Fractionation of Squalene from Amaranth Seed Oil

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ABSTRACT: Amaranth seed oil was fractionated in a bench-scale short-path distillation unit to obtain fractions rich in squalene. Fractionations were conducted with degummed amaranth oil, alkali-refined amaranth oil, and simulated amaranth oil. Squalene concentration was increased about sevenfold with a squalene recovery of 76.0% in the distillate when degummed amaranth oil was fractionated at 180°C and 3 mtorr vacuum. Free fatty acids codistilled with squalene, lowering the squalene content of the distillate, and resulted in a semisolid distillate at room temperature. Alkali-refining was subsequently used to reduce the free fatty acid content before fractionation. A simulated oil (7% squalene/93% soybean oil) and alkali-refined amaranth oil were fractionated at three temperatures (160, 170, and 180°C) and five vacuum settings (10, 100, 200, 400, and 600 mtorr). The highest squalene recoveries from simulated oil and alkali-refined amaranth oil were 73.4 and 67.8%, respectively, both at 180°C and 100 mtorr, which translates to 12.1- and 9.2-fold increases in squalene concentration, respectively. The squalene recovery of the alkali-refined amaranth oil at 180°C was not significantly different at 10 mtorr vs. 100 mtorr. The results of this study can be used as a component to assess the economic feasibility of fractionating amaranth seed for starch, oil, meal, and squalene.
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KEY WORDS: Alkali-refining, amaranth oil, distillation, fractionation, short-path distillation, squalene, squalene content, squalene recovery.

Squalene is important as a constituent in skin-care products and as an oxidation-resistant industrial lubricant (1). All squalene used in the United States, about 200–300 MT per year (Tajiri, S., private communication, Kannematsu USA Inc., New York, 1991), is imported, and the market price was about $15/kg in 1990 (2). Squalene is primarily derived from certain types of fish liver oil (3) in which the content is about 80%. Because of the concern for marine animal protection, attention has been focused on identifying crop sources of squalene. Two potential crop sources are olive oil (3,4) and amaranth seed oil (1,5). Olive oil contains about 0.3–0.7% squalene, and olive oil deodorizer distillates contain 10–30% squalene (3,4). The oil content of amaranth seed is only about 7%, but oil from amaranth grain contains 6–8% squalene (1,5,6), making it a particularly rich crop source of squalene.

The total U.S. production of amaranth was about 2,300 MT in 1995 (Hubbard, Edward S., private communication, Amaranth Resources, Inc., Albert Lea, Minnesota, 1995). Squalene recovery directly from amaranth seed is not economically feasible, but it could be advantageous to recover squalene from amaranth oil (7). Amaranth oil can be solvent-extracted from extruded collets of oil-rich embryonic tissue (or bran), which is a co-product of the milling of amaranth seed and its separation for starch production (6). The squalene in olive oil deodorizer distillate is recovered by converting the free fatty acids and esters in the distillates into their corresponding triglycerides. Those triglycerides are extracted with supercritical CO_2 at 110–170 bar pressure to obtain a highly enriched squalene fraction (4). This process requires zinc catalytic esterification and high pressure. Because amaranth oil contains a higher starting concentration of squalene than olive oil, enrichment of the squalene can perhaps be accomplished by simpler and more direct methods.

Short-path distillation is a high-vacuum process in which the condenser is located a few centimeters from the evaporator surface. The feed liquid is distributed on the evaporator surface as a thin falling film. This process can be conducted at different temperature and vacuum settings (8).

The objective of this study was to enrich squalene from amaranth seed oil by means of short-path distillation and to determine the relationship between process conditions and squalene enrichment and recovery.

EXPERIMENTAL PROCEDURES

Source of materials. Amaranth oil was prepared as previously described (6). The crude oil was degummed by heating the oil to about 90°C, adding 2% water (w/w), stirring the water/oil mixture, allowing the mixture to stand for 24 h, and centrifuging at 18,900 × *g* for 20 min. Soybean oil (brand name Wesson; Hunt-Wesson Inc., Fullerton, CA) was purchased locally. Squalene (99% purity) and palmitic acids (95% purity) were from the Sigma Chemical Co. (St. Louis, MO).

Short-path distillation. A glass short-path distillation unit (Model KDL4; UIC Inc., Joliet, IL) was used to fractionate

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the oil samples. The surface area of the evaporator was 0.043 m^2, and the surface area of the internal condenser was 0.022 m^2. The processing conditions of the distillation unit that can be varied are the heating-jacket temperature, system vacuum, and feed rate of the material to be processed. The roller-wiper speed inside the evaporator was fixed at 190 rpm (third mark). All samples were degassed under vacuum before distillation.

Squalene feed rate determination. Squalene was evaporated in the short-path distillation unit at three feed rates (0.9, 1.3, and 2.0 g/min), for a total of 50 g at each rate. Each feed rate was evaluated in duplicate, and the distillate was weighed. The heating-jacket temperature was set at 180°C, and the vacuum reached 3 mtorr by using the attached diffusion pump. The internal condenser was chilled with tap water at 3.6°C and 3.4 L/min.

Fractionation of degummed amaranth oil and simulated oil. A 200-g degummed amaranth oil sample was fractionated in the distillation unit to obtain squalene-rich distillate and squalene-lean residual oil. The fractionation was conducted at 180°C and 3 mtorr. Immediately before fractionating the 200-g sample, a 25-g sample was fractionated to offset the sample loss by adhesion to equipment surfaces.

Simulated amaranth oils, consisting of 7% squalene/3% palmitic acid/90% soybean oil (w/w/w) and 7% squalene/93% soybean oil (w/w), 50 g each, were fractionated at 180°C, 100 mtorr, and 2.8 g/min (85 drops/min). Owing to the difficulty of collecting distillate from the 3% palmitic acid mixture, the distillate was not quantitated.

Refining the amaranth oil. Degummed amaranth oil was alkali-refined by adding 2.5 g 20% caustic soda (w/w) solution per 100 g degummed amaranth oil. The oil and caustic mixture was agitated in a flask with a magnetic stir bar at room temperature (20°C). After standing 3–5 h, the oil–caustic mixture was mixed with 100 g of water and then agitated vigorously. Prior to centrifuging, 15 g NaCl was added to aid in breaking the emulsion. The upper oil phase that resulted after centrifuging the mixture at 18,900 × *g* for 20 min was isolated by using a separatory funnel.

Single-stage short-path distillation tests. Duplicate 50-g samples of the 7% squalene/93% soybean oil mixture were fractionated by short-path distillation at three heating-jacket temperatures (160, 170, and 180°C) and five vacuum settings (10, 100, 200, 400, and 600 mtorr). Duplicate 50-g samples of alkali-refined amaranth oil were also fractionated by short-path distillation at the same conditions. Both tests were conducted at 2.8 g/min feed rate.

Two-stage short-path distillation tests. Samples (50 g each) of 80% squalene/20% soybean oil mixture were fractionated in duplicate at 180°C with 10, 100, or 200 mtorr vacuum. Two 1000-g alkali-refined amaranth oil samples were fractionated at 180°C and 100 mtorr, and the two resulting distillates were run through the distillation unit at the same processing conditions as the first stage. The feed rate for each stage was 2.8 g/min.

Free fatty acid (FFA) analysis. The FFA content of the amaranth oil was measured by American Oil Chemists' Society (AOCS) Method Ca 5a-40 (9).

Squalene analysis. A Hewlett-Packard high-performance liquid chromatograph (HPLC) (Model 1090; Hewlett-Packard, Waldbronn, Germany) was used to determine the content of squalene in the oil samples as previously described (6). The squalene standard curve was generated from squalene–isopropanol (IPA) mixtures that contained from 4 to 10% (w/w) in 2% increments and from 10 to 80% (w/w) in 20% increments. Standards and samples of distillate and residue were diluted with IPA to a final strength of 5µL sample/50-mL final volume.

The squalene recovery for this test, and also for the other soybean oil/squalene mixtures and amaranth seed oil, was defined as:

$$\text{squalene recovery (\%)} = \frac{\text{distillate weight} \times \text{distillate squalene content}}{\text{sample weight} \times \text{sample squalene content}} \times 100\% \quad [1]$$

Similarly, the squalene loss in the residue was defined as:

$$\text{squalene loss (\%)} = \frac{\text{residual weight} \times \text{residual squalene content}}{\text{sample weight} \times \text{sample squalene content}} \times 100\% \quad [2]$$

Identification of components in distillates. Thin-layer chromatography (TLC) was used to identify the components in the distillates based on their relative mobility (R_f) values. The solvent was petroleum ether/ethyl ether/acetic acid, 90:10:1 (vol/vol/vol) (10). The standard samples on the plates were mono-, di-, and triglycerides, FFA, sterols, squalene, and tocopherols. Iodine was used to develop the plates.

Statistical analysis. Analysis of variance was used to determine the difference in mean values on at least duplicate runs of each treatment. Significance was determined at the $P < 0.05$ level (11).

RESULTS AND DISCUSSION

Squalene feed rate determination. Preliminary tests of the short-path distillation unit were performed with pure squalene to estimate the squalene evaporative and condensing capacities of the unit. The feed rate ranged from 0.9 to 2.0 g/min. Feed rates greater than 2.0 g/min (140 drops/min) were not evaluated because the drop rate was too high to be accurately measured. Because the sample was pure squalene, the recovery defined in Equation 1 was simply the distillate weight divided by starting sample weight. At a rate of 0.9 g/min, the average squalene recovery was 86.5 ± 0.1%. Recovery was 87.5 ± 0.7 and 84.9 ± 0.4%, at 1.3 and 2.0 g/min, respectively. It was concluded that recovery was only weakly dependent on flow rate in this range. The lower recovery at the 2.0 g/min feed rate was expected. The remainder of the squalene that was fed did not evaporate, although a small amount of condensate was found in the cold trap at all rates. Thus, the condenser was not able to prevent all vapor from escaping the main evaporator section.

Fractionation of degummed amaranth oil. It was hoped that squalene could be recovered in high yield and concentration from unrefined, unbleached amaranth oil. Not only would this simplify the process for obtaining squalene but it might also minimize squalene losses that were expected to occur during purification of the oil. Degummed amaranth oil was fractionated by short-path distillation at an average feed rate of 2.53 g/min over a 79-min period. This distillation was conducted at 3 mtorr and a jacket temperature of 180°C.

The squalene concentration was increased about sevenfold from 8.9% to 55.9 ± 0.8% with 76.0% of the squalene recovered in the distillate. About 9.5% of the squalene still remained in the residue, and the rest (14.5%) was apparently lost during processing, presumably through the vacuum system. The residue and distillate fractions represented 85.6 and 12.1% of the original sample weight, respectively. About 2.4% of the initial sample weight was lost. As mentioned above, small amounts of liquid were also found in the cold trap, but they were not quantitated.

During distillation, a soft, solid material which did not flow freely into the receiving flask was condensed on the internal condenser surface. Even at room temperature, this distillate was semisolid; thus, at the end of the test, this material was recovered from the condenser by replacing the cold condenser water with hot water at 60°C. The accumulation of distillate on the condenser surface progressively reduced the condenser capacity, contributing to the high squalene loss.

The melting point of squalene is −75°C, and it was assumed that it would not account for the solid consistency of the distillate. The FFA content of the distillate was 35.5%. The main fatty acids reported in amaranth oil are 13–20% palmitic, 19–34% oleic, and 37–62% linoleic (1), which have melting points 64, 4, and −12°C (12), respectively. Thus, the high FFA content, especially of palmitic acid, more likely contributed to the semisolid accumulating on the condenser surface.

Fractionation of squalene/soybean oil and squalene/soybean oil/palmitic acid mixtures. Fractionation of a high-FFA sample vs. a low-FFA sample was performed to help determine whether FFA contributed to the solid accumulation on the condenser. Because of limited availability of amaranth oil, this comparison was performed with a mixture of 3% palmitic acid/7% squalene/90% refined, bleached and deodorized (RBD) soybean oil and of 7% squalene/93% RBD soybean oil. HPLC analyses of the RBD soybean oil did not show a component at the 6.69-min retention time characteristic of squalene. The mixture of 7% squalene/93% soybean oil was a clear liquid at room temperature. The distillate of this mixture flowed freely from the condenser to the receiver. But the mixture of 7% squalene/3% palmitic acid/90% soybean oil was semisolid at room temperature. Thus, this mixture was heated to 60°C before it was fed into the distillation unit. Distillate from this mixture solidified on the surface of the condenser. It was assumed that free palmitic acid and perhaps other FFA impede the distillate flow. The removal of FFA was necessary to achieve successful distillation with this unit. Also, removing FFA before distillation should result in distillate with a higher squalene content than distillate from the degummed amaranth oil.

Refining the amaranth oil. Alkali refining can lower FFA to less than 0.05% for soybean oil. Squalene is part of the unsaponifiable matter and is not chemically altered by alkali (13). The FFA content of the alkali-refined amaranth oil was reduced from 4.2 ± 0.1% to less than 1%. Recovery of amaranth oil after alkali-refining averaged 85.3 ± 0.8%, and squalene recovery during this step was 83.6 ± 2.1%.

Single-stage short-path distillation. A mixture of 7% squalene/93% RBD soybean oil was fractionated to determine the relationship of squalene recovery and content in distillate with jacket temperature and evaporator vacuum. The squalene recovery in distillate generally increased with increased vacuum (decreasing absolute pressure) (Table 1). Because the boiling point of squalene decreased with increased vacuum (12), this was expected. The squalene contents of the distillates were not significantly different at the 10 and 100 mtorr vacuum settings for each jacket temperature. At each jacket temperature, squalene recovery decreased significantly with increased absolute pressure ($P < 0.05$) except from 10 to 100 mtorr at 180°C (Fig. 1). At 180°C, squalene recoveries at 10 and 100 mtorr were not significantly different, indicating no advantage to fractionating the sample at lower pressure.

Squalene was concentrated up to 12-fold with an 82.3% recovery at 170°C and 10 mtorr. Squalene recovery was significantly higher at these processing conditions than at the other processing conditions. When distillation was conducted at 180°C/10 mtorr, 180°C/100 mtorr and 160°C/10 mtorr, the squalene recoveries were not significantly different ($P < 0.05$, Table 1). Fluctuations in vacuum readings, up to 10% of the target, were observed owing to the difficulty of controlling pressure in this unit. The jacket temperature and feed rate remained uniform throughout the process.

The short-path distillation results with the alkali-refined amaranth oil were similar to the results with 7% squa-

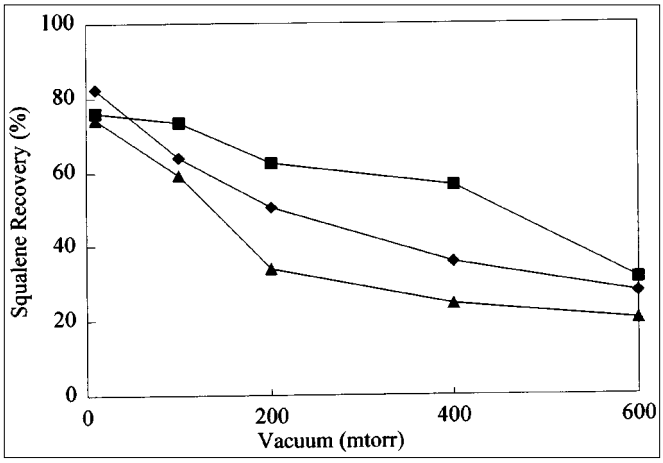

FIG. 1. Squalene recovery from 7% squalene/93% soybean oil by short-path distillation. Recoveries are at heating-jacket temperatures of ■, 180°C; ◆, 170°C; and ▲, 160°C.

TABLE 1
Distillate Squalene Content and Squalene Recovery with Various Processing Conditions for 7% Squalene/93% Soybean Oil and Alkali-Refined Amaranth Seed Oil[a]

Heating temperature (°C)	Vacuum (mTorr)	7% Squalene/93% soybean oil		Alkali-refined amaranth oil	
		Squalene content (%)	Squalene recovery (%)	Squalene content (%)	Squalene recovery (%)
180	10	85.8^{d}	75.9^{b}	76.9^{b,c,d}	65.6^{a,b}
180	100	85.2^{d}	73.4^{b}	77.9^{a,b}	67.8^{a}
180	200	94.0^{a,b}	62.6^{c}	79.9^{a}	65.6^{a,b}
180	400	96.7^{a}	56.5^{e}	71.3^{f}	38.3^{e}
180	600	90.5^{b,c}	31.2^{h}	67.4^{g}	28.5^{g}
170	10	91.8^{b,c}	82.3^{a}	79.2^{b,c}	67.1^{a}
170	100	90.2^{b,c}	64.0^{c}	80.2^{a}	63.4^{b}
170	200	88.4^{c,d}	50.5^{f}	78.7^{a,b,c}	49.3^{d}
170	400	92.8^{a,b}	35.6^{g}	76.4^{c,d}	31.8^{f}
170	600	93.4^{a,b}	27.5^{i}	71.9^{f}	20.9^{h}
160	10	92.3^{b,c}	74.2^{b}	71.6^{f}	53.8^{c}
160	100	93.4^{a,b}	59.2^{d}	78.6^{a,b,c}	55.9^{c}
160	200	89.7^{b,c}	33.8^{g}	75.3^{d,e}	34.5^{f}
160	400	93.2^{a,b}	24.5^{j}	73.5^{e,f}	21.2^{h}
160	600	91.9^{b,c}	20.2^{k}	67.3^{g}	12.7^{i}

[a]Values within a column followed by the same superscript are not significantly different at $P < 0.05$, based on Duncan's method.

lene/93% RBD soybean oil. The highest squalene recoveries from alkali-refined amaranth oil occurred at 180°C with 10, 100, or 200 mtorr vacuum and 170°C with 10 mtorr vacuum. At 180°C, there was no significant difference in squalene recovery between 10, 100, or 200 mtorr. At 170°C, squalene recovery decreased significantly with increased absolute pressure. Squalene recoveries at 10 and 100 mtorr vacuum were not significantly different from each other at 160°C, but were 8–12% lower than maximum recoveries at 180 and 170°C. The distillates of the alkali-refined amaranth oil were all free-flowing liquids. The distillates could be easily collected, and a negligible amount of distillate was retained on the condenser wall. The highest squalene recovery of alkali-refined amaranth oil was 67.8% at 180°C and 100 mtorr, and squalene concentration increased about 9.2-fold in one step (Fig. 2, Table 1). Use of the 7% squalene/93% RBD soybean oil mixture to simulate alkali-refined amaranth oil successfully predicted optimal conditions for enriching squalene from amaranth oil by short-path distillation. Lower squalene concentration in the amaranth oil distillate could be explained by the presence of FFA, tocopherol, and sterols, which were largely absent from deodorized soybean oil.

Representative HPLC profiles of alkali-refined amaranth oil, distillate and residue, are shown in Figure 3 (A–C). The peak retained at 6.68 to 6.69 min, which corresponded to squalene, increased 9.4-fold in area from seed oil to distillate, while the smaller peaks in the seed oil chromatogram were diminished or eliminated. Conversely, the squalene peak area in the residual oil decreased fivefold compared with that of the seed oil.

Compared with distillation of the degummed amaranth oil, distillation of the alkali-refined amaranth oil resulted in both higher squalene content in the distillate and higher squalene recovery. Fractionating squalene at 100 mtorr and 180°C should be feasible at industrial scale.

Two-stage short-path distillation. It was hypothesized that passing distillate from the first fractionation through the short-path distillation unit a second time could further enrich squalene. As a preliminary test of the hypothesis, 80% squalene/20% RBD soybean oil was fractionated at 180°C. As expected, both the residual oil weight and the squalene content of the residual oil from the 80% squalene/20% RBD soybean oil mixture increased with increased absolute pressure (Table 2). The squalene contents of the residues were relatively high, about 30 to 35%, which indicated that the squa-

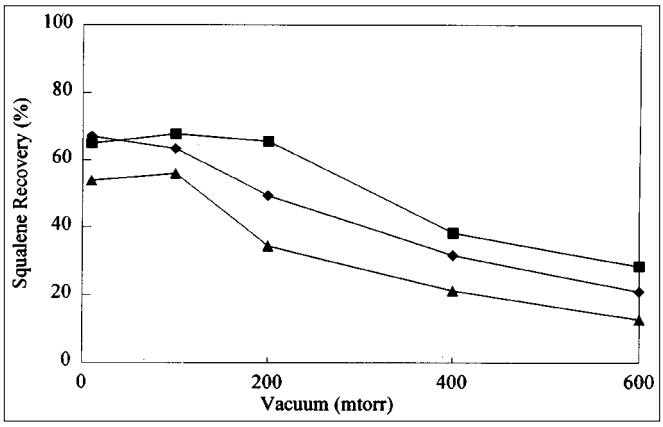

FIG. 2. Squalene recovery from alkali-refined amaranth oil by short-path distillation. Recoveries are at heating-jacket temperatures of ■, 180°C; ◆, 170°C; and ▲, 160°C.

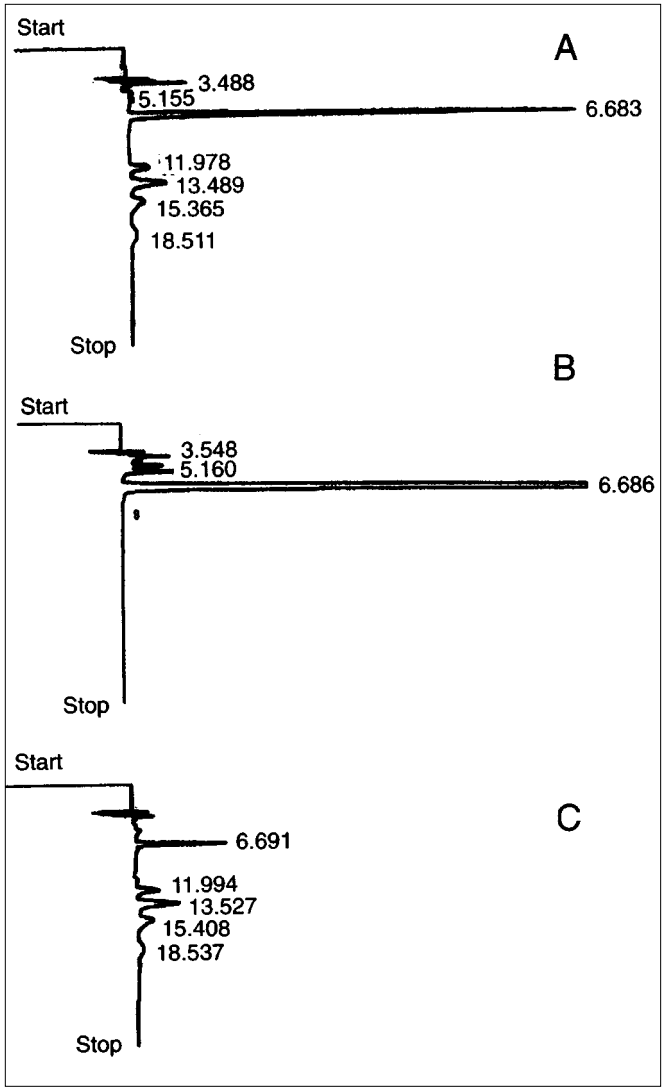

FIG. 3. High-performance liquid chromatography analysis profiles of (A) alkali-refined amaranth oil, (B) distillate of the short-path distillation, and (C) residual oil of short-path distillation. Squalene retention time was 6.69 min.

lene in the samples could not be vaporized completely by the evaporator. For the distillates, squalene recovery increased with decreased absolute pressure, and the squalene contents of the distillates were 91 to 93% at all pressures. Reducing the feed rate or using a unit that achieves greater residence time could increase squalene recovery in the distillate. In a commercial process, the residual oil should be recycled to the first distillation stage.

Distilling high-squalene-content amaranth oil distillate might suggest optimal processing conditions for further purification. The first distillation stage recovered 69.7 ± 1.6% squalene though some squalene was lost through the vacuum system. The squalene content of the distillate from the second stage was similar to the first stage (75.2 ± 1.1 and 77.0 ± 2.4%, respectively). Thus, the second stage did not achieve further enrichment of the squalene. However, the squalene

TABLE 2
Squalene Loss and Recovery in the Residual Oil and the Distillate, Respectively, Obtained by Short-Path Distillation of 80% Squalene/20% Soybean Oil

	Residual oil[a]		Distillate[a]	
Vacuum (mtorr)	Squalene content (%)	Squalene loss (%)	Squalene content (%)	Squalene recovery (%)
10	29.6 ± 4.9	9.5 ± 2.8	92.0 ± 2.9	88.4 ± 3.4
100	34.3 ± 0.7	18.1 ± 9.3	90.8 ± 0.7	78.9 ± 2.6
200	34.5 ± 0.3	15.2 ± 0.2	92.6 ± 4.0	74.6 ± 3.2

[a]Mean values ± SD.

content of the second-stage residue was much higher than that of the first stage (49.5 ± 10.6 and 1.1 ± 0.4%, respectively). The internal condenser was too small to condense the squalene vapor at the same feed rate as the first stage, or else the heating-jacket did not provide enough heat to vaporize the squalene completely. The distillate FFA contents of the two stages were similar, 6.9 ± 0.2%, whereas the second-stage residue had a slightly higher FFA content, 0.9 ± 0.1%, than that of the first stage, 0.6 ± 0.0%. Using a unit that achieves longer residence time and has larger condensing capacity could improve the recovery and reduce squalene vapor losses.

Analysis of components in distillates and residual. FFA, di- and triglycerides, tocopherols, and sterols were detected in the distillates of degummed and alkali-refined amaranth oil (Table 3). Results of the TLC analysis might suggest a strategy to remove these compounds for further purification of the squalene in the distillate.

FFA content in the distillate at 180°C and 100 mtorr was about 7%. If FFA was removed, the squalene purity increased from 78 to above 84%. The purity of squalene will be higher if di- and triglycerides in the distillate are also removed.

Squalene from amaranth oil can be enriched by short-path distillation. The squalene concentration was increased about sevenfold with 76.0% recovery when degummed amaranth oil was processed by short-path distillation at 180°C and 3 mtorr vacuum. The distillate was semisolid because of the high FFA content in the distillate. If the amaranth oil was alkali-refined before distillation, the squalene concentration in-

TABLE 3
R_f Values of the Components from the Distillates and Residual Samples of Degummed and Alkali-Refined Amaranth Oil

		Degummed amaranth oil		Alkali-refined amaranth oil	
Standard	R_f	Distillate	Residual	Distillate	Residual
Squalene	0.82	x[a]	x	x	x
Sterols	0.74	x		x	
Triglyceride	0.67	x	x	x	x
α-Tocopherol	0.43		x		x
γ-Tocopherol	0.40	x		x	
Free fatty acids	0.17	x		x	
Diglyceride	0.09	x	x	x	x
Monoglyceride	0.00				

[a]x, component present.

creased about 9.2-fold with 67.8% recovery in one step at 180°C and 100 mtorr vacuum. About 16.4% squalene was lost during refining. The squalene purity can be increased from 78% to higher than 84% if FFA and glycerides are removed from the distillate. The resulting purity may be sufficient for some skin-care products or industrial lubricants. Industrial-scale distillation of squalene from amaranth oil at 180°C would be less expensive at 100 mtorr compared with 10 mtorr.

The results of this study can be used to help assess the economic feasibility of processing amaranth seed into starch, oil and meal fractions, as well as squalene. Short-path distillation to fractionate squalene from amaranth oil does not require amaranth oil to be fully refined, and the squalene purity can be increased about 10-fold in one processing step.

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