The Analysis and Characterization of the Oil from the Seed of Citrullus vulgaris[1]

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CITRULLUS VULGARIS is a species which belongs to the cucurbitacae family. It is of African origin and is the familiar watermelon. The species also includes pickling varieties, the so-called citrons, which should not be confused with the true citron *(citrus medica)* (7).

This investigation includes the determination of the characteristics of the oil from the seeds of *citrullus vulgaris,* the large green citron, MA49242, Texas Agricultural Experiment Station designation, and a quantitative analysis of the constituent fatty acids present in the oil. The project was suggested by L. C. Curtis in conjunction with the oil seeds investigation sponsored by the National Cottonseed Products Association Inc., and the investigations were carried out by the Texas Agricultural Experiment Station under the supervision of J. Roy Quinby at the Chillicothe Sub-Station.

Experimental Procedures

1. *Extraction of Oil.* The whole seeds were ground to a fine meal, which was extracted by Skellysolve F in a glass percolator. This batch type of extraction was repeated until a few drops of the extract showed only a trace of oily residue after evaporation. After filtering, most of the solvent was removed by distilling under atmospheric pressure while heating on a steam bath, and the last traces of the solvent were removed by heating on a steam bath under reduced pressure. The oil obtained was light yellow.

2. *Determination of Characteristics of Oil.* The procedures employed in determining the physical and chemical characteristics were those of the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1) with some reference to the Official Methods of Analysis of the American Oil Chemists' Society (2) and Jamieson's "Vegetable Fats and Oils" (5).

The characteristics of the oil are recorded in Table I.

TABLE I

Characteristics of the Oil

Characteristics	Value
Specific gravity, 25°/25°	0.9216
Refractive index, $n_D^{25^\circ}$	1.4748
Iodine value (Wijs)	127.8
Saponification value	192.2
Acetyl value	16.05
Free fatty acids	2.75
Unsaponifiable matter, %	0.86
Oil in seed, %	19.3

3. *Analysis of the Constituent Fatty Acids.* The procedure of Hilditch (4) was followed in the preparation of mixed fatty acids from the oil, using a 98.5-g. sample of the oil. For the preliminary separation of mainly saturated and and mainly unsaturated fractions, the lead salt-alcohol method as modified by Hilditch was followed.

The methyl esters of the solid and liquid fatty acid fractions were prepared and fractional distillation was performed on a Todd fractionation column (8).

The solid ester fraction was fractionated into four fractions and the liquid ester fraction into five fractions. The iodine value (Wijs) and saponification value were determined for each fraction. The semimicro procedure of Marcali and Rieman (6) was followed for the determination of the saponification values.

The presence of linoleic acid was confirmed by the preparation of the tetrabromide, m. p. 114-114.3°C.

TABLE II

Fractionation of Methyl Esters of the Solid Esters

Fraction	Wt.	B.P. at 2 mm. Hg.	Sap. V.	I.V.	Esters of Fatty Acids			
					C_{14}	C_{16}	C_{18}	Oleate
	g.	°*C.*			*g.*	*g.*	*g.*	*g.*
S_1	4.23	130-150	269.2	5.88	0.35	3.59	......	0.29
S_2	7.63	150-164	285.0	23.90	......	3.33	2.17	2.13
S_3	3.42	164-175	289.1	5.51	......	1.06	2.14	0.22
S_4	1.15				......	......	1.15	......
Wt. esters	16.43				0.35	7.98	5.46	2.64
Wt. acids	15.60				0.33	7.56	5.20	2.51

TABLE III

Fractionation of Methyl Esters of the Liquid Acids

Fraction	Wt.	B.P. at 2 mm. Hg.	Sap. V.	I.V.	Saturated Monoethenoic Diethenoic					
					C_{14}	C_{16}	C_{14}	C_{16}	C_{18}	C_{18}
	g.	°*C.*			*g.*	*g.*	*g.*	*g.*	*g.*	*g.*
L_1	1.86	132-155	255.3	34.8	0.36	0.85	0.29	0.36	......	......
L_2	20.14	155.165	294.6	158.3	......	......	......	0.20	3.10	16.84
L_3	28.65	165-176	295.0	160.5	......	......	......	......	4.65	24.00
L_4	4.02	176-180	295.3	124.5	......	......	......	......	2.24	1.78
L_5	2.34				......	......	......	......	1.30	1.04
Wt. esters	57.01				0.36	0.85	0.29	0.56	11.29	43.66
Wt. acids	54.29				0.34	0.80	0.27	0.53	10.75	41.60

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[1] From the thesis of Chung Min T-Sao, submitted in partial fulfillment of the requirements for the M.S. degree at the Agricultural and Mechanical College of Texas, College Station, Texas.

TABLE IV

Composition of Mixed Fatty Acids

Acid	In Solid Fraction	In Liquid Fraction	Total
Myristic	0.4	0.5	0.9
Palmitic	9.3	1.2	10.5
Stearic	6.4	......	6.4
Tetradecenoic	......	0.4	0.4
Hexadecenoic	......	0.8	0.8
Oleic	3.1	16.0	19.1
Linoleic	......	61.9	61.9
Total	19.2	80.8	100.0

The distillation and analytical data of the solid and liquid fractions are respectively recorded in Tables II and III.

Discussion

The seed of *Citrullus vulgaris* (large green citron) yield a linoleic rich oil, which is light yellow in color and has an agreeable odor (3) and could be utilized as an edible oil. Curtis also points out that the yield of seed per acre is about four times the yield of seed from soybeans. The mixed fatty acids consist of myristic, palmitic, stearic, tetradecenoic, hexadecenoic, oleic, and linoleic acids. The seed contain about 19% of oil.

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Comparative Evaluation of Antioxidants for Carotene

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CONSIDERABLE success has been achieved in stabilizing carotene by means of phenolic antioxidants (1, 2, 3), three of which have been used in food products. Although aromatic amines such as diphenylamine have long been known to be effective antioxidants for carotene (6, 8), they have not been considered seriously for use in edible products for reasons of possible toxicity. In 1949 the use of N,N′-diphenyl-p-phenylenediamine was patented (4) for preserving carotene in alfalfa. Feeding tests with chicks showed that 10 to 15 times the amount recommended (0.015% in the dried meal) gave no toxic symptoms. This led to an increased interest in related compounds for carotene stabilization. In a recent report from this laboratory (7) a number of aromatic amines were tested as antioxidants for carotene in dehydrated alfalfa meal. In the present report a simplified system was employed to compare the antioxidant efficiency of a number of aromatic nitrogenous compounds in an attempt to obtain a more absolute evaluation. As far as possible the compounds tested were chosen to permit observation of change in activity with systematic change in structure.

Experimental

The procedure employed for testing the antioxidants was identical to that reported previously (3). A solution of purified crystalline beta-carotene in medicinal-grade mineral oil was employed as the substrate. The antioxidant compounds were dissolved in the oil solution and the time required for breakdown of 20% of the carotene at 75°C. was determined.

Results

Under the conditions of the test the control sample containing no added antioxidant lost 20% of its original carotene in about two hours. Since all the experiments reported here were carried out in the same way, the quantitative data are reported in a single table.

Aniline Derivatives. Aniline and all derivatives tested were either very weak antioxidants or were without effect. Of those tested, 2-aminobenzenethiol was most effective.

Naphthylamine Derivatives. Monoamino substitution on naphthalene produced moderately effective antioxidants. The corresponding phenyl derivatives (phenyl-alpha- and phenyl-beta-naphthylamine) were very effective antioxidants. Diamino substituted naphthalene was one of the most potent antioxidants tried in this study.

Secondary Amine Derivatives. Diphenylamine was much more effective than either monophenylamine (aniline) or triphenylamine. The effectiveness of diphenylamine could be greatly enhanced by suitable ring substitution. Thus monoalkyl substitution (4-heptyldiphenylamine) caused only slight antioxidant enhancement whereas dialkyl substitution (4,4′-diheptyl or 4,4′-dioctyldiphenylamine) more than doubled the antioxidant effectiveness. Hydroxy or alkoxy substitution (4-hydroxydiphenylamine, 4-isopropoxydiphenylamine) more than quadrupled the antioxidant efficiency. Amino substitution (4-aminodiphenylamine) enhanced the antioxidant effectiveness of the parent compound five-fold. p-Nitroso substitution more than tripled the activity of diphenylamine whereas nitro substitution caused the loss of antioxidant effect. Acetylation of diphenylamine destroyed most of the antioxidant effectiveness of the compound. Interposition of methylene groups between the nitrogen atom and the benzene ring (benzylaniline or dibenzylamine) greatly reduced the activity.

Phenylenediamine Derivatives. Both ortho- and para-phenylenediamines were effective antioxidants, the para isomer being the more effective of the two. An alkyl substituted derivative of m-phenylenediamine (2,4-diaminotoluene) had about the same effectiveness as p-phenylenediamine. Acetylation of one of the amino groups (p-aminoacetanilide) caused the loss of all the antioxidant activity. Derivatives of p-phe-

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.