Systems that Did Not Interact.—The following products did not react under the conditions specified and in every case were recovered unchanged unless otherwise noted. In every case the solvent used was liquid ammonia.

(1) Tetraphenylethylene[14] and 9-fluorylamine were heated at 60° for twenty hours.

(2) Diphenylbiphenyleneethylene[15] and 9-fluorylamine hydrochloride heated at 40° for six days and then maintained at room temperature for one month.

(3) Stilbene and 9-fluorylamine hydrochloride, allowed to stand at room temperature for four months.

(4) Hydrazobenzene and 9-fluorylamine hydrochloride, heated at 60° for six days and kept at room temperature for three weeks.

(5) Benzhydrylamine and methylene blue, heated at 60° for three days and kept at room temperature for four days, benzophenone was not detected upon hydrolysis of the products.

(6) Dibiphenyleneethylene and benzhydrylamine, allowed to stand at room temperature for one month.

(7) Benzhydrylamine hydrochloride and indigo, heated at 60° for twelve days, the indigo was decomposed; however, no benzophene was obtained on hydrolysis of the products.

Summary

1. Evidence has been obtained in favor of Wieland's theory of dehydrogenation as being one of the mechanisms of biological oxidation.

2. The dehydrogenation of 9-fluorylamine to fluorenone imide in liquid ammonia has been effected by dibiphenyleneethylene, benzalfluorene, azobenzene, indigo and methylene blue.

3. Dibiphenyleneethylene has been found to dehydrogenate hydrazobenzene to azobenzene.

[14] Schlenk and Bergmann, *Ann.*, **463**, 15 (1928).

[15] Kaufmann, *Ber.*, **29**, 75 (1896).

WASHINGTON, D. C.

[CONTRIBUTION FROM THE SEVERANCE CHEMICAL LABORATORY OF OBERLIN COLLEGE]

THE ISOLATION OF CAROTENE

BY HARRY N. HOLMES AND HENRY M. LEICESTER

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Since von Euler,[1] von Euler and Karrer and T. Moore[2] showed that carotene can replace vitamin A in the diet of a rat suffering from a deficiency of this substance, the problem of obtaining the pigment with greater ease than formerly has become important. It seems certain that animals convert the carotene of plant food into vitamin A, hence the occurrence of the vitamin in butter, egg yolk and cod liver oil.

The methods used in the past[3] for the isolation of carotene have, in

[1] Von Euler, *Helv. Chim. Acta*, **12**, 278 (1929).

[2] Moore, *J. Soc. Chem. Ind.*, **49**, 238 (1930); *Biochem. J.*, **24**, 692(1930).

[3] For a summary of such methods, see Palmer, "Carotinoids and Related Pigments," The Chemical Catalog Co. New York, **1922**, Chap. 8.

general, been based upon the extraction of fresh carrots with a fat solvent, or with petroleum ether. In all of these processes, the carrot pulp has been dried for some time before extraction. Further, since a large quantity of carrots is necessary to obtain a relatively small amount of carotene, and since only a moderate amount can be worked up at a time in the average laboratory, many carrots must be left in the air with consequent loss of this valuable pigment by oxidation. In working with a substance as easily oxidized as carotene, exposure to air should obviously be avoided as much as possible, if good yields are to be obtained.

A study was therefore undertaken to develop a laboratory method for the isolation of carotene which would minimize the exposure of the materials to air at all times.

Determinations were first made upon a number of substances for their carotene content. The colorimetric method of Willstätter and Stoll as given by Palmer[4] was used and for the green leafy plants, the modification of Schertz was employed.[5] The results obtained are shown in Table I.

TABLE I

RESULTS OF DETERMINATIONS

Source	Carotene, mg. per 100 g.	Xanthophyll, mg. per 100 g.
Alfalfa (cured)	2.3	9.0
Dehydrated spinach	1.5	3.9
Fresh spinach	4.8	8.6
Canned spinach (drained)	15.6	16.1
Yellow corn gluten	0.3	...
Canned carrots (drained)	6.5	...

These results show that dehydrated spinach and alfalfa have lost most of their carotene during drying. The low value for fresh spinach is surprising, and may be due to the fact that, as the vegetable was purchased on the open market, it may have been exposed to the air for some time. Also, since it was winter grown spinach, it may have had less sun and hence formed less pigment. The yellow corn gluten (a commercial stock food) is surprisingly low in the pigment, while canned spinach and canned carrots are relatively rich in this substance.

In view of these findings, the study was begun on the green, leafy plants. A method of treatment was developed which did away with the necessity of separating all the pigments together, and then removing the chlorophyll, as has previously been done. This improved method consists in permitting the green leaf to stand for three hours with enough 3 *N* sodium hydroxide to form a thick paste. This disintegrates the cellulose and hydrolyzes the chlorophyll to water-soluble chlorophyllines, but does not injure the carotenoids. The mixture is then diluted with an equal volume of water, and

[4] Palmer, Ref. 3, pp. 259–260.

[5] Schertz, *Plant Physiol.*, **3**, 211 (1928).

the whole mass is extracted with chloroform, taking care not to agitate to such an extent that troublesome emulsions result. The heavy solvent sinks to the bottom of a separatory funnel, and can easily be drawn off.

In this way a deep red solution of carotene and xanthophyll in chloroform uncontaminated by any chlorophyll can be obtained in a short time. Evaporation of the solvent leaves a fatty, orange mass. This can be dissolved in petroleum ether, and the usual separation of carotene and xanthophyll, by extracting the xanthophyll with 80–90% methyl alcohol, can be carried out.[6]

This method appears to be advantageous if a solution of carotenoids is desired for study, since it is rapid and the pigments are not mixed with other colored substances. It may also be used when the isolation of mixed carotene and xanthophyll is intended. However, the removal is not quite quantitative, and the subsequent treatment required to isolate carotene is troublesome for the average laboratory, if large amounts are desired. On a commercial basis this method seems attractive.

Therefore, it appeared best to turn again to carrots, long the principal source of the pigment. As shown in Table I, they do not contain as much of this substance as spinach, but they contain no interfering pigments, and the extraction is a relatively straightforward process.

The value of canned carrots as a laboratory source of carotene appears heretofore to have been overlooked. However, there are many striking advantages in their use. The carrots have been cooked very soon after picking, and in hermetically sealed containers. This eliminates almost all opportunities for oxidation to occur. The carrots thus prepared can be kept on hand indefinitely, and used only as convenient, without deterioration of the pigment. They are soft and easily crushed and worked up.

It is obvious that the same advantages apply to canned spinach as a source of carotene and xanthophyll.

The carrots employed were the Huxon Brand Diced Carrots, obtained in one gallon cans. The weight of solid in these cans ranged from 1800 to 2000 g. after the juice had been drained off. It was found convenient to work up four cans at a time.

The first step was to crush the carrots in a Carver Hydraulic Hand Press. A pressure of 8000 pounds to the square inch was sufficient to squeeze out almost all the juice. The two kilograms of carrots in a can were thus reduced to a weight of about 330 g. The juice pressed out contained practically no carotene and was discarded.

The press cake from four cans was ground up in a meat grinder and placed under 1.25 liters of acetone. After standing thus from eight to ten hours, the mixture was filtered on a Büchner funnel, and the pulp pressed once more, again with a pressure of 8000 lbs. per sq. in. This removed the remaining traces of water and permitted the later use of water-insoluble solvents for removal of carotene. This method of dehydra-

[6] Morrow, "Biochemical Laboratory Methods," John Wiley and Sons, Inc., New York, 1927, p. 309.

TABLE I

COMPARISON OF VARIOUS METHODS OF ISOLATING CAROTENE FROM CARROTS

Holmes and Leicester, 1931	Arnaud, 1886	Kohl, 1902	Euler and Nordenson, 1908	Escher, 1909	Strain, 1931	Guthrie, 1929
Canned carrots	Fresh carrots	Fresh carrots	Fresh carrots	Fresh carrots	Fresh carrots	Fresh carrots
	Grate	Boil	Boil	Dry at low heat	Dry rapidly	
Press	Press	Press	Press			
	Add $Pb(Ac)_2$ to juice					
	Filter ppt.					
Grind			Grind with sand	Grind	Grind	Grind
Wash with acetone		Wash with alcohol				Wash 2 times with acetone
Filter, press		Filter, press				
Grind		Grind				
	Dry ppt. and pulp *in vacuo*	Dry in air	Dry at 50° air			
			Extract twice with CS_2			
			Press			
			Distil off CS_2 with ether			
Extract with acetone, pet. ether	Extract with CS_2	Extract with ether in cont. extractor	Extract pulp with alcohol	Extract with pet. ether in cont. extractor	Extract with pet. ether	Extract 3 times with acetone
Transfer pigment to pet. ether			Transfer pigment to ether			
			Evap. all ether			
			Dissolve residue in pet. ether			
Conc. pet. ether	Conc. CS_2 soln.	Conc. ether		Conc. pet. ether	Conc. pet. ether	Add 1/3 volume H_2O and allow to crystallize
Add alc., filter			Add alc., filter			
Carotene cryst.	Crude carotene crystallizes	Crude carotene crystallizes	Carotene cryst.	Crude carotene crystallizes	Carotene crystallizes at 5°	

tion eliminated the necessity of air drying and the inevitable loss by oxidation. Guthrie[7] has also dehydrated carrots in a similar way.

The pulp, now a solid cake, was reground and allowed to stand for half an hour with 1.25 liters of fresh acetone. It was then filtered as before, and placed under one liter of petroleum ether. After an hour this solvent was filtered off and replaced by a liter of acetone. This also was allowed to stand for an hour, then was filtered and a final liter of petroleum ether was placed on the carrots. After another hour they were filtered and washed with 0.5 to 0.75 liter of petroleum ether. The pulp was then practically colorless, and further extraction was unnecessary. This alternation of solvents was used because it was found that less total volume of solvent had to be employed than if either had been used alone. Also, by dissolving the acetone extracts in the petroleum ether and washing the solvent out with water, the pigment it carried was transferred to the latter solvent, and less time was consumed during evaporation. The continuous extraction process might well be used on a commercial scale.

The petroleum ether was evaporated to about 75–100 cc. under reduced pressure, and then treated with a large excess of absolute alcohol, with violent stirring. A fatty substance precipitated and settled out. This was rapidly filtered off, the filtrate was placed in a flask in an atmosphere of nitrogen, and was allowed to stand for twenty-four hours in the ice box. The carotene crystallized out in dark red lustrous crystals.

From 100 gallons of canned carrots a quantity of carotene was obtained which, after standing over phosphorus pentoxide in a vacuum desiccator to remove solvent of crystallization, weighed 7.5 g. This is a yield of approximately 0.037 g. of pigment per kilogram of carrots, and compares very well with the yield of 0.025 g. per kilo obtained by Escher[8] and of 0.030 g. per kilo obtained by Arnaud.[9] After four precipitations from chloroform by methyl alcohol as recommended by Olcovitch and Matill,[10] the carotene melted at 173.6–174.6° (corr.).

Summary

1. The carotene content of several vegetables has been determined.

2. A rapid yet cheaper method for obtaining a solution of carotene and xanthophyll free from chlorophyll has been described.

3. The advantages of canned vegetables as sources of carotenoids have been pointed out.

4. A modified method for the cheaper isolation of carotene in good yield from canned carrots has been described.

OBERLIN, OHIO

[7] Guthrie, *Am. J. Bot.*, **16**, 716 (1929).

[8] *Cf.* Palmer, Ref. 3, p. 201.

[9] Arnaud, *Compt. rend.*, **102**, 1119 (1886).

[10] Olcovitch and Matill, *J. Biol. Chem.*, **91**, 105 (1931).