NOTE

A Comparison of Two Rapid Oat-Lipid Extraction Procedures in Terms of Fatty-Acid Profile[1]

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ABSTRACT

Two rapid lipid-extraction procedures were compared in terms of the fatty-acid profiles obtained for three oat cultivars known to differ in oil concentration and composition. The extraction procedures were a chloroform-methanol-water procedure and a Skellysolve B procedure. A more time-consuming, multiple-step extraction procedure was chosen as a comparison control for cultivar extraction because it gives nearly total lipid extraction of oats. Highly significant differences were present among procedures for palmitic, stearic, oleic, and linoleic acids. Ranking of cultivars within procedures was identical for stearic, oleic, linoleic, and linolenic acids. This indicates that all three procedures would be useful in ranking diverse materials. Variation due to cultivars was several times greater than that for procedures for the major fatty acids. Thus, genotypes are likely to be of greater significance than procedure in a ranking process. The Skellysolve procedure was least time consuming; however, extracts tended to be cloudy. The chloroform-methanol-water extraction was slightly more time consuming, samples were clear, and gas chromatograph column life was prolonged when samples extracted by this procedure were used.

Cereal Chem. 61(2):196-197

Oats (*Avena sativa* L.) have a higher lipid concentration than do other cereal grains (Weber 1973, Price and Parsons 1975). Research suggests that breeding for higher oil concentration (Baker and McKenzie 1972, Brown et al 1974, Frey and Hammond 1975, Youngs and Forsberg 1979, Thro 1982), for altered oil composition (Youngs and Puskulcu 1976, Thro 1982, Thro et al 1983), or both may be possible because these traits are under genetic control and because much variability for these traits exists in *Avena*. In such breeding efforts, rapid-analysis procedures will be required if large populations are to be screened. Groat oil concentration can be measured accurately and rapidly through nuclear magnetic resonance (NMR) or infrared spectroscopy techniques. However, determinations of oil composition (fatty-acid profile) are generally time consuming and are affected by the solvents used in the oil extraction (de la Roche et al 1973, Sahasrabudhe 1979). The objective of this study was to compare two rapid-extraction procedures in terms of fatty-acid profiles, and to evaluate each procedure for use in germ plasm screening programs. A more time-consuming multiple-step extraction procedure reported to give near quantitative lipid extraction (de la Roche et al 1977) was also used on samples and served as a control procedure.

MATERIALS AND METHODS

Plant Materials

Three oat (*Avena sativa* L.) cultivars differing in oil concentration were used: 'Dal' (8%, w/w), 'Sauk' (5%, w/w), and 'Exeter' (3%, w/w). Oil concentration was determined by NMR, and groats were dried to 5% moisture. Seed samples were obtained from 3-m-long rows of bulk-harvested oats grown in the Madison, WI, oat nursery in 1979. Seeds were mechanically dehulled, and whole, undamaged groats were stored at 0°C until oil was extracted.

Extraction Procedures

Two rapid-extraction procedures, a chloroform-methanol-water (CMW) procedure (a modified Bligh and Dyer [1959] procedure) and a Skellysolve B extraction procedure were compared to each other and to a multiple-step extraction procedure. Each procedure, using a 1-g whole-groat sample air-dried to 10% moisture, is described below.

Chloroform-methanol-water. The sample was added to 3.9 ml of water and 10 ml of methanol in a 30-ml glass centrifuge tube. The mixture was homogenized for 20–25 sec at room temperature with a Polytron homogenizer (Brinkman Instr., Westbury, NY). Chloroform (5 ml) was added to the homogenate, which then stood at room temperature for 15 min. Then 5 ml of chloroform and 5 ml of water were mixed with the homogenate, and the mixture was centrifuged for 15 min at 6,780 × *g*. The lower, lipid-containing chloroform layer of the resultant biphasic separation was removed by pipette and transferred to a 15-ml glass vial with a Teflon™-lined screw cap. The chloroform-lipid solution was passed through a pipette packed with glass wool as part of the transfer process to remove any particulate material. The vial was placed on a heating block at 30°C, and the chloroform was evaporated under a stream of nitrogen.

Skellysolve B. The sample was homogenized for 20–25 sec in 6 ml of Skellysolve B in a 15-ml glass centrifuge tube. The homogenate was held at room temperature for 15 min and mechanically mixed two times during the period. Following centrifugation for 15 min at 7,710 × *g*, the supernatant was decanted into a 15-ml vial, dried under nitrogen, and the residual lipid methylated as described below.

Multiple step. The sample was added to 6 ml of Skellysolve B in a 15-ml glass centrifuge tube. The mixture was homogenized for 20–25 sec, allowed to stand at room temperature for 15 min, and centrifuged for 10 min at 7,710 × *g*. The solvent was decanted and filtered through a glass-wool-packed pipette into a round-bottom flask. The meal was then extracted, for 15 min each, with 6 ml of a chloroform-methanol solution (2:1, v/v) and 6 ml of a chloroform-methanol-water solution (1:2:0.8, v/v). Each extraction was followed by the centrifugation-decant-filtration procedure outlined above. The combined filtrate was flash-evaporated to near dryness and was transferred to a 15-ml vial with 3–4 ml of chloroform-methanol (2:1, v/v), which was then removed under a stream of nitrogen. The dried lipid was methylated as described below.

Methylation and gas chromatography. Methyl ester derivatives of fatty acids were prepared in vials by transmethylation at 100°C for 10 min with 2 ml of 1.5*N* HCl in methanol. The resultant golden solution was shaken with 2 ml of hexane. After phase separation

[1] Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Research supported by the College of Agriculture and Life Sciences and by the USDA Oat Quality Laboratory, University of Wisconsin, Madison 53706. Part of a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree.

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TABLE I
Cultivar Fatty Acid Profiles and Ranks Within Extraction Procedures

Extraction Procedure	Cultivar	Palmitic (%)[a]	Stearic (%)[a]	Oleic (%)[a]	Linoleic (%)[a]	Linolenic (%)[a]
Chloroform-methanol-water	Dal	18.6 (2)	2.8 (1)	41.2 (1)	35.4 (2)	2.0 (2)
	Exeter	21.4 (1)	1.2 (2)	26.1 (3)	48.3 (1)	2.9 (1)
	Sauk	19.0 (2)	1.2 (2)	30.2 (2)	47.7 (1)	2.0 (2)
Skellysolve	Dal	20.0 (2)	3.8 (1)	42.7 (1)	31.4 (2)	2.0 (2)
	Exeter	22.3 (1)	2.1 (2)	28.9 (3)	43.6 (1)	3.0 (1)
	Sauk	20.8 (2)	1.7 (2)	33.4 (2)	42.1 (1)	2.0 (2)
Multiple-step	Dal	21.7 (2)	3.8 (1)	42.1 (1)	30.6 (2)	1.8 (2)
	Exeter	25.1 (1)	1.4 (2)	27.4 (3)	43.3 (1)	2.8 (1)
	Sauk	23.1 (1-2)	1.5 (2)	31.6 (2)	42.0 (1)	1.8 (2)
Least significant difference ($P = 0.05$)		2.1	0.6	2.5	3.1	0.6

[a] Percentages are averaged over six replications, with two subsamples per replication. Numbers in parentheses indicate ranking within each procedure.

(about 15 sec), approximately 1.5 ml of the upper hexane layer was removed and passed through a pipette containing a glass-wool plug overlaid by 1.5–2 cm of neutral silicic acid. Fatty-acid methyl esters were separated by gas-liquid chromatography using a Beckman GC 72-5 chromatograph (flame-ionization detector) equipped with a 3.2 mm (o.d.) × 1.8 m stainless-steel column fitted with a 15.2-cm precolumn and packed with 10% diethylene glycol succinate on 80/100 mesh Chromasorb WHP (Supelco, Inc., Bellefonte, PA). Precolumns were replaced after 16–25 injections, whereas the main column could be used for 60–80 sample analyses. Column temperature was 190° C and flow rates for H_2, N_2, and compressed air were 45, 80, and 260 ml/min, respectively. Duplicate chromatographic analyses were performed on each extracted sample, and chromatograms were integrated by triangulation. Identification of fatty-acid peaks was verified by use of authentic standards. The concentrations of five fatty acids, palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$), are reported here as percentages of the total triangulated area.

Data analysis. The experiment was analyzed as a three cultivar by three procedure factorial with six replications. Each replication was completed in one day and consisted of an analysis of all nine treatment combinations. Duplicate assays were analyzed as subsamples. Because stearic and linolenic acids were present in low concentrations (<4%), data for these fatty acids were transformed using a $\sqrt{X} + 0.5$ transformation (Steel and Torrie 1980). An analysis of variance was performed on the data for each of the five fatty acids.

RESULTS AND DISCUSSION

Highly significant differences were present among the nine cultivar-procedure combinations, among the three procedures (except for linolenic acid), and among the three cultivars. Procedure × cultivar interactions were nonsignificant. Cultivars were the major source of variation for stearic, oleic, linoleic, and linolenic acids, whereas variation due to cultivars and procedures was similar for palmitic acid. Subsample mean squares were significantly smaller than experimental error mean squares, except for linolenic acid, in which case these variances were similar.

Fatty-acid profiles for each cultivar, as determined by each procedure and averaged over replications and subsamples, are shown in Table I along with rankings based on a protected least significant difference at the 5% level. Whereas profiles determined for each cultivar differed depending on the extraction procedure used, ranking within each procedure was identical for stearic, oleic, linoleic, and linolenic acids. Slight variability in ranking occurred for palmitic acid.

These data indicate that if relative ranking of breeding materials is the goal, and if diverse populations are being screened, then genotypes are likely to be of greater significance in the ranking process than the extraction procedure used. If extraction procedures are assumed to be equivalent in ranking power, then time requirements and other factors take precedence in selecting a procedure for use as a screening tool.

Skellysolve extraction was most rapid, requiring less than 10 min per sample when groups of six to eight samples were extracted concomitantly (ie, 80 min was required for a set of eight samples). The CMW procedure required about 15 min per sample, and the multiple-step control procedure 25–30 min per sample. When time alone was used as the selection criterion, the Skellysolve procedure would be the procedure of choice. However, in our work, we have chosen to use the CMW procedure because samples prepared by this procedure were visibly less cloudy than those prepared by the other procedures and gas-chromatograph column-life was prolonged using CMW-extracted samples.

All of the procedures as reported here are unique in that groat samples were not ground before extraction. This saves time and eliminates an additional step. All of the procedures require chemicals or equipment that are readily available in most laboratories.

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[Received February 22, 1983. Accepted November 17, 1983]