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Quality Characterization and Oxidative Stability of Camellia Seed Oils Produced with Different Roasting Temperatures

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Abstract: In this study, the effects of roasting camellia (*Camellia oleifera* Abel.) seed oils at different temperatures (65℃, 100℃, 120℃, and 140℃) on the oxidative stability and composition of the oils were investigated. The results showed that, in terms of the quality of the oils, the roasting temperature influenced the total phenolic content (which ranged from 1.64～2.45 GAE mg/g for the different oils) and total flavonoid content (which ranged from 0.36～0.45 QE mg/g for the different oils), while the fatty acid profile and tocopherol content were not influenced by the roasting temperature. We also investigated the kinetic parameters of camellia seed oil during oxidation via Rancimat (at temperatures ranging from 110~140℃). It turned out that the natural logarithms of the oxidative stability index (OSI) varied linearly with respect to temperature (R^2: 0.958～0.997). This was done on the basis of the Arrhenius equation that indicates that the activation energies (*E*a) for oxidative stability are 65.7～78.4 KJ/mol. Simultaneously, we found that increasing the roasting temperature could increase the antioxidant stability of Maillard reaction products in camellia seed oil. The effects of roasting include the assurance that the camellia seed oil so produced will comply with the relevant governmental health codes and standards and have a longer shelf life.

Key words: camellia seed oil, roast, Rancimat test, oxidative stability

1 Introduction

The genus Camellia is native to East Asia, belongs to the Theaceae family, and comprises approximately 100 large evergreen shrub species worldwide, including *Camellia oleifera* Abel. Collectively, these species are economically important because a variety of products are made from them. For example, Camellia seeds are a good plant source of fat, and camellia seed oil is commonly used as a cooking oil and as a traditional medicine to treat stomach aches and burns in Chinese society[1]. The oil is antimicrobial and antiviral, has skin healing properties, protects the liver against CCl_4-induced oxidative damage, reduces cholesterol content in the body, and provides resistance to oxidative stress[1, 2]. More specifically, camellia seed oil enhances the hepatic antioxidant defense system and endogenous or exogenous defense mechanisms in order to achieve the prevention of liver damage, including via the downregulation of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase, as well as the upregulation of antioxidant enzymes, including glutathione peroxidase, glutathione reductase, and glutathione S-transferase[3]. In addition, still other research has shown that the four kaempferol glycosides that can be separated from camellia seed oil exhibit various anti-inflammatory effects, while seamin have antioxidant activity existing methanol distinction of camellia seed oil[4, 5]. Camellia seed oil was first recorded as a medical oil in the Compendium of Materia Medica and is recommended as a healthy edible oil by the FAO[6].

Seed oil extracts are mainly produced by solvent extraction and mechanical expression pressing. Among these two methods, the yield of the solvent extraction method is 80～90%, which is substantially higher than the mechanical compression yield of 40～52%[7]. Recently, cold-pressed oils have become increasingly popular with consumers due to their reputation as a good source of bioactive compo-

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nents. The main unsaponifiable components of seed oils are tocopherol, phenolic, and flavonoid, and these components can protect the oils and have antioxidant activities[8]. Roasting is an important pre-treatment stage in the production of cold-pressed oils insofar as the chemical composition and quality of such oils, which together determine the shelf life stability of the oils, is substantially enhanced by roasting[9]. The shelf life of cold-pressed oils typically ranges from 6～12 months, and studies have shown that roasting is the main reason for the increased oxidative stability of sesame oil (as a result of the sesamin formed during the roasting process) and that of rapeseed oil (as result of the canolol formed during the roasting process)[10, 11].

A variety of physicochemical changes occur to oils during lipid degradation, such as the main physical changes of increased viscosity and foaming, as well as color changes and a decreased smoke-point[12, 13]. Meanwhile, the main chemical changes consist of increases in the concentration of free fatty acids and polar components and decreases in unsaturated fatty acid content, flavor quality, and nutritive value due to the degradation of minor compounds that affect the organoleptic and nutraceutical properties of the oils[13]. Autoxidation is the most common form of deterioration that occurs during the storage of edible oils. Autoxidation consists of the reaction between oxygen and unsaturated fatty acids that occurs via an auto-catalytic process that, in turn, consists of a free radical chain mechanism. The high degree of unsaturation makes this oil highly susceptible to oxidation during processing and storage, due to such factors as heat, light, and oxygen[13, 14]. As a result of the oxidation of polyunsaturated fatty acids (PUFAs), the vitamins in the oil may lose some of their biological properties and may even form toxic products, some of which may be carcinogenic in nature[15]. On the other hand, sufficiently sophisticated processing and antioxidants can improve the oxidative stability of commercial products[12].

Camellia seed oil is a traditional product of Taiwan, but its biological activities have received only limited attention. As such, further analysis of the quality, nutritional characteristics, and purity of such oil is important for the purposes of commercial application. In this study, we made cold-pressed camellia seed oil products from seeds that had previously undergone roasting at temperatures of 65℃, 100℃, 120℃, and 140℃, after which we investigated the compositions and quality of the products in terms of their natural antioxidants and oxidative stability in order to determine the effects of the different roasting temperatures.

2 Materials and Methods

2.1 Materials

A fatty acid methyl ester (FAME) standard mixture, Supelco 37 Component FAME Mix, was purchased from Supelco (Sigma-Aldrich, Taufkirchen, Germany). α- tocopherol, gallic acid, quercetin, and Foline-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). The other analytical grade chemicals mentioned were purchased from CHEMICAL CO., LTD (Miaoli, Taiwan).

2.2 Production of camellia seed oils

Four separate portions of camellia seeds weighing 50 kg each were weighed out and then placed individually into the roasting machine to be roasted at one of four different temperatures (65℃, 100℃, 120℃, or 140℃) for 5 min. Each sample of roasted camellia seeds was then put into the extrusion pressing machine to press out the camellia seed oil, after which the camellia seed oil was filtered and, finally, collected (Fig. 1).

2.3 Fatty acid analysis

Each oil sample was analyzed for its fatty acid composition via GC/FID. The triacylglycerols were converted to methyl esters using the AOCS Official Method Ce 2-66[16]. The methyl esters were then separated using a column that was coated with DB-23 (30 m × 0.25 mm × 0.25 μm), with helium used as the carrier gas at a flow rate of 1.0 ml/min. The oven temperature was initially held for 8 min at 200℃, and was then increased at 10℃/min to 220℃ and held for 40 min. The FID was maintained at 270℃, and the injector (split mode 1:40, 4 mm liner) was maintained at 250℃. The fatty acid contents were determined using the normalization method.

2.4 Chemical analysis

The peroxide value (PV) of each oil sample was determined as prescribed by the official analytical methods of European Regulation EEC 2568/91[17]. The malondialdehyde (MDA) content of the samples was measured according to Jung *et al.*[18]. The acid value (AV) of the samples was determined using a titration with 0.1 N potassium hydroxide alcoholic solution.

The quantitative method for determining the tocopherol composition of each sample was described previously[15].

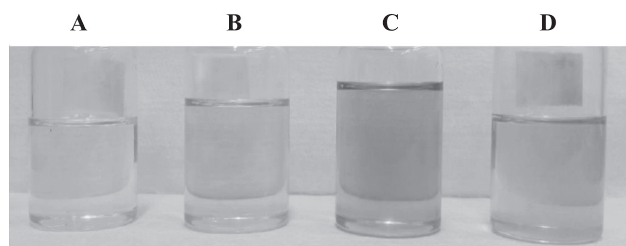

Fig. 1 Visual appearance of camellia seed oils produced with different roasting temperatures. [1] Camellia seed oil roasted at (A) 65℃, (B) 100℃, (C) 120℃ and (D) 140℃.

For each oil sample, a 20-μL aliquot of filtrate was injected into an injection port and separated by an HPLC system (L-2130 pump and L-2400 UV detector, Hitachi, Japan) attached to an RP-18GP250 column Mightysil (l = 250 mm; i.d. = 4.6 mm; thickness = 0.32 μm; Kanto Chemical Co., Inc., Japan). The calibration curves of each standard were established by plotting the peak area vs. the corresponding concentration, respectively.

Total phenolic and total flavonoid contents of each oil sample were analyzed via UV spectrophotometry[19]. Each sample (0.5 g) was diluted with acetone to a volume of 20 ml. The total phenolic content was measured as gallic acid equivalents mg/g of oil weight using Foline-Ciocalteu reagent, and the total flavonoid content was measured as quercetin equivalents mg/g of oil weight using the $AlCl_3$ colorimetric method.

The color of each oil sample was measured using a colorimeter (NE-4000, Nippon Denshku Industries Co, Ltd. Tokyo, Japan). After the instrument was standardized with a white plate (L0 = 97.51, a0 = −0.16, and b0 = 1.75), the colors of the samples were evaluated at room temperature. The Hunter L, a, and b values correspond to lightness, greenness (−a) or redness (+a), and blueness (−b) or yellowness (+b), respectively. The browning index was calculated using the following equation[20]:

$$\text{browning index} = [100 \times (x - 0.312)]/0.172$$

where $x = (a + 1.75L)/(5.645L + a - 3.012b)$.

2.5 Kinetic parameters of Rancimat test

The oxidative stability of each oil sample was determined at four different temperatures (110, 120, 130, and 140℃) based on the induction period (hours) that was recorded using a Rancimat 743 apparatus and 5 ± 0.05 g of the given oil sample with an air flow of 10 L/h. The OSIs of the different oil sample were automatically recorded and taken as the break point of the plotted curves (the intersection point of the two extrapolated parts of the curve).

The kinetic parameters of the oil samples were determined according to the method described previously[21]. The OSIs of the oil samples were automatically recorded and taken as the break point of the plotted curves (the intersection point of the two extrapolated parts of the curve). A kinetic rate constant was taken as the inverse of the given OSI (k, h^{-1}).

Temperature coefficients (T Coeff, K^{-1}) were determined from the slopes of the lines generated by regressing ln(k) vs. the absolute temperature (T, K):

Equation I: $\ln(k) = a(T) + b$

where a and b are the equation parameters.

Activation energies (Ea, kJ/mol) and pre-exponential or frequency factors (A, h^{-1}) were determined from the slopes and intercepts, respectively, of the lines generated by regressing ln(k) vs. 1/T using the Arrhenius equation:

Equation II: $\ln(k) = \ln(A) - (Ea/RT)$

where k is the reaction rate constant or reciprocal OSI (h^{-1}), and R is the molar gas constant (8.3143 J/mol K).

2.6 Statistical analysis

All the experiments were performed in triplicate and all data were expressed in the form of mean ± standard deviation of the mean (SD). An analysis of variance (ANOVA) performed with SPSS 10.0 (SPSS, Chicago, IL) was used to analyze the data obtained. Variances were analyzed using Statistical Analysis System (2000) software. In order to test the significance of the differences between paired means, Duncan's multiple range test was used. A confidence level of $p < 0.05$ was applied to judge the significance of each difference.

3 Results and Discussion

3.1 Fatty acid composition and quality indices

The roasted camellia seed oil samples had different fatty acid profiles that could be analyzed to determine the nature of their physicochemical and nutritional properties, in addition to providing quality information regarding the given oil. Other studies have shown that linoleic acid is the main fatty acid in walnuts, pumpkin seeds, evening primrose, and black cumin oils, whereas α-linolenic and oleic acid were the main fatty acids in flaxseed and rapeseed oils[22]. With respect to the fatty acid compositions of the camellia seed oils produced after roasting in this study, we found that the main fatty acids were oleic acid (68.04∼70.32%), palmitoleic acid (17.80∼17.93%), and stearic acid (5.36∼5.67%) in these camellia seed oils, whereas the content of PUFAs, monounsaturated fatty acids (MUFAs), and saturated fatty acids (SFAs) in walnut oils were previously found to be 1.99∼2.21%, 68.47∼70.87%, and 23.16∼23.60%, respectively (Table 1). Wang et al. (2017) found that camellia seed oil from Fujian in China had 17 fatty acids, with the content of oleic acid and linoleic acid being 46.33∼55.48% and 23.17∼29.40%, respectively[23].

The fatty acid composition of an oil affects its lipid oxidation and the final quality of oils and fats, in addition to having effects on human health. The high level of oleic acid provides health-promoting effects that include the lowering of blood pressure, cholesterol, and triglyceride levels, and can thus help to prevent cardiovascular diseases, cancer, hypertension, and autoimmune disorders[24]. The main components of vegetative oil were first identified as important items in the Codex Alimentarius. However, camellia seed oil is not listed in the Standard for Named Vegetable Oils (CODEX STAN 210-1999) at present.

Table 1 Fatty acid compositions (%) of camellia seed oils produced with different roasting temperatures.

	Camellia seed oil roasted at			
	65℃	100℃	120℃	140℃
C14:1	0.15	0.14	0.16	0.16
C16:0	17.80	17.93	17.84	17.93
C16:1	0.40	0.29	0.32	0.34
C18:0	5.36	5.67	5.58	5.50
C18:1	70.32	68.04	69.55	68.71
C20:2	1.99	2.06	2.01	2.21
other	3.98	5.87	4.54	5.15
SFA	23.16	23.60	23.42	23.43
MUFA	70.87	68.47	70.03	69.21
PUFA	1.99	2.06	2.01	2.21

[1] Data presented are in mean (n=3)
[2] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

3.2 Quality indices

Camellia seed oil is oxidized at high temperatures, and the secondary products of lipid oxidation can impair the sensory qualities and acceptability of products among consumers. Therefore, the exact roasting temperature used in the production of camellia seed oil is of great importance. Relatedly, the process of lipid oxidation can be evaluated by the monitoring of a diverse series of primary and secondary oxidation products over time.

In this study, the AVs of the camellia seed oil produced with different roasting temperatures ranged from 2.2～2.4 mg KOH/g, indicating that AV was not significantly influenced by the roasting temperature (Table 2). According to the Codex Standard for edible fats and oils, cold-pressed oils should have an AV of less than 4.0 mg KOH/g oil. Meanwhile, the study results showed that the PVs of the camellia seed oil produced with different roasting temperatures ranged from 6.3～9.4 mEq O_2/kg, indicating that PV was significantly influenced by the roasting temperature. According to the Codex Standard, the maximum permissible PV of cold-pressed oils is 15 mEq O_2/kg. PV and AV are used in the industry's on-line quality control index. PV has a significant correlation to the off-flavor compounds created during the initial oxidation, while AV represents the content of free fatty acids which are easily oxidized to hydroperoxides[25].

The hydroperoxides of lipid oxidation begin to form at the initial stages before reaching a maximum, and then decline during the latter stages, a phenomenon that is reflected in the related levels of peroxides and aldehydes[26]. We found that the MDA contents of the camellia seed oils produced with roasting at 65℃, 100℃, 120℃, and 140℃ were 0.85, 0.99, 0.53, and 0.35, respectively (Table 2), indicating that the oxidation products formed were aldehydes. The different roasting temperatures may have resulted in different oxidation and transformation processes that were in turn reflected in the production of peroxides and aldehydes with completely different behaviors. From an aldehyde, a polymerization can occur with dioxygen, which can produce alkanes, aldehydes with short chains, acids, and epoxides[26, 27].

3.3 Antioxidant compositions

Unsaponifiable compounds have antioxidant capability from vegetative oil. Santos *et al*. evaluated the thermal stability of commercial antioxidants in oil roasted at 110℃,

Table 2 Quality indices and antioxidant compositions of camellia seed oils produced with different roasting temperatures.

	Camellia seed oil roasted at			
	65℃	100℃	120℃	140℃
	Quality indices			
AV (mg KOH/g)	2.3 ± 0.1^{a}	2.4 ± 0.1^{a}	2.3 ± 0.2^{a}	2.2 ± 0.1^{a}
PV (mEq O_2/kg)	6.3 ± 0.1^{a}	7.6 ± 0.3^{b}	8.9 ± 0.2^{c}	9.4 ± 0.3^{d}
MDA (mg/Kg)	0.85 ± 0.02^{c}	0.99 ± 0.05^{d}	0.53 ± 0.03^{b}	0.35 ± 0.02^{a}
	Antioxidant compositions			
α-tocopherol (g/kg)	0.22 ± 0.03^{a}	0.20 ± 0.04^{a}	0.23 ± 0.03^{a}	0.23 ± 0.02^{a}
total phenol (mg/kg)	1.64 ± 0.12^{a}	2.45 ± 0.08^{c}	2.30 ± 0.15^{c}	2.15 ± 0.16^{b}
total flavonoid (mg/kg)	0.43 ± 0.03^{b}	0.54 ± 0.02^{c}	0.47 ± 0.02^{b}	0.36 ± 0.02^{a}

[1] Data presented are in mean ± SD (n=3) which with different letters are significantly different at $p < 0.05$.
[2] AV: acid value; PV: peroxide value; MDA: malondialdehyde.

which displayed stabilities in the following order: α-tocopherol＞caffeic acid＞ferulic acid＞gallic acid[28]. These compounds are primary antioxidants which either delay the oxidation step by reacting with free radicals or inhibit the propagation step by reacting with peroxy or alkoxy radicals, thus preventing the degradation of vegetative oils. The effects of natural antioxidants on the resistance of oils to degradation has been well documented.

We found that the tocopherol levels of the camellia seed oils produced with roasting at 65℃, 100℃, 120℃, and 140℃ in this study were 0.22, 0.20, 0.23, and 0.23 g/kg, respectively; the total phenolic contents were 1.64, 2.45, 2.30, and 2.15 GAE mg/kg, respectively; and the total flavonoid contents were 0.43, 0.54, 0.47, and 0.36 QE mg/kg, respectively (Table 2).

In this study, the results indicated that the total phenolic and total flavonoid contents of the camellia seed oils were influenced with roasting. The effects of the heating include the release of unsaponifiable compounds from vegetal cells[29]. We found that the roasting at 100℃ resulted in higher total phenolic and total flavonoid contents than roasting at 65℃. Besides, roasting at 120℃ and 140℃ were significantly reduced, which roasted caused by residual heat.

Camellia seed oils have a dark yellow color which is probably caused by Maillard-type reactions during roasting (Fig. 1). We found that the browning index values of the camellia seed oils produced with roasting at 65℃, 100℃, 120℃, and 140℃ were 3.64, 6.11, 7.84, and 7.03, respectively. The temperature was also crucial, indicating that roasted at 100～140℃ produced more carbonyl group than at 65 ℃, which promote Maillard reaction (Table 3). Maillard reaction products exhibit antioxidant action, and this has been confirmed in many model systems, as well as in certain fat-containing foodstuffs[30]. In this study, roasting led to an increase in the antioxidant stability and antioxidant activity of the oils produced. This increase in oxidative stability and antioxidant activity is probably also influenced by reaction products. Maillard reaction products with antioxidant properties generated during roasting may also contribute to the higher stability of oil against oxidation[31].

3.4 Oxidative stability

A previous study showed that food moisture, atmospheric oxygen, and high temperatures can produce various chemical changes and decrease antioxidant activity, such as via the steam distillation of antioxidants, as well as the oxidation of phenolic compounds and decreases in their pro-oxidative activity due to reaction with fried materials and polymerization[12]. Lipid oxidation is one of the main factors which affects the shelf life of vegetative oils. The determination of oxidation stability is a time-consuming process when the analysis is performed at room temperature. Therefore, the Rancimat method is typically used as a method of determining the oxidation stability of an oil within a shorter period of time. The Rancimat method is based on the production of volatile compounds of degraded products, with its analysis being based upon the changes observed in the electrical conductivity of distilled water[21].

The OSI of the camellia seed oils produced with roasting at 65℃, 100℃, 120℃, and 140℃ are presented in Fig. 2.

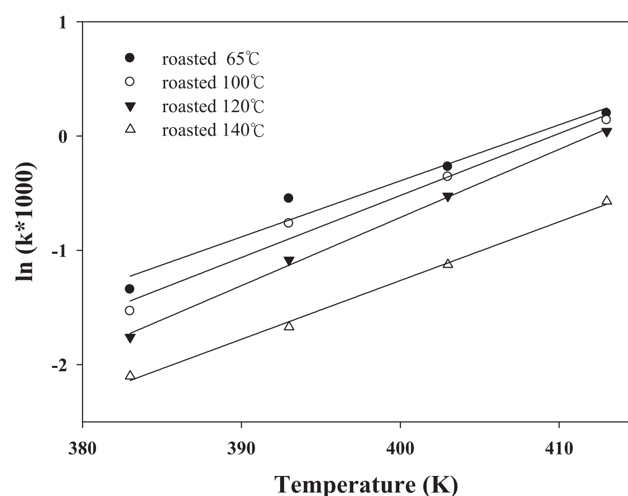

Fig. 2 Semi-logarithmic relationship between k and T values for lipid oxidation of camellia seed oils produced with different roasting temperatures.

Table 3 Color and browning index values of camellia seed oils produced with different roasting temperatures.

	Camellia seed oil roasted at			
	65℃	100℃	120℃	140℃
L	94.15	94.56	96.61	96.15
a	− 1.18	0.30	0.95	0.65
b	4.30	5.53	6.80	6.23
Browning index	3.64[a]	6.11[b]	7.84[d]	7.03[c]

[1] Data presented are in mean (n=3) which with different letters are significantly different at $p < 0.05$.

Table 4 Regression parameters for Arrhenius relationships between the reaction rate constant and the temperature for camellia seed oils produced with different roasting temperatures.

	Camellia seed oil roasted at			
	65℃	100℃	120℃	140℃
	ln(k)=a(1/T)+b			
a	−7.95	−8.79	−9.64	−8.30
b	19.07	20.98	22.89	19.03
R^2	0.96	0.99	1.00	0.99
*E*a (kj/mol)	65.7	71.5	78.4	67.5

For use of the Rancimat test at temperatures of 110℃, 120℃, 130℃, and 140℃, the induction times were 3.83, 1.73, 1.31, and 0.82 h, respectively, for the camellia seed oil sample produced with roasting at 65℃; 4.63, 2.15, 1.43, and 0.87 h, respectively, for the camellia oil sample produced by roasting at 100℃; 5.81, 2.96, 1.69, and 0.96 h, respectively, for the camellia seed oil produced with roasting at 120℃; and 8.18, 5.32, 3.08, and 1.77 h, respectively, for the camellia seed oil produced with roasting at 140℃. The results showed that the different temperatures used affected the degree of oxygen solubility in a given oil sample. Specifically, the oxygen solubility was decreased by almost 25% for each 10℃ rise in temperature[32]. In this study, there was a semi-logarithmic relationship with Equation (1) for all the oil samples, including a linear dependency with good correlation of determination. The R^2 values for the camellia seed oils produced with roasting at 65℃, 100℃, 120℃, and 140℃ were 0.958, 0.981, 0.997, and 0.996, respectively (Table 4). The *E*a value of an oil is also a property of interest, as it demonstrates the delay of the initial oxidation reaction due to the bond scission that takes place to form primary oxidation products[33].

The *E*a values of the camellia seed oil produced with roasting at 65℃, 100℃, 120℃, and 140℃ were 65.7, 71.5, 78.4 and 67.5 kJ/mol, respectively. In a previous study, *E*a values of between 74.03～77.76 kJ/mol were obtained for linseed oil[34]. The *E*a values of roasted and unroasted argan oil, meanwhile, were 90.71 and 94.12 because these oils have high levels of β-carotene and the other compounds, such as phospholipids and phosphorus, that improve resistance to lipid oxidation[9]. The *E*a value of a given vegetative oil is influenced by the degree of unsaturated compounds present in the oil.

Adhvaryu *et al*. showed that, the linoleic and linolenic acid content were lower with *E*a variety for lipid oxidation while higher oleic acid content would increase. Similarly, an increased SFA would improve resistance to lipid oxidation (increasing the *E*a value)[35]. Flaxseed oils change in terms of their stability and quality during storage, and it was previously found that the stability deterioration was higher when the share of linolenic acid was higher (r = 0.81)[36].

The oxidation stability of edible oils depends on the different antioxidants in the oils. For example, the antioxidant activity of tocopherols makes an oil able to scavenge reactive oxygen species and lipids, while polyphenol additions to edible oil have the effect of mitigating the heat-induced formation of toxicants, mainly by inhibiting oxidative pathways and trapping reactive intermediates[12]. Subjecting an edible oil to high roasting temperature induces moisture reduction and the release of hydrophilic phenolics, while the high polar phase of an oil increases the speed of the degradation process[37].

4 Conclusions

Camellia seed oil has been found to comply completely with the international nutritional standards of omega meals. Camellia seed oil is rich in tocopherol, phenolic, and flavonoid content, and these components are very important for the nutritional composition and shelf life of this oil. Quality of roasted camellia seed oil were comply with Codex Standard, the roasted seed oil which has released from vegetal cells, and reducing polar phase of oil and Maillard reaction products fromed. Oxidative stability is the main parameter for evaluating camellia seed oil quality and its susceptibility to oxidative degeneration, which in turn determines its shelf life.

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