DOI 10.1002/ejlt.200401044 *Eur. J. Lipid Sci. Technol.* **107** (2005) 74–79

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Storage of sunflower-seeds: variation on the wax content of the oil

Storage conditions of oil seeds before industrial extraction might influence the quality of the crude oil. The objective of this work was to study the influence of sunflower seed storage conditions (temperature and time) on the quality of the resulting oil in terms of its wax content and composition. Sunflower seeds were stored under different conditions, 10, 21 and 37 °C, in sealed recipients. Extractions of the seeds with hexane were made to obtain the oil at different storage times. The amount of oil extracted (25–40%) showed no significant differences with storage conditions. Wax content of the samples was determined with two different methods (laser polarized turbidimetry and microscopy), and results showed that wax concentration increased with storage conditions (time and temperature). Composition of wax components, determined using capillary gas chromatography, during storage was approximately constant for C_{35}–C_{39} and showed significant differences for C_{40}–C_{48} components. Waxes with high carbon number cause more turbidity than waxes with low carbon number, due to their higher melting point, resulting in a low-quality crude oil and therefore in variations in processing conditions during the oil refining. According to the data showed in this study, seed storage at low temperatures during short periods of time may be the more adequate conditions to obtain high-quality oil.

Keywords: Solid phase extraction, storage temperature, storage time, sunflower oil, wax content.

1 Introduction

The quality and stability of vegetable oils such as sunflower oils are affected by the presence of minor components. One of these components is wax, esters of C_{36} to C_{48}, which are found mainly on the surface of the hull. Morrison [1] demonstrated a relationship between hull content of the seed and wax content of the hull, variety and planting location [2]. Furthermore, the introduction of new varieties of sunflower seeds with high oil yields has brought about the need to increase the efficiency of the wax separation process, in order to avoid turbidity of the refined oil [3]. This turbidity is caused by crystallization of waxes due to their low solubility in oil [4]; therefore, they must be eliminated in the refining process (winterization). Unfortunately, winterization of sunflower seed oil is quite difficult because of the low wax content (0.02–0.35%) in the oil, together with the presence of gums that interfere in the wax crystallization [5].

Practices for harvesting oilseeds to maximize quality in yield plus storage and transportation procedures to minimize seed damage are almost as important as processing technology for the production of wholesome, high-quality, minimum-cost final products. Therefore, some of the world's major oilseed crops are used to illustrate the important factors during harvesting and storage [6, 7]. The principal factors that might affect the storage of the seed are moisture, temperature, humidity and bacteria. Seeds stored at high humidity and temperature may produce an increase in seeds temperature owing to the growth of bacteria. Very high temperatures increase the free fatty acids content resulting in low-quality oil. High humidity and temperature values during seed storage result in an increase of carbon dioxide and water production from sugar and starch. While temperature and humidity conditions increase, there is an exponential increment on seed respiration. High temperatures promote the action of enzymes, resulting in low quality of protein and oil (high content of free fatty acids, undesirable flavors, etc.) [8].

Considering that waxes have to be eliminated from the sunflower oil during the winterization process, since they are responsible for the turbidity present in the oil; and taking into account that storage conditions may affect the quality of the oil, the aim of the present work is to analyze the effect of sunflower seed storage conditions (time and temperature) on wax content and wax composition of the crude oil, in order to optimize the winterization step during sunflower oil refining.

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2 Materials and methods

2.1 Sunflower seeds, storage conditions

Sunflower seeds were provided by Molinos Río de La Plata S.A. (Avellaneda, Buenos Aires, Argentina). The seeds were stored in sealed recipients at three different temperatures, 10, 21 and 37 °C, for one year; samples were taken at different periods of time to determine both oil content and wax concentration in the crude oils, together with their wax composition.

2.2 Oil extraction

Seeds stored under the storage conditions described above were milled. The oil was extracted from the seeds using a soxhlet equipment (semi-continuous extraction) with hexane as the extracting solvent. Seeds were in contact with the solvent during 2 h, and afterwards the hexane was eliminated by evaporation (60 °C, 60 min, approximately). The extraction procedure was done in triplicate, and the quantity of the oil obtained was calculated.

2.3 Isothermal crystallization of waxes

Wax content of the oils obtained from seeds stored under different time and temperature conditions was analyzed by means of a laser turbidimeter. The isothermal crystallization of waxes present in the oil was induced as described by Martini and Añón [9]. The equipment was used by Herrera [10] to study crystallization in other lipid systems, and it was demonstrated to be applicable in the determination of wax concentration in vegetable oils [9]. Induction times of crystallization (interval between the moment crystallization temperature is reached and the start of the crystallization) of the oil samples were determined at 12 and 22 °C in order to determine their wax concentration.

2.4 Microscopic method

A Leitz microscope model Ortholux II (Ernest Leitz Co., Wetzlar, Germany) with a controlled temperature plate was used to follow the solubility of waxes in the oil as the temperature increased. The temperature was controlled through a programmable cryostat (Lauda UK 30, Werk Lauda-Königshofen, Germany). Ethylene glycol in water (3:1) was used as refrigerant fluid. When the plate reached 5 °C, the sample and a 0.05 mm i.d. Copper-Constantan thermocouple connected to a two-channel Gilson potentiometer (Gilson France S.A., Villiers le Bel, France) were placed between a slide and the cover-slide. Wax concentration was determined as described previously by Rivarola et al. [3] and Petruccelli and Añón [11].

2.5 Wax-free oil

Wax-free oil was obtained from commercial oil (Molinos Rio de la Plata S.A., Argentina) after centrifugation (60 min, 0 °C, 3000 × g). Waxes that crystallize at 0 °C or more were eliminated by this procedure. This oil was used to dilute the samples and enable the determination of wax concentration with the turbidimetric method.

2.6 Solid-phase extraction

Waxes were isolated from triacylglycerides by solid-phase extraction (SPE) [12]. A silica column was used to obtain the purified waxes (10 g; Alltech, Deerfield, IL, USA). The oil was heated (1.5 h at 110 °C), and 0.4 g was mixed with the dye Sudan II (one drop, 1% in $CHCl_3$). The mixture was heated for another 30 min to eliminate the $CHCl_3$ of the dye; the dried sample was then transferred into the silica gel column rinsed with $CHCl_3$. The test tube was washed three times with 50 μL $CHCl_3$, and these solutions were transferred into the rinsed silica gel column. The sample was eluted with $CHCl_3$; waxes were eluted before the dye appearance, while the triacylglyceride fraction was eluted with the dye. This fraction was discarded and the first eluate (which contained the wax fraction) was dried by heating at 60 °C and recovered with 500 μL $CHCl_3$. Finally 2 μL of the solution was injected into the gas chromatograph, with the addition of a suitable internal standard (0.2 μg/μL, C_{34}, stearic acid palmityl ester; Sigma Chemical Co., St. Louis, MO, USA).

2.7 Capillary gas chromatography

For Capillary gas chromatography (c-GC) a Varian 3400 equipped with a flame ionization detector (FID) was used. The operating conditions were as follows: carrier gas, helium (3 mL/min); oven temperature program, 2 min at T_i = 240 °C, raised at 2 °C/min to T_f = 340 °C, and 25 min at 340 °C; injector temperature, 340 °C; split ratio, 1 : 95; FID temperature, 350 °C; capillary column, AT-5, length 15 m × 0.25 mm i.d., film thickness 0.25 μm (Alltech, Deerfield, IL, USA). A calibration curve was done using C_{34}, stearic acid palmityl ester; C_{38}, stearic acid arachidyl ester; C_{40}, arachidic acid arachidyl ester; C_{42}, arachidic acid behenyl ester; C_{44}, behenic acid behenyl ester (Sigma Chemical Co., St. Louis, MO, USA) as standards [13]. Composition of wax components was determined by injecting 2 μL of the wax extracted as described before.

2.8 Statistical analysis

All the data reported below were analyzed using Student's *t*-test ($p < 0.05$).

3 Results and discussion

Sunflower seeds were stored at different temperatures (10, 21 and 37 °C), and samples were taken at 0, 10, 48, 124, 236 and 306 d of storage. The oil was extracted from the seeds as described in Section 2. Fig. 1 shows the oil content obtained for the different samples. Although there was a slight variation in the oil content of the seeds (25–40%), there was not a clear tendency in that variation, neither with time nor with storage temperature. Differences found in oil content values were not significantly different ($p < 0.05$).

Wax content of these samples was determined using a polarized laser turbidimeter as described in Section 2. Samples were diluted with wax-free sunflower oil and crystallized at 12 and 22 °C to determine their induction times of crystallization (τ) and, therefore, their concentration. The induction times of all samples and the isothermal crystallization conditions obtained are shown in Tab. 1.

Fig. 2A shows the wax concentration of each sample determined with the turbidimetric method. This figure shows an increase in wax concentration both with temperature and storage time. The initial wax concentration was approximately 1000 ppm for all storage conditions. After 50 d, the wax concentration slightly increased, and then, at 125 d of storage, it decreased to approximately the initial value. After this time, the wax concentration significantly increased for samples stored at 21 and 37 °C and slightly decreased or remained constant for storage at 10 °C. The highest wax concentration value obtained

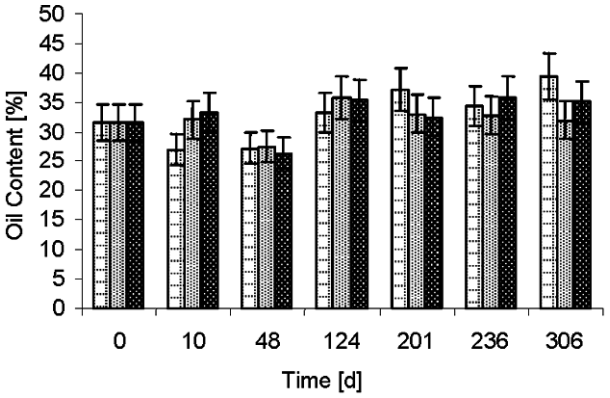

Fig. 1. Oil content obtained from sunflower seeds stored under different storage conditions: ▫ ▩ ■ 10, 21 and 37 °C, respectively.

Tab. 1. Induction times of crystallization of oil samples.

Storage conditions		Dil. [p/p]	Tc [°C]	τ [min]
Time [d]	Temperature [°C]			
0	10	1 : 100	12	42.9
10		1 : 100	12	24.6
48		1 : 10	22	0.2
124		1 : 10	22	1.5
201		1 : 20	22	9.6
236		1 : 20	12	0.5
306		1 : 20	22	9.6
0	21	1 : 100	12	42.9
10		1 : 100	12	12.3
48		1 : 10	22	0.9
124		1 : 10	22	3.1
201		1 : 20	22	8.2
236		1 : 20	22	6.8
306		1 : 20	22	7.4
0	37	1 : 100	12	42.9
10		1 : 50	12	8.1
48		1 : 10	22	1.1
124		1 : 25	12	2.6
201		1 : 20	22	5.3
236		1 : 20	22	4.6
306		1 : 20	22	4.8

was observed for the oils extracted from seeds stored at 37 °C (2000 ppm), while lower values were found for the oils obtained from seeds stored at 21 (1600 ppm) and 10 °C (1400 ppm). This increase in wax concentration both with time and storage temperature could be explained basically by two different mechanisms. On the one hand, seeds could be damaged during storage due to high temperatures, resulting in structural changes. Therefore, waxes can be easily extracted from the damaged cells on the seed, resulting in a higher wax concentration in the oil. On the other hand, it is well known that temperature promotes enzyme activity. As all biosynthetic pathways, wax synthesis is mediated by enzymes, and a higher enzymatic activity may cause an increase in the wax synthesis rate, resulting in higher amounts of waxes during storage at high temperatures.

Wax concentration of the samples was also determined using the microscopic method described in Section 2 (Fig. 2B). Wax concentration slightly increased during the first 10 d of storage for samples stored at different temperatures. Then, the wax concentration decreased approximately to its initial value and remained constant until 125 d of storage. After this time there was a significant increase of wax concentration for all samples. The maximum values obtained using the microscopic method were 3000, 2000 and 1900 ppm for samples stored at 37,

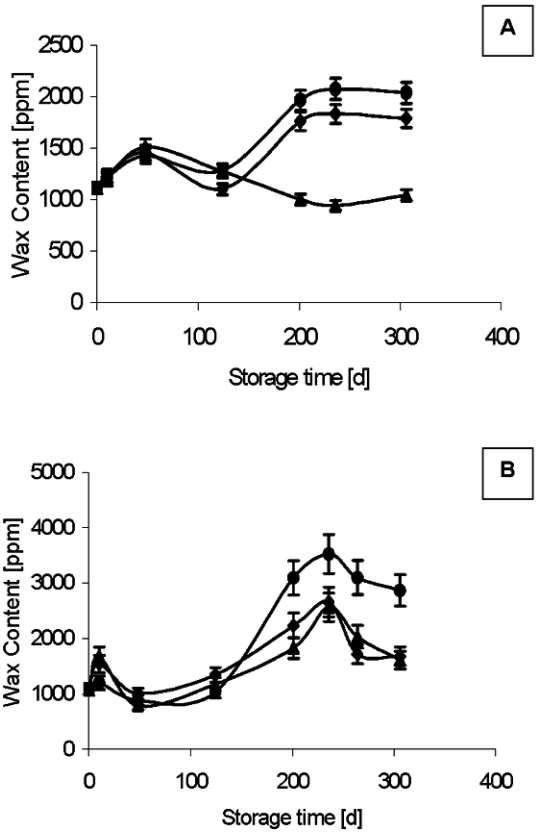

Fig. 2. Wax content of oil samples obtained from sunflower seeds stored under different storage conditions (time and temperature). (▲) 10 °C, (◆) 21 °C, and (●) 37 °C, determined with the turbidimetric method (**A**) and by microscopy (**B**).

21 and 10 °C, respectively. Although some differences were found between the wax concentration values obtained with the different methods (turbidimetric and microscopic), an increase in wax concentration with time and storage temperature was observed in both cases (Figs. 2A and B).

Differences in wax determinations between methods are more significant when oils with high wax concentrations were analyzed (2500 ppm), as reported elsewhere [9]. These differences could be due to the presence of impurities that might delay the melting process of waxes, resulting in higher wax concentration values when using the microscopic method than those expected when using other analytical methods. These components did not seem to affect the wax crystallization process, showing more accurate results during the turbidimetric measurements. Regardless of the method used to determine wax concentration, a clear tendency is observed. Wax concentrations in oils obtained from seeds stored at high temperatures are higher than the values obtained from seeds stored at lower temperature. Additionally, seeds stored for longer periods of time generated oils with higher wax content and therefore higher turbidity.

Wax composition was analyzed using gas chromatography [4, 13]. Waxes were isolated from the triacylglyceride fraction by means of a solid-phase extraction (SPE) technique [12]. The first eluate of this extraction contained the wax components of the oil. After isolating the waxes from the oil sample, they were injected into the chromatograph with the addition of an internal standard as described in Section 2. Wax molecules present in the oils analyzed had between 36 and 48 atoms of carbon. The longer the wax chain (higher number of carbon atoms), the longer was the retention time in the chromatographic column.

Wax components of C_{36} and C_{38} were found in low proportion (less than 5%), and less than 1–3% of C_{37} and C_{39} were obtained for all storage conditions assayed (data not shown). Components of even carbon number were found in higher proportion (C_{40}–C_{48}), together with C_{41} waxes and high-molecular weight esters that eluted after C_{48} waxes [14–16].

Fig. 3 shows the variation of wax composition (percentages of C_{40}, C_{41}, C_{42}, C_{44}, C_{46} and C_{48}) during storage conditions (time and temperature). These results show that the percentages of C_{40}, C_{42} and C_{44} wax components present in the oil obtained from stored seeds were about the same or lower than the ones obtained from non-stored seeds, for almost all storage days. Waxes of C_{41}, C_{46} and C_{48} had an opposite behavior; this means that the proportion of these components increased with longer storage time. When storage temperature was analyzed, a slight increase in C_{40}, C_{42} and C_{44} was observed with high storage temperature, especially for long storage periods, while C_{41} percentage decreased at high storage temperature and C_{46} and C_{48} showed a maximum at 21 °C.

According to the results obtained, variations in the percentages of C_{40}, C_{42} and C_{44} were inversely correlated with the variations of C_{41}, C_{46} and C_{48}. The inverse relationship between these components might be due to the action of hydrolases during storage. Ester bonds of waxes may be affected by these enzymes, especially at high temperatures, producing alcohols and fatty acids [17]. Taking into account that in the seed cell the enzymatic pool is still active, waxes may be used as substrates of the enzymes involved in wax biosynthesis (elongases, reductases, hydroxylases, etc.), resulting in changes in wax composition. It can also be seen that there is a higher activity of decarboxylases and hydrolases, especially at 10 and 21 °C, resulting in higher values of C_{41}. It seems that the production of C_{44}, C_{46} and C_{48} waxes needs less time than the synthesis of mole-

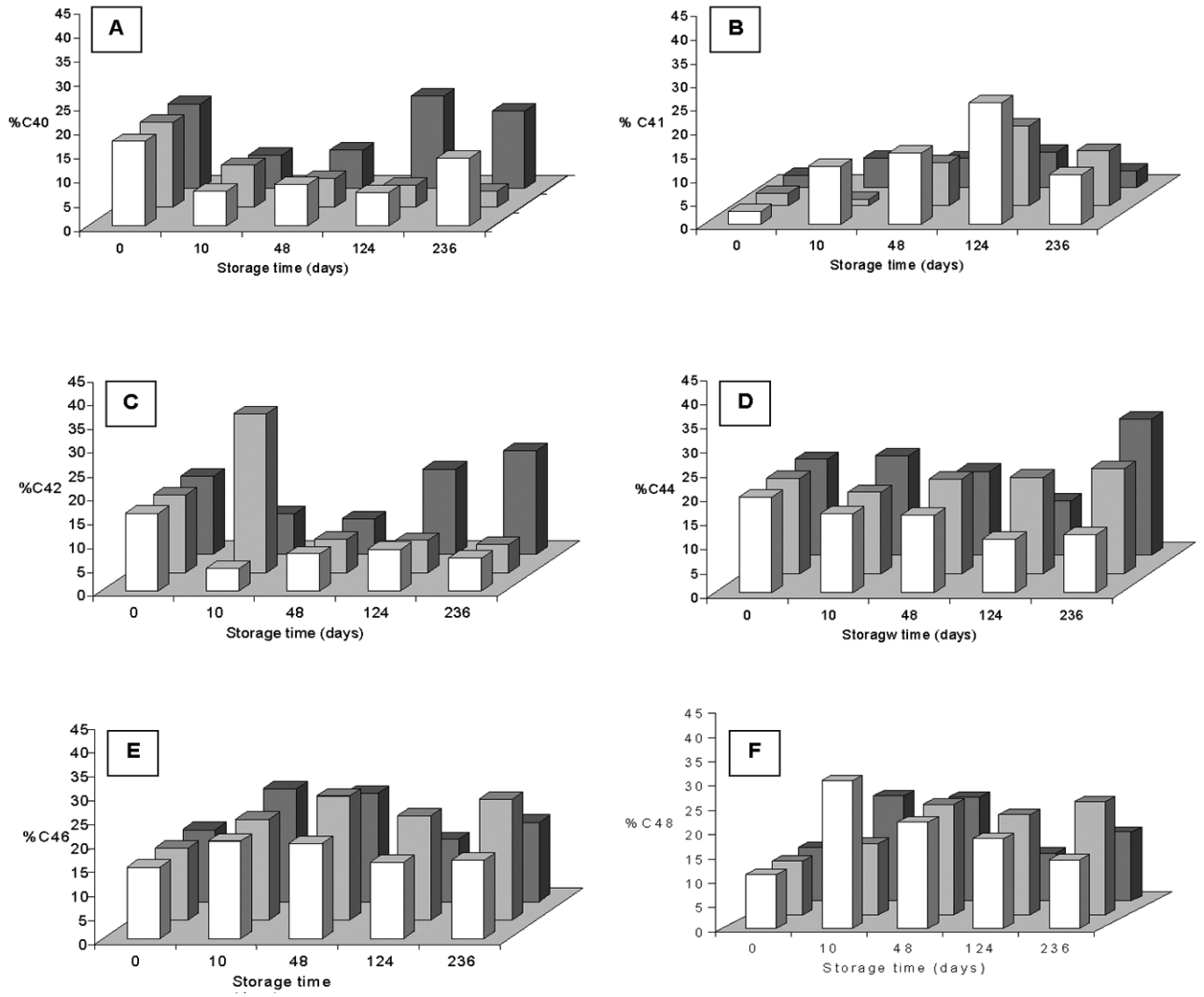

Fig. 3. Wax composition expressed in percentage values (%): (**A–F**) for C_{40}, C_{41}, C_{42}, C_{44}, C_{46} and C_{48}, respectively, of oils obtained from seeds stored at 10 °C (□), 21 °C (▒) and 37 °C (■).

cules with C_{40} and C_{42}. The enzymes responsible for these reactions are elongases, and they seem to be more active at 21 and 37 °C.

In conclusion, the data shown in this work suggests that storage conditions increase wax concentration in the oil and cause variations on wax composition (waxes richer in components of higher carbon numbers when seeds are stored at high temperatures and for long periods of time). These two parameters (wax concentration and composition) influence oil quality. Oils with a high amount of waxes need a stricter winterization process to pass the cold test, since consumers will not accept turbid oils. On the other hand, wax composition also influences the winterization step, since waxes with high number of carbons cause more turbidity (as they have higher melting points than waxes with a lower number of carbons), resulting in variations in oil processing conditions. Therefore, according to the data shown in this study, in order to optimize the refining process, storage at low temperatures and for short periods of time may be the more adequate conditions to obtain high-quality oil.

Acknowledgment

Thanks to the National Research Council of Argentina for the Ph.D. Fellowship granted to Silvana Martini. Dr. Añón is Superior Researcher of the National Research Council of Argentina. This work was performed under financial support from the project PID-BID 0357 (FONCyT-ANPCyT, Argentina).

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[Received: July 9, 2004; accepted: December 30, 2004]

