Bench-Scale Processing of Amaranth Seed for Oil

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ABSTRACT: Amaranth seed (*Amaranthus hypochondriacus* cv. K432) was processed to obtain oil, reported to be a promising source of squalene. The amaranth seed was ground using a stone mill, then separated into oil-rich embryonic tissue (or "bran") and starchy perisperm. Amaranth bran was much more stable than rice bran when free fatty acid (FFA) content and peroxide value were monitored. Milling at a gap of 0.755 mm did not result in excessive damage to the starch in the perisperm fraction and yielded a bran fraction that contained more than three-fourths of the oil and a starchy fraction consisting of more than two-thirds of the seed weight. The bran particles were too fine for effective bench-scale extraction of the oil. Consequently the bran was extruded into collets prior to extraction. Two extrusion settings were evaluated regarding the rate of moisture injection, while the bran feed rates were constant. There was no significant difference in appearance or size between the two dried collets. Collets were extracted with hexane using an Armfield Extraction/Desolventizing Unit (Model FT 29, Armfield, Ltd., Hampshire, England). Oil recovery averaged 97.7 and 80.0%, respectively. Oil was extracted at high yield from the bran when the bran was extruded into collets. Oil can be obtained as a coproduct of amaranth starch by milling and separating the fractions of amaranth seed. Milling, extrusion, and extraction did not decrease significantly the squalene content in amaranth oil, but increased FFA content and peroxide value and changed tocopherol content of the oil.

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KEY WORDS: Amaranth seed, amaranth starch, bench-scale processing, bran, collet, coproduct, extraction, extrusion, perisperm, separation.

Amaranth grain contains about 6–9% oil, and this oil is rich in squalene compared to other vegetable oils (1–3). Squalene and its hydrogenated form, squalane, are relatively high-value lipids with many applications in the pharmaceutical and cosmetics industries. All squalene and squalane used in the United States is imported. The solvent extraction of oil from whole amaranth flour, using a Butt-type extractor, and the refining of the crude oil have been described (2). However, commercial production of squalene from amaranth oil would probably be most feasible as a coproduct of amaranth starch, which has unique, desirable characteristics (4). Amaranth starch can be used as an ingredient in cereal-based foods (5).

Production of amaranth oil and starch requires that the oil-rich embryonic tissue (henceforth referred to as "bran") of the amaranth be separated from the starchy perisperm prior to solvent extraction. Fractionation of amaranth seed using a stone mill has been described (1), but the processing of the bran for oil has not. The fine particle size of amaranth bran precludes its direct solvent extraction using typical industrial equipment. Furthermore, the oil in the bran might be susceptible to rapid enzymic degradation, similar to rice bran oil. It was thought that both of these process problems could be overcome by extrusion of the amaranth bran prior to solvent extraction, similar to processing of rice bran for oil (6).

The objectives of this study were to (i) develop a process to extract oil from amaranth seed using methods that would permit the fractionation of bran from perisperm prior to solvent extraction and (ii) characterize the raw products and determine whether squalene and tocopherol contents in amaranth oil were affected by the selected methods.

EXPERIMENTAL PROCEDURES

Amaranth seed (*Amaranthus hypochondriacus* cv. K432) was obtained from Nu-World Amaranth, Inc. (Naperville, IL). The light brown seed contained 12.3% moisture (wet basis, or w.b.). Rice bran, shipped on the same day of milling by overnight delivery from Riceland Foods, Inc. (Stuttgart, AR), which contained 12% moisture (w.b.), was used to conduct the storage stability comparison with the amaranth bran.

Milling. Amaranth seeds were milled using a Morehouse stone mill (Model MS-1, Morehouse-Cowles, Inc., Los Angeles, CA) at five milling gaps ranging from 0.71 to 0.89 mm in 0.045-mm increments. The milling gap was checked manually with calipers. Seeds were metered to the mill at a rate of 0.5 kg/min using a screw conveyor. Duplicate 100 g samples were milled at each gap. Coarse particles of perisperm were manually separated from the finer bran using a U.S. No. 24 screen.

Amaranth seed for oil extraction and the bran storage stability study was milled using a 0.755-mm gap and separated by a laboratory flour rebolter (Allis-Chalmers Manufacturing Co., Milwaukee, WI) using a U.S. No. 24 screen. The screen was contained within a cabinet that rotated eccentrically at

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about 220 rpm. The rebolter separated 2.5 kg milled seed in about 10 min.

Bran storage stability test. Amaranth bran and rice bran (2,400 g each) were stored at three temperatures: 20°C, 3°C, and −4°C. Duplicate 200 g samples were analyzed for free fatty acid (FFA) content and peroxide value at 0, 1, 7, 14, and 28 d using American Oil Chemists' Society (AOCS) Methods Ca 5a-40 and Cd 8-53 (7), respectively.

Extrusion. Amaranth bran was extruded into porous collets using a corotating twin-screw extruder (Model TX 52; Wenger Manufacturing, Sabetha, KS), and having a length/diameter ratio of 25:1. The die assembly included a transition die spacer and a final die having two 6-mm diameter extrusion die openings. The feed rate of bran was 77 kg/h (170 lb/h), and the material was mixed in the conditioning cylinder with a water injection rate of 5.3 kg/h and a steam injection rate of 11–12 kg/h. The pressures in the eighth and ninth heads (numbered from barrel entrance) were 2.1 MPa (300 psi) and 3.4 MPa (500 psi), respectively. Heads 4 through 9 were maintained at 100–115°C, and heads 2 and 3 were maintained at 50 and 75°C, respectively. For the test 1, barrel water and steam injection rates were 4.6 kg/h and 4.2 kg/h; barrel water and steam injection rates were 3.1 kg/h and 7.2 kg/h for the test 2. The collets were cut at the die into 2.5-cm lengths, then dried in a cabinet dryer (Standard Industries, Fargo, ND) at 50°C for 18 h to a final moisture content of 8.5% (w.b.).

Extraction. A bench-scale solvent extraction/desolventizing unit (Model FT 29, Armfield, Ltd., Hampshire, England) was used to extract oil from duplicate 15 kg samples of bran. The bran was either in the form of extruded collets, or the nonextruded bran was wrapped as 500-g packages in Whatman No. 1 filter paper (Whatman, International Ltd., Maidstone, England). A single batch extraction was performed as follows: 15 kg bran was immersed in 40 L hexane for 10 min, then the miscella was drained to a separate vessel. Miscella was then desolventized for 1 h at 65°C and atmospheric pressure with indirect heating using steam at 1.5 KPa. The hexane vapors were condensed using cooling water at 18°C. When the miscella temperature was above 65°C, vacuum was applied to minimize the hexane residual in the crude oil until the vacuum pressure reached 500 Pa; then the crude oil was drained out. Four cycles of immersion, draining, desolventizing, and crude oil draining were performed with each batch of bran. The crude oil obtained from the extraction process was filtered under vacuum using Whatman No. 3 filter paper without further desolventizing.

Analysis of collets. Collet diameter was determined at the midpoint with calipers using 30 dried collets from each of the two extrusion tests. Volume expansion of collets was calculated from the collet diameter (d_c), and dye-opening diameter (d_d), by assuming that collets expanded uniformly in all three dimensions. Therefore, the volume expansion is expressed as $VE = (d_c/d_d)^3 \times 100\%$. Bulk density was determined in triplicate based on the method of the Federal Grain Inspection Service (8).

Proximate analysis. Moisture and protein were determined in triplicate by using American Association of Cereal Chemists (AACC) Methods 44-15A and 46-11A (9), respectively. Oil was determined by AACC Method 30-25 (9), with the following modifications: (i) hexane (b.p. 69°C) was used instead of petroleum ether (b.p. 37–65°C), (ii) whole amaranth seeds were dried at 130°C for 1 h, and (iii) the solvent from the oil was removed with a Brinkmann Model R Rotovapor (Brinkmann Instruments, Inc., Westbury, NY). Extractions were performed in triplicate.

Starch quality analysis. The starch damage was determined using a Megazyme Kit (Megazyme Australia, Sydney, Australia) based on the method of Gibson *et al.* (10). The starch paste viscosities were measured with a Rapid Visco Analyzer (Newport Scientific Pty. Ltd., Narrabeen, Australia) from the method of Uriyapongson and Rayas-Duarte (5).

Squalene analysis. Dried oil samples were dissolved in isopropanol and diluted (final dilution 1:1000) with methanol/isopropanol/acetic acid, 91.95:8:0.05, (vol/vol/vol). Squalene content was determined using a Waters high-performance liquid chromatograph (HPLC) (Model 1090; Hewlett-Packard, Waldbronn, Germany) with a C18 Whatman column (5 μ, 4.6 × 280 mm, ISI-344), a Waters Autosampler Model 717, and a Waters Integrator Model 730. The mobile phase was methanol/isopropanol/acetic acid, 91.95:8:0.05 (vol/vol/vol). The analyses were performed at room temperature, using a flow rate of 1.2 mL/min and Waters Detector Model 440 at 214 nm [range 0.1 absorbance units full scale (AUFS)]. The squalene standard (99% purity) was from Kodak Co. (Minneapolis, MN).

Total tocopherol analysis. The oil sample extracts were used at a dilution 1:25 in isopropanol. The HPLC, column, and detector used for the analysis of α-, δ-, and γ-tocopherols were the same as described in the squalene analysis. The mobile phase was methanol/isopropanol/acetic acid, 91.95:8:0.05 (vol/vol/vol), at a flow rate of 1.2 mL/min. Absorbance at 280 nm (range 0.010 AUFS) was recorded. Tocopherols standards were purchased from Kodak Co.

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

RESULTS AND DISCUSSION

Milling. A preliminary milling study was performed to determine optimum milling gap of the stone mill. The selected range of milling gaps from 0.71 to 0.89 mm were chosen based on a previously published study using a wider range of milling gaps (1). It was expected that a milling gap smaller than 0.71 mm would remove too much perisperm with the bran, and that a gap larger than 0.89 mm would result in too low a yield of the bran fraction. Similar to the previous study of Becker *et al.* (1), the percentage weight of the bran fraction decreased with increasing milling gap (Fig. 1). Milling gap also significantly affected the distribution of oil between

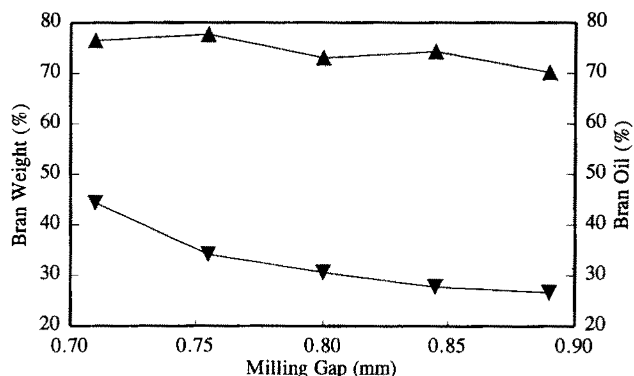

FIG. 1. Weight distribution of the amaranth bran and percentage total oil in the bran at different milling gaps: ▲, percentage total oil in the bran fraction; ▼, percentage weight of the bran.

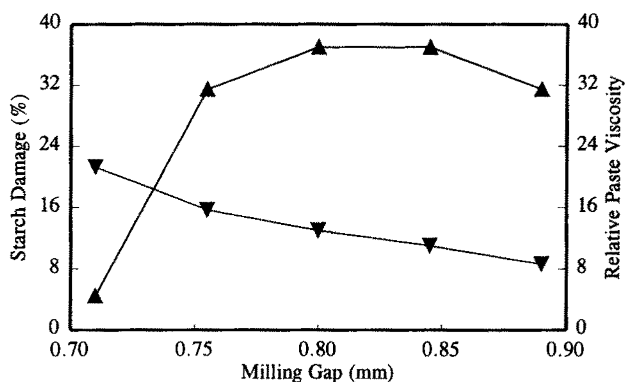

FIG. 2. Amaranth starch damage and the relative paste viscosity at different milling gaps: ▼, starch damage; ▲, relative paste viscosity.

the bran and perisperm fraction (see Fig. 1), and the oil content of the bran fraction (Table 1).

When the milling gap was increased from 0.71 to 0.755 mm, percentage weight of the bran decreased significantly, but the percentage of oil remaining in the bran fraction did not decrease significantly ($P < 0.05$), because the oil content of the bran increased. Also, when the milling gap was increased further to 0.845 mm, there was still no significant decrease in the percentage of oil remaining in the bran relative to the 0.71-mm gap ($P < 0.05$). The decrease in the percentage weight of bran from 0.755 to 0.89 mm was not significant ($P < 0.05$).

The effect of the milling gap on starch quality, as determined by the percentage of starch damage and starch paste viscosity, was also studied (Fig. 2). During the milling process, the temperature of the milled amaranth was about 40–50°C at a gap of 0.80 mm or greater, but increased with progressively smaller gaps. The temperature was 60°C at the 0.755-mm milling gap, increasing to 90°C at the 0.71-mm milling gap. The stone mill used in this study was not equipped with a water-cooling jacket; thus it was not possible to avoid such high temperatures without reducing the feed rate of seed to the mill. The sharp decrease in paste viscosity and increase in starch damage indicated reduced starch quality, probably resulting from mechanical and thermal damage to the starch granules.

As a result of this milling study, a milling gap of 0.755 mm was chosen to mill the bulk of the amaranth. Milling at this gap did not result in excessive damage to the starch in the perisperm fraction. Also, this gap yielded a bran fraction that contains more than three-fourths of the oil, and it also yielded a starchy perisperm fraction consisting of more than two-thirds of the seed weight. During the subsequent bulk milling and fractionation for the extrusion and solvent-extraction study, the oil content of the bran fraction was increased more than twofold relative to the whole seed (Table 2).

Stability of oil in amaranth bran. The triglycerides in rice bran are rapidly degraded by the lipolytic enzymes in the bran (12). Consequently, either the rice bran oil must be extracted promptly or the lipase must be inactivated. The storage stability of oil in amaranth bran was compared with that of rice bran to determine whether similar precautions are needed with amaranth bran. The effect on amaranth oil quality of bran storage time after milling was determined by monitoring FFA and peroxide value (Figs. 3 and 4). When amaranth and rice brans were both stored at room temperature (20°C), the rice bran oil had a significantly higher FFA content ($P < 0.05$) and peroxide value ($P < 0.05$) from the first day to day 28. Amaranth bran stored at −3°C had significantly less FFA than that of the amaranth bran stored at 3°C ($P < 0.05$). Unlike rice bran, amaranth bran stored at −3°C showed negligible deterioration after 28 d as determined by FFA and peroxide value.

TABLE 1
Percentage Weights and Oil Content of the Amaranth Perisperm and Bran Milled at Various Milling Gaps[a]

Milling gap (mm)	Perisperm weight (%)	Bran weight (%)	Percentage weight of oil in bran	Oil content in bran (%)
0.710	55.7[a]	44.4[a]	76.5[a,b]	16.3[a,b]
0.755	65.8[b]	34.2[b]	77.7[a]	18.4[a]
0.800	69.4[b]	30.6[b]	73.0[a,b]	15.6[b]
0.845	72.3[b]	27.7[b]	74.3[a,b]	16.1[a,b]
0.890	73.3[b]	26.7[b]	70.2[b]	12.3[c]

[a]Values within a column followed by the same letter (a–c) are not significantly different at $P < 0.05$, using Duncan's method.

TABLE 2
Compositions of Amaranth Before and After Milling, Extrusion and Extraction Processes

	Component (%)[a]			
	Whole seed[b]	Bran fraction[b]	Bran collet[b]	Defatted bran[b]
Oil	6.1 ± 0.04	14.7 ± 0.3	13.0 ± 0.1	1.9 ± 0.9
Starch	43.8 ± 4.2	14.4 ± 5.9	13.5 ± 2.2	19.0 ± 3.0
Protein[c]	14.7 ± 0.4	31.9 ± 0.9	29.7 ± 0.6	27.8 ± 4.1
Ash[c]	3.1 ± 0.07	6.6 ± 0.5	6.1 ± 0.2	7.0 ± 0.4

[a]The remainder was crude fiber and moisture (Ref. 14).
[b]Values are listed as mean ± standard deviation.
[c]14% moisture content (w.b.).

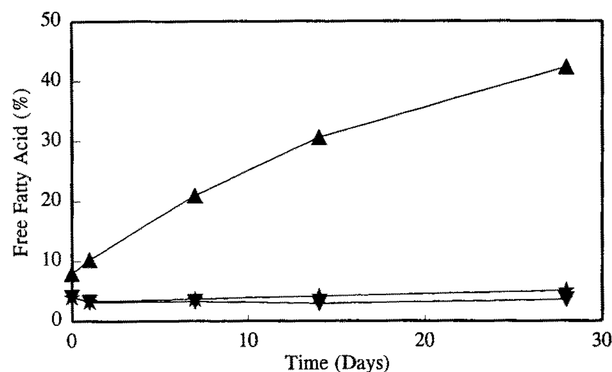

FIG. 3. Change of free fatty acid content with storage time for rice bran and amaranth bran at 20°C and amaranth bran at 3°C: ▲, rice bran stored at 20°C; ★, amaranth bran stored at 20°C; ▼, amaranth bran stored at 3°C.

The stability of amaranth bran may be an advantage for its potential utilization.

Extrusion. Although extrusion of the amaranth bran was not needed for the purpose of stabilizing the oil, extrusion was still needed to agglomerate the fine bran particles into expanded collets. The fine bran particles would be easily flushed away by the solvent in typical commercial extraction equipment or would allow poor percolation of solvent causing low extraction efficiency. Single-screw extruders can produce a material with better extraction and solvent drainage characteristics than flakes have (13). The twin-screw extruder available for this study was expected to give results comparable to a single-screw extruder.

Two moisture injection rates (4.6 kg/h water and 4.2 kg/h steam for test 1; 3.1 kg/h water and 7.2 kg/h steam for test 2) were used to study the effect of steam injection rate on expansion of collets, extractability of oil, and oil quality. A sample of conditioned bran taken from the conditioning cylinder had a moisture content of 23.9% (w.b.). The moisture content at the die plate for test 1 was 24.5 ± 0.5% (w.b.); for test 2 it was 23.2 ± 0.5% (w.b.). After drying to 8.5% (w.b.) moisture, the average collet diameter of 30 collets was 6.7 mm for both tests, which corresponded to a volume expansion of 39%. Although the measurements of the collet diameter were similar, the bulk densities of the collets were 586 ± 1.6 and 535 ± 2.4 kg/m^3 for tests 1 and 2, respectively.

Extraction. About 1.8–2.0 L dark brown oil was obtained by extraction of each 15-kg sample of bran. In addition to the extruded collet samples, oil was extracted from a sample of nonextruded bran wrapped in filter paper to facilitate solvent extraction. Oil was extracted from the nonextruded bran to determine if extrusion resulted in a difference in the quality of the final extracted oil.

Under otherwise identical extraction conditions, 97.7 ± 0.2% of oil was recovered from the test-1 collets, 80.0 ± 0.5% from the test-2 collets, and 84.4 ± 0.06% from the nonextruded bran. With the collets from test 2, inspection of the bed of defatted collets revealed pockets of collets with high residual oil close to the bottom of the bed, as evidenced by a dark color compared to completely defatted collets. This was true of both duplicates using test-2 collets. The collets from test 2 might have contained a higher percentage of fines, which interfered with solvent percolation; however, the percentage of fines was not measured. The lower yield, compared with test-1 extruded collets, associated with nonextruded bran, probably resulted from the relatively large mass transfer resistance associated with wrapping the bran in filter paper.

Effects of processing on chemical characteristics of intermediate and final products. Milling and separation yielded a bran fraction enriched in oil and protein content relative to the whole seed (see Table 2) and yielded a perisperm fraction enriched in starch. Extrusion appeared to result in some loss of oil. Ash content of the bran fraction increased about twofold compared to that of whole seed.

Table 3 compares the squalene content of oil from whole seed, bran fraction, and collets extracted using the modified AACC method, and that of oil extracted from collets by the bench-scale extractor. Milling, extrusion, and extraction did not appear to cause any significant changes in squalene content of the oil ($P < 0.05$). Squalene is known to be stable at

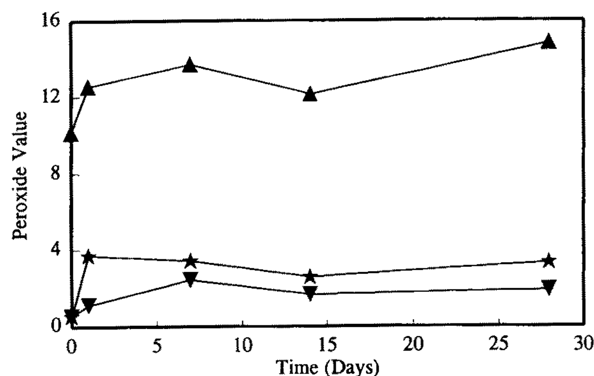

FIG. 4. Change of the peroxide value with storage time for rice bran and amaranth bran at 20°C and amaranth bran at 3°C: ▲, rice bran stored at 20°C; ★, amaranth bran stored at 20°C; ▼, amaranth bran stored at 3°C.

TABLE 3
Characteristics of Amaranth Oil Affected by Various Processes[a]

	Squalene (%)	Total tocopherol (ppm)	Free fatty acids (%)	Peroxide value
Whole seed	7.2[a]	374[a,b,c]	3.2[a]	0.6[a]
Bran fraction	7.0[a]	360[c]	3.2[a]	0.6[a]
Crude oil 1[b]	7.2[a]	385[a]	4.7[b]	12.3[b]
Crude oil 2[c]	7.7[a]	376[a,b]	4.5[b]	9.5[b]
Crude oil 3[d]	7.4[a]	370[b,c]	6.0[c]	5.4[c]

[a]Values within a column followed by the same letter (a–c) are not significantly different at $P < 0.05$, using Duncan's method.
[b]Oil extracted from the collets processed under extrusion test 1 (barrel water and steam injection rates were 4.6 and 4.2 kg/h).
[c]Oil extracted from the collets processed under extrusion test 2 (barrel water and steam injection rates were 3.1 and 7.2 kg/h).
[d]Oil extracted from the bran wrapped in the filter paper.

high temperatures, hence its desirability as an industrial lubricant (14). Significant differences were found in tocopherol content ($P < 0.05$), FFA content ($P < 0.05$), and peroxide value of the crude oil ($P < 0.05$). The changes in these characteristics indicated that the degree of oxidation and hydrolysis of the oil generally increased with each additional process step, as expected. The increase in peroxide value probably resulted mainly from oxidation of unsaturated acids.

In conclusion, oil can be readily extracted at high yield from amaranth bran by extruding the bran into collets. Thus oil can be obtained as a coproduct of amaranth starch by milling and separating the perisperm and bran fractions of amaranth seed. Oil in the amaranth bran is much more stable during storage than is oil in rice bran. Milling, extrusion, and extraction did not change squalene content of amaranth oil significantly, but resulted in increased FFA content and peroxide value and caused the changes in tocopherol content of the oil.

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