Autoxidation of Dehydrated Sweet Potato Flakes. The Effect of Solvent Extraction on Flake Stability

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Extraction of dehydrated sweet potato flakes with solvents of different dielectric constant decreased the rate of autoxidation. Results suggest that surface lipids and carotene which can be removed by certain solvents are more rapidly oxidized than bound lipids. Chloroform–methanol (2:1) apparently removed all surface lipids. Chloroform, though not as efficient as the mixed solvent, was a better extractant than hexane. Taste panels indicate that extraction with Freon 11 increased stability without changing the flavor of reconstituted flakes.

A serious drawback to production of dehydrated sweet potato flakes (flakes) has been storage instability. Unless stored in atmospheres of low oxygen tension, off-flavor development and color loss occur. Antioxidants have been used in attempts to eliminate the deleterious effects of oxidative attack (Deobald and McLemore, 1964) but these materials are of marginal effectiveness. Failure of antioxidants to prevent autoxidation has made it necessary to package flakes in atmospheres containing low oxygen levels and this necessity imposes severe restrictions on packaging with resulting marketing difficulties.

Autoxidation manifests itself in the destruction of carotenes and the simultaneous development of pronounced off-flavors (Deobald and McLemore, 1964). When flakes are stored in air, from 20 to 40% of the carotene is destroyed rapidly for the first 30 or so days and then the rate of destruction decreases considerably. It has been postulated (Walter *et al.*, 1970) that this dramatic change in the rate of carotene destruction is due to protection of part of the carotene from oxidative attack by substances native to the sweet potato or by the formation of substances which retard autoxidation. Work now in progress using oxygen uptake and carotene destruction to follow autoxidation indicates that autoretardation is not involved.

When ^{14}C-β-carotene is incorporated into flakes, it is found that the specific radioactivity of the carotene is not constant (Walter and Purcell, 1971) indicating that carotene and presumably other lipids are present as more than one fraction. This finding when viewed in the light of the resistance to oxidative attack of a large part of the carotene in flakes suggested that removal of lipids and carotene which are readily attacked by oxygen might lead to a stabilization of the flakes to oxidative attack. It is the purpose of this paper to describe the effects of solvent extraction on the autoxidation of dehydrated sweet potato flakes.

MATERIALS

Solvents. Only reagent grade solvents were used with the exception of high purity *n*-hexane (Phillips Petroleum Co.). All solvents were dried overnight with Drierite, carefully distilled, and stored in brown bottles in the dark. Reagent ethyl ether (3 l.) was stirred for 4 hr with 400 ml of 10% sulfuric acid containing 40 g of ferrous sulfate before distillation.

Dehydrated Sweet Potato Flakes. Cured Centennial sweet potatoes from the 1970 crop were processed into flakes as previously described (Purcell and Walter, 1968). The flakes were sized between 20 and 80 mesh screens.

METHODS

Solvent Extraction. Triplicate 60-g samples of flakes were placed in separate 500-ml Erlenmeyer flasks. To each sample was added 200 ml of one of the following solvents: hexane, chloroform, or chloroform–methanol (2:1 v/v). The flasks were tightly sealed and placed on a rotary shaker set at 200 rpm. After 8 hr the flasks were removed. The supernatant was removed by filtration and fresh solvent was added. This procedure was repeated twice more. The extraction period was 24 hr and the total volume of each solvent was about 800 ml. The extracted flakes were placed in a vacuum oven (30°) and held at 1.0 mm overnight to remove any residual solvent. These flakes were used for the autoxidation study below. The solvent extracts for each treatment were combined and evaporated to dryness *in vacuo*, giving lipid samples for each solvent used as an extractant. The crude lipid samples obtained were weighed on a Sartorius Model 2463 balance and stored under nitrogen at −5° until analyzed.

Carotene Autoxidation Study. Samples, 3 g each, of the nonextracted flakes (control) and flakes from each solvent treatment were placed in 120-ml jars and tightly capped. The jars were stored at 21–23° in a desiccated box and opened periodically to assure adequate oxygen levels. Duplicate samples were analyzed for carotene content at the start of the storage study and single samples analzyed periodically. The amount of carotene in each sample was determined after exhaustive extraction (Purcell, 1962) by multiplying the absorbance at 450 nm by 4 to obtain the concentration in milligrams per liter. The results were used to measure the amount of carotene destroyed for each sample for each sampling time.

Control Lipid Extraction. A 10-g sample of control flakes was blended with 40 ml of boiling water and 160 ml of hot 95% ethanol for 2 min. After allowing the slurry to settle it was filtered and the mat extracted exhaustively with chloroform–methanol (2:1 v/v). The extracts were combined, evaporated, and freeze-dried. The amount of control crude lipids was obtained by weighing the lyophilized residue.

Separation of Lipids into Classes. Each crude lipid sample was freed from nonlipid contaminants by passage through a Sephadex G-25 column (Wuthier, 1963). After purification,

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carotene was determined spectrophotometrically and the amount of total lipid was determined gravimetrically. A portion of each purified lipid sample was separated into neutral, glycolipid, and phospholipid fractions on 1 × 15 cm silicic acid columns (Walter *et al.*, 1971). After evaporation of the solvent, the quantities of each fraction were determined gravimetrically. Homogeneity of each fraction was checked using thin-layer chromatography and selected diagnostic spray reagents. The amount of each lipid class from each solvent treatment was then related to that found for the control lipid sample.

Lipid Thin-Layer Chromatography. The lipid concentrations were adjusted to approximately 1% in chloroform before spotting onto plates. Tlc was performed on silica gel HP-coated glass plates activated 1 hr at 110° before use. Neutral lipids were separated by the method of Nagy and Nordby (1970). Polar lipids were studied with the solvent system chloroform–methanol–water (65:25:4 v/v/v). In this system glycolipids and phospholipids were readily distinguishable, while neutral lipids moved with the solvent front.

Visualization of the lipids after development was accomplished by: spraying with 50% sulfuric acid and charring at 150° for all lipids; specific phospholipid spray of Dittmer and Lester (1964); α-naphthol spray specific for glycolipids (Siakatos and Rouser, 1965).

Fatty Acid Analysis. The major fatty acid content of the total lipid fraction from each solvent extraction, as well as that of the control, was determined by converting an aliquot of each portion into methyl esters (FAME). FAME were prepared by transesterification in 5% methanolic hydrogen chloride followed by purification on tlc. The purified FAME were subjected to gas chromatography (glc) and quantitated as described previously (Walter *et al.*, 1971).

Lipid Unsaturation. Unsaturation of the lipids was determined by microhydrogenation. For this procedure the Brown[2] Micro-Hydro-Analyzer (Delmar Scientific Laboratories, Maywood, Ill.) was used. The data obtained were in micromoles of hydrogen reacted.

Taste Panel Evaluation. Freshly made flakes were divided into three lots. Lot 1 was sealed in No. 2½ cans in air. Lot 2 was stored in similar size cans containing an atmosphere of less than 1.0% oxygen and 99.0% nitrogen. The limit of useful measurement for oxygen was 1%. Lot 3 was extracted with difluoro–dichloromethane (Freon 11) and canned in the same manner as was lot 1. After 80 days storage at 26°, the flakes were reconstituted with warm water in the ratio of 4 parts water to 1 part flakes. The three samples (lots 1–3) were then presented to a panel of 16 members on two successive days. The panelists were asked to indicate which sample was different and whether the different sample was preferable or inferior to the similar samples.

RESULTS AND DISCUSSION

Effect of Solvent Extraction on Carotene Oxidation. Destruction of carotene in flakes extracted with either hexane, chloroform, or chloroform–methanol (2:1 v/v) was compared to that in untreated flakes. Control flakes lost 22% of their carotene by 31 days, while hexane-extracted flakes (HEF) lost 8.1% during the same time period. After 31 days, carotene was lost from both control and HEF much more slowly (Figure 1). Chloroform-extracted flakes (CEF) appear to have lost carotene slightly more rapidly during the first 31 days (4.7% lost) than for the remainder of the storage period. Chloroform–methanol-extracted flakes (CMEF) lost carotene at about the same rate throughout the storage period. Autoxidation in flakes appears to occur *via* a rapid oxidation of surface carotenes and a concurrent but much slower destruction of subsurface material.

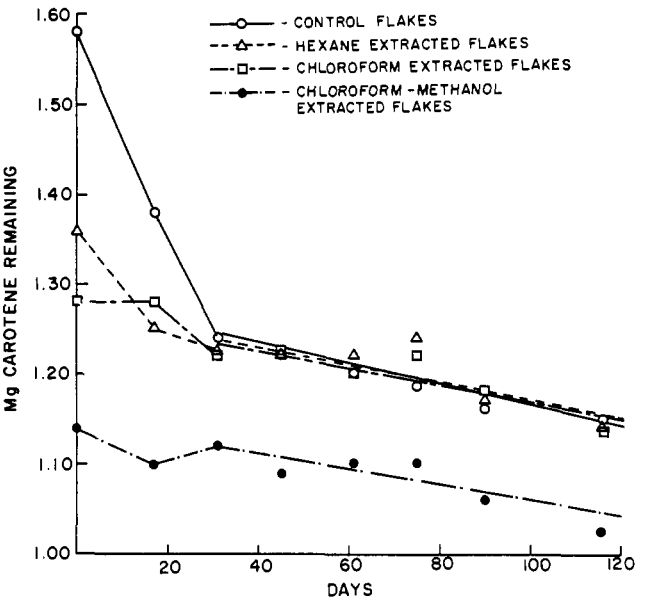

Figure 1. Carotene destruction in dehydrated sweet potato flakes during storage at 21–23° in air. Carotene determined as β-carotene in 3.0-g sample. Regression lines plotted for data from 31 days to the end of the storage period

Rapid carotene destruction apparently is complete by 31 days. Regression lines for carotene loss from 31 days to the end of the storage period are given in Figure 1. Chloroform seems able to remove most of the readily oxidizable surface carotene, so that little remains for rapid destruction. Chloroform–methanol removes all of this surface material as well as some subsurface carotene which is destroyed at a very slow rate. After the initial rapid destruction of surface carotene, oxidation rates decrease considerably, becoming similar for control and solvent-extracted flakes.

A comprehensive taste panel study has not yet been conducted. However, the aroma of each sample was noted as it was opened. It seemed that the control sample had a distinct hay-like off-aroma typical of oxidized sweet potato flakes after 31 days, and after 90 days it had a strong rancid odor. The HEF sample began to develop the hay-like off-aroma after about 90 days, but it was not intense even after 116 days. The CEF and CMEF samples did not develop the oxidized sweet potato flake off-aroma at all.

A preliminary taste panel was conducted by comparing untreated flakes with flakes extracted with Freon 11. This fluorinated hydrocarbon has a polarity similar to chloroform and would be expected to extract about the same amount of lipids. When flakes reconstituted after 80 days were presented to the panel, the members were not able to distinguish between extracted flakes stored in air and control flakes stored in nitrogen, while controls stored in air were uniformly rejected. These results indicate that solvent extraction did not remove flavor compounds and precursors vital to the flavor of flakes and that solvent extraction delayed off-flavor development in flakes canned in air.

Lipids Removed by Solvent Extraction. Each solvent extraction removed carotene and relatively larger amounts of other lipids. Increasing solvent polarity increased the amount of lipids extracted from the flakes (Table I). This is not due to a lack of solubility of the more polar lipids. In experiments with commercial lecithin, hexane, the least polar

Table I. Lipids Removed from Dehydrated Sweet Potato Flakes by Solvent Extraction

Solvent treatment	Portion of total removed, %		Lipid class extracted,[a] %		
	Lipid	Carotene	Neutral	Glycolipids	Phospholipid
Hexane-extracted flakes	10.9	17.0	17.6	6.8	5.1
Chloroform-extracted flakes	14.9	19.7	21.3	13.0	7.2
Chloroform–Methanol-extracted flakes	24.3	28.7	29.9	21.7	18.4

[a] Fractionation of solvent-extracted lipids by silicic acid chromatography.

solvent, was shown to be capable of dissolving more of the polar lipid than was extracted from the flakes by the more polar chloroform–methanol. The inference here is that some lipids of the flakes are bound to other constituents and solvents of higher dielectric constant (ϵ) are necessary to solvate them. Some 75.7% of the lipids and 71.3% of the carotenes were not extracted by even the most polar solvent used, a further indication of lipid binding.

Fractionation of the extracted lipids shows that hexane removed mostly neutral lipids (Table I). Chloroform extracted more total lipid with proportionately more glycolipids, while chloroform–methanol removed even more of all three classes with relatively more of the more polar lipids. Each solvent removes other materials along with the lipids. Less than 0.1% nonlipid material was removed by hexane and chloroform, while 1.8% was removed by the chloroform–methanol extraction. These data suggest that the lipids of flakes exist in different environments. As ϵ of the solvent increases, bound lipids are solvated and removed. Hexane is capable of extracting only those lipids which are found on the surface. Chloroform solvates slightly more lipids as well as very small amounts of nonlipid material (carbohydrates.)

It is suggested from Figure 1 that chloroform removes most of those lipids, which are highly susceptible to autoxidation. These are designated as surface lipids and they contain a slightly higher carotene percentage relative to that of the other extracted lipids. Chloroform–methanol removes surface lipids as well as those more tightly bound and perhaps slightly coated with nonlipid material. The relative amount of these lipids, called coated, is obtained by subtracting the weight of chloroform–methanol-extracted lipids from that of chloroform-extracted lipids and converting the result into percent, relative to the total weight of lipids in the control (nonsolvent-extracted samples). The remaining lipids, designated as bound, are more completely protected from autoxidation. The coated lipids as well as those tightly bound are probably held by a combination of weak physical bonds and simple mechanical entrapment. These data are presented in Table II.

Table II. Distribution and Unsaturation Values for Lipids and Carotene in Dehydrated Sweet Potato Flakes

Lipid fraction	% total in each fraction		Unsaturation values[d]
	Lipids	Carotene	
Surface[a]	14.9	19.7	0.66
Coated[b]	9.4	9.0	0.70
Bound[c]	75.7	71.3	2.22

[a] Chloroform-extracted. [b] Chloroform–methanol-extracted less chloroform-extracted. [c] Nonextractable with chloroform–methanol. [d] Millimoles of hydrogen absorbed by lipids from 60 g of flakes.

A histochemical study of flakes has shown (Purcell, 1971) that in flakes a portion of the carotene and other lipids is found on the surface of carbohydrate matrices. Presumably a large portion of this lipid fraction is removed by chloroform. The remainder of the carotene and lipids are trapped or bound inside the carbohydrate matrix and thus are protected from oxidative attack. Other workers (Maywald and Schoch, 1957) have shown that oxygen-sensitive material such as xanthophyll oil or vitamin A can be protected from oxygen attack by emulsifying these materials in a cooked paste of a suitable modified starch and drying the emulsion as a thin film or flake. About 85% of the dry weight of sweet potatoes is carbohydrate and a large portion of this is made up of dextrins and starch. Histochemical studies confirm that lipids of flakes are bound in a carbohydrate matrix.

In order to determine the susceptibility of the solvent-extracted lipids toward autoxidation, studies were made of the unsaturation (Table II) and the fatty acid content (Table III). Relative amounts of unsaturated fatty acids are similar for surface lipids and total lipids, suggesting that bound lipids have essentially the same composition as surface lipids. About 76% of the unsaturated fatty acids occur in bound lipids and should be oxidized at the same rate as those in the surface lipids. However, bound lipids are oxidized at a much lower rate than surface lipids, suggesting that environment is more important than composition.

Thin-layer chromatography of the extracted lipids showed that although there were differences in the total amount of lipids and amounts in each fraction (neutral lipids, glycolipids, and phospholipids), there were no differences in the individual lipids comprising each fraction, that is, the same spots were present in each comparable fraction.

The preceding data indicate that autoxidation of dehydrated sweet potato flakes occurs in a bimodal fashion. Carotene and possibly other lipids remaining on the surface after processing into flakes are rapidly attacked by oxygen, causing loss of color and development of off-flavor. A major portion of carotene and lipids are oxidized at a much slower rate. Probably these lipids are bound in some manner in a dense carbohydrate matrix made up of sugars, starches, dextrins, and other carbohydrates.

Table III. Fatty Acid Composition of Solvent-Extracted Lipids from Centennial Sweet Potato Flakes

Solvent Treatment	% (by weight) of each component[a]										
	14:0	15:0	16:2	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0
Control	0.5	0.5	0.3	28.4	0.8	0.8	5.2	3.0	49.4	9.6	1.7
Hexane-extracted	0.7	0.7	0.5	32.4	1.1	0.7	5.4	2.3	44.7	9.2	2.2
Chloroform-extracted	0.7	0.6	0.5	27.5	1.5	1.1	5.6	2.9	47.9	10.4	2.0
Chloroform–methanol-extracted	0.7	0.8	0.5	27.7	1.0	1.0	5.6	2.1	49.7	9.0	2.0

[a] Results based on the average of two gas chromatographic analyses of two replicate transesterifications.

Removal of surface lipids by solvent extraction results in stabilization of flakes toward autoxidation, thereby extending the shelf-life. Solvents such as chloroform–methanol, chloroform, and Freon 11 appear able to remove those lipids most available for oxidative attack. Possibly other solvents would be equally effective. The entire question of solvent residues and legal aspects thereof remains to be studied. An added economic advantage to solvent extraction lies in the fact that the extracted carotene could be recovered and used as a vitamin A supplement.

The mechanism by which bound lipids are protected is not clear. Maywald and Schoch (1957) proposed that no mechanism in their study of protection afforded lipids by modified starches. Others (Schlenk *et al.*, 1955) used cyclodextrins to protect autoxidizable materials. They postulated that protection was due either to prevention of a free radical chain mechanism due to fixation of the lipid by host molecules or by the inability of oxygen to diffuse into the carbohydrate matrix. The concept of a hydrogen-bonded carbohydrate "microregion" has been used by Flink and Karel (1970) to explain retention of volatiles during freeze-drying. Possibly such a phenomenon could inhibit oxygen penetration into dehydrated sweet potato flakes. From our study it is not possible to determine which of the effects cited above are responsible for the protective influence on those lipids which are resistant to oxidative attack. Very probably all play a role.

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Adaption of Artificial Rumen and Simulated Abomasal and Intestinal Fluids in Estimating Solubility of Radionuclides; Solubility of ^{59}Fe and Secretion into Goats Milk

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An *in vitro* procedure was developed to study the solubility of radionuclides and essential minerals in the ruminal-gastrointestinal tract and estimate *in vivo* uptake and secretion. For purposes of validating this procedure, the *in vitro* solubility of two forms of ^{59}Fe is correlated with ^{59}Fe secretion *via* milk in the goat. When ^{59}Fe was administered as completely soluble $FeCl_3$, 17.5% remained soluble following the artificial rumen incubation period, 66% during the abomasal period, and 47, 28, and 17.3% when held at pH 4, 5, and 6, respectively, in the intestinal phase. Iron-59 administered as $Fe_4[Fe_3(CN)_6]_3$ was 2.4% soluble in rumen fluid, 9.2% in the abomasum, and 7.1 and 4.2% at pH 5 and 6, respectively, in the intestinal phase. The average 14-day secretion of ^{59}Fe in milk from $FeCl_3$ was 29.2×10^{-3}% of the dose, while from $Fe_4[Fe_3(CN)_6]_3$ it was 6.07×10^{-3}%. The average milk secretion of the portion of ^{59}Fe soluble in the duodenum was 0.0787% from $FeCl_3$ and 0.0723% from $Fe_4[Fe_3(CN)_6]_3$.

Ingested fallout radionuclides are frequently bound to particulate material such as dust or soil. Dissociation of the radionuclide from the particulate material must take place in the digestive tract in order for the radionuclide to become soluble and available for absorption.

The secretion in milk of a radionuclide, administered in a purified soluble form, can be determined. However, the results of metabolism trials, using purified radionuclides, may not represent the metabolism and milk secretion of actual fallout radionuclides. If the solubility and the percentage of transfer of the soluble form of a fallout radionuclide to milk were known, the percentage of transfer of this radionuclide from its fallout form to milk could be predicted with reasonable accuracy. *In vitro* procedures are a convenient method of studying chemical reactions and biological activity in the rumen.

The artificial rumen and simulated abomasal and intestinal fluids procedure has been developed and utilized by Barth and Bruckner (1969a,b) to evaluate the effectiveness of binding agents for the reduction of radionuclides in milk. This study was designed to validate a modification of this procedure to study the solubility of comparatively insoluble and partially soluble radionuclides in the ruminant digestive tract. Concurrently, basic factors affecting the availability of iron and subsequent secretion into milk were determined. These were

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