Food Sci. Biotechnol. 25(1): 105-110 (2016)
DOI 10.1007/s10068-016-0015-9

Food Science and Biotechnology

Physicochemical Properties and Oxidative Stabilities of Mealworm (*Tenebrio molitor*) Oils Under Different Roasting Conditions

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Received August 24, 2015
Revised October 19, 2015
Accepted October 20, 2015
Published online February 29, 2016

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pISSN 1226-7708
eISSN 2092-6456

Abstract Physicochemical properties and oxidative stabilities of mealworm (*Tenebrio molitor*) oils under different roasting conditions were investigated. Oils were extracted using n-hexane from mealworms roasted at 200°C for 0, 5, 10, and 15 min and physicochemical properties and oxidative stabilities of oils were analyzed. Roasting increased the color intensity and the oleic acid and δ-tocopherol contents, but decreased linoleic acid, and α- and γ-tocopherol contents. An improvement in oxidative stability was observed in roasted mealworm oils, demonstrated by induction time and peroxide values. Mealworm oil contained abundant essential fatty acids and exhibited a superior oxidative stability.

Keywords: mealworm, oil, roasting, physicochemical property, oxidative stability

Introduction

Due to a rapidly changing economy and population growth, demand for new protein resources as an alternative to meat production has emerged. A novel protein source is predicted to replace approximately 40% of red bovine meat in the next few decades (1). Food insecurity, environmental problems, and the rising cost of animal products have created an increased interest in consumption of edible insects (2), which are a beneficial food resource due to environmental and economical impacts. Insects emit considerably less greenhouse gas than most livestock, require smaller spaces, and have a high fecundity with fast-growth (3). Moreover, insects are highly nutritious, especially in protein, energy, vitamins and minerals. More than 1,900 species of insects are reported to be used as a food source, including beetles (31%), caterpillars (18%), bees, wasps, and ants (14%) (2).

Mealworm (*Tenebrio molitor*) larvae are distributed worldwide and are commercially used for animal feed. In addition, the mealworm is an edible insect consumed in many countries, including China and the Netherlands, and is being accepted as a food ingredient in a growing number of countries. The Ministry of Food and Drug Safety in South Korea permitted legal use of mealworms in food products in 2014. Mealworms consist of approximately 50% protein on a dry basis and meet the most essential human requirements for amino acids (4), and are drawing attention as a protein-rich food source.

However, in spite of having a high fat content of approximately 30% on a dry basis, little attention has been given to the fat of mealworms. Mealworms are known for large amounts of omega-3 and omega-6 fatty acids (5) levels that are comparable with fish. Thus, mealworm oil could be valuable for use as an essential fatty acid resource. No report of oil extraction and analysis of mealworm oil currently exists.

The roasting process can cause flavor changes and increase palatability (6) of most foods, including mealworms. In addition, roasting is considered to be an important treatment prior to oil extraction. Effects of roasting on changes in chemical composition and oxidative stability of extracted oils were extensively studied with pumpkin seed oil and sesame seed oil (7,8). The roasting process is thought to improve oxidative stability due to deactivation of undesirable enzymes and by formation of Maillard reaction products, which can terminate lipid oxidation reactions (9). The purpose of this study was to investigate the effect of roasting time on the physicochemical properties and oxidative stabilities of oils extracted from mealworms (*T. molitor*).

Materials and Methods

Preparation of mealworm oil Freeze-dried mealworms that had

been raised at 25°C and 70% relative humidity for less than 10 weeks were purchased from farms in Hwaseong, Korea in February of 2015. Mealworms then placed into an oven (FDO-7102; Daeyung Bakery Machinery Co., Seoul, Korea) at 200°C and roasted for 5, 10, and 15 min before grinding (HR-2860; Philips, Seoul, Korea). Oils were extracted from resulting powder twice using n-hexane (1:5, w/v) for 3 h at 170 rpm shaker (SI600R; Lab Companion, Daejeon, Korea). Extracts were filtered (No. 1 filter paper; Whatman, Buckinghamshire, UK) and the solvent was distilled off under vacuum in a rotary evaporator (RE111; Büchi, Flawil, Switzerland) at 37°C. Crude mealworm oil was centrifuged at 1,350x*g* for 10 min (Combi-514R; Hanil Science Industrial, Incheon, Korea) and supernatants were used as oils. For storage experiments, crude mealworm oil samples were stored at 50°C incubator (BI-1000M; Jeiotech, Daejeon, Korea) for up to 50 days.

Specific gravity, viscosity, and saponification and iodine values of mealworm oil Oil specific gravity was measured following the American Oil Chemists' Society (AOCS) 2013 method (10). Oil viscosity was determined at 25°C using a digital viscometer (Digital Viscometer DV-IP; Brookfield, Middleboro, MA, USA) with an LV3 spindle at a speed of 60 rpm. The saponification value was determined based on addition of 25 mL of a 0.5 N ethanolic KOH solution to 0.5 g of oil, followed by heating under a reflux condenser for 30 min. After cooling, oil was titrated using 0.5 N HCl with a phenolphthalein indicator. For determination of the iodine value, 0.3 g of oil was dissolved in 10 mL of chloroform, then 25 mL of a Wijs solution was added. The resulting solution was kept in the dark for 30 min, then mixed with 20 mL of a 20% KI solution and 100 mL of distilled water, followed by titration with 0.1 N sodium thiosulfate and a 1% starch indicator.

Color value and the browning index of mealworm oil Color was determined based on measurement of Hunter *L* (lightness), *a* (redness), and *b* (yellowness) values using a colorimeter (CM-5; Konica Minolta, Tokyo, Japan). The browning index was measured based on dilution of oil with hexane (1:20, w/v). The absorbance of the solution was measured (Optizen 2120UV; Mecasys, Daejeon, Korea) at 420 nm to represent the browning level (11).

Analysis of the fatty acid composition Analysis of the fatty acid composition was performed using GC (Agilent 6890; Agilent Technologies, Santa Clara, CA, USA). To produce methyl esters of fatty acid, 0.25 g of oil was mixed with 6 mL of 0.5 N methanol sodium hydroxide in a conical tube and heated in a water bath (BS-21; Jeiotech) set at 80°C for 10 min. Oil was then cooled on ice for 3 min and 7 mL of 14% boron trifluoride methanol was then added. The resulting solution was heated at 80°C (BS-21; Jeiotech) for 2 min, then allowed to cool on ice for 3 min before addition of 5 mL of *n*-hexane. The oil was then heated for 1 min, then the top layer was transferred to a vial for analysis. The GC apparatus (Agilent 6890; Agilent Technologies) was equipped with a capillary column (DB-23, 30 mx0.25 mmx0.25 μm) (J&W Scientific, Folsom, CA, USA) and a 260 flame ionization detector. Helium gas at a flow rate of 1.3 mL/min was used as a carrier gas. The amount injected was 1 μL and the split ratio was 50:1. The fatty acid composition was measured as a relative percentage of the total peak area.

Analysis of tocopherol Analysis of tocopherol was performed using HPLC (Ultimate 3000; Thermo Scientific Dionex, Hudson, NH, USA). A 120 mg of oil sample was dissolved in 1 mL of 2-propanol (12) and the filtered solution using 0.45 μm hydrophobic syringe filter (Advantec MFS; Advantec, Tokyo, Japan) was injected onto a C_{18} Inno column (4.60×250 mm, 5.0 μm) (Innopia, Seongnam, Korea). The mobile phase was 100% methanol at 1 mL/min. A fluorescence detector was used and the eluate was monitored at 295 nm.

Induction time of mealworm oil The induction time of oil was determined using a Rancimat device (Rancimat 734; Metrohm, Herisau, Switzerland). Three g of oil was weighed in a reaction vessel, then placed in the heating block to be oxidized with air at a flow rate of 20 L/h at a temperature of 110°C. Volatile components of oxidized oil were trapped in 60 mL of distilled water and the induction time was measured automatically with changes in water conductivity.

Oxidative stability of mealworm oil Peroxide and acid values were measured for determination of oxidative stability. For measurement of the peroxide value, 2 g of oil was dissolved in 25 mL of an acetic acid-chloroform (3:2, v/v) solution, then 1 mL of a saturated potassium iodide solution was added prior to standing in the dark for 10 min. Then, 30 mL of distilled water and a 1% starch solution were added and the mixture was titrated with 0.01 N sodium thiosulphate until the blue color disappeared. The acid value was determined based on titration of 2 g of oil dissolved in 100 mL of ethanol-ether (1:2, v/v) with 0.1 N ethanol potassium hydroxide. One hundred μL of a 1% phenolphthalein solution was used as an indicator.

Statistical Analysis Data were evaluated using an analysis of variance (ANOVA) and statistical significance was determined using Duncan's multiple range test at a level of $p<0.05$ (IBM SPSS Statistics 21.0; IBM, Armonk, NY, USA).

Results and Discussion

Extraction yield, specific gravity, and viscosity of mealworm oil Extraction yields and specific gravity and viscosity values of extracted mealworm oil are shown in Table 1. Extraction yields, which ranged from 31.54 to 33.64%, did not exhibit significant ($p>0.05$) differences between oil samples. Yields were in agreement with the dry basis fat content of mealworms of 35.42% (13).

The specific gravity of 0.8930 for unroasted mealworm oil significantly

Table 1. Extraction yields, specific gravity, viscosity, saponification and iodine values, and induction times of mealworm oils under different roasting times at 200°C

	Roasting time			
	Unroasted	5 min	10 min	15 min
Extraction Yield (%)	33.50±2.39[a1)]	31.54±1.90[a]	33.64±2.92[a]	31.77±2.49[a]
Specific gravity	0.8930±0.0063[a]	0.8909±0.0000[a]	0.8567±0.0001[c]	0.8777±0.0056[b]
Viscosity (cp)	306.67±11.55[a]	266.67±11.55[b]	246.67±11.55[b]	326.67±11.55[a]
Viscosity after storage (cp)[2)]	1093.33±23.09[a]	980.00±20.00[c]	1053.33±23.09 [b]	1086.67±11.55[ab]
Saponification value (mg KOH/g oil)	225.84±1.89[a]	221.43±2.02[a]	224.84±1.47[a]	219.44±9.70[a]
Iodine value (g iodine/ 100 g oil)	90.91±0.70[a]	89.89±0.81[a]	85.26±0.66[c]	88.25±0.35[b]
Induction time (h)	10.56±0.06[c]	17.69±0.41[b]	64.29±0.13[a]	64.57±0.50[a]

[1)]Values are means±standard deviation (SD) (n=3). Values with different superscript letters in each row are significantly different (p<0.05).
[2)]Observed after 50 days of storage at 50°C

Table 2. Color values and browning indices of mealworm oils under different roasting times at 200°C

Roasting time	Hunter color value[1)]			Browning index[2)]
	L	*a*	*b*	
Unroasted	29.09±0.74[a3)]	-3.72±0.16[d]	11.92±0.74[b]	0.205±0.005[d]
5 min	21.78±0.21[b]	-1.92±0.04[c]	12.12±0.67[b]	0.289±0.006[c]
10 min	18.75±0.50[c]	3.71±0.43[b]	23.29±0.88[a]	0.622±0.004[b]
15 min	8.49±0.37[d]	8.41±0.17[a]	10.71±0.73[c]	0.839±0.002[a]

[1)]Values are means±SD (n=6). *L*, lightness; *a*, redness; *b*, yellowness
[2)]Values are means±SD (n=3). Absorbance at 420 nm.
[3)]Values with different superscript letters in each column are significantly different (p<0.05).

decreased after 5 min of roasting (p<0.05). A specific gravity of 0.8567 was the lowest value after 10 min of roasting. Overall specific gravity values were relatively low, compared with common vegetable oils reported as 0.9188 for corn oil, 0.9193 for soybean oil, and 0.9073 for rapeseed oil (14). Reportedly, the specific gravity of oil is proportionately influenced by the degree of unsaturated fatty acids and short chain fatty acids (15).

The roasting time did not significantly (p>0.05) affect viscosity, with the lowest value after 10 min of roasting (246.67 cp) and the highest value after 15 min of roasting (326.67 cp). Viscosity values of all oil samples gradually increased during storage, regardless of roasting time. After 50 days of storage, viscosity values increased to 1093.33, 980.00, 1053.33, and 1086.67 cp for unroasted, 5, 10, and 15 min roasted oils, respectively. Thus, there was probably formation of viscous substances during storage of mealworm oil. Bouaid *et al*. (16) reported that the viscosity of oil can increase during storage due to formation of oxygen containing molecules and oxidized polymeric compounds that cause formation of gums and sediments.

Saponification and iodine values The saponification value represents the amount of potassium hydroxide required to saponify 1 g of oil (17). A high saponification value indicates the presence of short chain length or low Mw fatty acids. The saponification value ranged from 225.84 mg/g for unroasted oil samples to 219.44 mg/g for 15 min roasted samples. Roasting did not cause a significant (p<0.05) difference between saponification values of any oil samples. The saponification value of mealworm oil was relatively high, compared with other edible oils. Saponification values for safflower seed and palm oils are 190.23 and 205.00 mg/g, respectively (18).

The iodine value of unroasted mealworm oil was 90.91 g/100 g and the iodine values of roasted oil samples ranged from 85.26 to 89.89 g/100 g. More than 10 min of roasting caused a significant (p<0.05) decrease in the iodine value, compared with controls, which indicated a decrease in numbers of double bonds. A similar tendency was observed in sunflower seed oil, attributable to a reduction in the number of unsaturated sites as a result of oxidation and polymerization during heat treatment (19).

Color value and the browning index Hunter color values are shown in Table 2. Roasting led to significant (p<0.05) decrease in *L* values, compared with controls, with a value of 29.09 for unroasted oils and 8.49 for 15 min roasted oils. The *a* value significantly (p<0.05) increased with roasting, compared with controls, from −3.72 to 8.41. The *b* value was the highest after 10 min of roasting and was the lowest after 15 min of roasting.

The browning index significantly (p<0.05) increased with roasting time, compared with controls. Color development was probably due to browning substances produced during the roasting process, in agreement with other thermally processed oils, including sesame oil (20). Browning substance formation is mainly due to Maillard reaction, caramelization, and phospholipid degradation. Other thermal processes, such as microwave heating, have been reported to cause an increase in color intensity as a result of phospholipid degradation (19).

Table 3. Fatty acid composition (%) of mealworm oils under different roasting times at 200°C

	Roasting time			
	Unroasted	5 min	10 min	15 min
Laurate (C12:0)	0.205±0.015$^{b1)}$	0.196±0.003^{b}	0.246±0.021^{a}	0.238±0.001^{a}
Myristate (C14:0)	2.375±0.054^{b}	2.315±0.008^{b}	2.782±0.044^{a}	2.699±0.025^{a}
Palmitate (C16:0)	17.770±0.043^{a}	17.594±0.009^{c}	17.661±0.047^{bc}	17.719±0.006^{ab}
Palmitoleate (C16:1)	1.407±0.013^{a}	1.006±0.522^{a}	1.603±0.015^{a}	1.564±0.009^{a}
Stearate (C18:0)	3.228±0.085^{b}	3.451±0.005^{a}	3.204±0.057^{b}	3.401±0.025^{a}
Oleate (C18:1)	39.712±0.218^{c}	40.371±0.010^{b}	41.580±0.212^{a}	41.821±0.113^{a}
Linoleate (C18:2)	29.799±0.136^{a}	29.062±0.009^{b}	27.638±0.084^{c}	26.451±0.152^{d}
Linolenate (C18:3)	1.739±0.007^{a}	1.615±0.001^{b}	1.360±0.011^{c}	1.231±0.006^{d}
Arachidate (C20:0)	0.180±0.044^{a}	0.163±0.005^{a}	0.182±0.040^{a}	0.207±0.064^{a}
11-Eicosenoate (C20:1)	0.235±0.003^{a}	0.253±0.003^{a}	0.199±0.001^{b}	0.198±0.002^{b}
Total SFA$^{2)}$	23.758±0.152^{b}	23.718±0.003^{b}	24.075±0.129^{a}	24.264±0.008^{a}
Total UFA	72.893±0.331^{a}	72.307±0.543^{a}	72.381±0.269^{a}	71.265±0.260^{b}

$^{1)}$Values are means±SD (n=3). Values with different superscript letters in each row are significantly different (p<0.05).
$^{2)}$SFA, saturated fatty acid; UFA, unsaturated fatty acid

Table 4. Tocopherol contents (mg/kg of oil) of mealworm oils under different roasting times at 200°C

	Roasting time			
	Unroasted	5 min	10 min	15 min
α-Tocopherol	5.74±0.16$^{a1)}$	5.61±0.06^{a}	4.86±0.19^{b}	4.42±0.06^{c}
γ-Tocopherol	186.02±5.99^{a}	165.75±1.86^{b}	169.97±1.86^{b}	169.16±3.02^{b}
δ-Tocopherol	3.91±0.02^{d}	4.37±0.39^{c}	5.35±0.11^{b}	6.66±0.82^{a}
Total tocopherol$^{2)}$	195.67±5.85^{a}	175.74±1.91^{b}	180.19±2.36^{b}	180.23±2.94^{b}

$^{1)}$Values are means±SD (n=3). Values with different superscript letters in each row are significantly different (p<0.05).
$^{2)}$Calculated as the sum of α-, γ-, and δ-tocopherol.

Fatty acid composition Fatty acid compositions of mealworm oils with roasting are shown in Table 3. The primary fatty acids of mealworm oil were oleic (39.71-41.82%), linoleic (26.45-29.80%), palmitic (17.59-17.77%), and stearic (3.20-3.45%) acids. The total unsaturated and saturated fatty acid contents were 71.14-73.45% and 23.22-24.71% respectively, similar to previously reported results for mealworms (4). It is worth paying attention to the availability of essential fatty acids in mealworm oil, which are also present in fish and vegetable seed oils. The high content of omega-6 fatty acids is also notable in that omega-6 plays a crucial role in normal human development and brain function.

As roasting time increased, the content of oleic acid increased significantly (p<0.05) while the contents of linoleic and linolenic acids decreased significantly (p<0.05), compared with controls. Similar changes in contents of these fatty acids have been reported (19) with microwave roasting. On the other hand, previous studies have reported that roasting does not affect fatty acid compositions in rice germ and safflower seed oils (21,22).

Tocopherol composition Contents of α-, γ-, and δ-tocopherols in mealworm oil are shown in Table 4. Unroasted oil contained 5.74, 186.02, and 3.91 mg/kg of α-, γ-, and δ-tocopherol, respectively. Previously, it was reported that mealworm contained 19 mg/kg of α-tocopherol (23), which is higher than the amount reported in this study. No analyses of γ- and δ-tocopherol in mealworm oil have been reported. Thus, γ-tocopherol was the primary tocopherol in mealworm oil.

Roasting caused significant (p<0.05) decreases in α- and γ-tocopherol contents of mealworm oil, whereas the δ-tocopherol content increased significantly (p<0.05) with roasting time, compared with controls. The 15 min roasted oil exhibited α-, γ-, and δ-tocopherol contents of 4.42, 169.16, and 6.66 mg/kg, respectively. Thermal degradation or changes in tocopherol extractability from mealworms might have occurred during roasting, causing changes in tocopherol contents. Decreases in tocopherol contents with roasting were observed in sunflower seed oil (19), and gradual increases in tocopherol content of rice germ oil have been reported (21). Durmaz and Gökmen (24) reported that changes in tocopherol distribution depended on the variety of oil material and the intensity and type of heat treatment.

Oxidative stability Induction time refers to a period of time before the chain reaction of oil oxidation begins to accelerate and, thus, a longer induction time indicates superior oxidative stability. Induction times of mealworm oils are shown in Table 1. Unroasted mealworm oil exhibited a 10.56 h induction time. Induction times measured using the Rancimat test for other oils under identical experimental conditions were 7.65 h for peanut oil and 4.40 h for sunflower oil

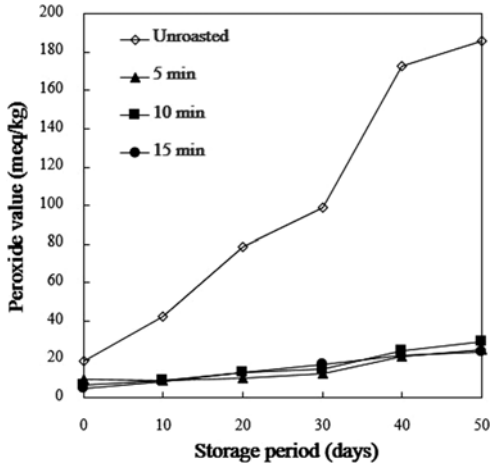

Fig. 1. Peroxide values of mealworm oils under different roasting times at 200°C during storage at 50°C.

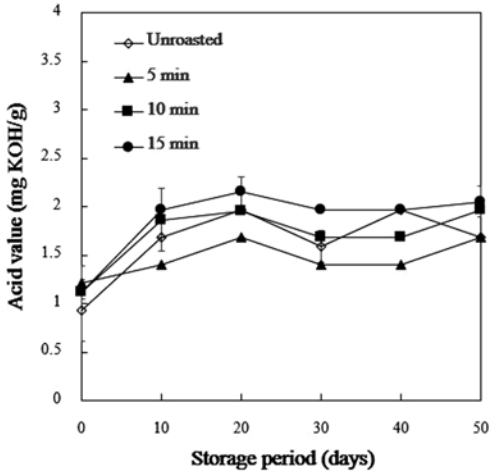

Fig. 2. Acid values of mealworm oils under different roasting times at 200°C during storage at 50°C.

(25), which demonstrates superior oxidative stability of mealworm oil. A significant (p<0.05) increase in induction time was observed within 10 min of roasting, compared with controls, with no significant (p>0.05) change for 15 min roasted oil, compared with controls.

Peroxide values of oil stored for up to 50 days at 50°C are shown in Fig. 1. The peroxide value of unroasted oil was initially 19.01 meq/kg at storage time=0 while 5, 10, and 15 min roasted oils exhibited initial values of 9.94, 6.82, 4.98 meq/kg, respectively. During storage, accumulation of primary oxidation products in unroasted mealworm oil caused a rapid increase in the peroxide value to 185.82 meq/kg after 50 days of storage. Even though roasted oils showed a significant (p<0.05) increase in peroxide values during storage, compared with controls, increases in peroxide values of roasted oils were minor, compared with unroasted oil. However, the duration of roasting did not significantly (p>0.05) affect the peroxide value. A similar tendency of primary oxidation product formation was observed in a study of roasted terebinth (*Pistaciaterebinthus*) oil (24).

The acid value is an important quality parameter of edible oils that is used to measure the content of free fatty acids (26). The acid value of unroasted oil was 0.93 mg KOH/g and values of roasted oils were from 1.12 to 1.22 mg KOH/g (Fig. 2). Acid values for all oils significantly (p<0.05) increased during the first 10 days of storage, compared with controls, but showed no significant (p>0.05) changes thereafter, compared with controls. Roasting did not result in any significant (p>0.05) differences in acid values between any oils. Oils in this study were extracted from mealworms that had been freeze dried by the supplier prior to subsequent roasting. Therefore, hydrolysis of oil might not have occurred due to a low water content. Kim *et al*. (27) also reported that freeze dried and pan fried mealworms displayed only slight increases in acid values, compared with raw mealworms.

The roasting process in this study had a retardation effect for mealworm oil oxidation, in agreement with previously reported oxidative stability results for roasted oils (21,23), mainly due to browning substances produced during the roasting process. Zamora and Hidalgo (28) demonstrated that Maillard reaction products, including non-enzymatically browned proteins, are effective for reducing levels of lipid oxidation. In addition, an increase in δ-tocopherol levels with roasting could improve oxidative stability, although α- and γ-tocopherol levels decreased. Reportedly, anti-oxidant activity is the strongest in δ-tocopherol, among all tocopherols (29). Inactivation of enzymes that promote oxidation in mealworms after heat treatment also could have influenced oxidative stability.

To conclude, the physicochemical properties and oxidative stabilities of mealworm oils with roasting were investigated. Duration of roasting time did not affect the physical properties of oils, but changes in fatty acid compositions and tocopherol contents were observed. The roasting process improved oil oxidative stability. Fifteen min of roasting did not increase the oxidative stability and produced an excessively dark color. Therefore, 10 min of roasting was sufficient. Abundant unsaturated and essential fatty acid contents and superior oxidative stabilities of mealworm oils was observed. Thus, further development of processing technologies and commercial use of mealworm oil are indicated.

Acknowledgment This research was supported by the Rural Development Administration in Korea (PJ01002204).

Disclosure The authors declare no conflict of interest.

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