

nating flavor of coffee but a strongly oxidized or astringent flavor conceivably could adversely influence otherwise good coffee or milk substituted foods.

Sterols in nondairy powdered creamers associated with the cholesterol or related sterol peaks may be attributed to the presence of activated ergosterol or to cholesterol contaminants from added casein or emulsifiers.

Filsoof *et al.* (1973) observed that the fatty acids of 14 nondairy imitation milks were predominately unsaturated. In these four nondairy powdered creamers, representing four major brands, the fatty acids were very highly saturated, 95.0 to 97.8%.

Creamers have not been considered seriously as a vehicle for supplying food nutrients, but coffee drinkers do acquire nutrients through the addition of fresh cream, milk, or creamers. Also, some manufacturers recommend that their powdered nondairy creamers can replace milk in some menu items. The nutritional aspect, therefore, merits some attention because the list of ingredients on the

label does not fully indicate its composition. In the present study nondairy creamers contained highly saturated fats and mainly plant sterols and cow's milk powdered creamers contained partly saturated fat and animal sterols. Depending upon the amount of whitened coffee consumed, the choice of creamer affects the nutrient intake.

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Lipid Autoxidation in Precooked Dehydrated Sweet Potato Flakes Stored in Air

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Destruction of carotene and fatty acids during autoxidation of precooked dehydrated sweet potato flakes was studied in relation to oxygen uptake. Lipids of the oxidized flakes were separated into surface and bound components before analysis. Major fatty acids were identified and quantitated by gas chromatography. Carotene was determined spectrophotometrically. Results showed that surface carotene was lost about 100 times more rapidly than bound carotene. In addition it was observed that surface fatty acids were not

destroyed nearly as rapidly as carotene but much faster than bound fatty acids. More oxygen was absorbed than could be accounted for by peroxide formation, indicating that other oxygen-consuming reactions are involved in flake autoxidation. The data confirmed an earlier observation that autoxidation of sweet potato flakes occurs *via* a dual reaction mechanism, with surface and bound lipids oxidizing concurrently but at very different rates.

Precooked dehydrated sweet potato flakes (flakes) undergo rapid oxidative deterioration unless stored in atmospheres of low oxygen content. The usual course of events in flake autoxidation is a fairly rapid loss of a part of the carotene and the simultaneous development of unpleasant off-flavors. Due to the ineffectiveness of antioxidants in controlling off-flavor development, Deobald and McLemore (1964) concluded that the only suitable alternative is packaging in a nitrogen atmosphere.

Subsequently Walter *et al.* (1972) proposed that solvent extraction of flakes to remove surface carotene and lipids should retard development of off-flavor. Their report suggested that autoxidation of flakes occurs in a bimodal fashion, with surface lipids being attacked at a faster rate than lipids which are bound. Removal of the surface lipids with a suitable solvent would thus leave only bound lipids which are oxidized at a much slower rate and would result in increased shelf life of flakes.

Sweet potato lipid, although present in small amounts (1.5-2.5%, dry basis), is highly unsaturated and thus would be expected to be very susceptible to oxidative attack. In addition to containing the unsaturated fatty

acids, linoleic and linolenic, which comprise about 55% of the total fatty acids (Walter *et al.*, 1971), Centennial sweet potatoes contain a relatively large amount of carotenes, primarily β -carotene. Since carotene destruction in autoxidizing flakes is obvious and fairly easy to follow, this property has been used by several groups as a probe to evaluate the progress of the oxidation (Deobald *et al.*, 1964; Walter *et al.*, 1970). No information has been published concerning the fate of fats.

We undertook in our study to determine the manner in which fat autoxidation occurs during storage in air. Of special interest is the difference in the rate of oxidation for bound and surface lipids. The study reported here has attempted to relate oxygen uptake, carotene, and fat destruction data for surface and bound lipids.

MATERIALS AND METHODS

Samples. Precooked dehydrated sweet potato flakes were prepared from the 1971 crop as previously described (Purcell and Walter, 1968). This paper reports the results obtained from studying the oxidative deterioration of two separate batches of flakes: S-1, Centennial roots from a root maintenance collection harvested October 1971 and processed immediately after being cured, moisture 3.5%; and S-2, Centennial roots purchased on the open market about 5 months after harvest and processed immediately, moisture 3.9%.

Oxygen Absorption. The Warburg technique was used

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for this determination of oxygen absorption (Umbreit *et al.*, 1964). Flake samples were weighed into flasks of known volume, and a weighed strip of filter paper impregnated with sodium hydroxide was added to absorb carbon dioxide. Each flask was then attached to a calibrated manometer and placed in a water bath at $31 \pm 0.1^\circ$. Readings were taken daily in the early stages of the test and less often as the reaction progressed. All readings were corrected by using thermal barometers and the data were expressed as micromoles of oxygen absorbed per gram of fat. At suitable intervals the flasks were removed from the bath and purged with dry air so that oxygen did not become depleted. Periodically samples were removed and analyzed as described below.

Lipid Extraction. After removal from the reaction bath, each sample was extracted with dry redistilled chloroform on a rotary shaker for 4 hr. Supernatant was removed and fresh solvent added twice during the extraction period. The supernatants were combined and evaporated on a rotary evaporator. Crude surface lipids thus obtained were freed of nonlipid contaminants by the method of Bligh and Dyer (1959).

Solvent was removed from the extracted flakes under vacuum at 35° and 1 mm for 2 hr. The sample was then reconstituted with boiling water and ethanol and the bound lipids were extracted as previously described (Walter *et al.*, 1972). The crude bound lipids were purified as described above.

After being weighed, the lipid samples were diluted to appropriate volumes with chloroform and stored under nitrogen at -10° until needed. Each analytical procedure described below was performed on an aliquot of lipid solution.

Carotene Pigment Loss and Ultraviolet Spectral Absorption. A measured volume of the lipid solution was transferred to a volumetric flask. The chloroform was evaporated with a stream of nitrogen and the sample reconstituted with "optical" hexane (Purcell *et al.*, 1959). The absorbancy was measured with a Cary model 15 spectrophotometer at 436 and 450 nm for calculation of carotene concentration (Purcell, 1962) and at 233 nm for changes in diene conjugation (Privett and Blank, 1962).

Peroxide Values. Peroxide values were determined by using the modified method of Wheeler (Swern, 1961). The results are expressed as mmol/kg of fat.

Composition of Fatty Acids. Duplicate measured volumes of solution containing known amounts of lipids (5–10 mg) were placed in 10-ml ampoules. To each was added 1.0 ml of a hexane solution containing a known amount of methyl heptadecanoate which was to serve as an internal standard. The solvent was evaporated and replaced by 0.2 ml of dry benzene and 4.0 ml of anhydrous methanolic hydrogen chloride (5%). The ampoules were cooled to -50° , flushed with nitrogen, and sealed. After being sealed, the samples were heated in a water bath at $80\text{--}85^\circ$ for 3.5 hr, cooled to room temperature, and extracted as previously described (Walter *et al.*, 1971). Fatty acid methyl esters (FAME) were separated from contaminating materials by thin-layer chromatography. The crude FAME were applied to plates of silica gel HR (Brinkmann Instruments) and the plates developed with benzene-hexane (7:4 v/v). The methyl ester band was removed, the esters were extracted and, after solvent evaporation, adjusted to 1.0 ml with hexane. These purified FAME were then analyzed by gas-liquid chromatography (glc).

The glc instrument used was a Packard 800 series equipped with dual flame ionizers and dual 6 ft \times $\frac{1}{8}$ in. stainless steel columns packed with 10% DEGS on Chromosorb G (100–120 mesh, Applied Science Laboratories, State College, Pa.). The injector port and detector oven temperatures were 230° . After an initial hold at 170° for 5 min, the column oven was programmed to 180° at $1^\circ/\text{min}$ and held at the final temperature for 17 min. The flow

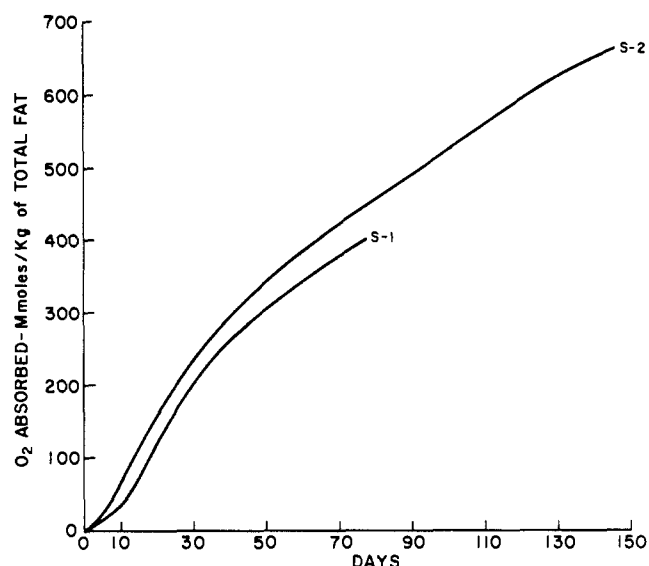


Figure 1. Oxygen uptake for sweet potato flakes at 31° .

rate of the nitrogen carrier gas was 30 ml/min. Individual fatty acids were identified by comparing relative retention times (to methyl palmitate) with those of authentic reference standards.

Transesterification studies on samples of triolein showed that essentially 100% yield of methyl oleate was obtained. Similar results were reported for trilinolein (Goldstein and Wick, 1969). Using AOCS standard 15-A (Nu-Chek Prep, Elysian, Minn.) the percentage composition was confirmed within $\pm 3\%$ precision and accuracy. Gas chromatographic detector response was linear over the range studied.

Duplicate 3- μ l samples were chromatographed and the mean peak areas were obtained by planimetry. The methyl heptadecanoate standard, a portion of which was mixed with each lipid sample to serve as an internal standard, was itself chromatographed in quadruplicate for each series of samples. The mean peak area from this determination served as an external standard. Division of the internal standard peak area by the external standard peak area provided a measure of recovery of esters from the preparation and purification steps. The area of each fatty acid methyl ester was then adjusted for this change, and the corrected areas were then converted into weights relative to the weight-area relationship obtained for the external standard. Results with triolein showed that conversion to the methyl ester was complete; thus, it was possible to calculate the amounts of the fatty acid methyl esters found in flake surface and bound lipids. Precision for the entire procedure was found to be $\pm 5.8\%$.

RESULTS AND DISCUSSION

Oxygen Uptake Studies. Oxygen absorption curves for two lots of flakes are given in Figure 1. Although lots were processed from roots procured from different sources, they showed the same general pattern of oxygen uptake. Absorption occurred slowly at first, then more rapidly, and finally with decreasing speed. For example, sample S-1 had absorption rates of 3.3, 6.5, 6.3, and 5.2 mmol of O₂/kg of fat/day after 10, 22, 36, and 77 days, respectively. These data appear to indicate that this oxidation is of an autocatalytic nature similar to oxidative deterioration of fats but showing no definite induction period. However, due to the nature of flake making and necessary handling after processing into flakes, an induction period of less than 24 hr would not be measured. Chipault and Hawkins (1971) studying freeze-dried beef encountered autoxidation in which no induction period occurred, even though

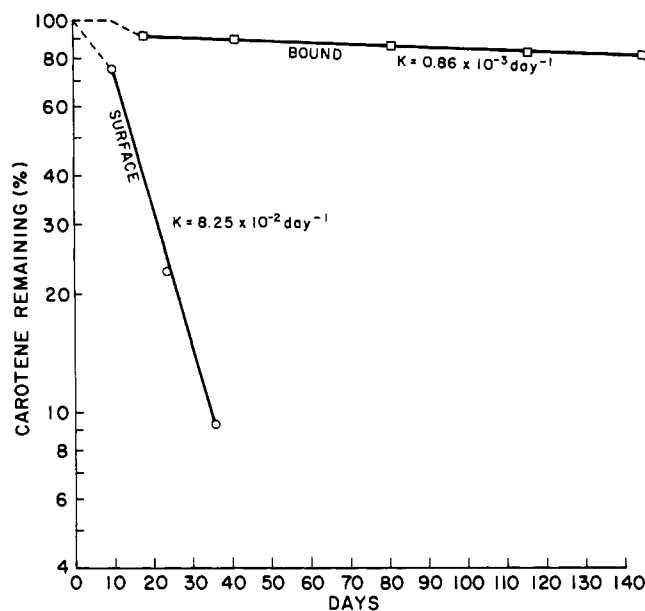


Figure 2. First-order plot for carotene destruction in bound and surface lipids from sweet potato flakes in air at 31°C.

Table I. Autoxidation of Precooked Dehydrated Sweet Potato Flakes at 31°C^a

Time, days	O ₂ ^b absorbed, mmol/kg of fat	Peroxide ^c value, mmol/kg of fat	Extinction coefficient, E _{1%^{1cm}} ^{c, d} 233 nm
0		0.0	1.04
17.9	146	145.6	1.18
40.9	298	74.3	1.48
80.9	457	71.3	1.27
115.9	577	80.4	1.31
145.9	671	117.3	1.06

^a Study S-2. ^b Absorbed by flakes but expressed as fat content of flakes. ^c Obtained from fat extracted from flakes. ^d In optical hexane.

oxygen uptake curves for the later phases of the reaction exhibited autocatalytic behavior.

Peroxide Values and Diene Conjugation. Peroxide values and diene conjugation followed by iodometry and changes in absorbance at 233 nm, respectively, are compared to oxygen uptake values in Table I. It is possible that early in the autoxidation oxygen uptake closely parallels increases in both peroxide value and diene conjugation, as has been reported for the initial stages of methyl linoleate autoxidation (Lundberg and Chipault, 1947; Privett and Blank, 1962). In our study after 17.9 days, however, the three indices of autoxidation did not behave in a manner typical of pure methyl linoleate oxidation.

The theoretical ratio of oxygen uptake to peroxide value is 0.5 where peroxide value is expressed as mequiv/g of fat and oxygen uptake is μmol of O₂/g of fat (Quast and Karel, 1972). We found that the ratio for sweet potato flakes at 17.9 days was 1.0, indicating that significant amounts of peroxides were degraded into other compounds. This ratio increased through 115.9 days. Inconsistency between oxygen uptake and peroxide values is not unprecedented for dehydrated foods undergoing autoxidation. Martinez and Labuza (1968) found poor correlation between oxygen uptake and peroxide values in freeze-dried salmon. Similar findings have been reported for freeze-dried beef (Chipault and Hawkins, 1971) and fish meal (Waissbluth *et al.*, 1971).

The precooked dehydrated sweet potato flakes used in the study reported here contained 2.4% lipid which was

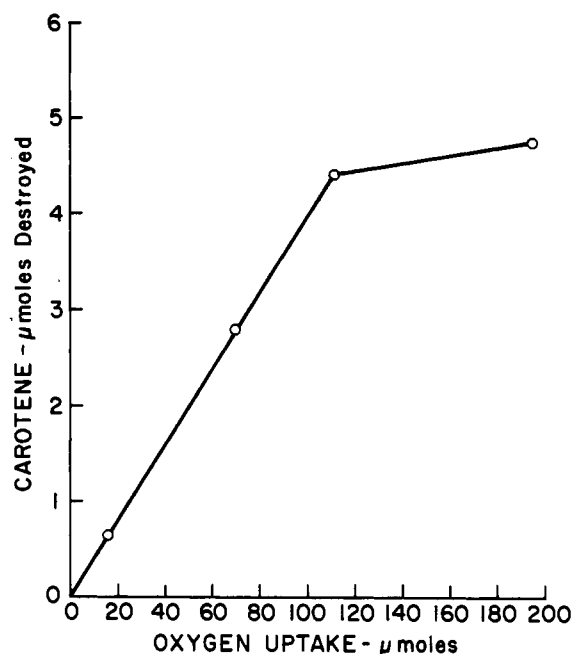


Figure 3. Carotene destruction relative to oxygen uptake for sweet potato flakes stored in air at 31°C.

composed of 60% unsaturated fatty acids and relatively large amounts of β -carotene. Considering this composition, it is not surprising that high peroxide values were noted. Due to the small amount of fat present in flakes, a large sample was required to obtain enough lipid material for peroxides to be detected. It is for this reason that peroxides have not been measured previously.

Lipid Analyses. Flake lipids were quantitatively separated into surface and bound components. Analytical results indicated that surface lipids contained 11.9% of the total carotene and fatty acids. This separation permitted investigation of relative rates of oxidation of the two fractions.

Carotene destruction was followed by measurement of the decreases in absorbance at 450 nm. With the data plotted as log of the percent carotene remaining *vs.* time (Figure 2), it was noted that carotene destruction obeyed first-order kinetics through 90% disappearance. This indicated that the rate of carotene loss depended only on the concentration of substrate remaining. Since the oxygen concentration is so large that its value does not change significantly, this term does not appear in the rate expression.

Both surface and bound carotene appeared to obey first-order kinetics but at much different rates (Figure 2). Surface carotene was destroyed almost 100 times more rapidly than was bound carotene. The rate of $8.25 \times 10^{-2} \text{ day}^{-1}$ for surface carotene destruction compares reasonably well with that of Chou and Breene (1972), who found rates of from 8.8 to $5.0 \times 10^{-2} \text{ day}^{-1}$, depending upon the water activity in an Avicel-carotene model system.

Flakes stored in air rapidly lose from 20 to 40% of their carotene during the first 30 or so days and then the rate of destruction slows considerably so that a plateau phase is attained (Walter *et al.*, 1972). The relationship between oxygen uptake and carotene loss which we observed in our study (Figure 3) indicates that during the rapid phase of the carotene destruction, the pigment loss is directly proportional to the oxygen uptake. But it is evident that on a molar basis considerably more oxygen is absorbed than carotene destroyed. However, carotenes are highly unsaturated and thus more than one site is available for oxidative attack. In addition, the possibility exists that some of the oxidative products themselves are further oxidized.

Table II. Major Fatty Acid Composition of Precooked Dehydrated Sweet Potato Flakes Stored in Air^{a,b}

	Fatty acid			
	Palmitic (16:0)	Stearic (18:0)	Linoleic (18:2)	Linolenic (18:3)
Surface lipid ^c				
Start	7.65	0.95	11.70	1.75
17.9 days	7.30	0.85	9.40	1.25
40.9 days	6.60	0.85	7.00	0.90
80.9 days	6.00	0.75	5.70	0.55
115.9 days	5.60	0.65	5.54	0.61
145.9 days	5.35	0.60	5.05	0.60
Bound lipids ^c				
Start	57.10	6.07	100.70	10.70
17.9 days	60.90	6.39	90.95	9.40
40.9 days	58.17	6.78	87.44	9.64
80.9 days	60.67	6.42	92.43	10.35
115.9 days	58.89	6.42	86.72	9.28
145.9 days	57.42	5.71	92.73	11.41

^a Study S-2, 31°. ^b Results are means obtained from replicate transesterifications. ^c Determined as mg of fatty acid methyl esters from 20 g of flakes using methyl heptadecanoate as internal standard.

Table III. Unsaturation Ratios^a for Major Fatty Acids of Precooked Dehydrated Sweet Potato Flakes Stored at 31° in Air

Time, days	Surface lipids	Bound lipids	Composite ^b
Start	1.60	1.75	1.74
10	1.63	1.75	1.74
22	1.44	1.75	1.72
36	1.19	1.77	1.72
77	0.96	1.58	1.52

^a (Linoleic + linolenic)/(palmitic + stearic). ^b Unsaturation ratios for surface lipids + bound lipids.

Since flakes are a complex system, still other possibilities exist for oxidative attack. At any rate, carotene loss in flakes provides an excellent index of autoxidation until the plateau phase is reached. This happened at about 30 days in our study. After the plateau phase was reached and carotene destruction slowed considerably, the oxygen uptake continued at an undiminished rate, indicating that fatty acids and other food components were continuing to be oxidized.

Fatty Acid Deterioration. Previous studies had shown that palmitic (16:0), stearic (18:0), linoleic (18:2), and linolenic (18:3) acids make up about 93% of the total fatty acids (Walter *et al.*, 1971). Thus, only changes in these compounds were followed. Fatty acid (FA) destruction was found to be much slower than carotene loss. Both S-1 and S-2 showed that significant losses of surface FA occurred after 17.9 days, while bound FA loss was so slow as to be almost undetectable (Table II). As would be expected, surface 18:3 was destroyed more rapidly than 18:2. The linoleic-linolenic ratio in flakes is about 7:1 and consequently more of the 18:2 was destroyed. On a percent basis, however, the rate of 18:3 destruction was greater. The saturated fatty acids found on the surface were also destroyed at a slow but significant rate, while those isolated from the bound lipid fraction remain intact. This was probably due to the active reaction occurring among the other surface lipids. Brodnitz *et al.* (1968) have also observed that saturated fatty acids can be autoxidized under mild conditions when in the presence of impurities or products from an active autoxidation.

One of the criteria used to evaluate autoxidation in white potatoes was change in the unsaturation ratio (Buttery *et al.*, 1961) which is defined as the ratio of the sums

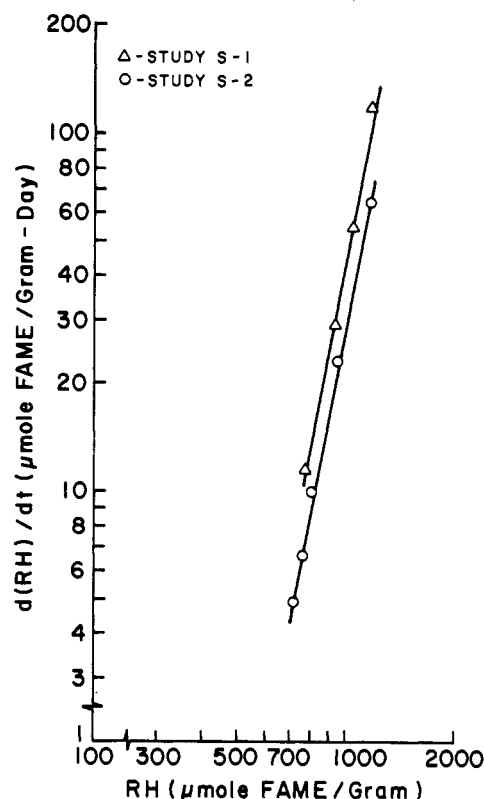


Figure 4. Rate of disappearance of major surface fatty acids at 31° as a function of the concentration of surface fatty acids. The data are expressed per gram of surface lipid obtained from sweet potato flakes.

of the amounts of linoleic and linolenic acids to the sums of the amount of stearic and palmitic acids. Unsaturation ratios for study S-1 are given in Table III. The data are similar to those for carotene destruction in that the bound unsaturation ratio declined very little while the surface ratio exhibited a steady decline during storage. A decline in the unsaturation ratio is indicative of unsaturated fatty acid destruction. When the composite ratio (surface + bound) was calculated (Table III), the ratios closely paralleled those of the bound lipid fraction. This is not surprising since surface fatty acids comprise only about 11% of the total.

White potato granules are reported to develop off-flavors in proportion to decreases in the unsaturation ratio. In one study, for example, the first detectable flavor difference noted for the granules occurred after 29 days storage in air and this corresponded to a change in unsaturation ratio of 11% (Buttery *et al.*, 1961). We found that sweet potato flakes stored in this way have a strong off-flavor by 29 days and yet the composite unsaturation ratio has decreased less than 2%. In light of these differences, it could be suggested that initial off-flavor in sweet potato flakes is caused by oxidation of lipids other than those containing unsaturated fatty acids.

Since oxygen uptake and peroxide values did not correlate, the peroxide value cannot be used in the rate expression for autoxidation. Waissbluth *et al.* (1971) reported that for oxidizing fish meal the rate of destruction of unsaturated fatty acids is independent of peroxide concentration and the overall rate could be expressed as $d(RH)/dt = -k(RH)^n$, where (RH) is the concentration of unsaturated fatty acid and n an exponent with values between 4.0 and 5.0. Fatty acids from surface lipids appear to fit this equation reasonably well, with n of 3.7 and 3.6 for S-1 and S-2, respectively (Figure 4). Attempts to fit bound lipid fatty acid changes in the same expression were not success-

ful due to insignificant concentration changes which occurred during the storage period.

The observation that surface fatty acids in concert with surface carotene are destroyed concurrently but more rapidly than the bound materials confirms the earlier suggestion that autoxidation in flakes is a bimodal reaction (Walter *et al.*, 1972). A plausible explanation has been suggested (Purcell, 1973). Briefly stated, during processing, most of the lipids are trapped in a dense carbohydrate matrix which in some manner retards the progress of the autoxidative reaction. The remaining lipids being spread over the flake surface are not protected and so are readily available for oxidative attack. Reaction products from autoxidation of surface lipids are very probably responsible for off-flavor development and the resulting short shelf life of sweet potato flakes.

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Nitrite in Meat. Effect of Various Compounds on Loss of Nitrite

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Various compounds endogenous to meat or added to cured meats, and known to react with nitrite, were tested in meat slurries for their effects on nitrite loss. The reductants, ascorbate and cysteine, and the amino acid, histidine, caused the only important losses when added to endogenous or normally used concentrations. Reduced nicotinamide adenine dinucleotide caused a short term reduction of nitrite, but the loss was not

permanent. Kinetic data show that loss of nitrite is related to the reduction reaction which produces nitric oxide. Production of the latter accounts for a large part of total nitrite loss and evidence indicates that most or all of the remaining nitrite not accounted for is involved in the formation of nitroso-reductant intermediates or products.

In the United States, sodium nitrite is added to cured meat products at levels up to 2.25 mM (dry mix) or 3.62 mM (10% pumped pickle). During curing, cooking, and aging, nitrite content is greatly reduced and sometimes completely eliminated (AMIF, 1971). Mirna and Hofmann (1969) made a significant discovery with regard to nitrite loss when they found that the loss of added nitrite in meat was equaled by a reduction in free sulfhydryl groups. The sulfhydryl content of meat is about 20 mM and is therefore sufficient to completely eliminate nitrite. Other observations however, raise questions about this loss. In practice, even with molar excesses of reductants over nitrite, the latter is not usually completely eliminated. Ascorbate, which is commonly added to cured meats, is even more reactive toward nitrite on a molar basis in forming of nitric oxide than is cysteine (Fox and Ackerman, 1968) suggesting that the roles of ascorbate and cysteine in

causing nitrite loss should be compared. Walters and Taylor (1964) reported that not all nitrite loss in meat could be accounted for as nitric oxide, which is as yet the only identified product of the reaction of either cysteine or ascorbate with nitrite (Kelley and Watts, 1957). The relation between nitrite loss and nitric oxide formation with either ascorbate or cysteine has not been quantitated. Mirna and Hofmann (1969) observed the formation of spectrally absorbing nitrosothiol compounds, which they suggested might be involved in nitrite disappearance, possibly from the oxidation of sulfhydryl by nitrite. The oxidation of ascorbate by oxidants other than nitrite has been shown to proceed through the formation of spectrally identifiable intermediates (Herbert *et al.*, 1933; Schauenstein *et al.*, 1948; Fujimura and Ikeda, 1957; Bielski *et al.*, 1971), which suggests that spectral identification of ascorbate or cysteine intermediates might be used to determine the mechanism of nitrite loss.

This investigation was undertaken to determine: first, the relative importance of the cysteine (sulfhydryl) and ascorbate reactions in the loss of nitrite; second, if spectrally distinct compounds are formed during the ascor-

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