Table III. Nitrogen Fractions and Dry Matter in Shredded Sorgo Samples

Fraction	G. of N/1000 G. of Dry Tissue
Total nitrogen	4.41
Soluble nitrogen	3.19
Nitrogen soluble in copper hydroxide	1.96
Ammonia plus amide nitrogen	0.417
Harmful nitrogen (1.960 − 0.417)	1.543
Dry matter	32.87%

shredded samples, while with cold-water digestion the sucrose in the chips was only 40% of that in shredded samples.

The extracts from the chips with hot water filtered in approximately 5 minutes, whereas the extracts from the shredded samples required approximately 30 minutes.. The filtrates obtained by hot-water digestion gave a very light, straw-colored solution, whereas the clear extract from the shredded samples was a light amber color. This slight coloration of the clarified filtrates did not interfere with the saccharimeter readings.

Determination of Harmful Nitrogen. The methods used for the determination of harmful nitrogen in sorgo are essentially those developed by the European investigators for sugar beets. The term "harmful nitrogen" is here applied to the nitrogen not precipitated by copper hydroxide minus the total ammonia nitrogen (ammonia plus amides). Briefly, the methods used for the nitrogen studies are as follows:

Approximately 350 ml. of water was added to 100 grams of shredded sample in a calibrated 500-ml., wide-mouthed Erlenmeyer flask and placed under a vacuum to remove the air from the tissue, then transferred to a water bath which was held at 85° C. After the sample had reached the temperature of the bath, 50 ml. of copper sulfate solution (60 grams of copper sulfate made up to 1000 ml. with water) and 50 ml. of sodium hydroxide (0.312N) were added and thoroughly mixed. The samples were allowed to digest for 15 minutes with frequent shaking, after which they were cooled to room temperature and made up to 500 ml. and filtered, clear, through dry paper pulp. Aliquots were removed from the clear extract to determine the nitrogen not precipitated by copper hydroxide. The ammonia-plus-amide nitrogen was determined in aliquots, made 1N with sulfuric acid, after hydrolysis for 2 hours. The samples were neutralized with sodium hydroxide and then made alkaline with an alkaline borate mixture (0.5N sodium hydroxide in 5% borax) and the ammonia distilled off at atmospheric pressure. Total nitrogen was determined on a 5-gram sample of the shreds. The total soluble nitrogen content of the samples was determined by placing 20 grams of sample in a 200-ml. Kohlrausch flask with 180 ml. of water, removing the air by vacuum, and then placing the flask in a water bath at 90° C. After the sample had attained the temperature of the bath, sufficient 1N acetic acid was added to bring the pH to 4.7. The extract was cooled to room temperature, made up to volume, and filtered through dry paper pulp. The nitrogen determinations were carried out using official methods which did not include the nitrates (*1*). The nitrogen fractions are shown in Table III.

The harmful nitrogen content of the sorgo canes used in these tests is only approximately one half that found in sugar beets grown in the same general locality in 1944 and 1945.

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XANTHOPHYLL OIL STABILIZATION

Protective Starch Matrix for Xanthophyll Oil and Vitamin A

EILEEN C. MAYWALD and THOMAS J. SCHOCH

George M. Moffett Research Laboratories, Corn Products Refining Co., Argo, Ill.

Oxygen-sensitive materials such as corn xanthophyll oil, vitamin A, or white phosphorus can be protected against air oxidation by emulsifying the materials in a cooked paste of a suitable modified starch, and drying the emulsion as a thin film or flake. The starchy base must be a good protective colloid and must give molecularly dispersed solutions, free from swollen granules or retrograded starch. Suitable starch types include hydrolyzed cornstarch of 5 to 20 dextrose equivalent, high-soluble canary dextrins, thin-boiling waxy starches, and thin-boiling oxidized or etherified starches.

THE FOOD AND AGRICULTURAL INDUSTRIES need means of protecting water-insoluble substances from deterioration by air oxidation or by evaporation. The stabilization of vitamin preparations for human use or for stock feed supplements, the protection of essential oils and flavoring agents, and the production of dry insecticidal dusting powder are examples. There are methods for protecting such materials as vitamin A and flavoring oils, many of which merely involve soaking these substances into a suitable carrier—e.g., flour or a roll-flaked cereal base. However, the added substance is still accessible to the air and the degree of protection is low. Olsen and Seltzer (*2*) described the emulsification of citrus oils in a warm gelatin solution, cooling to produce a rigid gel, then mechanically subdividing the latter and drying the gel particles to give a stable dispersion of the oil in a solid gelatin matrix. Taylor (*4*) protected oxygen-sensitive vitamin A and D concentrates in a somewhat similar fashion. More recently, Schlenk, Sand, and Tillotson (*3*) reported the protection of vitamin A by the formation of a molecular complex with the cyclic Schardinger α- and β-dextrins.

Corn xanthophyll oil, a by-product isolated from corn gluten during the manufacture of zein, contains approximately 1 gram of xanthophyll and 0.5 gram of carotene per pound. This study was undertaken to find a cheap and simple method for protecting xanthophyll oil in a starchy matrix, primarily for use as a poultry feed supplement. Protection is provided only by certain specific types of starch products

which give a homogeneous matrix, substantially free from swollen granules and retrograded starch substance. The described methods appear to have general usefulness for the protection or incorporation of a wide variety of water insoluble substances, either liquid or solid.

Methods and Materials

Formulation with Corn Xanthophyll Oil. Commercial xanthophyll oil from corn was used in evaluating the degree of protection against oxidation afforded by various starches. As a general mode of formulation, 100 grams of the starch product (containing 8 to 10% moisture) was slurried in an appropriate volume of water, as specified for each starch type in Table I. Twenty-five grams of commercial corn sirup (43° Baumé, 42 dextrose equivalent) was added as a plasticizer, and the mixture cooked for 30 minutes in a boiling water bath to gelatinize and dissolve the starch, being stirred continuously with a propeller-type agitator. The mixture was cooled to 50° C. with stirring, 40 grams of xanthophyll oil was slowly added, and emulsification effected by high-speed agitation. With optimal starch types, stirring for a few minutes gave smooth, heavy-bodied pastes. In these cases, microscopic examination showed the oil to be dispersed in uniform droplets approximately 10 microns in diameter. The paste was then spread in a thin layer (0.02 inch thick) on glass plates and allowed to dry spontaneously at room temperature. In one instance (sample 2b, Table I), the paste was coated on a stainless steel plate and rapidly dried on top of a boiling water bath, to approximate a roll-dried product. Depending on the starch type, the paste dried as loose orange-yellow scales (readily brushed off the glass plate), or as a tightly adherent film (removed by scraping with a razor blade). Microscopic examination of the dried products showed a replication of the state of emulsification in the original paste. Thus the preferred starch types showed uniform oil globules approximately 10 microns in diameter embedded in the dry starchy matrix. Inferior starch types showed considerable coalescence of oil globules, wide variation in diameter, and in extreme cases bleeding of the oil to the surface of the flake or film.

Accelerated Oxidation Tests. The dried products were crushed to pass a 30-mesh screen, then spread in a thin layer on Petri dishes, and placed in the air oven at 75° C. Samples were withdrawn after heating for 1 and 4 weeks. With certain of the more unstable preparations (samples 23 and 24, Table I), the deterioration of pigment was so rapid that analyses had to be run after heating for only 2 days.

Table I. Effectiveness of Various Starches for Protection of Xanthophyll Oil

(Formulations contain 100 grams of starch, 25 grams of corn sirup, and 40 grams of xanthophyll oil, with the indicated amount of water)

No.	Starch[a]	Type	Water, Ml.	Pigment Extracted by Naphtha, %	Survival of Pigment at 75° C., %	
					1 week	4 weeks
1	18 D.E. cornstarch hydrolysis product. Solubles = 100%	E	70	22	79	79
2a	Light yellow corn dextrin, by roasting with acid catalyst. Solubles = 98%	A	80	3	84	74
2b	Same paste, dried on heated metal plate	A	80	4	87	76
3	British gum, tan in color, by roasting with acid catalyst. Solubles = 80%	A	140	2	79	74
4	Dark yellow corn dextrin, by roasting with acid catalyst. Solubles = 97%	A	50	9	82	73
5	85-Fluidity thin-boiling waxy maize starch	D	220	4	78	69
6	18 D.E. cornstarch hydrolysis product, subsequently dextrinized. Solubles = 100%	E-A	50	6	79	66
7	50-Fluidity thin-boiling waxy maize starch	D	320	11	65	51
8	5 D.E. cornstarch hydrolysis product. Solubles = 95%	E	90	15	62	51
9	80-Fluidity hydroxyethyl cornstarch, 0.05 D.S.	C	300	6	68	46
10	Hypochlorite-oxidized cornstarch, 55-Scott	B	260	10	62	44
11	26-Fluidity sulfonic-carboxylic starch ester	C	420	9	75	40
12	Corn gum, light tan color, by roasting neutral starch. Solubles = 10%	A	200	9	58	42
13	Hypochlorite-oxidized cornstarch, 90-Scott	B	260	12	56	39
14	45-Fluidity cornstarch acetate, 1.8% esterified acetic acid	C	400	11	52	38
15	Corn gum, light tan color, by roasting alkaline starch Solubles = 2%	A	720	9	41	35
16	Thick-boiling hydroxyethyl potato starch, 0.25 D.S.	C	500	34	41	31
17	Mildly oxidized cornstarch	B	360	20	38	22
18	44-Fluidity acid-modified potato starch	H	320	16	42	12
19	75-Fluidity acid-modified cornstarch	H	260	35	25	13
20	White dextrin from cornstarch. Solubles = 65%	I	110	34	25	12
21	White dextrin from cornstarch. Solubles = 25%	I	120	37	15	8
22	White dextrin from cornstarch. Solubles = 4%	I	170	25	10	0
23	Unmodified cornstarch	F	2000	89	13 (2 days)	
24	90-Fluidity acid-modified cornstarch	H	240	100	2 (2 days)	

[a] For explanation of such commercial terms as fluidity, Scott viscosity, dextrose equivalent (D.E.), degree of substitution (D.S.), and dextrin solubility, see Kerr (*7*).

Analysis for Carotenoids. Total carotenoids were determined at 450 mμ with the Coleman No. 14 spectrophotometer, calibrated against pure β-carotene (Eastman 3702, recrystallized before use). No attempt was made to distinguish between xanthophyll and carotene pigments. Two techniques were used to estimate the extent of protection provided by the starchy matrix: estimation of the extraneous or surface pigment on the freshly prepared unheated samples, and evaluation of total surviving carotenoid after heating

Table II. Effect of Various Plasticizers on Xanthophyll Oil Protection

Plasticizer	Survival of Pigment at 75° C., %	
	1 week	4 weeks
None	46	28
Glycerol	34	15
Urea	62	41
Corn sirup	62	44

for 1 and 4 weeks at 75° C. The extraneous pigment was determined by stirring an appropriate sample of the freshly dried product for 30 minutes with naphtha (Skellysolve B), and spectrophotometrically determining the amount of pigment extracted. This represents the pigment not protected within the starchy matrix. To determine the total pigment surviving after aging at 75° C., a 500-mg. sample was placed in a 150-ml. standard-taper extraction flask and wetted with a few milliliters of benzene. Twenty milliliters of aqueous 5.V potassium hydroxide was added, and the flask was gently swirled in the boiling water bath until the sample was completely dissolved. A saturated solution of potassium hydroxide in ethyl alcohol, 25 ml., was then added, while the flask was swirled to avoid precipitation of the starch as a doughy mass. This solubilization procedure is necessary in order to release both xanthophyll oil and vitamin from the starchy matrix. Then the mixture was gently refluxed for 25 minutes, transferred to a separatory funnel, and extracted three or four times with naphtha. The combined naphtha extracts were made up to volume and the total carotenoid was determined spectrophotometrically. Results in Table I are expressed as percentage survival of the original pigment present in the freshly prepared samples, corrected to dry basis. All analyses were run in duplicate, with a precision of ±1.3% (average deviation from the mean).

Effect of Plasticizers. A starch plasticizer is not imperative, but its use may enhance the protective ability of the matrix and give a dried product of less friable character. The effect of several plasticizing agents on the stability of carotenoid was determined, using a hypochlorite-oxidized cornstarch (sample 10, Table I) formulated as previously described. The following amounts of plasticizer were used per 100 grams of starch: Corn sirup, 25 grams; urea, 20 grams; and glycerol, 20 grams. Approximately equal stability was realized with either corn sirup or urea (Table II); however, the latter adjunct caused considerable browning at 75° C. Glycerol markedly decreased the protective action of the starch matrix; hence corn sirup was used as the preferred plasticizer in all formulations. The effect of different proportions of corn sirup on the protection afforded by hypochlorite-oxidized cornstarch was studied (Table III). Increasing amounts of corn sirup improved the stability, but the flaked products containing higher levels of corn sirup developed tackiness.

Table III. Effect of Various Proportions of Corn Sirup on Xanthophyll Protection

Corn Sirup, %[a]	Survival of Pigment at 75° C., %	
	1 week	4 weeks
None	46	28
20	62	44
40	70	54
60	81	70
80	76	62

[a] As percentage corn sirup (dry basis) calculated on dry starch basis.

Formulation with Vitamin A Concentrate. In a three-necked 500-ml. flask, a mixture of 100 grams of hypochlorite-oxidized cornstarch (sample 10, Table I), 25 grams of corn sirup, and 240 ml. of water was cooked for 15 minutes in a boiling water bath with continuous agitation. The paste was then cooled to 40° C., while the flask was purged with a stream of carbon dioxide. Twenty grams of a commercial concentrate of vitamin A palmitate in oil (containing approximately 1,000,000 U.S.P. units per gram) was then added in rapid dropwise fashion with vigorous agitation. This gave a light yellow emulsion of the consistency and color of mayonnaise; microscopic examination showed a uniform oil globule size of approximately 5 microns. The emulsion was dried in a thin layer on glass, giving lustrous lemon-yellow flakes in which the embedded oil droplets were of the same size and uniformity as in the original paste. A similar formulation was prepared using 85-fluidity waxy maize starch (sample 5, Table I). The flaked products were aged at room temperature (not 75° C.) for 3 weeks and 3 months, then analyzed for vitamin A content by the 1945 Association of Official Agricultural Chemists method. Analyses were run by the Laboratory of Vitamin Technology, Chicago; results are shown in Table IV.

Formulation with White Phosphorus. As an extreme case, the method has been applied to the emulsification and protection of white phosphorus in a starchy matrix. One hundred grams of hypochlorite-oxidized cornstarch (sample 10, Table I) and 50 grams of corn sirup were cooked in 290 ml. of water in a three-necked flask equipped with propeller stirrer. The paste was then cooled to 30° C., 10 grams of stick white phosphorus added, and the flask flooded with a continuous stream of carbon dioxide. The mixture was reheated to 80° to 90° C. in the water bath, while being stirred to emulsify the molten phosphorus. Finally, the emulsion was cooled to 30° C. to solidify the phosphorus droplets, coated out on a stainless steel plate with an 0.05-inch applicator, and dried at room temperature. The opaque yellowish film was crushed to small flakes, causing some spontaneous smoking. The product was stored at room temperature in contact with air. After various periods of aging, weighed samples were dispersed in hot water and titrated immediately with standard alkali; acidity was calculated as per cent of oxidized phosphorus. This product (containing approximately 8% of phosphorus) showed an initial acidity corresponding to 0.38% of phosphorus. This did not change during 19 days of storage and increased only slightly (to 0.41%) after 14 weeks, representing approximately 95% protection of the phosphorus. A second product was prepared in similar fashion, but with twice the amount of phosphorus (20 grams). This product could not be crushed without inflaming. It contained approximately 15% of phosphorus, and had an initial titratable acidity corresponding to 1.4% of phosphorus. The acidity did not increase in 12 days of storage, representing approximately 90% protection of the phosphorus.

Results and Discussion

Over 50 different starch products have been tested for the protection of xanthophyll oil. The majority of these are standard commercial products of the starch industry. A representative group is listed in Table I in the approximate order of their protective action, ranging from 79% survival of carotenoid after 4 weeks of heating to complete destruction in 2 days. The proportion of pigment extracted from the freshly prepared products by naphtha is inversely related to the percentage survival. It appears that naphtha removes the extraneous pigment which is not completely enclosed within the starchy matrix and is therefore exposed to air oxidation.

Table IV. Stability of Vitamin A Preparations at Room Temperature

(As U.S.P. units per gram of product)

Time	Type of Starchy Matrix	
	Hypochlorite-oxidized	85-fluidity waxy maize
Initial	116,000	109,000
3 weeks	101,000	105,000
3 months	106,000	118,000

The qualities of the starch necessary for protection depend primarily on the molecular architecture within the matrix—i.e., the size and configuration of the starchy molecules and the extent and strength of associative bonding. For example, a well-cooked paste of unmodified thick-boiling starch is composed chiefly of greatly swollen granules or fragments of granules. When xanthophyll oil is dispersed in such a paste and the emulsion is examined under the microscope, the oil droplets are found to be located between the swollen granules. The starch does not function as a protective colloid and the oil droplets are observed to undergo a continuous coalescence. When such an emulsion is dried to a film, the individual swollen granules shrink down without enclosing and protecting the oil droplets. The latter may therefore be squeezed out to the surface of the film during drying, or submicroscopic air channels may develop between the shrunken granules, causing oxidative deterioration of oil droplets still embedded within the film. The original cooked starch system should be virtually free of swollen granules, preferably approaching a molecularly dispersed solution.

Another characteristic of certain starch types which is detrimental to a protective matrix is the tendency to retrograde—i.e., the development of associative bonding between molecules. For example, a corn white dextrin may be cooked in water to give a molecularly dispersed solution. However, the dextrin retrogrades during cooling of the paste and drying of the film, as evidenced by such physical changes as increased opacity, formation of insoluble particles visible under the microscope, and the development of either a gel structure or a thick mushy consistency. Much of the starch substance is tied up as insoluble aggregates and is not available to enclose and protect the oil droplets. Retrogradation is largely due to the linear fraction of the starch. Thus the thin-boiling acid-modified cornstarches show very inferior protective action (samples 19 and 24, Table I), while the corresponding thin-boiling waxy starches which contain no linear fraction provide good protection (samples 5 and 7, Table I). Modified cornstarches in which the linear structure has been distorted or destroyed (oxidized or derivatized starches, torrefaction dextrins) likewise give efficient protection.

Several other considerations are necessary for the formation of a good matrix. The starch product must be used at relatively high concentration and therefore must have a substantially reduced viscosity. Hence thick-boiling derivatized starches have low protective capacity (sample 16, Table I). Even a highly effective product will give inferior results if used at too low a solution concentration. Under these conditions, the starch loses its protective colloid action, and the oil droplets coalesce. Also, the starchy product must still retain high polymeric properties in order to disperse and protect the xanthophyll oil. Thus corn sirups of 40 to 60 dextrose equivalent, while useful as supplementary plasticizers, were completely ineffectual for the formation of a protective matrix. These products did not emulsify the xanthophyll oil and gave gummy films which could not be dried. In contrast, cornstarch hydrolysis products in the range of 5 to 20 dextrose equivalent were highly effective, apparently because they retained the colloidal and film-forming characteristics of a high polymer.

The effectiveness of the various starch types is summarized in the following listing. The same key letters are likewise used to identify these starch types in Table I.

Effective Starch Types

A. The torrefaction or roasted dextrins, which are soluble in cold water (80% or higher), have good stability in solution, and give no blue color with iodine.

B. Thin-boiling hypochlorite-oxidized starches, having a Scott hot paste viscosity of 90 or thinner (on the basis of 100 grams in 280 ml. of water).

C. Etherified and esterified starches, including the hydroxyethyl ether and the acetate and sulfocarboxylic esters, preferably of a degree of substitution of 0.1 or higher. These must be rendered thin-boiling by oxidation or acid modification, in order to assist dissolution of the granule and to permit the use of higher starch concentrations.

D. Thin-boiling waxy starches.

E. Starch hydrolysis products in the range of 5 to 20 dextrose equivalent, produced by acidic or enzymatic conversion. These should have high solubility in cold water and good stability against retrogradation, and should give red to plum colorations with iodine.

Ineffective Starch Types

F. Unmodified starches.

G. Chemically cross-bonded or inhibited starches, derivatized with such agents as phosphorus oxychloride or epichlorohydrin. These products (even when of waxy origin) retain their swollen granule structure after prolonged boiling.

H. Thin-boiling acid-modified starches of normal linear content, which do not give molecular dispersions on cooking, or which retrograde or gel.

I. White dextrins from starches of normal linear content, which tend to retrograde or gel.

J. Corn sirups of 30 to 60 dextrose equivalent, which do not give stable emulsions and which cannot be satisfactorily dried.

To demonstrate the low stability of a surface coating, approximately 20% xanthophyll oil was sprayed on a prepasted and roll-dried cornstarch. The pigment did not survive a single day of heating at 75° C. Similar instability was observed with a commercial bakers' coloring agent, which appeared to be a simple admixture of xanthophyll pigment and wheat flour. Both products developed a strongly rancid odor during heating, an effect which did not occur with an adequate protective matrix.

A large batch of emulsion of the following composition was dried in a commercial countercurrent spray dryer: 55-Scott hypochlorite-oxidized corn starch, 50 pounds; corn sirup, 12.5 pounds; water, 375 pounds; xanthophyll oil, 12.5 pounds. The product was a fine, intensely yellow powder and microscopic examination showed no evidence of a dispersed oil phase. Extraction with naphtha removed 5% of the total pigment. Survival of carotenoid after 1 and 4 weeks at 75° C. was 69 and 21%, respectively. This represents only moderate protection, probably because of the high proportion of water used in the formulation and the submicroscopic dispersion of the xanthophyll oil during spray-drying. In general, it seems preferable to dry the emulsion on a suitable belt or drum dryer. Thus a sample of xanthophyll oil emulsion dried on a heated stainless steel sheet showed the same stability as a similar preparation dried spontaneously at room temperature (compare samples 2a and 2b, Table I).

The two vitamin A preparations showed no significant change on storage at room temperature (Table IV). While these data are much less extensive than for xanthophyll oil, the pattern of protection is presumed to be similar. The protection of white phosphorus was included in these studies primarily as an extreme and rather spectacular example of a protective matrix.

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