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INVESTIGACIÓN

# Changes in pumpkin seed oil during heating.

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**RESUMEN**

**Cambios en aceite de semilla de calabaza durante el calentamiento**

Se calentó aceite de semilla de calabaza en continuo e intermitentemente durante 10 horas a 180±5°C y se evaluaron los cambios ocurridos. Aumentaron los ácidos grasos libres (FFA), índice de peróxido (PV), color, compuestos polares y viscosidad, mientras que disminuyó el índice de iodo y la insaturación. El calentamiento intermitente produjo el efecto más pronunciado sobre estos cambios. La determinación del perfil de ácidos grasos mostró cambios no significativos en los ácidos palmítico (C16:0), araquídico (C20:0) y oleico (C18:1) durante el calentamiento. Se observaron disminuciones significativas estadísticamente en el contenido en ácido linoleico (C18:2), que causó un aumento significativo en el contenido en ácido esteárico (C18:0). Sólo se detectó α-tocoferol, que se redujo a un 33% durante la purificación y decoloración. El calentamiento durante 10 horas en continuo e intermitente causó una pérdida adicional del 14% y 23% respectivamente. Se obtuvieron altas correlaciones entre FFA y compuestos polares, PV y compuestos polares, viscosidad y compuestos polares, IV e insaturación y ácido linoleico y compuestos polares.

PALABRAS-CLAVE: *Aceite de semilla de calabaza — Calentamiento continuo — Calentamiento intermitente — Propiedades físico-químicas (cambios).*

**SUMMARY**

**Changes in pumpkin seed oil during heating**

Purified pumpkin seed oil was heated either continuously or intermittently for 10 hours at 180±5°C, and occurring changes were evaluated. Free fatty acids (FFA), peroxide value (PV), colour, polar compounds and viscosity increased, while iodine value and unsaturation decreased. Intermittent heating had the more pronounced effect on these changes. Fatty acid profile determination showed non-significant changes in palmitic (C16:0), arachidic (C20:0) and oleic (C18:1) acids during heating. Statistically significant decreases were observed in linoleic (C18:2) acid content. These decreases also caused significant increases in stearic (C18:0) acid content. Of the tocopherols, only α-tocopherol was detected, which was reduced during purification and bleaching to 33%. The 10-hour continuous and intermittent heating caused an additional 14 and 23 % loss respectively. High correlations were obtained between FFA and polar compounds, PV and polar compounds, viscosity and polar compounds, IV and unsaturation, and linoleic acid and polar compounds.

KEY-WORDS: *Continuous heating — Intermittent heating — Physico-chemical properties (changes) - Pumpkin seed oil.*

## 1. INTRODUCTION

Many *Cucurbitaceae* produce seeds rich in oil and protein. Although none of these seeds has been utilized on an industrial scale, many are used as sources of cooking oil and protein in some countries in Africa and the Middle East (1-7). In Greece no oil or protein from such seeds has been produced, but pumpkin seeds *(Cucurbita pepo* and *Cucurbita maxima)* are consumed in significant amounts as a snack in the form of salted roasted seeds, constituting a food rich in oil and protein. Compositionally, pumpkin seeds contain by weight 25-55% oil and 23-35% protein (5)(6)(8)(9). The oil is highly unsaturated, containing mainly linoleic and oleic acids, edible and might be an acceptable substitute for highly unsaturated oils in diets (5)(6).

During the last two decades a resurgence of interest has developed in the exploitation of seeds from various cucurbits, as useful, nutritious foodstuff (10). The composition of oil from cucurbit seeds, as well as work on some of the chemical components and oil edibility has been reported (2)(4-6)(8)(11). So far there are only data available on oil composition and edibility, while little is known on stability and behaviour of cucurbit seed oil during heating. The stability of crude and purified oil extracted from unroasted and roasted seeds of *Citrullus vulgaris* has been investigated by Ogunsua and Badifu (12).

Although suggestions for cucurbit seed oil utilization have been made, it is unknown how these oils will behave under various processing conditions including heating. Fats and oils are a major source of calories in human diet and extensively are used in the frying process. During frying, the oil or fat is kept at high temperatures, and also is exposed to air, water and the constituents of food being fried. As a result thermal, oxidative and hydrolytic decomposition of the oil takes place, leading to the formation of volatile and non-volatile products which affect functional, organoleptic and nutritional qualities of oil (13-16).

In the past, a considerable amount of work has been devoted to the physical and chemical changes occurring in frying oils such as cottonseed, soybean, sunflower, corn, olive oil and canola oil (17-24). Little work is available on lesser known vegetable oils behaviour during heating. In practice, heating and deep frying are extensively used at home and in food-service establishments, and thus, the oil is heated continuously and intermittently. The purpose of the present investigation was to study some physical and

chemical changes which occur in pumpkin seed oil during heating.

## 2. MATERIALS AND METHODS

### Sample collection and preparation

Sun-dried seeds of *Cucurbita pepo* and *Cucurbita maxima* were purchased from local farmers in Prangi-Didymotikhon area, Evros, Greece. The seeds were shelled by hand and then were ground to a powder by a Brabender mill to pass through a 1.00 mm sieve. Batches of ground seeds were extracted for 15 hr with petroleum ether (b.p. 40-60°C) in 2L Soxhlet extractors. The solvent was evaporated under reduced pressure, and the oils from different batches were combined and kept in sealed bottles under refrigeration (0-4°C) for further processing.

### Laboratory purification of the oil

A total of 3600g of pumpkin seed oil were degummed using the following procedure. Fifty grams of oil were put in 100 mL capacity pyrex test tubes and then were immersed in a water-bath adjusted at 90°C. Afterwards, a 3% (w/w) water at 80°C and 0.3% (w/w) phosphoric acid were added under continuous stirring. The mixture was stirred for 10 min, cooled, centrifuged for 5 min at 3000 rpm, and decanted to obtain degummed oil. A 1.5% oil loss was observed.

Batches of 500g of oil were put into 1L beakers, heated at 80°C, and then 8.2 mL of 4.125 M NaOH were added under continuous stirring. Stirring was continued for 40 min to coagulate the soap, and then left to stand for soap separation. Most of the neutralized oil was obtained by decanting, and the remaining soap-stock was centrifuged at 3000 rpm for further oil separation. Afterwards, the oil was transferred into a separatory funnel and was washed with 15% (w/w) distilled water (80°C) by shaking vigorously the separatory funnel, and allowed to stand for 15 min. The lower aqueous layer was separated and discarded. Oil acidity was lowered from 1.75% (as oleic) in crude oil to 0.08% in final neutralized oil. Oil loss amounted 2.54% and the neutralization coefficient was 1.45.

The purified oil was heated at 105°C for moisture removal, and afterwards, was bleached by adding 3% Tonsil Optimum earth technical powder, plus 2% activated carbon. The mixture was stirred for 15 min and then filtered under vacuum using a Whatman No 1 filter paper. The colour of the oil as measured by a Lovibond colourimeter was changed from the initial 27.9 red and 75 yellow units to 12.8 red and 16 yellow units. Losses due to decolourization amounted 1.94%.

### Heating procedures

Two methods of heating were applied, intermittent and continuous, after dividing the oil in two equal parts of 1690g. During the intermittent heating, the oil was put in an uncovered stainless steel frying pan with an internal diameter of 20 cm and a total capacity of 2 liters. The pan was heated to 180±5°C using an electrical cooking stove. The length of time required to heat the oil to this temperature was between 11 and 15 minutes. After 2 hours of heating the frying pan was removed from the stove and left to cool in the pan to room temperature (28°C). This took 2 hours. A 150g sample was transferred into a 250g glass bottle, nitrogen was added and then sealed and stored at -20°C until required for analysis. The lid of the frying pan was replaced and the oil was left at room temperature over-night. The heating was continued the next day, and this was repeated for a total of five days. The samples were taken every 2 hours after heating, kept under nitrogen and stored at -20°C until analyzed.

During continuous heating, the oil was similarly heated at 180±5°C for 10 hours. Every two hours a 150g sample was taken and handled under conditions just described above.

### Analytical procedures

Free fatty acids (FFA) expressed as g of oleic acid/100g oil, peroxide value (PV) expressed as meq/kg oil, and polar compounds expressed as % by weight, were determined using the IUPAC methods (25). Iodine value (IV) was determined using the Wijs method as described by Pearsons (26). Colour (photometric colour index) was determined using the AOCS cc13c-50 method (27). The viscosity was measured using a Brookfield LVT rotational viscometer equipped with a No 2 spindle rotated at 60 rpm.

Fatty acids were characterized by gas-liquid chromatography (GLC). The fatty acid methyl esters (FAME) were prepared according to the following method. About 25 mg of oil was accurately weighed into a screw cap tube and 1.5 mL 0.5M methanolic sodium hydroxide was added, mixed and heated at 100°C for 7 minutes. After cooling 2 mL of boron trifluoride was added and heated for 5 minutes. The tube was cooled to 30-40°C and 1 mL iso-octane was added, capped and shaken using a whirli mix for 30 seconds. 5 mL of saturated sodium chloride solution was immediately added and the tube was shaken again. The tube contents were allowed to separate and the top (iso-octane containing FAME) layer was removed and the lower layer was re-extracted with an additional 1 mL iso-octane. The two iso-octane extracts were combined, dried over anhydrous sodium sulfate if necessary, and concentrated to approximately 1 mL using a nitrogen stream.

The analysis of FAME were performed with a Varian 3700 gas-liquid chromatograph equipped with a hydrogen flame ionization detector using a 0.3X300 cm stainless steel column packed with 10% DEGS on Unisorb 80/100 mesh. Chromatographic conditions were as follows: injector temperature 150°C, and then increased by 4°C/min to 230°C, detector temperature 240°C. Methyl esters were identified and quantified by comparing the retention time and peak area of the unknowns with those of FAME standards (British Greyhound, Chromatography and Allied Chemicals).

Tocopherols were determined by high performance liquid chromatography (HPLC) using a modification of

Carpenter's method (28). One gram of oil was accurately weighed into a 3 dram sample vial wrapped in foil paper to prevent oxidation. The oil was dissolved in 5 mL n-hexane, HPLC grade, before injection. A 20 µL sample was injected into a Waters 600E HPLC system with a Lichrosorb Si60 column with the following characteristics: unmodified neutral silica gel; specific area about 500 $m^2/g$; specific pore volume about 0.7 $cm^3/g$; geometry irregular. Detection was made with a Waters 486 Tunable Absorbance detector at 295 nm. Iso-propanol:n-hexane:absolute ethanol (2:97.5:0.5) at 1 mL/min was used as the mobile phase. A INTRA integrator/recorder was used for the determination of the standard calibration curves and for the calculation of the amounts of tocopherols in the oil samples. Tocopherols obtained from Merck Ltd, were used as standards.

## 3. RESULTS AND DISCUSSION

The extracted pumpkin seed oil was liquid at room temperature. Its colour was dark brown with a green tint and its odour faintly nut-like. The crude oil had an acidity of 1.75%, an iodine value of 103 and a peroxide value of 2.2 meq/kg. These values were in agreement with those previously reported (5)(6). GLC analyses of unheated oil showed that pumpkin seed oil consisted of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acids. The major fatty acid was linoleic in an average concentration of 47%, followed by oleic, 34%. These findings were in line with those reported in the past (5)(6)(8). It should be noted that pumpkin seed oil is compositionally similar to other highly unsaturated vegetable oils such as corn, cotton and soybean oil.

The changes in the physical and chemical parameters in purified pumpkin seed oil during continuous and intermittent heating are shown in Table I. During heating the oil exposed in varying degrees to adverse conditions which produced certain undesirable changes. These included an increase in free fatty acids, peroxide value, polar compounds, colour and viscosity, and a decrease in iodine value. As can be seen, intermittent heating had the most pronounced effect, encouraging the destruction of the oil through oxidation, degradation and hydrolysis. A similar behaviour has been reported for cottonseed, corn, soybean and olive oil (14)(17(22)(29).

The mechanism of the thermal degradation of an oil is usually very complex, and involves formation of FFA through hydrolysis, formation of hydroperoxides, epoxides, hydroxides and ketones through oxidation (oxygen uptake), and cross-linking via oxygen bridges and carbon-carbon bonds leading to dimer and polymer formation (30). Oil degradation proceeds in different ways according to the dominance of one or another of parameters such as unsaturation of fatty acids, concentration and number of antioxidants, temperature, oxygen concentration, metals in oil and substrate, and nature of food being cooked (29).

A significant increase in FFA content during both the continuous and intermittent heating was observed (Table I). Although all acidic components were titrated during FFA determination, it was assumed that only fatty acids were measured. The increase in FFA could be attributed to oxidation and hydrolysis which produce FFA. As no water was added to the oil during heating, it may be concluded that the glyceride reacted with water which was formed from oxidation products and free radicals to form free fatty acids (21)(31). Moreover, fatty acid content is a dynamic value, since at the same time that the acids are being produced they have sufficient vapour pressure at frying temperatures to evaporate from the surface (22). Intermittent heating of the oil resulted in a higher rate of fatty acid production than the continuous heating (0.035 vs. 0.023 kg oleic/100kg oil.h), due to hydrolysis and oxidation. In addition, comparison of mean values for FFA in samples heated for equal times, continuously and intermittently, using Student's t-test, showed that intermittent heating resulted in significantly higher FFA development; e.g. at 2 hours ($p < 0.018$), 4 hr ($p < 0.001$), 6 hr ($p < 0.003$), 8 hr ($p < 0.001$) and 10 hr ($p < 0.003$). It should be pointed out that fatty acids catalyze the further hydrolysis of triglycerides (32). Handel and Guerrieri (22) reported that the level of fatty acids in the oil affected acid value by sifting the evaporation rate equilibrium. Although it can have limitations, FFA or acid value have been promoted as an appropriate measure of frying oil decomposition and their determination is a widely used practice because it is quick and can be performed by semi-skilled staff. However, though acidity is commonly used as an indicator of oil quality, it should not be relied upon as the sole measure.

The iodine value of purified pumpkin seed oil was decreased with heating time. Small not statistically significant, but observable differences were measured up to a heating time of 6 hours. Significant decreases in IV were observed at heating times of 8 hours ($p<0.005$ for continuous and $p<0.013$ for intermittent heating, respectively) and 10 hours ($p<0.003$ for continuous and $p < 0.0004$ for intermittent heating respectively). Comparison of continuously and intermittently heated oil samples for equal times showed not significant differences up to 8 hours of heating. Significant ($p < 0.025$) differences were observed between oil samples continuously and intermittently heated for 10 hours. IV decreases are indicative of the increased rate of oxidation during heating, either by continuous or the intermittent method, and could be attributed to oxidation and polymerization reactions involving the double bonds (33).

Table I shows the changes in peroxide value. The peroxide value development is mainly due to preferential oxidation of polyunsaturated fatty acids (PUFA) C18:2n-6 and C18:3n-3. It should be noted that oxidation of an oil at high temperature differs from oxidation of the same oil at low temperature. Not only are the reactions speeded up at high temperatures but also quite different reactions take place. Purified pumpkin seed oil showed a significant increase in PV, especially after 6 hours of continuous heating and 4 hours of intermittent heating. Intermittently heated oil showed a higher apparent rate of PV increase than the continuously heated oil, 0.917 meq/kg.h vs. 0.474 meq/kg.h, respectively. Peroxides, under the heating conditions used are unstable, and react to form secondary oxidation products. Normally, an increase at the initial stage of heating would be expected to be followed by a decrease with further heating, because the

hydroperoxides tend to decompose at 180°C to form secondary oxidation products (34). Unexpectedly, the results showed that the PV increased during the total heating. This increase could be attributed to conjugation known to precede hydroperoxide formation in the secondary stage and polymerization of partially oxidized oil in the tertiary state of auto-oxidation (35). Also, the overall increase in PV is connected with the cooling period of the oil. The length of time required to cool the oil at room temperature was 2 hours. During the cooling period the oil samples were exposed to air at high temperatures and the hydroperoxides were formed again (36).

No significant changes in colour were observed during heating up to 4 hours both continuously and intermittently,

Table I
**Physical and chemical changes in pumpkin seed oil during continuous and intermittent heating at 180±5°C.**

| Parameter | Heating time, hours | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Continuous | | | | | Intermittent | | | | |
| | 0 | 2 | 4 | 6 | 8 | 10 | 2 | 4 | 6 | 8 | 10 |
| Free Fatty Acids (% as oleic) | 0.08 (0.010) | 0.21 (0.011) | 0.23 (0.015) | 0.24 (0.020) | 0.31 (0.012) | 0.34 (0.026) | 0.25 (0.020) | 0.31 (0.012) | 0.34 (0.026) | 0.40 (0.021) | 0.47 (0.036) |
| Iodine Value (Wijs) | 102.74 (0.310) | 102.59 (0.730) | 102.41 (0.765) | 102.21 (0.492) | 101.74 (0.233) | 101.13 (0.397) | 102.53 (1.287) | 102.26 (0.324) | 101.92 (0.796) | 101.27 (0.680) | 100.28 (0.353) |
| Peroxide Value (meq/kg) | 2.17 (0.157) | 2.42 (0.292) | 2.86 (0.607) | 3.83 (0.299) | 5.13 (0.921) | 6.99 (0.841) | 2.65 (0.535) | 3.63 (0.340) | 5.13 (0.669) | 7.93 (0.409) | 11.54 (0.320) |
| Polar Compounds (% w/w) | 2.84 (0.166) | 3.26 (0.144) | 3.78 (0.348) | 5.02 (0.749) | 7.34 (0.424) | 10.92 (0.664) | 3.37 (0.060) | 3.99 (0.564) | 6.32 (0.372) | 9.97 (0.952) | 17.67 (0.884) |
| Colour | 4.12 (0.079) | 4.14 (0.135) | 4.17 (0.209) | 4.32 (0.084) | 4.52 (0.094) | 4.87 (0.107) | 4.14 (0.065) | 4.20 (0.171) | 4.42 (0.068) | 5.04 (0.093) | 6.00 (0.160) |
| Viscosity (mPa.s) | 72.34 (0.519) | 72.39 (0.332) | 72.47 (0.538) | 72.63 (0.382) | 73.00 (0.369) | 73.35 (0.252) | 72.43 (0.342) | 72.62 (0.168) | 72.97 (0.204) | 73.46 (0.243) | 74.10 (0.574) |

()=standard deviation; average of 3 samples with 3 to 5 replicates

as compared to unheated oil (Table I). Photometric colour index for oil samples heated continuously for 2 hours were significantly different from those after 8 ($p<0.008$) and 10 ($p<0.0009$) hours heating. Such differences were also observed between samples heated for 4 & 8 hours ($p<0.028$) and 4 & 10 hours ($p<0.003$), 6 & 8 ($p < 0.025$) and 6 & 10 ($p < 0.001$) hours, and 8 & 10 ($p < 0.006$) hours. A similar behaviour was also observed during intermittent heating. In addition, significant differences in colour were observed after continuous and intermittent heating for 8 ($p < 0.001$) and 10 ($p < 0.003$) hours (Table I). The increase in colour has been attributed to α,β-unsaturated carbonyl compounds which are intermediates to give non-volatile decomposition products containing carbonyl group and have the ability to absorb energy of the magnitude of visible light. The increase in colour (Table I) indicated the degradation of pumpkin seed oil and the breakdown of peroxides, resulting in the formation of secondary products when heated continuously or intermittently.

Accumulation of polar compounds is indicative of both hydrolysis and oxidation. Continuous and intermittent heating resulted in an increase in polar compounds (Table I). Differences in polar compound content were observed between heating time intervals, while comparison between continuous and intermittent heating times showed significant differences for heating times of 6 ($p < 0.027$), 8 ($p < 0.006$) and 10 ($p < 0.0002$) hours. During the heating process a wide variety of chemical reactions result in the formation of compounds with high molecular weight and polarity. These compounds are non-volatile and steadily increase in concentration with heating time (37). These present results confirm this observation. The rate of polar compound increase was higher during intermittent heating as compared to continuous heating (1.374 vs. 0.771 kg/100 kg.h). This could be due to the higher degree of oxidation and hydrolysis of intermittently heated oil, which is evident from PV and FFA measurements (Table I). Handel and Guerrieri (22) reported that FFA affect polar compound formation and fatty acid addition sometimes increased and

sometimes decreased the rate of polar compound formation. As can be seen from Fig. 1, FFA were highly correlated (r =0.838, p< 0.001) to polar compounds. Billek et al. (38) have stated that any heated cooking oil with 27% or more polar compounds should be discarded. Using this criterion and taking into consideration the rate of polar compound increase, purified pumpkin seed oil will reach this percentage after 33 hours of continuous heating and/or after 19 hours of intermittent heating as described here, showing that pumpkin seed oil will behave similarly to other cooking oils such as corn, cotton or soybean oil. Moreover, polar compounds were highly correlated with peroxide value during both continuous (r=0.9964, p<0.001) and intermittent (r=0.9898, p<0.001) heating. As PV may be decreased due to peroxide breakdown and FFA may evaporate during heating, the analysis of percentage polar compounds is considered to be one of the most reliable indicators of the state of oil deterioration (15).

As can be seen from Table I, oil viscosity was increased during both continuous and intermittent heating. The results showed that the increase in viscosity was significant in the case of continuous heating between 0 & 10 hours (p<0.019), 2 & 10 hours (p<0.008), 2 & 8 hours (p<0.05), 4 & 10 hours (p<0.031), and 6 & 10 hours (p<0.026), while in the case of intermittent heating between 0 & 10 hours (p<0.008), 0 & 8 hours (p<0.014), 2 & 10 hours (p<0.006), 2 & 8 hours (p<0.007), 2 & 6 hours (p<0.039), 4 & 10 hours (p<0.006), 4 & 8 hours (p<0.004), 4 & 6 hours (p<0.042), 6 & 10 hours (p<0.016) and 6 & 8 hours (p<0.028). Alim and Morton (33) reported that the build-up of the non-volatile decomposition products causes an increase in the viscosity. It should be noted that oxidized fatty acids can remain in the triglyceride molecule and cross-link with each other (30). This leads to dimeric and even higher polymeric triglycerides. Cross-linking does not necessarily takes place only via oxygen bridges. Particularly, during heating in the absence of oxygen, new carbon-carbon bonds between two different fatty acids can be formed leading to the formation of dimeric acids either within one or between two triglyceride molecules, which affect the viscosity of the oil. A high correlation between viscosity and polar compounds was observed (Fig. 2). This correlation was higher (r=0.9997, p<0.001), if only the data collected during continuous heating were used.

Only α-tocopherol was detected in pumpkin seed oil. The initial content of α-tocopherol in crude pumpkin seed oil was 73 ppm. Higher values of α-tocopherol amounting 190 ppm, have been reported by Ogunsua and Badifu (12) for melon seed oil *(C.vulgaris)*. The α-tocopherol content in purified oil was 68 ppm corresponding to a retention of 93%. Bleaching led to a further reduction in α-tocopherol content to a value of 56 ppm, corresponding to a 77% retention. Ogunsua and Badifu (12) reported a retention of 52% in bleached melon seed oil. After 10 hours of heating, α-tocopherol content dropped to 48 ppm (85.7% retention) after continuous and 43 ppm (76.8% retention) after intermittent heating. Miyagawa et al. (39) reported that the decomposition of tocopherols is due to oxidation, and that the order of antioxidant activity is α-> γ- > δ-tocopherol. Kiritsakis (40) reported that the higher oxidative stability of olive oil comparatively to other vegetable oils is related to some extent to the presence of α-tocopherol. α-tocopherol is one of the most reactive naturally occurring singlet oxygen acceptors, and is the most reactive among the four tocopherols. Thus, α-tocopherol offered some protection during heating, but the reduction in its amounts in the oil is not necessarily in direct relation to the index for the other chemical properties.

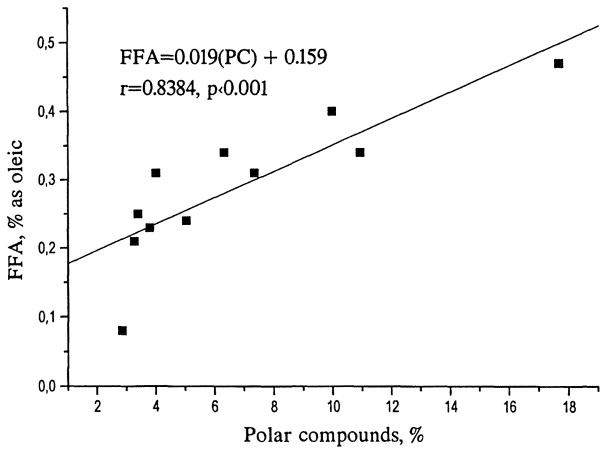


Figure 1.
Correlation between FFA and polar compounds in heated pumpkin seed oil.

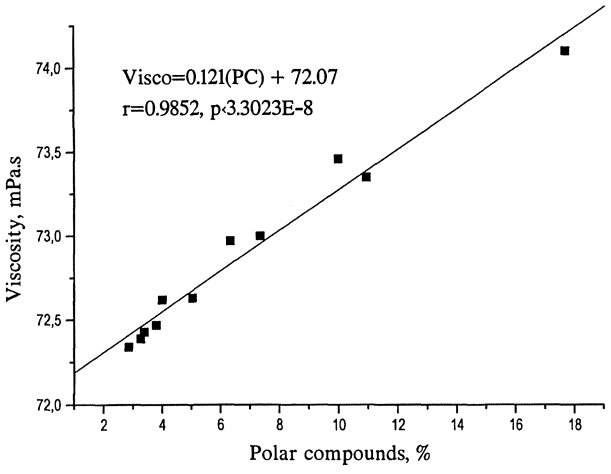


Figure 2.
Correlation between viscosity and polar compounds in heated pumpkin seed oil.

In general, the nature of oil affects the degree of degradation and oils containing more PUFA are considered to be more susceptible to deterioration. The rate of oxidation of PUFA can be described by the changes in IV and fatty acid composition. PUFA in purified pumpkin seed oil amounted 47.3% (Table II) and the saturated/unsaturated ratio was 0.224. A reduction in oil unsaturation was observed with heating time, while saturated/unsaturated ratio was increased reaching a value of 0.243 and 0.246 after 10 hours of continuous and intermittent heating, respectively. Although, after 10 hours of heating, the saturated fatty acid content of the oil was increased (Table II), not significant differences at the 95% level (Student's t-test) were observed to occur in palmitic (C16:0) and arachidic (C20:0) acids. Such differences did exist in stearic (C18:0) acid content of oil samples heated intermittently for 10 hours ($p<0.039$). Similarly, significant differences in stearic acid content were observed after intermittent heating for the time intervals of 2-10 hours ($p<0.040$), 4-10 hours ($p<0.032$), and 10 hours ($p<0.047$) of continuous and intermittent heating as well. This apparent increase in saturated fatty acid content is due to the PUFA decrease, as results expressed as percentages, while usually there is scarcely any or no actual change at all in saturates or in the monounsaturates (41). As can be seen from Table II, not significant changes in oleic (C18:1) acid content were observed. During both continuous and intermittent heating, significant differences in linoleic (C18:2) acid content were observed. Considering the continuous heating statistically significant differences were observed for the following time intervals: 0-10 hours ($p<0.03$), 2-10 hours ($p<0.016$), 4-10 hours ($p<0.019$), 6-10 hours ($p<0.041$), while during intermittent heating: 0-10 hours ($p<0.004$), 0-8 hours ($p < 0.020$), 2-10 hours ($p < 0.0009$), 2-8 hours ($p < 0.007$), 4-10 hours ($p < 0.003$), and 4-8 hours ($p<0.026$). By comparison of heating time intervals between continuous and intermittent heating, significant differences in linoleic acid content were observed between 10 ($p<0.015$) and 8 ($p<0.046$) hours, and also between 8 hours of continuous heating and 10 hours of intermittent heating ($p<0.002$), 6 hours of continuous and 10 hours of intermittent heating ($p<0.002$), and 4 hours of intermittent and 10 hours of continuous heating ($p<0.046$). This reduction in linoleic acid content could be attributed to oxidation and degradation, as the rate of degradation of linoleic is 100 times higher than that of stearic and 10 times higher than that of oleic acid (29). Moreover, the decrease in unsaturation of the oil was highly correlated with the

Table II
**Fatty acid profile in purified pumpkin seed oil during continuous and intermittent heating at 180±5°C.**

| Fatty Acid | Heating time, hours | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Continuous | | | | | Intermittent | | | | |
| | 0 | 2 | 4 | 6 | 8 | 10 | 2 | 4 | 6 | 8 | 10 |
| C16:0 | 11.90 (0.416) | 11.90 (0.677) | 11.94 (0.445) | 11.98 (0.579) | 12.13 (0.503) | 12.31 (0.516) | 11.92 (0.392) | 11.95 (0.535) | 11.99 (0.596) | 12.10 (0.395) | 12.35 (0.573) |
| C18:0 | 6.20 (0.437) | 6.22 (0.336) | 6.24 (0.313) | 6.27 (0.604) | 6.72 (0.440) | 6.96 (0.495) | 6.21 (0.439) | 6.27 (0.308) | 6.31 (0.481) | 6.84 (0.383) | 6.99 (0.380) |
| C18:1 | 34.40 (0.296) | 34.24 (0.223) | 34.34 (0.356) | 34.44 (0.909) | 34.04 (0.531) | 34.05 (0.319) | 34.25 (0.774) | 34.42 (0.397) | 34.65 (0.448) | 34.36 (0.617) | 34.69 (0.394) |
| C18:2 | 46.90 (0.805) | 46.74 (0.488) | 46.56 (0.394) | 46.32 (0.406) | 46.01 (0.365) | 45.50 (0.462) | 46.71 (0.440) | 46.45 (0.583) | 46.04 (0.452) | 45.41 (0.298) | 44.44 (0.304) |
| C18:3 | 0.40 (0.115) | 0.40 (0.149) | 0.38 (0.106) | 0.36 (0.252) | 0.35 (0.184) | 0.31 (0.185) | 0.39 (0.180) | 0.36 (0.193) | 0.33 (0.171) | 0.29 (0.190) | 0.24 (0.187) |
| C20:0 | 0.20 (0.106) | 0.20 (0.079) | 0.19 (0.140) | 0.19 (0.166) | 0.17 (0.149) | 0.15 (0.131) | 0.20 (0.115) | 0.18 (0.089) | 0.17 (0.089) | 0.16 (0.132) | 0.16 (0.154) |
| Unknown | - | 0.30 | 0.35 | 0.44 | 0.58 | 0.72 | 0.32 | 0.37 | 0.51 | 0.84 | 1.13 |
| Saturated/ Unsaturated | 0.224 | 0.225 | 0.226 | 0.227 | 0.238 | 0.243 | 0.225 | 0.227 | 0.228 | 0.239 | 0.246 |

()=standard deviation

decrease in IV, giving r=0.983 ($p<0.001$). A high negative correlation between the decrease in linoleic acid and the increase in polar compounds ($r=-0.989$, $p < 0.001$) was also observed. The above findings show that deterioration of pumpkin seed oil during heating is due to the oxidative degradation of the linoleate present in large amounts in this oil. It should be noted that the extent of oxidative changes during frying of various foods will depend not only on the oil composition, but also on that of the fried food. Furthermore, the water present in the fried food will accelerate the thermal oxidation and hydrolytic cleavage of the oil. Of course, the addition of various antioxidants will increase the stability of pumpkin seed oil.

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