Purification and Uses of a Xanthophyl—Containing Oil Derived From Corn

L. C. SWALLEN and J. B. GOTTFRIED
Corn Products Refining Company, Argo, Illinois

The pigments of yellow corn consist primarily of various xanthophylls together with small amounts of carotin. For feeding purposes yellow corn is often preferred to white corn. While in many cases specific reasons have not been given to this preference, it is without doubt due to the presence of these pigments. In particular we have information of a qualitative nature that the pigments are important in producing a desirable yellow color of the skin and legs of poultry. It has also been indicated by Peterson, Hughes, and Scott (2) that these xanthophyll pigments are essential for proper growth and development of poultry. It is considered advisable, therefore, to prepare a concentrate of this pigment for mixing with feeds in order to enhance the amounts of xanthophyll pigments present. Carotin, being present in relatively small amounts, is considered as having only a secondary importance in this case.

In the processing of corn for the production of starch, the xanthophyll pigment remains attached almost quantitatively to the insoluble protein (gluten). The gluten, which is separated as a feeding material, therefore contains a high concentration of the pigment. If the gluten is treated with an oil solvent, the oil and pigment are extracted together and a further degree of concentration of the pigment may be obtained. The relationships between the amounts of xanthophyll pigments in the substances mentioned above are given in Table I.

TABLE I

Yellow Corn	.004%
Gluten Meal (41% Protein)	.035%
Oil extracted from gluten	.40% to .45%

The latter concentration is the highest which has been obtained without the use of complicated chemical procedures.

The xanthophyll concentrate may be obtained conveniently as a by product in the production of zein, the alcohol soluble protein of corn. In the process of producing zein, gluten meal of high protein content (55% dry basis) is extracted with an alcoholic solvent, preferably 85% by volume isopropyl alcohol, which extracts the zein, the oil, and the xanthophyll pigments. After proper means of clarification the alcoholic solution is treated with a hydrocarbon, preferably hexane. The bulk of the protein is precipitated as a viscous concentrated solution while the oil and xanthophyll pigments remain in the solvent layer consisting of a mixture of the alcohol and the hydrocarbon. If this solvent mixture is distilled directly to recover the solvents, the residual oil will contain a considerable amount of protein. However, if the solvent mixture is first treated with a regulated amount of water, a solvent layer containing primarily the hydrocarbon will be obtained which will contain practically all of the oil and pigment. The aqueous alcohol layer will contain the residual protein. By distillation of the hydrocarbon solvent, the oil may be recovered. As a matter of simplicity in equipment and operating, it is preferable to distill with steam. The oil is therefore obtained in the form of a mixture with water. The small concentration of protein still present and some of the constituents of the oil tend to produce a degree of emulsification so that a simple settling process is not sufficient for the recovery of a dry oil in good yield. It has been found that if this mixture is run through a centrifuge designed for the separation of liquids, the oil will be recovered in practically dry form and may be obtained in a yield of about 95% based on that present in the still slop. Any protein or solid material is deposited in the centrifuge and does not contaminate the product. While it is possible to facilitate the separation of the oil by the use of salt or an acid it is not necessary to use these materials and a simple centrifuging at an elevated temperature (170 to 180° F.) is sufficient to produce a satisfactory separation.

Experimental

For the determination of xanthophyll pigments in the various materials mentioned in the above discussion the dry corn or gluten was ground to pass a 50 mesh screen and allowed to stand in contact with 8 to 10 times its weight of a mixture of equal parts of toluene and isopropyl alcohol for at least 24 hours. The mixture was filtered and washed with fresh solvent mixture until all of the color was removed. Extractions made in this manner are complete while if the extraction is carried out in a Soxhlet extractor, the extraction is not only slow but does not give a complete yield of the pigments since some of these are destroyed in the heating process. The solutions obtained as described above were diluted so that the colors could be compared in a DuBosq colorimeter with an aqueous solution containing .2% potassium dicromate. The dicromate solution was standardized against a known sample of xanthophyll. The standardization of the dicromate did not follow the curve given by Palmer (1). The reason for this difference was not investigated in detail but may have been due to the nature of the solvent mixture used. It is known that the pigment contains some carotin which has a somewhat different absorption from the xanthophyll. However, the amount of carotin is considered to be small enough that for practical purposes it may be considered along with the xanthophyll in the calculations. The results obtained in the determination of the xanthophyll are given in Table I.

In the preparation of concentrated xanthophyll oil, gluten containing 55% protein was extracted with 85% isopropyl alcohol for approximately 2 hours at 60° C. The resulting extract was separated from the residual meal, cooled to 15° C., and filtered. The clarified solution was treated with an equal volume of hexane (Skellysolve B). The mixture was separated in a continuous centrifuge. The lower layer consisting of a concentrated solution of zein was used for the preparation of the pure protein. The solvent layer

was diluted with sufficient water to yield an aqueous alcohol of about 50% concentration by volume. The pigments remained dissolved almost entirely in the hydrocarbon layer which was separated by gravity and steam distilled in a continuous still. The slop which flowed from the still continuously contained about 5% of oil. It was run through a National Acme Centrifuge designed for the separation of liquids using an average feed rate of 210 gallons per hour, a maximum rate of 720 gallons per hour. The oil was recovered in about 95% yield. It contained 6.7% moisture during the period of most rapid flow through the centrifuge.

The xanthophyll oil is a viscous material which if not thoroughly dried may assume a pasty character due to emulsified water. It is almost black in color but gives a yellow solution on dilution with suitable solvents.

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1. Palmer, L. S. Carotinoids and Related pigments: the Chromolipoids pp. 259-260, Chemical Catalog Co., New York, 1922.
2. Peterson, W. J., Hughes, J. S., and Scott, H. M. "The Role of Xanthophylls in the Ration of the Fowl." Presented before the Division of Biological Chemistry, Boston, Mass., Sept. 1939.

Abstracts

Oils and Fats

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M. M. PISKUR and SARAH HICKS

APPLICATION OF NONCORROSIVE METALS TO DEODORIZING OF VEGETABLE OILS. Alan Porter Lee. *Proc. Inst. Prod. Tech. 1941*, 219-23 (1941). Several samples of edible cottonseed oil deodorized in stainless-steel equip. have been preserved in partially filled clear glass bottles with exposure to the effects of light and air for periods up to 6 months without developing rancidity to extent determinable by taste or odor. Development of a series of comparative induction-period tests to obtain quant. data for checking these reported results is planned.

TRANSFORMING CARBOHYDRATES INTO FAT WITH THE AID OF MICROORGANISMS. Hugo Fredholm. *Kgl. Lantbrudsakad. Tid. 80*, 341-9 (1941) (English summary). Production of fat by Oospora (1) when cultivated on whey has been studied, with special attention given to the symbiosis with lactic acid bacteria. The optimum temp. was 28°. Generally autolysis began on the 7th to 9th day. The amt. of fat reached its max. on the 5th to 6th day. I utilizes the N compds. of autoclaved whey. Two parts of dry matter contg. 25.5% fat are obtained from 4.25 parts of lactose + lactic acid, when I is grown in pure culture. Better results are obtained when I is grown symbiotically with certain bacteria of the family Lactobacteriaceae, viz., Streptococcus lactis (II), Streptococcus cremoris (III) and Leuconostoc citrovorum (IV). With II, III, and IV, 1.84 parts of dry matter, contg. 40% fat, is obtained from 4.15 parts of lactose + lactic acid. The extd. fat has a yellowish color. At 15° it is semiliquid. *(Chem. Abs.)*

THE EFFECT OF FEEDING SOME FAT SOLUBLE DYES TO MILKING COWS UPON THE COLOR OF MILK FAT. C. F. Huffman and C. W. Duncan (*Mich. Sta. Quart. Bul. 24*, No. 1, 54-55 [1941]). *Expt. Sta. Record, 86*, 82. When trials were conducted with Sudan III, Sudan IV, brilliant green, and perfect purple, each fed at the rate of 15 gm. dissolved in 0.5 lb. of soybean oil, the Sudan III or Sudan IV imparted a pronounced pink color to butterfat, most intense 24 hr. after feeding. Some color persisted up to 144 hr. after feeding, and some color persisted for 132 hr. The perfect purple gave a pronounced green color to the fat, which was most pronounced at 36 hr. and persisted at a diminishing rate for more than 84 hr. Nigrosine black, fed at a 45-gm. level, imparted a pink color to the butterfat. The possibility of using these fat-soluble dyes in studying the relation of food fat to milk is indicated.

INFLUENCE OF SOME DIETARY FACTORS ON THE DEVELOPMENT OF RANCIDITY IN THE FAT OF THE WHITE RAT. Andrea Overman. *J. Biol. Chem. 142*, 441-4 (1942). Rats were fed a synthetic diet; one group received no supplement, a second group received 1 mg. of ascorbic acid daily, and a third group received 1 mg. of hydroquinone daily. The fats from each series were analyzed for rancidity at stated intervals. The results indicate that the difference in resistance to reacidity are due partly to thinness or fatness of the animal, and partly to the diet. Ascorbic acid feeding, together with a low per cent of gain in wt. resulted in a significant increase in resistance of the fat to rancidity.

THE RESPONSE OF LIPID METABOLISM TO ALTERATIONS IN NUTRITIONAL STATE. II. THE EFFECTS OF OVERNUTRITION ON THE POSTABSORPTIVE LEVELS OF THE BLOOD LIPIDS OF THE DOG. C. Entenman and I. L. Chaikoff. *J. Biol. Chem. 142*, 129-37 (1942). The expl. production of obesity (in which dogs were made to increase their wts. by as much as 80%) led to little or no rise in the total cholesterol content of the blood. There was a tendency for total fatty acids and phospholipids to rise in the obese dog, but this response was not uniform in the animals studied. Raw pancreas (which readily influences the blood lipids of the completely depancreatized dog maintained with insulin) failed to produce significant changes in the blood lipid constituents in the *normal* dog. Fasting appears to produce a more pronounced fall in the blood lipids in the obese dog than in the dog of normal nutritional state.

A NOTE ON THE EPIDERMIS OF THE RAT ON A FAT-FREE DIET. R. Williamson. *Biochem. J. 35*, 1002-5 (1941). The epidermis of rats on fat-free diets becomes thicker, and more differentiated than that of normal rats, the stratum granulosum becoming especially distinct and the horny layer thick. When rats were fed on fat-free diets with supplements of unsaturated acids the epidermis was found to be nearly normal.

FAT-DEFICIENCY DISEASE OF RATS. THE STORAGE OF FAT IN THE FAT-STARVED RAT. I. S. MacLean and E. M. Hume. *Biochem. J. 35*, 990-5 (1941). Rats were kept on a fat-free diet for periods varying from 172