LIPID AUTOXIDATION AND AMINO ACID CHANGES IN PROTEIN–ENRICHED SWEET POTATO FLAKES

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ABSTRACT

Three sweet potato flake formulations, containing (1) soy flour plus methionine, (2) casein and (3) no supplementary protein, were prepared and stored at room temperature (21–23°C) in air for 16 months. The formulations were analyzed periodically to ascertain changes in carotene content, amino acid levels and water-binding capacity. On the basis of carotene degradation as a measure of lipid autoxidation, it was found that after an induction period of 19 days required by the flakes supplemented with soy-methionine, all formulations were oxidized in an identical manner. Among the amino acids, only glutamic acid was lost from all three formulations, and isoleucine levels decreased in the control formulation only. Lysine concentration in the fortified flakes decreased while histidine decreased in the soy-methionine formulation. Storage had no effect on the water-binding capacity of any of the three formulations.

INTRODUCTION

SWEET POTATO FLAKES stored in air undergo autoxidation in which carotene and fatty acids are destroyed (Walter and Purcell, 1974). The autoxidation of such lipids in foods can mediate destruction of other compounds present, some with high nutritional value. Research with both model systems and dehydrated foods has shown that protein interacts with lipid oxidation products thus undergoing both denaturation and loss of biological value (Labuza, 1972).

No information is available concerning the effect of autoxidizing lipids on the amino acid composition of dehydrated sweet potato flakes. A previous study has shown that dehydrated sweet potato flakes and flakes fortified with soy flour, cottonseed flour, and wheat gluten, retained essential amino acids when stored at 23°C and 40°C under nitrogen (Walter et al., 1978). However, water-binding capacity of flakes stored at 40°C decreased. The purpose of the present study was to investigate the relationship between lipid autoxidation and changes in amino acid content of sweet potato flakes, flakes fortified with casein, and flakes containing soy flour plus methionine. In addition, it was desired to determine if autoxidation modified other food components in such a way as to change water-binding capacity.

MATERIALS & METHODS

JEWEL SWEET POTATOES, which had been cured for 1 wk and then stored for 1 month (Purcell et al., 1976), were washed, hand-peeled and cut into 2-inch cubes. Three 1.8-kg batches (25% dry matter) were cooked in an autoclave at 121°C for 30 min, cooled, mixed with additives described below, and blended into a smooth puree. Each formulation was dried into flakes on a 12 × 19 inch, double drum dryer heated with steam at 80 psi. The flakes were retained on the drum for about 30 sec.

All formulations contained 0.3g sodium bisulfite, 0.1g sodium sulfite and 1.8g sodium acid pyrophosphate to prevent discoloration (Hoover, 1963). The soy-methionine formulation contained 129g defatted soy flour (Central Soya Company), 2.0g DL methionine, and 65g sucrose. The casein formulation contained 120g casein (NB Company) and 65g sucrose. The control formulation contained no other additives.

The flakes were ground to a size between 40- and 80-mesh, and 25-g samples sealed in air in #303 cans. Headspace volume was about 500 ml, thus assuring adequate oxygen levels for autoxidation. The cans were held at room temperature (21–23°C) until sampled. Each formulation was analyzed immediately after preparation and at 1, 2, 4, 6, 8, 12.25 and 16 months of storage.

Amino acid analysis

Duplicate samples of each formulation were acid hydrolyzed and amino acid content was measured with a Beckman Model 119, automatic amino acid analyzer (Walter et al., 1978). Data from the analyzer was integrated and reduced to the desired format by a Texas Instruments Model 960A minicomputer.

Analyses for cysteine and tryptophan were carried out on an enzymatic hydrolysate of the flakes, as previously described (Walter et al., 1978). This analysis was performed only on the freshly prepared formulations.

Carotene analysis

Duplicate 3.0-g samples of each formulation were reconstituted with 60 ml boiling water and set aside in the dark unitl cool. One gram of filter aid and 50 ml methanol were added and the mixture was blended in a high speed blendor. The slurry was filtered and the mat exhaustively extracted with hexane-acetone (1:1). The filtrate was placed in a 500 ml separatory funnel. The lower layer was passed into a second separatory funnel containing 200 ml of peroxide-free ethyl ether, then discarded. The upper layer in both separatory funnels was washed three times with 200 ml water. The lower layers were discarded and the organic extracts were combined and evaporated *in vacuo*. The residue containing carotenoids was redissolved and diluted to 250 ml with hexane. Since the solutions were cloudy, they were treated with 5g of anhydrous sodium sulfate and placed in the dark to clarify before the absorbancy was measured at 436 and 450 nm for calculation of the carotene concentration (Purcell, 1962).

Viscosity

Two 5-g samples of each formulation were reconstituted with 20 ml of boiling water and stirred for 1.0 min. Each sample was covered and held at room temperature for 3 hr. Apparent viscosity was determined on a Haake Rotovisko model RV-1 rotary viscometer (Walter et al., 1976).

Statistical methods

Effects of formulation on amino acid composition of flakes were determined by analysis of variance (ANOVA) and Duncan's multiple range test. Effects of storage time on amino acid levels were studied by regression analysis of data for individual formulations and of pooled data. Data were tested for both linear and quadratic regression. Similarly, the dependent variable apparent viscosity was subjected to zero time analysis of variance for determination of formulation effects, and to linear and quadratic regression analysis of variance for detection of viscosity changes over time.

Moisture and protein content

Moisture levels were determined by measuring weight changes occurring when the samples were dried to constant weight at 95°C. Protein content (N × 6.25) was determined by the Kjeldahl method with copper and selenium catalysts.

RESULTS & DISCUSSION

Flake properties

Initial moisture levels ranged from 4.02% for the control formulation to 5.30% for the soy-methionine formulation (Table 1). Pro-vitamin A (β-carotene) was lower in the fortified flakes than in the control due to the diluting effect of the additives. The casein formulation, although it contained the least amount of carotene, still provided three times the vitamin

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Table 1—Initial composition of control and fortified sweet potato flakes

Flake formulation	Protein[a] (%)	β-Carotene (mg/g flakes)	Moisture (%)
Control	6.21	0.43	4.02
Soy-methionine	13.25	0.32	5.30
Casein	19.36	0.29	4.61

[a] Kjeldahl nitrogen X 6.25

A RDA in a 100-g serving. As expected, fortification increased the protein two- to threefold.

Each formulation had its own characteristic amino acid pattern (Table 2). Amino acid balance, as manifested by the ratio of essential to nonessential amino acids for the control and soy-methionine formulations, has been reported previously (Walter et al., 1978) as 0.72 for control and 0.68 for soy-methionine formulations. This study has shown that the casein formulation ratio was slightly higher with a value of 0.75. Of the essential amino acids, only total sulfur is deficient with 97% of FAO/WHO (1973) reference value. Thus, both casein and soy flour plus methionine are suitable supplementary materials for sweet potato flakes with respect to amino acid balance and quality.

An examination of the apparent viscosities of the reconstituted formulations at several rotor speeds (Fig. 1) indicated that the control formulation had a greater water-binding capacity (i.e. higher apparent viscosity) than did either fortified formulation. The casein formulation bound slightly more water than the soy-methionine formulation and exhibited pseudoplastic flow patterns similar to the flow patterns of other fortified flake formulations previously reported (Walter et al., 1978).

Stored flakes

One measure of the progress of autoxidation in sweet potato flakes is the extent of carotene destruction. Loss of carotene (Fig. 2) began immediately in the control and casein formulations while loss from the soy-methionine flakes began after 19 days had elapsed. This evidence indicated that the soy flour contained an inhibitory substance which retarded lipid autoxidation for this initial period. The rate of destruction in all three formulations increased for 2 months, then moderated. After 4 months, destruction was very slow.

It has been reported previously (Walter and Purcell, 1974) that lipids of sweet potato flakes exist as surface and bound fractions. Surface lipids are oxidized first; of these, carotene is destroyed rapidly, whereas linoleic and linolenic acids of the surface fraction react more slowly. The bound lipid fraction is surrounded by a dense carbohydrate matrix which serves as an oxygen barrier and as a result, the carotene and fatty acids in this fraction are oxidized at an extremely slow rate. Thus, flakes exhibit a pattern of rapid carotene and fat destruction followed by a period of very slow loss. Fortification did not change the mechanism of carotene autoxidation except that the soy flour did cause a short induction period after which all formulations oxidized at about the same rate.

Amino acid changes. Regression analyses of data pooled over all formulations revealed only two instances of significant storage time effects. Lysine content changed according to a second order polynomial (quadratic) reaction rate (Table 3). The initial decrease indicated that lysine was more stable than the other amino acids during the 0 to 16 wk storage period. Phenylalanine content increased linearly with time (Table 3), again indicating high relative storage stability.

The levels of several amino acids (e.g. glutamic acid) displayed significant changes during storage for individual formulations, but not in pooled analyses for all samples. Linear models of these storage changes produced the best fit (Table 4). Since the amino acid levels were calculated on the basis of grams amino acid per 16g of amino acid nitrogen recovered, a

Table 2—Initial amino acid composition[a] of control and supplemented sweet potato flakes[b]

Amino acid	Formulation		
	Control	Soy-methionine	Casein
Aspartic acid	27.0a	19.1b	14.9c
Threonine	7.0a	5.4b	5.8c
Serine	7.6a	6.8b	7.5ab
Glutamic acid	14.1a	20.7b	28.4c
Proline	5.1a	5.5a	11.5b
Glycine	5.0a	4.6a	2.6b
Alanine	8.5a	6.1b	5.0c
Cysteine[c]	0.5a	0.3a	0.2a
Valine	8.3a	6.7b	8.6a
Methionine	1.9a	4.6b	3.2c
Isoleucine	6.0a	5.8a	6.7b
Leucine	9.4a	9.5a	11.9b
Tyrosine	4.5a	3.5b	5.7c
Phenylalanine	7.4a	6.2b	6.6ab
Lysine	6.1a	6.0a	6.6a
Histidine	2.1a	3.4a	2.0a
Arginine	4.0a	6.3b	3.3a
Tryptophan[c]	1.5a	1.1a	1.2a

[a] Grams of amino acid per 16g amino acid nitrogen recovered.
[b] Values followed by the same letter in a horizontal row are not significantly different ($P < 0.05$).
[c] Values determined colorimetrically on enzyme hydrolysate.

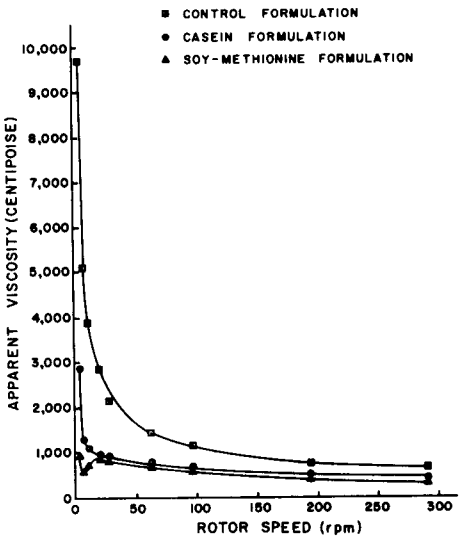

Fig. 1—Apparent viscosity of reconstituted sweet potato flake formulations as a function of rotor speed.

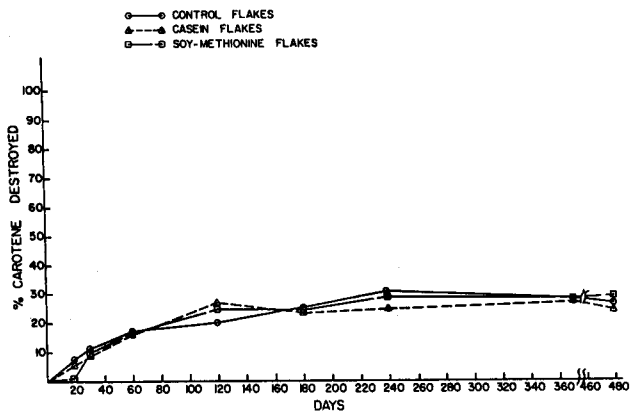

Fig. 2—Carotene loss from control and fortified sweet potato flakes stored in air.

Table 3—Quadratic and linear regression analyses of variance of lysine and phenylalanine composition of control and fortified (pooled) sweet potato flakes stored in air

		Lysine				Phenylalanine			
Source[a]	d.f.	MS	F	R^2	Slope[b]	MS	F	R^2	Slope[b]
Quadratic									
T	1	11.1405	15.55**	0.4211	0.0616**	2.4105	9.63**	0.1799	0.0121
T^2	1	11.7880	16.45**		−0.0013**	0.0052	0.02		−0.00003
Error	44	0.7165				0.2504			
Linear									
T	1	11.1405	11.57**	0.2046	−0.0222**	2.4105	9.84**	0.1795	0.0103**
Error	45	0.9625				0.2449			

[a] T = time (weeks)
[b] Slope = grams of amino acid · 16g amino acid nitrogen recovered^{-1} · week^{-1}.
** $P < 0.01$

Table 4—Regression analysis of variance of the amino acid composition of control, soy-methionine and casein formulation sweet potato flakes stored in air

			Control			Soy-methionine			Casein		
Amino acid	Source[a]	d.f.	MS	F	Slope[b]	MS	F	Slope[b]	MS	F	Slope[b]
Glutamic	T	1	5.6348	8.64*	−0.0273*	13.2487	16.59**	−0.0421**	27.466	17.51**	−0.0602**
acid	Error	14	0.6519			0.7984			1.568		
Isoleucine	T	1	0.2871	7.40*	−0.0062*						
	Error	14	0.0388								
Phenyl-	T	1				1.1708	11.06**	0.0125**	1.3855	9.978**	0.0135**
alanine	Error	14				0.1059			0.1389		
Lysine	T	1				5.6780	28.21**	−0.0275**	3.8233	5.77*	−0.0225*
	Error	14				0.2013			0.6626		
Glycine	T	1				0.5878	7.81*	0.0089*	0.2951	4.73*	0.0062*
	Error	14				0.0753			0.0624		
Arginine	T	1				1.0321	10.61**	0.0117**	0.9389	6.51*	0.0111*
	Error	14				0.0973			0.1442		
Tyrosine	T	1				0.6338	24.09**	0.0092**	0.5653	18.87**	0.0086**
	Error	14				0.0263			0.02996		
Histidine	T	1				1.4568	11.28**	−0.0152**	1.1233	5.41*	0.0122*
	Error	14				0.1291			0.2076		

[a] T = Time (weeks).
[b] Slope = grams amino acid · 16g amino acid nitrogen recovered^{-1} · week^{-1}.
* $P < 0.05$
** $P < 0.01$

positive regression coefficient indicated that the amino acid was more stable than the other amino acids. Conversely, a negative regression coefficient indicated a relatively less stable amino acid.

Storage changes were fewest in the control formulation with isoleucine decreasing only in this formulation. Glutamic acid was lost from all formulations. In the two fortified formulations, lysine decreased while phenylalanine, glycine, arginine, and tyrosine increased. This increase is probably the result of greater relative stability. Histidine decreased in soy-methionine but increased in casein.

Lipid autoxidation in foods generates chemical species such as hydroperoxides, carbonyls, epoxides, alcohols, and acids which are capable of reacting with proteins. Acidic and basic amino acid residues as well as those containing hydroxy and sulfur groups should be susceptible to reaction with autoxidation products. Our data however do not show any pattern of loss based on the functional groups of the amino acid residues. The only acidic amino acid lost was glutamic acid and it was lost from all formulations. Lysine and histidine, basic amino acids, were destroyed in one or both fortified formulations. None of the hydroxy or sulfur containing amino acids were destroyed.

Water-binding capacity. A previous study (Walter et al., 1978) on flakes stored under nitrogen at 23°C suggested that the water-binding capacity appeared to increase with time as indicated by an increase in viscosity of the reconstituted flakes. However, due to the nature of the data, there was some doubt as to whether the increase was real (i.e. statistically significant). The present study indicates no consistent significant changes of viscosity for either pooled data or those for individual formulations. Although the storage conditions of the two studies were not identical, we feel that our data strengthen the argument presented by Walter et al. (1978) that the water-binding capacity does not change when flakes are stored at 23°C.

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