The Composition of the Oil Extracted From 14 Different Varieties of Andropogon Sorghum var. Vulgaris[1]

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MORE than 40 distinct varieties of *Andropogon Sorghum* var. *vulgaris* are now grown in the United States (1). They differ in time of maturity, in height and succulency of the stalk, and in size and color of the grain. The seed range from 2-4 mm. in diameter and vary in color from chalky white to dark red. Varieties yielding succulent stalks and small seed are used as forage while those producing dry stalks and large seed are grown for grain (2). In 1943 approximately 50% of this grain was used on the farm as feed, the remainder or 55,186,000 bushels was sold for commercial use (3).

In a previous paper (4) it was reported that one of the varieties of grain sorghum (Western Blackhull Kafir) contained 0.5% wax and 2.5% oil. The wax was found to be similar in composition to carnauba wax and the oil similar to corn oil. Since these results may not have been representative of the numerous existing varieties of sorghum grains, this present paper reports the results of subsequent analyses. Four forage, 10 grain type sorghums,[3] and 2 samples of corn[4] were subjected to fractional solvent extraction (4) and the yield and characteristics of the extracted wax and oil compared.

Experimental

Separation of the Wax and Oil: The wax was removed from the hull by extracting the unground grain with Skellysolve B and the oil removed from the germ and endosperm by extracting the ground grain with Skellysolve F. The wax was extracted from a 1,200-g. sample of grain with 1,000 ml. of Skellysolve B under reflux for one hour. The hot solvent was decanted through an 8" Buchner funnel and the grain washed twice with 250 ml. of hot Skellysolve B. The combined washings and filtrate were poured into a 2-liter Erlenmeyer flask and approximately 1,000 ml. of the solvent removed by distillation. The remainder was stored at —20° C. overnight and the precipitated wax removed with the aid of a 4" Buchner funnel fitted with a tared piece of filter paper. The filtrate was freed from Skellysolve B and the residue washed into a tared 100-ml. beaker with a few ml. of hot Skellysolve B. The weight of residual wax was added to the weight of the crystalline wax collected on the filter paper and the percentage of wax obtained from the various grains calculated (Table I). The melting points of the crystalline wax collected on the filter paper were also determined.

The oil was extracted from the wax-free grain with Skellysolve F. In order to facilitate grinding 120 ml. of water was added to every 1,200 g. of grain. The grain was then stored at 4° C. for 12 hours, ground, and extracted with Skellysolve F in a large Soxhlet extractor for at least 48 hours. The extract was freed from Skellysolve F, weighed, and the percentage of oil obtained from the various grains determined (Table I). The stability of the extracted oils was compared with corn oil by the rapid oxygen method of Mattill *et al.* (5).

TABLE I

Extraction of Wax and Oil

Variety	Wax		Oil		
	Extracted	Melting point	Extracted	Refractive index	Nonsaponifiable
	%	°C.	%	*at 25° C.*	%
Forage Type					
Early Sumac	0.29	84	2.92	1.4689	2.26
Kansas Orange	.39	82	3.16	1.4694	2.18
Leoti x Atlas	.43	83	2.90	1.4698	2.26
Leoti Red	.32	80	3.12	1.4692	2.11
Grain Type					
Club	.22	84	2.41	1.4700	2.69
Cody	.28	84	2.54	1.4688	2.52
Early Kalo	.28	81	3.02	1.4698	2.46
Feterita	.27	81	2.68	1.4688	3.04
Finney Milo	.24	83	2.68	1.4688	2.27
Midland	.26	81	2.62	1.4692	2.46
Pink Kafir	.29	82	3.00	1.4686	2.51
Red Kafir	.26	83	2.88	1.4704	2.54
Western Blkhl kafir	.40	83	2.50	1.4697	3.12
Western Blkhl kafir[5]	.50	81	2.50	1.4718	3.23
Westland	.21	80	2.49	1.4705	2.04
Average	.30	81	2.76	1.4695	2.51
Corn					
Waxy Corn	.01	....	3.98	1.4714	2.12
Yellow Corn	.03	....	4.72	1.4700	2.83
Average	.02	....	4.35	1.4707	2.47

Characteristics and Composition of the Mixed Fatty Acids Obtained From the Various Oils: Five-g. samples of oil were saponified, diluted with water and extracted with Skellysolve F to remove the unsaponifiable material. The soaps were then neutralized with dilute hydrochloric acid, the fatty acids extracted with Skellysolve F, washed with water, dried with sodium sulphate, and freed from solvent. The melting point, neutralization, iodine (Wijs), and thiocyanogen values (Table II) were determined according to standard procedures (6). The percentages of linoleic (l) and oleic (o) acid were calculated from spectrophotometric analyses according to the method of Mitchell (7) as well as from the iodine and thiocyanogen values (8) substituted into the equations:

$$(1)\quad 181.0\,(l) + 89.4\,(o) = \text{Wt. of sample} \times \text{Iodine value}$$

$$(2)\quad 96.8\,(l) + 89.4\,(o) = \text{Wt. of sample} \times \text{Thiocyanogen value.}$$

The percentages of palmitic (p) and stearic (s) acid were then obtained from:

$$(3)\quad (l) + (o) + (s) + (p) = \text{Wt. of sample}$$

$$(4)\quad \frac{(l)}{280} + \frac{(o)}{282} + \frac{(s)}{284} + \frac{(p)}{256} = \frac{\text{Wt. of sample}}{\text{Neutralization equivalent}}$$

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[3] Harvested in 1944. Obtained through the courtesy of A. F. Swanson, Fort Hays Expt. Sta., Hays, Kansas.

[4] Obtained through the courtesy of E. G. Heyne, Dept. of Agronomy. The yellow corn was grown on the college farm. The white corn was obtained from The Iowa Expt. Sta., Ames, Iowa.

TABLE II

Characteristics and Composition of the Mixed Fatty Acids

Source of Oil	Melting point	Neutralization equivalent	Iodine value	Thiocyanogen value	Linoleic		Oleic		Palmitic	Stearic
					Spectra. method	I_2SCN method	Spectra. method	I_2SCN method	I_2SCN method	I_2SCN method
Forage Type	°C.				%	%	%	%	%	%
Early Sumac	28.0	279.0	120.3	83.1	43.4	44.2	46.6	45.4	7.0	3.2
Kansas Orange	28.0	279.8	118.2	80.9	45.3	44.3	39.4	42.1	8.3	5.2
Leoti x Atlas	29.0	278.0	120.7	80.6	46.1	47.7	41.2	38.6	9.5	4.2
Leoti Red	29.8	278.0	113.7	78.7	43.6	41.6	38.7	42.8	9.8	5.8
~~Grain Type~~										
Club Kafir	27.8	279.0	120.8	81.6	45.2	46.6	43.4	40.6	8.0	4.7
Cody	31.2	278.6	119.1	81.7	44.5	44.4	43.0	43.2	7.5	5.1
Early Kalo	28.2	279.1	122.8	82.2	48.7	48.2	38.8	39.6	8.8	3.4
Feterita	29.6	279.0	116.5	80.7	41.2	42.5	46.6	44.0	7.4	6.0
Finney Milo	31.2	278.5	126.9	83.4	52.7	51.7	35.0	37.2	6.0	5.2
Midland	26.2	279.4	122.6	81.9	46.8	48.4	42.0	39.4	7.8	4.5
Pink Kafir	29.6	279.0	119.9	81.4	46.1	45.7	40.6	41.8	7.1	5.3
Red Kafir	30.6	279.0	120.3	80.8	45.7	46.9	41.8	39.8	7.5	5.6
Western Blkhl	27.2	278.5	125.2	83.2	48.4	49.9	41.8	39.1	8.0	3.0
Western Blkhl[5]	28.0	279.0	120.7	80.0	47.4	48.3	38.4	36.8	9.5	5.1
Westland	29.6	278.6	125.6	82.7	49.2	51.0	40.7	37.0	6.5	5.5
Average	28.9	278.8	120.8	81.5	46.3	46.7	38.6	40.5	7.8	4.7
Corn										
Waxy Corn	26.8	279.5	128.0	81.3	56.7	55.2	31.2	31.0	8.3	5.1
Yellow Corn	26.2	278.5	128.2	82.4	53.8	54.4	31.9	33.2	8.0	4.4
Corn Oil[6]	24.8	278.5	129.7	82.2	56.2	56.4	31.0	30.7	8.7	4.1
Average	25.9	278.8	128.6	81.9	55.5	55.3	31.3	31.6	8.3	4.5

The values of (o) and (l) substituted into equation (3) and (4) were those obtained from equations (1) and (2).

Extraction of Sorghum Grain With Various Solvents: In order to obtain a maximum yield of wax and oil, samples of Western Blackhull kafir [5] were extracted in various ways and with different solvents. The wax was removed by extracting the whole grain with either Skellysolve B or chloroform. Fifteen-g. samples of grain and 25 ml. of solvent were poured into a 250-ml. Erlenmeyer flask and allowed to stand at room temperature or refluxed on a steam bath for varying lengths of time. The extract was filtered into a tared beaker, freed from solvent, and weighed. With Skellysolve B as a solvent, an 8-hour extraction at room temperature yielded as much extract as a 1-hr. extraction under reflux. Chloroform gave similar results. When the whole grain was refluxed from 1-8 hrs. with Skellysolve B, 0.51-.87% of the grain proved to be extractable; with chloroform, 0.70-1.21% was removed. However, the extracts which contained more than 0.5% of the original material consisted of wax contaminated with varying amounts of oil. The purity of the wax extracted appeared to vary with the solvent and the time of extraction; hence a refluxing time of 1 hr. and Skellysolve B was chosen for obtaining a maximum amount of wax containing a minimum of oil.

Ground wax-free Western Blackhull kafir was then extracted with various solvents in order to obtain a maximum yield of oil. Ten-g. samples were extracted in small Soxhlet extractors for 48 hours, the extract filtered into weighed beakers and freed from solvent. Extraction with Skellysolve F yielded the smallest and tetrachloroethane the largest percentage of extract, or 2.28 and 7.15% respectively (Table III).

However, extraction with all of the solvents except Skellysolve F, Skellysolve B, ethyl ether, and carbon tetrachloride gave an extract containing a viscous brownish residue which was insoluble in Skellysolve F. Furthermore, extraction of the pigmented sorghum grains with absolute alcohol, for example, yielded 4.74-8.46% of extract varying in color from light brown to dark red, but only 2.01-3.46% of this extract was soluble in Skellysolve F.

TABLE III

Extraction of Wax Free Ground Grain With Various Solvents

Solvent	Extract
	%
Acetone	3.66
Alcohol-absolute	5.46
Benzene	3.56
Carbon tetrachloride	3.12
Chloroform	3.62
Dioxane	3.63
Ethyl ether	3.22
Skellysolve F	2.28
Skellysolve B	2.89
Tetrachloroethane	7.15
Trichloroethylene	4.39

Results: Differences in both the amount and characteristics of the wax and oil extracted from 14 varieties of sorghum grain were noted (Table II).

The yield of wax varied between 0.21-.50% and the melting point of the wax ranged from 80-84° C. The yield of oil varied between 2.41-3.16%. The induction period of the sorghum grain oils varied between 74-87 hours as compared with 70 hours for the oil from waxy corn and 53 for a commercial corn oil. These results indicated that sorghum grain oils are at least as stable as corn oil.[6] The oils had refractive indices of 1.4686-1.4718, and contained from 2.04-3.23% of nonsaponifiable material.

Analyses of the mixed fatty acids obtained from the various oils indicated that some difference in the ratio of linoleic to oleic acid existed. Oils from Early Sumac, Kansas Orange, Leoti x Atlas, Leoti Red, Cody, and Feterita contained approximately the same amount of linoleic and oleic acids; oils from the other varieties contained approximately 5 units % more linoleic than oleic acid. The mixed fatty acids of the oils had melting points of 26.2-31.2° C., neutralization equivalents of 278.0-279.8, iodine values of 113.7-125.9, and thiocyanogen values of 78.7-83.4. The mixed fatty acids contained 41.2-52.7% linoleic, 35.0-46.6% oleic, 6.0-9.8% palmitic, and 3.0-6.0% stearic acid. The percentages of linoleic and oleic acid as determined by the spectrophotometric method

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[5] Harvested in 1943. Fort Hays Expt. Sta., Hays, Kansas.

[6] Mazola, Corn Products Refining Co., Argo, Illinois.

agreed well with those obtained from calculations involving the iodine and thiocyanogen values; variations for linoleic acid were within 2 unit % while for oleic acid they were within 4 units %.

Discussion

The extraction of 14 varieties of sorghum grain under standardized conditions revealed variations between varieties of approximately 50% in the yield of wax and 20% in the yield of oil. These variations may have been influenced by soil fertility and climatic conditions as well as by genetic factors although all 14 varieties were grown during the same season in experimental plots under identical conditions.

Variations in climatic conditions might, however, influence the amounts of wax secreted in a given variety. Knaggs (9) noted such a relationship in the yield of carnauba wax; the carnauba palm reached its greatest productivity in periods of drought. The Western Blackhull kafir (1944 crop) reported in this paper contained 20% less wax than the sample from the 1943 crop reported previously (4). The annual rainfall during these two years was 29.7 and 16.1″ respectively (10).

The slight variations in melting points indicate that the sorghum grain waxes probably do not differ greatly in composition. There was not enough wax obtained from the two samples of corn to determine an accurate melting point. Jamieson (11), however, reported a melting point of 81° C. for corn wax.

All of the waxes extracted with Skellysolve B were white in color. The pigment in sorghum grain can, if desired, be extracted with alcohol (12) or dilute alkali (13).

The characteristics of the oil extracted from the different varieties were similar to that of the Western Blackhull kafir previously reported (4). The percentage of nonsaponifiable matter, however, was approximately 0.5 units % larger.

Summary

Fourteen varieties of *Andropogon Sorghum* var. *vulgaris* were subjected to fractional solvent extraction. An average yield of 0.32% wax and 2.76% of oil was obtained.

The 14 sorghum grain oils varied from a light amber to green in color. They had an average refractive index of 1.4695 at 25° C. and contained 2.51% nonsaponifiable matter. The mixed fatty acids obtained from the oils had an average melting point of 28.9° C., a neutralization equivalent of 278.8, iodine value of 120.8, and thiocyanogen value of 81.5. The composition of the mixed fatty acids were calculated from the iodine and thiocyanogen values. The mixed fatty acids contained an average of 46.5% linoleic, 39.5% oleic, 7.8% palmitic, and 4.7% stearic acid.

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Report of the Vitamin Committee
1945-46

THERE are many scientific and commercial problems in the field of oil-soluble vitamins which need to be solved. The most urgent seemed to be the development of analytical methods which can be generally agreed upon. Methods for vitamin A and E were selected for first attention. Since vitamin A is better known scientifically and is probably more significant commercially at the present time, our attention was restricted to it during the last year.

A generally recognized need is for a vitamin A reference standard that can be useful for physical and chemical as well as biological tests. The present International Standard is a solution of carotene while the U. S. P. Standards have been a series of cod liver oils. Carotene, of course, is an entirely different substance from the vitamin A found in liver oils and in concentrates suitable for fortifying margarine, pharmaceuticals, etc. The cod liver oils are somewhat unstable and often give chemical reactions that are not typical of vitamin A. Our recommendation, given in detail in Table I, is that a solution of vitamin A acetate in vegetable oil be used as the reference standard.

There are at least two physicochemical methods that give fairly satisfactory determinations of vitamin A in certain high potency fish liver oils and concentrates. Unfortunately, they must be applied with considerable discrimination. The committee believes that it may be possible to work out a rigorous method which would cover most products of commercial interest. A proposed outline for such a method is shown in Table II. Much experimental work will have to be done to establish some of the points still in dispute.

Margarine is one of the most important commercial products containing vitamin A, and at the present