relative humidity). A control sample (time 0) of wheat germ was stored in the refrigerator at 5°C in a plastic recipient. All the tests were performed in triplicate.

After each period of storage in the stove, the wheat germ oil was extracted in a Soxhlet apparatus, as previously described.

**Oil Analyses**

The oils obtained in the control and storage samples were characterized for free fatty acid content, peroxide rate, tocopherol concentration and fatty acid composition.

**Oil Quality Rate**

The free fatty acid content (free acidity expressed as the percentage of oleic acid) and peroxide rates were determined according to the AOCS (1998) and IUPAC (1992) Standard Methods.

**Tocopherol Concentration**

The tocopherol concentration in wheat germ oil was determined by high performance liquid chromatography (HPLC) with normal phase, following the IUPAC 2.432 (1992) and AOCS Ce 8-89 (1998) Standard Methods. A Hewlett Packard Series 1050 equipment with fluorescence detector was used (excitation wavelength: 292 nm, and emission wavelength: 330 nm), with a HiCHROM column, Lichrosorb Si 60, 250 x 4.6 mm i.d. and 5 μm particle size. Hexane: isopropanol (99.5:0.5 v/v) was used as mobile phase, with a flow rate of 1.5 ml/min. The identification of peaks in the chromatogram was carried out considering the retention times and/or the use of patterns of the different tocopherol isomers. The quantification of the different tocopherol isomers was performed by the external standard method using α-tocopherol as reference (Sigma T3251, 95% purity; AOCS Standard Method Ce 8-89, 1998) and the results were expressed in μg tocopherol/g oil.

**Fatty Acid Composition**

The fatty acid composition was determined by gas-liquid chromatography (Izquierdo et al., 2009). Methyl esters were obtained using acetyl chloride in methanol and chloroform as solvent. A Varian 3400 chromatograph equipped with an Alltech capillary column (30 m x 0.25 mm) and a Flame Ionization Detector (FID) with a split ratio of 95/1 was used. Furnace, injector and detector temperatures were 210°C, 250°C and 275°C, respectively. N\textsubscript{2} was used as carrier gas. The identification of the peaks in the chromatogram was carried out considering retention times and using the patterns of methyl esters.
Kinetic Data Analysis

In order to determine the degradation reaction order of α- and β-tocopherol, zero and first-order kinetics were hypothesised by applying the general reaction rate expression \( -dC/dt = kC^n \), where \( C \) is the tocopherol concentration (\( \mu g/g \)), \( k \) is the reaction rate constant (days\(^{-1}\)), \( t \) is the reaction time (days), and \( n \) is the order of the reaction. The regression coefficients were 0.70 \( \sim \) 0.78 for the zero-order kinetics, and 0.87 \( \sim \) 0.98 for the first-order kinetics. Thus, the first-order kinetic equation was used in this study: \( \ln [C] = [C_0] - k_{exp} t \), where \([C]\) is the residual tocopherol content during heating, \([C_0]\) is the tocopherol content at the beginning of heating (\( t = 0 \)), \( t \) is storage time at 45ºC, and \( k_{exp} \) is the rate constant of the first-order kinetics.

Data Analysis

The data obtained were evaluated by statistical analysis of variance using the program InfoStat 6.0 (2003) according to Tukey’s test (\( P<0.05 \)).

Relations among variables of storage and oil properties were determined by the software SigmaPlot Scientific Graphing System, version 4.10 (Jeandel Corporation, SSPS Inc. 1986-1997).

RESULTS AND DISCUSSION

Initial Sample Characterization

The wheat germ used for the experimental tests presented a moisture content of 13.7% and an oil percentage of 10.2% dry base (d.b.) within the 8-14% range reported by other authors for this product (Sonntag, 1979; Pomeranz, 1988). This sample, taken as control, presented a free acidity of 3.9% (expressed as oleic acid) and no peroxides were detected. As regards its fatty acid composition, it presented linoleic acid as its main component (59.4%), followed by 18.8% of palmitic acid, 14.3% of oleic acid, and linolenic and stearic acids in a lower proportion (7.0% and 0.5%, respectively). These percentages were in agreement with those reported in the literature (Wang and Jonson, 2001; Megahad and El Kinawy, 2002). In addition, the sample showed a concentration of total tocopherols of 3134 \( \mu g/g \), higher than that reported by Gustone et al. (1994), with the highest percentage corresponding to α-tocopherol (67%). The presence of β- and γ-tocopherol (30.5% and 2.4%, respectively) was also observed. In contrast with the reports by Gustone et al. (1994), δ-tocopherol was not detected.

Effects of Storage Conditions

Free Fatty Acids

Table 1 shows that the amount of free fatty acid increased with storage time for both temperatures tested, with a higher increase for the higher temperature. The analysis of variance allowed us to confirm the significant effect of both the storage temperature and days of storage on the free acidity of the wheat germ oil (\( P<0.0001 \)). However, no significant temperature x days of storage interaction was detected (\( P \geq 0.0628 \)).

Similar to the results reported by Megahad and El Kinawy (2002), an increase in the free fatty acid concentration was observed in the oil during wheat germ storage. The effect was significant during all the storage time, showing an exponential variation represented by the following equation (\( R^2: 0.9991, P<0.0001 \)): \( Y = a + b t^c \), where \( Y \) is the free acidity (% oleic acid), \( t \) is the storage time (days), and the constants: \( a=3.8603 \) (\( P<0.0001 \)), \( b=1.4860 \) (\( P=0.0003 \)) and \( c=0.4316 \) (\( P<0.0001 \)). These results confirm the need to stabilize the wheat germ after the industrial milling process, disabling the enzymes present in it (i.e., lipase) (Kapranichkov et al., 2004).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>27</th>
<th>45</th>
<th>A.T.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.9 ± 0.71</td>
<td>3.9 ± 0.71</td>
<td>4.98</td>
</tr>
<tr>
<td>7</td>
<td>6.2 ± 0.68</td>
<td>8.4 ± 0.47</td>
<td>5.15</td>
</tr>
<tr>
<td>14</td>
<td>6.7 ± 1.13</td>
<td>10.1 ± 1.67</td>
<td>5.50</td>
</tr>
<tr>
<td>21</td>
<td>7.8 ± 0.88</td>
<td>11.2 ± 0.12</td>
<td>6.90</td>
</tr>
<tr>
<td>28</td>
<td>8.8 ± 0.11</td>
<td>11.5 ± 1.10</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>9.8 ± 1.97</td>
<td>11.7 ± 0.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Peroxide Rate

In the oil corresponding to the wheat germ sample stored at 27°C, no formation of peroxides was detected during all the storage time, as was also reported by Megahad and El Kinawy (2002). However, in the case of the wheat germ samples stored at 45°C, peroxides were observed in the oil after 21 days of storage (1.85 meq/kg), the formation of peroxides being significant after 28 days of storage (P=0.0007, Figure 1), reaching a maximum value of 6.11 meq/kg at the end of the storage period. This can be attributed to the initial propagation stage of the lipid auto-oxidation phenomenon, with chain reactions, and thus a rapid increase of peroxide formation.

Tocopherol Concentration

A decrease in total tocopherol concentration was observed in the oil when increasing the storage temperature of the wheat germ (Figure 2). Nevertheless, this difference between storage temperatures was not significant (P>0.0855). At the same time, the statistical analysis of variance allowed us to detect significant differences among storage days (P<0.0001), without a significant temperature x storage time interaction (P≥0.0602). Figure 2 shows that the total tocopherol concentration decreased as the storage time elapsed.

The concentrations of α- and γ-tocopherol showed a similar behavior to that observed for total tocopherols. For both antioxidants, the analysis of variance showed only one significant effect of the storage time (P<0.0001, Figures 3 and 4), whereas the influence of temperature and the temperature x time interaction were not significant (P≥ 0.0501 and ≥ 0.5996 for α-tocopherol, respectively, and P≥ 0.4447 and P≥ 0.2574, for γ-tocopherol, respectively). The concentration of α- and γ-tocopherol decreased on average 35% and 65%, respectively. Similar results were found by Garcia-Pascual et al. (2003) when analyzing the influence of the storage conditions on the oil quality of different varieties of almond, observing a decrease in the α-tocopherol concentration with time and no significant influence of the storage temperature (8°C and 36°C). The decrease in the concentration of α-tocopherol affects mainly the oil quality from the view point of its biological properties, since this isomer exhibits the highest vitamin E activity. At the same time, the decrease in the concentration of γ-tocopherol affects specially the oil stability, being one of the isomers with the highest antioxidant activity (Burton and Ingold, 1981; Velasco and Fernández-Martínez, 2002).

On the other hand, the concentration of β-tocopherol in wheat germ oil was affected both by temperature and the storage time of the germ (P<0.0001), being also significant the interaction between both parameters (P<0.0001) (Figure 5).

Figure 1: Peroxide rates (meq/kg) of wheat germ oils obtained for the germ stored at 45°C for different periods of time. Values followed by different letters indicate significant differences.
The influence of temperature on the reaction rate, which manifests itself as an increase of the rate constant with increasing temperature, was analyzed using the Arrhenius equation: \( \ln k_{\text{exp}} = \ln A - \frac{E^*}{R \cdot T} \), where \( R \) is the gas constant (1.9872 cal mol\(^{-1}\) K\(^{-1}\)) and \( T \) is the absolute temperature (K).

Figure 6 shows the first-order kinetic degradation of total tocopherol, \( \alpha \)-tocopherol, \( \beta \)-tocopherol and \( \gamma \)-tocopherol (\( \mu \)g tocopherol/g oil) of wheat germ oil for the germ stored at 45°C for different time periods. Similar trends were obtained by storing at 27°C.

The degradation rate constants (\( k_{\text{exp}} \)) of \( \alpha \)-tocopherol estimated from the linear regression data were 11.3 \( \times \) 10\(^{-3}\) days\(^{-1}\) (\( r = 0.979 \)) and 11.7 \( \times \) 10\(^{-3}\) days\(^{-1}\) (\( r = 0.984 \)) at 27 and 45°C, respectively. The experimental activation energy (\( E^* \)) estimated for the oxidative degradation of \( \alpha \)-tocopherol was 0.4 kcal/mole.
On the other hand, the degradation rate constants of \( \gamma \)-tocopherol estimated from the linear regression data were 6.2 \( 10^{-3} \) days\(^{-1} \) (r= 0.897) and 57.7 \( 10^{-3} \) days\(^{-1} \) (r= 0.985) at 27 and 45°C, respectively. The experimental activation energy estimated for the oxidative degradation of \( \gamma \)-tocopherol was 13.8 kcal/mol.

Considering that the experimental tests were carried out for two storage temperatures, the present work presents a first approximation of the E* value for \( \alpha \)- and \( \gamma \)-tocopherol degradation, future studies that consider other storage temperatures and that extend their ranges being necessary in order to confirm the values obtained.

As shown in the plot of \( \beta \)-tocopherol concentration vs. storage time, the degradation rate constants were 9.5 \( 10^{-3} \) days\(^{-1} \) (r= 0.981) and 14.0 \( 10^{-3} \) days\(^{-1} \) (r= 0.989) at 27 and 45°C, respectively. The estimated experimental activation energy for the oxidative degradation of \( \beta \)-tocopherol was 4.083 kcal/mol. At higher temperatures (45°C) and longer storage times, the oxidative degradation of \( \beta \)-tocopherol increased.

These results suggest that the degradation of \( \beta \)-tocopherol was more affected by the change of heating temperature than that of \( \alpha \)-tocopherol.

**Fatty Acid Composition**

The storage conditions analyzed did not significantly affect the fatty acid composition of the wheat germ oil (P \( \geq \) 0.05). Table 2 shows the average fatty acid composition of wheat germ oil for the set of samples (27°C and 45°C) for the different storage times, having similar percentages with respect to the control samples. The maximum coefficient of variation observed was 6.9% for stearic acid. The polyunsaturated acids (linoleic and linolenic) presented very low variations (coefficients of variation of 0.41 and 2.99%, respectively).

![Figure 6: First-order kinetic degradation of total tocopherol, \( \alpha \)-tocopherol, \( \beta \)-tocopherol and \( \gamma \)-tocopherol (\( \mu g \) tocopherol/g oil) of wheat germ oil for the germ stored at 45°C for different time periods and of the control sample (time 0).](image)

**Table 2: Average fatty acid composition of the wheat germ oil for the set of samples of wheat germ stored at 27°C and 45°C, for different periods of time.**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Palmitic</th>
<th>Stearic</th>
<th>Oleic</th>
<th>Linoleic</th>
<th>Linolenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>18.96 ± 0.10</td>
<td>0.48 ± 0.04</td>
<td>14.31 ± 0.25</td>
<td>59.39 ± 0.11</td>
<td>6.88 ± 0.05</td>
</tr>
<tr>
<td>14</td>
<td>18.86 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>14.03 ± 0.08</td>
<td>59.73 ± 0.11</td>
<td>6.94 ± 0.01</td>
</tr>
<tr>
<td>21</td>
<td>19.02 ± 0.43</td>
<td>0.48 ± 0.01</td>
<td>14.06 ± 0.39</td>
<td>59.44 ± 0.11</td>
<td>7.03 ± 0.07</td>
</tr>
<tr>
<td>28</td>
<td>19.04 ± 0.51</td>
<td>0.47 ± 0.02</td>
<td>14.08 ± 0.30</td>
<td>59.52 ± 0.59</td>
<td>6.91 ± 0.23</td>
</tr>
<tr>
<td>35</td>
<td>19.27 ± 0.33</td>
<td>0.46 ± 0.08</td>
<td>14.22 ± 0.40</td>
<td>59.35 ± 0.28</td>
<td>6.71 ± 0.52</td>
</tr>
</tbody>
</table>
CONCLUSIONS

At higher temperatures and storage times, an increase in the concentration of free fatty acids and peroxide values was observed, both being important variables for the control of the hydrolytic and oxidative rancidification of oil during the wheat germ storage.

The concentration of total tocopherols in oil decreased significantly during the storage time of the germ. However, no significant effect was observed for the temperatures analyzed (27°C and 45°C; 35 storage days).

Both the isomer with the highest concentration and biological vitamin E activity (α-tocopherol) and the one present in a lower proportion, but with a higher “in vitro” antioxidant activity (γ-tocopherol), showed a behavior similar to that observed for total tocopherols. However, the concentration of β-tocopherol was affected by both storage variables analyzed.

In the temperature ranges studied, tocopherols decreased as a function of storage time, following first-order kinetics. The β-tocopherol reaction rate constant k increased with temperature.

The fatty acid composition was not affected significantly by the storage conditions applied.

According to the results obtained, it is suggested to store the wheat germ at approximately room temperature during relatively short periods of time in order to preserve the oil quality. At the same time, it becomes interesting to carry out further studies to assess the effect of the stabilization process of the wheat germ on the concentration of total tocopherols and the corresponding isomers and the disability of the enzymes present.

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