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Journal of FOOD PROCESSING AND PRESERVATION

Edited by T. P. Labuza, University of Minnesota

FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT 06880 USA

VOLUME 1, NUMBER 3 JULY 1977

JOURNAL OF FOOD PROCESSING AND PRESERVATION

All articles for publication and inquiries regarding publication should be sent to Prof. T. P. Labuza, University of Minnesota, Department of Food Science and Nutrition, St. Paul, Mn. 55108 USA.

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., 265 Post Road West, Westport, Connecticut USA.

One volume of four issues will be published annually. The price per volume is $45.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $55.00 per year via surface mail, and $62.00 per year via airmail.

Subscriptions for individuals for their own personal use are $25.00 per volume which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $35.00 per year via surface mail, and $42.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

The *Journal of Food Processing and Preservation* is published quarterly by Food & Nutrition Press, Inc. — Office of publication is 265 Post Road West, Westport, Connecticut 06880 USA.

Application to mail at second class postage rates is pending at Westport, Ct. 06880

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Journal of
FOOD PROCESSING AND PRESERVATION

VOLUME 1

Editor: T. P. LABUZA

Assistant Editor: ALLA L. PETRENKO

FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT USA

ISSN: 0145-8892

Printed in the United States of America

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MICROBIAL STABILITY AND REMANUFACTURING CHARACTERISTICS OF HIGH SOLIDS TOMATO CONCENTRATES

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Received for Publication July 29, 1977

ABSTRACT

*High solids concentrates prepared from VF*145-7879 *tomatoes showed microbiological stability without heat preservation at* A_W = 0.879 *through* 480 *days of incubation at* 30°*C. The concentrates with* A_W = 0.882, *however, spoiled within* 360 *days of incubation. The* A_W *values corresponded to* 55.13% *and* 52.86% *total solids, respectively.*

The high concentrates prepared from other varieties which were unusually soiled and had excessive microbial contamination spoiled within 5 *days at* A_W *levels as low as* 0.853 *(*50.74% *total solids), but remained microbiologically stable at* A_W = 0.819 *(*63.64% *total solids) through* 480 *days. The effects of pH and the temperature of extraction are discussed.*

The consistency of the reconstituted high concentrates indicated a trend, i.e. with higher concentration, the concentrate remanufactured into catsup had slightly poorer consistency.

INTRODUCTION

Tomatoes are one of the most important food crops in California in terms of production and gross income. The 1975 California crop was worth more than 450 million dollars (USDA 1977). The majority of the crop was processed into juice, puree, catsup, canned whole tomatoes and paste.

Commercial concentration of tomato pulp into paste accounted for an estimated 32.2% of the 1975 California crop (Canners League of

Journal of Food Processing and Preservation 1 (1977) 191–206.

California 1977). These pastes varied in concentration from 26 to 46°Brix, and were primarily produced for remanufacturing purposes. The tomato concentrates either received a thermal treatment to acquire biological stability, or were held at − 18°C until they were used as an ingredient in the manufacture of other foods.

In the preservation of tomato concentrates, the various factors which interact and limit the type of microorganisms capable of proliferating should be considered. The factors which could be considered are the pH and buffering capacity of the foods, nutritive potential, E_h (critical redox potential) and O_2 partial pressure during storage, A_W (water activity), amount of antimicrobial compounds present, and storage time and temperature (Mossel 1975).

Mossel (1971) discussed how the aciduric bacteria predominate and cause spoilage in acid foods, how yeasts and molds spoil high-sugar foods (at low A_W), and how organisms which metabolize or tolerate preservatives may spoil foods with preservatives. The pH of concentrates would inhibit the growth of organisms of public health concern, such as *Cl. botulinum*, at pH <4.5, and *B. coagulans* spore germination would be inhibited at pH <4.24 (Rice and Pederson 1954). Spores, in general, would be inhibited at A_W <0.95 (Ingram 1969). Faville *et al.* (1951) showed that organisms which could grow in single strength orange juice could not grow in the concentrate, and they were in agreement with Hockenhull (1973) in that microorganisms required higher storage temperature at lower A_W because the organisms require additional energy to balance the osmotic pressure (Mossel 1975). The buffering capacity of food is important to the extent that pH should not change easily, thus providing a more compatible environment for faster growth. Mossel (1971) indicated that the nutritional requirements of organisms become more specific at higher incubation temperature, at lower A_W and for injured cells, and that fruit concentrates would be a poor growth medium for proteolytic organisms or for organisms which require large amounts of B vitamins for growth. Mossel (1971) pointed out the findings of other scientists, and indicated that the partial pressure of O_2 (air < 10 mm or pO_2< 2 mm) would control aerobic growth of microorganisms and that CO_2 had microbiostatic properties. Luh *et al.* (1958) studied the formation of the natural preservative hydroxymethylfurfural (HMF) in tomato concentrates, and Ingram *et al.* (1955) demonstrated the inhibitory influence of this chemical on the growth of *S. cerevisiae*.

The nutritive potential, pH, buffering capacity and the formation of antimicrobial HMF make tomato concentrates a likely candidate for procuring microbiological stability without heating, by controlling the

environment (A_W, pO_2 and/or conditions of storage) so that microorganisms cannot grow.

Birnbaum *et al.* (1977) indicated that although stability without heat preservation was questionable within a model system of high solids tomato serum concentrates, the microbial activity in whole tomato concentrates might be controlled by high solids levels, i.e. low A_W.

The aim of this investigation was to determine if the activity of spoilage-causing organisms could be arrested in whole tomato concentrates by reducing water activity, i.e. increasing solids levels.

Since the bulk of tomato concentrate produced is used as an ingredient in the formulation of consistency-oriented food products, e.g., tomato catsup, limited tests were conducted to evaluate the remanufactured consistency and color of high solids concentrates which were prepared from a commercial puree.

MATERIALS AND METHODS

Preparation of Tomato Concentrates

VF-145-7879, Petomech II, and VF-134 varieties of tomatoes were selected to represent varieties known to have respectively low, medium and high amounts of water-insoluble solids. The tomatoes were mechanically harvested and sorted as for a commercial delivery. The tomatoes were delivered to the processing laboratory where they were yard-stored overnight (approximately 16 hr), unless otherwise indicated.

The tomatoes received two alternating soak and spray washes and the excessively damaged and defective fruits not considered suitable for processing were removed. One third of the tomatoes were comminuted through a Rietz disintegrator (Rietz Mfg. Co., Santa Rosa, CA), heated by steam injection to 104.4°C, and held 45 sec (Birnbaum *et al.* 1977). The two remaining thirds were also comminuted. One was heated to 82.2°C, and the other extracted at ambient temperature without heating. The above treatments correspond to hot, medium and cold break-processing of tomatoes. The extracted pulp was cooled as needed to $\leqslant$40.6°C and concentrated in a 1.486 m^2 wiped film evaporator (Pfaudler Co., Rochester, N.Y.) to total solids of 25–66%, as shown in Tables 1–5. Concentrates at each solids level were hand packed at ambient temperature into dry, unsterilized flip-top 202 × 308 tomato juice cans. Ten cans from each concentration level were sealed without vacuum and incubated at 30°C until definite swelling of the cans was observed. The incubation temperature of 30°C approximated the

Table 1. Analytical data on concentrates prepared from VF-145-7879 tomatoes harvested on October 7, and washed with chlorinated water

	% Total Solids	°Brix	Total Acidity (Meq. H^+/100g)	Serum Color	Agtron E-5M Color Value	A_W (equil. RH)	Microbial Count on OSA	Microbial Spoilage	Spoilage in
Raw Material (pH 4.38)	6.57	6.1	5.55		24.0				
			Hot Break at 104.4° C						
Pulp	5.55	5.1	4.24	—	32.0	—	<1		
Concentrate 1	32.82	32.7	24.54	—	31.0	0.955	9.00×10^1	100%	2 days
2	37.16	38.0	28.46	0.417	31.0	0.948	2.28×10^2	100%	2 days
3	44.97	46.2	34.47	0.419	30.0	0.922	9.30×10^1	100%	6 days
4	50.05	51.0	38.61	0.469	31.0	0.900	2.46×10^2	100%	180 days
5	54.54	55.0	42.55	0.449	30.5	0.870	2.34×10^2	None	480 days
6	65.73	68.3	51.85	0.463	31.0	0.805	6.30×10^1	None	480 days
			Medium Break at 82.2° C						
Pulp	5.64	5.3	4.45	—	30.0	—	3.60×10^1		
Concentrate 1	31.89	32.9	25.69	0.408	28.5	0.950	1.77×10^3	100%	2 days
2	37.47	38.9	29.39	0.400	29.0	0.944	1.61×10^3	100%	3 days
3	38.97	40.0	30.58	0.380	30.0	0.946	9.12×10^2	100%	5 days
4	47.84	49.2	39.63	0.454	29.5	0.910	4.90×10^2	100%	10-19 days
5	55.74	57.2	46.86	0.430	30.0	0.868	3.68×10^2	None	480 days
			Cold Break at Ambient Temperature						
Pulp	6.22	6.1	5.42	—	25.0	—	8.37×10^5		
Concentrate 1	34.69	34.6	30.64	0.389	25.5	0.941	1.29×10^6	100%	2 days
2	43.02	44.0	37.21	0.403	26.0	0.921	1.76×10^5	100%	3 days
3	46.25	47.5	40.25	0.382	26.0	0.906	3.30×10^5	100%	9-12 days
4	59.85	61.2	51.23	0.425	28.0	0.846	7.78×10^4	None	480 days

Table 2. Analytical data on concentrates prepared from VF-145-7879 variety tomatoes harvested on October 8, and washed in unchlorinated water

	% Total Solids	°Brix	Total Acidity (Meq. H^+/100g)	Serum Color	Agtron E-5M Color Value	A_W (equil. RH)	Microbial Count on OSA	Microbial Spoilage	Spoilage in
Raw Material (pH 4.35)	5.94	5.2	4.84		25.5				
			Hot Break at 104.4°C						
Pulp	5.29	4.7	3.98	—	32.5	—	1.31×10^2		
Concentrate 1	31.98	31.6	24.64	0.460	30.0	0.972	2.07×10^3	100%	1 day
2	35.41	35.5	27.09	0.461	30.5	0.950	1.72×10^3	100%	2 days
3	36.56	36.5	27.23	0.451	30.0	0.943	4.74×10^3	100%	2 days
4	47.14	48.0	36.87	0.462	30.0	0.909	3.67×10^3	100%	4 days
5	56.87	56.7	42.78	0.480	30.5	0.865	2.50×10^3	None	480 days
			Medium Break at 82.2°C						
Pulp	5.24	4.8	4.54	—	30.0	—	2.08×10^3		
Concentrate 1	30.46	30.1	26.06	0.397	27.0	0.964	9.05×10^3	100%	1 day
2	34.90	35.4	30.79	0.434	28.0	0.940	6.85×10^3	100%	2 days
3	39.18	39.4	34.35	0.447	28.5	0.930	6.32×10^3	100%	2 days
4	44.59	45.0	39.08	0.463	29.0	0.925	4.58×10^3	100%	4 days
			Cold Break at Ambient Temperature						
			Not available						

Table 3. Analytical data on concentrates prepared from VF-145-7879 variety tomatoes harvested on October 8, stored 40 hr and washed in unchlorinated water

	% Total Solids	°Brix	Total Acidity (Meq. H^+/100g)	Serum Color	Agtron E-5M Color Value	A_W (equil. RH)	Microbial Count on OSA	Microbial Spoilage	Spoilage in
Raw Material (pH 4.39)	6.16	5.6	4.95		25.0				
			Hot Break at 104.4° C						
Pulp	5.63	5.0	3.91	—	32.5	—	6.50×10^2		
Concentrate 1	27.86	27.8	26.80	0.492	31.5	0.965	3.62×10^4	100%	1 day
2	40.18	40.3	27.95	0.535	31.0	0.934	1.97×10^4	100%	2-3 days
3	48.84	48.8	34.17	0.540	31.0	0.907	1.81×10^4	100%	5 days
4	55.13	55.5	38.26	0.555	31.0	0.879	1.90×10^4	None	480 days
5	60.67	64.4	43.73	0.590	31.0	0.840	6.48×10^3	None	480 days
			Medium Break at 82.2° C						
Pulp	5.78	5.3	4.52	—	30.0	—	5.75×10^2		
Concentrate 1	31.67	30.8	24.94	0.485	30.0	0.958	6.68×10^3	100%	2 days
2	39.16	37.7	30.86	0.521	29.5	0.933	2.58×10^3	100%	3 days
3	47.90	47.4	37.62	0.538	30.0	0.903	3.65×10^3	100%	6-14 days
4	60.55	61.2	47.84	0.572	30.0	0.843	4.28×10^3	None	480 days
			Cold Break at Ambient Temperature						
Pulp	6.26	5.8	4.90	—	25.5	—	8.02×10^6		
Concentrate 1	35.08	35.2	27.76	0.313	29.0	0.931	1.80×10^6	100%	1 day
2	43.17	43.6	33.83	0.320	29.5	0.920	9.84×10^6	100%	1 day
3	52.86	54.3	41.22	0.391	30.0	0.882	1.96×10^6	100%	360 days
4	62.59	64.2	48.01	0.434	30.5	0.837	2.95×10^5	None	480 days

Table 4. Analytical data on concentrates prepared from VF-134 variety tomatoes harvested on October 13, and washed in unchlorinated water

	% Total Solids	°Brix	Total Acidity (Meq. H^+/100g)	Serum Color	Agtron E-5M Color Value	A_W (equil. RH)	Microbial Count on OSA	Microbial Spoilage	Spoilage in
Raw Material (pH 4.46)	6.32	5.8	3.59		25.0				
			Hot Break at 104.4°C						
Pulp	5.68	4.9	2.97	—	—	—	1.80×10^4		
Concentrate 1	32.40	31.5	20.84	0.521	30.0	0.960	3.89×10^5	100%	1 day
2	37.30	38.0	19.18	0.585	29.5	0.944	1.90×10^5	100%	1 day
3	43.95	44.0	23.09	0.606	30.5	0.925	4.80×10^5	100%	1 day
4	50.74	—	26.52	—	—	0.853	1.72×10^5	100%	4-5 days
			Medium Break at 82.2°C						
			Not available						
			Cold Break at Ambient Temperature						
Pulp	6.23	5.5	3.90	—	—	—	4.28×10^6		
Concentrate 1	33.43	33.8	21.77	0.358	25.0	0.947	2.88×10^5	100%	1 day
2	41.33	41.9	27.78	0.416	25.0	0.930	9.42×10^5	100%	4 days
3	44.10	45.1	28.86	0.429	25.0	0.916	1.27×10^5	100%	5-10 days
4	63.64	65.5	41.20	0.534	31.0	0.819	1.20×10^5	None	480 days

Table 5. Analytical data on concentrates prepared from Petomech II variety tomatoes harvested October 14, and washed in unchlorinated water

	% Total Solids	°Brix	Total Acidity (Meq. H^+/100g)	Serum Color	Agtron E-5M Color Value	A_W (equil. RH)	Microbial Count on OSA	Microbial Spoilage	Spoilage in
Raw Material (pH 4.40)	6.48	5.9	4.49		24.5				
			Hot Break at 104.4° C						
Pulp	5.88	5.0	3.22	—	—	—	6.18×10^2		
Concentrate 1	24.54	23.8	16.81	0.463	29.5	0.975	4.29×10^4	100%	1 day
2	34.06	33.6	19.74	0.521	29.0	0.960	1.41×10^4	100%	1 day
3	39.98	40.0	22.66	0.570	30.5	0.942	1.39×10^4	100%	1 day
4	48.20	54.0	27.90	0.589	30.0	0.910	2.38×10^4	100%	3 days
5	53.03	52.6	30.24	0.580	30.0	0.895	4.16×10^4	100%	9 days
			Medium Break at 82.2° C						
Pulp	5.79	5.1	3.67	—	—	—	2.88×10^5		
Concentrate 1	25.40	23.4	24.42	0.479	26.0	0.973	2.75×10^4	100%	1 day
2	35.60	32.3	24.51	0.589	27.0	0.950	3.25×10^4	100%	1 day
3	39.91	37.3	27.22	0.622	27.5	0.938	2.22×10^5	100%	1 day
4	47.46	45.7	32.52	0.710	28.5	0.911	2.80×10^5	100%	3-5 days
5	52.06	50.8	35.43	0.638	29.0	0.891	3.72×10^5	100%	12 days
			Cold Break at Ambient Temperature						
Pulp	6.30	5.6	4.36	—	—	—	7.20×10^6		
Concentrate 1	25.69	25.1	15.91	0.334	24.0	0.972	2.63×10^6	100%	1 day
2	32.41	32.0	23.58	0.337	25.0	0.953	1.52×10^6	100%	1 day
3	44.87	45.4	32.34	0.370	25.0	0.916	1.48×10^5	100%	5-9 days
4	54.36	54.8	38.68	0.473	26.5	0.880	8.25×10^4	100%	360 days

average summer temperature in the area, and caused less browning in the concentrates than could be expected at higher storage temperatures (Luh *et al.* 1958). Representative samples of the concentrates were stored at 0°C until analyzed.

Microbiological Analysis

Since industry uses OSA (Orange Serum Agar, Difco) to determine the number of viable spoilage-causing organisms in tomato paste, this medium was chosen for determining the original numbers of viable spoilage organisms in the juice and the resulting concentrates. Samples were pour-plated on the day of production and incubated 48 hr at 37°C.

Spoilage in the incubated canned samples was monitored by observing the cans for bulging which was indicated, when minimal, by the rising of the flip-top ring. The bulged cans were removed from incubation and the headspace gases were flame-tested for the presence of hydrogen. The combustion of headspace gases indicated the presence of hydrogen, i.e. the probability of a hydrogen swell. The spoiled concentrates had odors, and it was hoped that the odor of the spoiled cans would reflect the types of spoilage, i.e. by lactics, yeast, or butyric acid anaerobes, all of which generate predominantly noncombustible CO_2 (NCA 1968a).

Total Solids, pH and Total Acidity

Total solids were determined in quadruplicate, total acidity in triplicate, and pH as a single measurement according to the methods of AOAC (1975).

Percent total solids were calculated as

$$\% \text{ total solids} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

where W_1 = tare weight of drying dish and DE (diatomaceous earth)
W_2 = weight of drying dish, DE and wet sample
W_3 = weight of drying dish, DE and dried sample

Total acidity was calculated as

$$\text{TA (milliequivalent } H^+ \text{ per 100 g sample)} = \frac{\text{ml NaOH} \times \text{N} \times 100}{\text{sample weight in grams}}$$

°Brix Values

The °B values of the juice and concentrates were determined using the sugar scale on a Zeiss-Option Abbe-Refractometer maintained at 20°C with a constant temperature water bath. The °B values were used for estimating the solids levels during concentration.

Color of Concentrates

The concentrates were diluted to 8.5°B for color measurement in the Agtron E-5M (Magnuson Eng. Inc., San Jose, CA) reflectance meter. Pulp color was measured at natural °B. Lower Agtron E-5M values indicate better color. The color of the incubated concentrates was not evaluated.

Water Activity

Water activity measurements were made using a Beckman Hygroline meter (EZFBA-4-02-05-16) equipped with a Sina Equi-Hygro-Scope Humidity Sensor (ezFBA/cPP). Samples were filled into 40 × 12 mm plastic dishes, leveled to obtain a smooth surface, then placed into the sensor. Each sample was allowed to equilibrate (no change in value for 15 min) before readings were recorded. The reliability of the instrument was monitored by alternating standard saturated salt solutions with the samples. The accuracy of the instrument was within ± 1% relative humidity. Temperature of the measurements was 25°C.

Serum Color

Absorption measurements were taken at 420 nm against a water blank in a Bausch-Lomb Spectronic 70. The tomato serum was prepared from 5°B dilutions of the concentrates. The diluted concentrates were centrifuged, and the resulting serum was filtered through adjacent Gelman glass fiber (GF-E) and 0.2μ membrane filters. The measurements were made in a 1 cm Pyrex cell. Increases in absorption relate to thermal degradation, i.e. the formation of HMF (Luh *et al.* 1958).

Consistency of High Concentrates

For this phase of the study, 12.8°B commercial tomato puree was concentrated to various solids levels. Bostwick values were measured on 12°B dilutions, and the remanufactured consistency characteristics of the concentrates from each level were evaluated by converting the concentrates into catsup that contained 33% total solids with a Bostwick value of 6 cm. The procedure developed by Marsh *et al.* (1977)

determined the percentage of tomato solids required in the catsup formulation to achieve the fixed total solids and Bostwick values of a standard laboratory batch. Lower Bostwick values or lower lbs tomato solids requirement per 100 lb catsup indicate higher consistency.

The analytical work was not performed on the incubated concentrates.

RESULTS AND DISCUSSION

As in previous findings (Birnbaum *et al.* 1977), all concentrates at $<50.05\%$ solids levels ($A_W > 0.900$) spoiled after only a few days of incubation. The spoilage in these concentrates was characterized by the rapid formation of large amounts of noncombustible gases and the odor of lactic or butyric acids.

As A_W reached 0.900, the rate of development of spoilage slowed in the concentrates made from VF-145-7879 tomatoes and became evident only after 180 days of incubation (Table 1). Microbiological stability (through 480 days) was achieved in the VF-145-7879 concentrates at $A_W = 0.879$. The concentrates with $A_W = 0.882$, however, spoiled by the end of 360 days of incubation (Table 3). These samples spoiled slowly and formed only small amounts of gas. These samples had a fermented odor characteristic of spoilage caused by yeasts. None of the experimental packs showed mold growth.

Table 1 shows data for VF-145-7879 tomatoes which were washed in chlorinated water, while Tables 2–5 provide data on tomatoes which were given an unchlorinated water wash. The addition of chlorine to the wash water brought about an approximate tenfold reduction in the initial numbers of microorganisms in the concentrate (Tables 1 and 2). The microbial counts on samples prepared from the same variety stored an additional 24 hr varied (Table 3) but generally were higher than those stored only overnight (Table 2). The trend corresponds to that reported by Leonard *et al.* (1977).

The initial microbial counts and the spoilage pattern, as related to A_W and solids values, changed in the samples that were prepared from VF-134 and Petomech II tomatoes. These tomatoes, which contained higher water-insoluble solids, were harvested shortly after a rain. The tomatoes were encased in mud which the experimental equipment failed to wash off completely. The condition is reflected in the higher initial counts of microorganisms in the hot and medium break samples (Tables 4 and 5). There were high microbial counts and rapid spoilage for $A_W \geqslant 0.853$ (Table 4) in all samples except one, where spoilage

occurred at A_W = 0.880 in 360 days (Table 5). In the spoiled samples gas formation was rapid, and the odor of the concentrates was characteristic of spoilage by butyric acid anaerobes. Concentrates which were prepared from hot or medium break pulps spoiled faster at comparable A_W ranges than did the concentrates which were prepared from cold break pulp. These observations indicate that spoilage was heat activated, i.e. caused by an outgrowth of activated spores. The pH of these tomatoes was within an acceptable range for germination and growth of such spores, but the corresponding A_W values were as low as 0.852 which is below the limiting A_W = 0.95 defined by Ingram (1969), or A_W = 0.93, at which several workers managed to germinate spores of *Cl. botulinum* in NaCl (Hockenhull 1973). It appears that further work will be necessary to explain or clarify the above differences.

Since the experiment was designed to procure microbiological stability by decreasing A_W , a wide range of conditions was permitted in finding an approximate A_W value at which the tomato pastes would not spoil. According to the data, microbiological stability has been achieved at several A_W values, which varied according to the severity of the test conditions. Under the worst of conditions, i.e. harvesting after a rain, after substantial storage and washing without chlorination, microbial stability was achieved at A_W = 0.819 (63.65% total solids). When conditions were less severe, i.e. soil adherence was seasonally normal, microbiological stability was achieved repeatedly at and below A_W = 0.879, a level where pastes prepared from tomatoes harvested after the rain routinely spoiled. The role of water-insoluble solids in the spoilage pattern could not be established, because the rain and the resulting adherence of soil happened as an unexpected variable that changed the sanitary condition of the tomatoes (Sand 1973).

Temperature of the breaking procedure used to inactivate the pectic enzymes and/or extract the seeds and skins affected microbial counts in that the counts in heated pulps were consistently lower than in pulps which were extracted cold (Tables 1–5). The fluctuations in initial counts in the concentrates are believed to be the result of exposure to the conditions of concentration, to vacuum and to the temperature of the evaporating surface (138°C on the steam side), although the discharge temperature of the concentrates from the evaporator did not exceed 38°C at any level.

The pH was not controlled in this experiment, and the values varied in the raw material between 4.35–4.46. The pH of undiluted concentrates decreased as concentration increased. According to NCA (1968b), in addition to H^+ concentration, the pH of a product may reflect H^+ ion activity, thus it may be assumed that H^+ ion activity increased with increasing concentration of solids.

The economic feasibility of producing, storing, transporting and remanufacturing high concentrates involves considerations of energy sources and consumption, storage stability, and remanufactured quality. Luh *et al.* (1958) studied the storage stability of tomato pastes, and their findings may be a starting point in evaluating the storage stability of high concentrates. The economics of energy use was beyond the scope of this research, but some exploratory work on the remanufacturing quality of high concentrates was begun. The data are given in Table 6.

Analytical data on the concentrates prepared from commercial puree (pH 4.33) follow trends similar to those for concentrates prepared directly from raw tomatoes. There is a general increase in serum color with increasing concentration. The highest concentration from commercial puree was 68.59% total solids (A_W = 0.760) as compared to 65.73% (A_W = 0.805) prepared from fresh tomatoes (Table 1). Unheated 12°B dilutions of the concentrates from puree all had lower consistency (higher Bostwick values) than the 12°B dilution of the feedstock from which they were produced (Table 6). When heated, however, the diluted concentrates have been observed to regain most of their original consistency.

Catsup produced under standard conditions from the various concentrates also indicated that the remanufactured consistency decreased slightly at the various levels of concentration. This is shown in Table 6 in terms of the amounts of tomato solids required to produce 100 lb catsup at 6 cm Bostwick consistency. The variations shown are within the range observed for unintentional changes in the processing variables, and more data will be necessary to draw final conclusions.

The Agtron E-5M values obtained on the dilutions of pastes prepared from fresh tomatoes (Tables 1–5) show less change in color with concentration than the pastes produced from the commercial puree (Table 6). It appears that color changes observed in the pastes produced from canned commercial puree were compounded and accelerated by the heat treatment and handling that the canned material received.

Similarly to orange juice concentrates, tomato concentrates also undergo browning at elevated temperatures (Luh *et al.* 1958); thus storage temperature lower than 30°C would be advantageous, considering microbiological stability and the retention of quality (Faville *et al.* 1951; Luh *et al.* 1958; Hockenhull 1973). Although the temperature of incubation was representative of the average summer conditions in the Davis, CA area, it was not optimum for storing tomato concentrates. The quality attributes of the incubated samples were not evaluated.

Table 6. Analytical data on concentrates prepared from 12.8° B commercial puree, and the corresponding standards catsups

	Concentrate							Catsup	
	Total Solids	°Brix	Total Acidity (Meq. H^+/100g)	Serum Color Absorption @ 420 nm	Agtron E-5M Color Value	A_W equil. RH	Bostwick[1] cm @ 30 sec 12° B	lb Tomato Solids/100 lb	Agtron E Color Value
Puree Feed Stock (pH 4.33)	13.87	12.8	13.18	0.375	41	0.990	7.4	—	—
Concentrate A	29.23	27.6	27.59	0.427	42	0.970	8.2	14.19	33.0
B	43.33	43.8	40.51	0.500	43	0.921	8.3	14.45	35.0
C	54.60	55.0	51.09	0.528	45	0.877	8.1	14.68	37.0
D	57.74	—	53.67	0.535	46	0.867	7.7	14.98	37.0
E	68.59	69.9	65.80	0.580	50	0.760	9.2	14.82	37.0

[1] Unheated

Standard catsups produced from the commercial puree concentrated to various levels of solids exhibited a smaller range of Agtron E-5M values than did the concentrates. The apparent recovery of Agtron E-5M color value in the catsup is normal and is due to an interaction of tomato concentrate, vinegar, sugar, spices and heat used in the formulation.

CONCLUSIONS

NCA (1968a) listed the potential spoilage organisms in tomato products as species of the genus *Lactobacillus*, butyric acid anaerobes such as *C. pasteurianum* and *C. thermosaccharolyticum*, yeasts and the flat sour *B. coagulans*. The OSA plate counts on the samples did not necessarily enumerate all of the organisms mentioned but gave a comparative account, and reflected changes in wash water treatment, storage, harvest condition after the rain, and the breaking procedures.

Chlorination of the wash water and application of heat to inactive enzymes each demonstrated bactericidal effect in reducing the initial number of potential spoilage organisms.

The data, based upon the natural microbial flora and conditions of a single growing season and area, indicate that within the experimental procedures used, tomato concentrates with appropriately high levels of solids remained microbiologically stable without thermal preservation. The remanufacturing quality of the high concentrates decreased slightly, but additional work is needed to show whether the indicated decrease would be significant. Additional work is needed for evaluating the variations in the natural microbial flora and the chemical composition of tomatoes as they would influence the control of spoilage by A_W. Since tomato concentrates undergo browning with heat treatment and at elevated storage temperatures, quality retention and biological stability (storage life) should be studied at $<30°C$, i.e. possibly considering cold storage.

ACKNOWLEDGMENT

Appreciation is expressed to Langeberg Co-Op Ltd. for grants-in-aid to partially support this project.

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NUTRITIVE VALUE OF PROPIONIBACTERIA AND LACTOSE-FERMENTING YEAST GROWN IN WHEY[1]

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Received for Publication August 25, 1977

ABSTRACT

The nutritive values of propionic acid bacteria (Pb) grown in whey media as mixed cultures and as a 2-stage fermentation with a lactose-fermenting yeast (Y-Pb) were determined.

Yields were 14 *g of cells per liter for Pb cultures and* 16 *g/liter for Y-Pb fermentations. When the fermentation medium and cells were dried together, total solids equalled* 28 *g/liter. The BOD of the whey was reduced over* 90% *in both fermentations. Adequate yields of vitamins, particularly* B_{12}, *were obtained in both fermentations. The* in vitro *enzymatic digestibility of the cultures was increased by mechanical disruption of cells; however, this cell breakage did not affect Biological Value (BV). The essential amino acid composition was equal to or better than the FAO Standard Reference Protein. The BV of the Y-Pb was* 25% *higher than Pb and* 10–40% *higher than other microbial cell masses, including Torula yeast, commercial yeast and BP yeast from n-paraffin.*

INTRODUCTION

Whey, the by-product of cheese manufacture, creates a world-wide

[1] This work was supported in part by PL 480 Grant No. FG-Po-293 (Project PL-ARS-11) from the U.S. Department of Agriculture to the Academy of Agriculture, Poznan, Poland.

problem of waste disposal of considerable proportions. Fresh untreated whey is used in animal feeding in some countries, but high transportation costs and rapid spoilage seriously limit its use (Schingoethe 1976). Dry whey is widely used in animal feeds and human foods, and dried whey added to cereals increases their nutritional value (Womack and Vaughan 1972). Whey proteins isolated by various methods are increasing in use as protein supplements (Al-ani *et al.* 1972).

Considerable effort has gone into the use of whey as a medium for different microorganisms in order to obtain biomasses (cell concentrates) that are high in proteins (Al-ani *et al.* 1972; Vananuvat and Kinsella 1975). Other workers have explored the potential of using whey for the biosynthesis of Vitamin B_{12} and vitamin-protein concentrates by propionic acid bacteria (Bullerman and Berry 1966; Leviton and Hargrove 1952).

This investigation was undertaken to explore the possibilities of using propionic acid bacteria, alone and in conjunction with yeast, to produce a cell mass rich in protein and vitamins, while reducing the Biochemical Oxygen Demand (BOD) of the residual fluid to more nearly acceptable levels.

MATERIALS AND METHODS

Cultures

Twenty-five strains of *Propionibacterium* selected from the culture collection of the Institute of Food Science were grown in whey medium to determine their growth, lactose and lactate utilization, and Vitamin B_{12} production. The three cultures selected for this investigation were *P. shermanii 1*, *P. petersonnii J* and *P. freudenreichii freudenreichii J.*

Nine strains of yeast from the culture collection were examined for the completeness and rapidity with which they fermented the lactose in whey medium. Three strains selected were *Kluyveromyces fragilis CBS* 397, *Torula sp.* (B), and *Mycoderma sp.* (S).

Media

Propionobacteria Stock Medium. Aqueous yeast extract, 1000 ml; enzymatic hydrolysate of casein (0.99 g N/100 ml), 50 ml; KH_2PO_4, 1 g; sodium lactate, 10 g; NaCl, 0.5 g; agar, 30 g; pH 6.8.

Yeast Stock Culture Medium. Aqueous yeast extract, 1000 ml; glucose, 40 g; peptone, 5 g; agar, 25 g; pH 5.8–6.0.

Inoculum Growth Medium. Acid hydrolysate of casein (2%N), 100 ml; enzymatic hydrolysate of casein (1.0%N), 200 ml; K_3PO_4, 0.88 g; NaH_2PO_4, 0.88 g; $MgCl \cdot 6H_2O$, 0.4 g; $CaSO_4 \cdot 7H_2O$, 12 mg; distilled H_2O to make 940 ml. The following solutions were sterilized by tyndallization (flowing steam for 60 min on 3 successive days) and added to the above medium: 0.1% $FeSO_4 \cdot 7H_2O$, 10 ml; 0.025% Ca panthothenate, 10 ml. 0.003% biotin, 10 ml; 60% glucose, 30 ml.

Propionibacteria (Pb) Fermentation Medium. Cheese whey (from Cottage-type cheese: Total solids, 5.6%; lactose, 4.0–4.1%, protein (Nx6.25) 0.75%, pH approximately 4.4, 1000 ml; $CaSO_4$, 12 mg; adjusted to pH 6.8 with NH_4OH.

Yeast-propionibacteria (Y-Pb) Fermentation Medium. Cheese whey supplemented with 0.5%, $(NH_4)_2SO_4$ and 0.5% K_2HPO_4 and adjusted to pH 5.7 with NH_4OH.

Dry Weight

Unless otherwise noted dry weights were obtained from freeze-dried samples.

Fermentation

Propionibacteria. Equal volumes of 48 hr cultures of *P. shermanii 1*, *P. petersonii J.*, and *P. freudenreichii J* were mixed and an inoculum equal to 20% of the volume of whey medium used. Five liters of medium in a 6-liter Erlenmeyer flask were incubated at 30°C for 8 days; pH was adjusted daily to 6.8–7.2 with NH_4OH. On the 6th day 5, 6-dimethylbenzimidazole (16 mg/liter) was added as Vitamin B_{12} precursor. Cells were centrifuged at 1900 × g for 1 hr, washed with distilled water and re-centrifuged.

Yeast-propionibacteria. *Kluyveromcyes fragilis* CBS 397 was the yeast of choice since it had been shown to almost completely utilize the lactose in whey medium. The Y-Pb fermentation medium (20 liter in 50 liter glass fermentor) was inoculated with 20% by volume of a 12 hr culture of *K. fragilis CBS 397* and incubated at 30°C with continuous aeration. (Air flow rate: 50 liters/liter culture medium/hour passed through sparger at bottom of fermentor). After 8–10 hr the lactose content had decreased to 0.5% and the medium was heated to boiling for 5 min. After cooling the pH was adjusted to 7.0–7.2 and 16 mg $CaSO_4$/liter added. Forty-eight hour cultures of the three propionic acid bacteria were mixed and added (1 liter/7 liter medium); fermentation was continued for 72 hr at 30°C; pH was adjusted daily to 6.8. Four hours before termination of fermentation 5, 6-dimethylben-

zimidazole (16 mg/liter) was added. The yeast-bacteria cell mass was spun down at 1900 × g.

Chemical Methods

Total Nitrogen. Determined by the Kjeldahl method. Protein was calculated as 6.25 × Kjeldahl N determined on trichloroacetic acid precipitate of the cell mass.

Lactose. Determined by the Association of Official Analytical Chemists procedure (1965) and BOD was determined by the standard American Public Health Association procedure (1971).

Isolation of Corrinoids (B_{12}). Approximately 60 g of fresh cell mass (from 1 liter culture) was suspended in 250 ml distilled water. 100 mg KCN was added, and the pH adjusted to 4.0. The sample was heated to 80°C for 15 min, cooled, centrifuged, the sediment washed and re-centrifuged. Corrinoids were extracted from the centrifugate with several phenol treatments, and the combined phenol extracts were washed and filtered. The clear phenol filtrate was extracted with an equal volume of butanol-chloroform (1:1 V/V). The corrinoids were removed from the butanol-chloroform by extraction with small amounts of distilled water and then concentrated by evaporation under reduced pressure.

Separation of Corrinoids. The isolated corrinoids were separated electrophoretically by the method of Holdsworth (1953).

Amino Acids. The amino acids were determined on acid hydrolysates by single column resin chromatography in an automatic amino acid analyzer (Beckman-Multichrom 4225) following the standard procedure. In this procedure cystine is converted to cysteine, and a part of it may be further oxidized to cysteic acid. Thus the values reported may be low when only the cysteine peaks are measured. However, since the same procedure was used throughout this study, the amino acid compositions are still comparable, and it is the comparable values, not absolute values with which we are concerned.

Nucleic Acids. Determined by the methods of Mauron (1970).

Nutritional Evaluation

In Vitro Enzymatic Digestibility of Protein. Determined by two methods: (1) pepsin and pancreatin, (2) Pronase (protease from *Streptomyces griseus).* These methods are described by Mauron (1970) and modified by Stasinska *et al.* (1975).

Net Protein Utilization (NPU). Determined by the Bender and Miller procedure (1953) on groups of 30-day old rats (2 male, 2 female per

group, each diet in duplicate). For ten days animals were fed a standard diet supplemented with 10% protein supplied by one of the biomasses.

$$\text{NPU} = \frac{\text{Food N} - (\text{fecal N} - \text{endogenous fecal N}) - (\text{urinary N} - \text{endogenous urinary N}) \times 100}{\text{Food N}}$$

True Digestibility Coefficient (TDC). Determined on duplicate groups of rats (4 male, 4 female, 150 g body weight per group). Test diets were same as for NPU and were fed for 10 days. Food consumption and fecal nitrogen were determined from the 5th through 10th day.

$$\text{TDC} = \frac{\text{Food N} - (\text{Food N} - \text{metabolic N}) \times 100}{\text{Food N}}$$

$$\text{Biological value (BV)} = \frac{\text{NPU} \times 100}{\text{TDC}}$$

Vitamin Assays

Corrinoids. Total corrinoids were assayed microbiologically with *Escherichia coli* 113-3 (Trojanowska *et al.* 1975). Spectrophotometric determinations were also made by converting corrinoids to di-CN-form (2 hr incubation with KCN). For quantitation the extinction coefficient $E^{1\%}_{1cm} = 105$ was applied.

Thiamine was assayed microbiologically with *Lactobacillus fermenti* 36 (Myskowska *et al.* 1963). Other vitamins were assayed by Association of Vitamin Chemists Methods (1966).

RESULTS

The cell yields and lactose utilization by the three cultures used in this investigation are shown in Table 1. The *P. shermanii 1* and *P. petersonii J* had been selected because they gave the highest lactose utilization of the 25 cultures tested as well as good B_{12} production; *P. freudenreichii J* was chosen because of its lactate utilization. The data show that a combination of the three in mixed culture gave almost complete utilization of the lactose, as well as a 25% increase in cell yield and a 20% increase in B_{12}.

In Table 2 the cell yield (dry weight) of the Y-Pb is shown to be about 15% higher than the propionibacteria alone while the total pro-

Table 1. Cell yields and lactose utilization by selected strains of propionibacteria in pure and mixed culture

Propionibacterium Species	Dry Wt. of Cells g/liter	Lactose Utilized %	B_{12} Produced μg/g Dry Wt.
P. shermanii 1	10.9[a]	66.1	360
P. petersonii J	9.7	61.3	460
P. freudenreichii J	7.8	18.7	170
Mix of all three	13.7	95.0	550

[a]All values are mean values of at least duplicates

Table 2. Yield of cells, protein and nucleic acids from different cultures grown on whey medium

Cell Mass[a]	Wet Weight g/liter	Dry Weight g/liter	Protein % Dry Wt.	Nucleic Acid	
				DNA % Dry Wt.	RNA % Dry Wt.
1	62.2[b]	13.7	58.9	3.4	4.3
2	—	28.1	54.1	2.3	2.6
3	63.3	15.8	56.2	2.5	3.1

[a](1) Propionibacteria (freeze dried)
(2) Propionibacteria, whole culture (spray dried)
(3) Yeast-propionibacteria (freeze dried)
[b]All values are mean values of at least duplicates.

tein is about 10% higher. When the culture supernatant is dried along with the cells, however, protein content increases by approximately 50%. The lactose is almost completely utilized and the BOD of the whey is reduced over 90% (Table 3) to a residual that could be more easily handled by a secondary treatment. The differences in vitamin content among the cultures were relatively small (Table 4). The lower vitamin B_{12}, riboflavin and folic acid in the Y-Pb culture than in the Pb culture alone reflects the higher yields produced by the latter.

The digestibility of proteins can be estimated by comparing the α-amino N released by enzymatic digestion with that obtained by acid hydrolysis. Only about 15–20% of both cell masses was hydrolyzed by a pepsin-pancreatic enzyme preparation (Table 5). Disintegration of the cells improved the digestibility of both preparations but was still near 30%. Breakdown was slightly greater with the microbial protease, Pronase.

Table 6 indicates only minor differences in the percentages among

Table 3. Lactose utilization and Biochemical Oxygen Demand reduction of whey medium by different cultures

Cell Mass[a]	Lactose		BOD of Medium	
	Before Fermentation %	After Fermentation %	Before Fermentation %	After Fermentation %
1	4.0[b]	0.2	33,500	2,500
2	3.8	0.2	—	—
3	4.0	0.1	32,000	2,700

[a](1) Propionibacteria (freeze dried)
(2) Propionibacteria, whole culture (spray dried)
(3) Yeast-propionibacteria (freeze dried)
[b]All values are mean values of at least duplicates

Table 4. Vitamin content of different cultures grown in whey medium

Cell Mass[a]	B_{12}	Thiamine	Riboflavin	Niacin	Folic Acid	Ca Pantothenate	Biotin
	mg/100 g						
1	55.0[b]	0.6	2.2	31.3	1.8	2.7	0.15
2	26.1	0.5	2.1	29.6	1.1	2.2	0.12
3	24.7	0.8	0.6	27.3	0.6	3.6	0.21

[a](1) Propionibacteria (freeze dried)
(2) Propionibacteria, whole culture (spray dried)
(3) Yeast-propionibacteria (freeze dried)
[b]All values are mean values of at least duplicates

the ten essential amino acids shown for Pb and Y-Pb. Seven other amino acids also were comparable. It is also shown in this table that the essential amino acid composition of the Pb and Y-Pb cells are compared quite favorably with the ideal protein described by FAO and the analyses of several other commonly used proteins.

As a final investigation of its nutritive value, the Y-Pb cell mass was compared in a rat-feeding trial with a commercial food yeast preparation available in Poland (Table 7). Both the NPU and BV were 25–35% higher in the Y-Pb culture than in the food yeast. These values compare favorably with other microbial proteins as summarized by Lipinsky and Litchfield (1974). Disintegration of the cells only slightly improved utilization by the rats.

Table 5. Enzyme digestibility of different biomasses grown on whey medium

Cell Mass[a]	α-NH_2 N	Enzyme Hydrolysis	
		Pepsin + Pancreatic Pronase	
	g/100g Dry Wt.	%	%
1	8.8[b]	20.2	21.6
1A	8.6	33.7	35.0
2	7.5	18.8	30.8
3	7.8	13.5	23.6
3A	7.9	17.4	31.0

[a](1) Propionibacteria
(1A) Propionibacteria cells disintegrated by means of Hughes-type press (Giec and Skupin 1974)
(2) Propionibacteria, whole culture
(3) Yeast-propionibacteria
(3A) Y-Pb cells disintegrated
[b]Values from acid hydrolysis. 100% hydrolysis assumed. All values are mean values of at least duplicates

Table 6. Comparison of essential amino acids in different proteins

Amino Acid	Propioni-bacteria	Yeast-Propioni-bacteria	FAO[a]	Whey Protein Concen-trate[b]	Soya Bean Meal[c]	BP Yeast L[c]	S. fragilis[d]
			—g/16gN—				
Isoleucine	3.9	4.0	4.2	5.7	5.4	5.3	6.1
Leucine	9.4	9.8	4.8	12.3	7.7	7.8	9.6
Phenylalanine	2.3	2.5	2.8	3.8	5.1	4.8	5.4
Tyrosine	4.0	3.3	2.8	3.2	2.7	4.0	3.4
Valine	4.7	4.3	4.2	6.1	5.0	5.8	7.8
Lysine	6.7	7.9	4.2	10.3	6.5	7.8	10.2
Cystine	1.3	2.6	2.0	2.3	1.4	0.9	—
Methionine	3.3	2.4	2.2	2.3	1.4	1.6	1.2
Threonine	4.3	4.4	2.8	5.8	4.0	5.4	6.5

[a] FAO/WHO (1973)
[b] McDonough *et al.* (1976)
[c] Shacklady (1975)
[d] Wasserman (1961)

DISCUSSION

It is well-known that propionibacteria are excellent producers of Vitamin B_{12} and other vitamins but their poor growth has limited

Table 7. Biological indices of Yeast-propionibacteria cell mass and of commercial Torula yeast

	NPU[a]	TDC[b]	BV[c]
Yeast-propioni-bacteria	54.6[d]	78.8	69.3
Yeast-propioni-bacteria, disintegrated	61.7	80.1	70.0
Commercial food yeast in Poland	39.8	81.4	48.9

[a] Net protein utilization
[b] True digestibility coefficient
[c] Biological value
[d] All values are mean values of at least duplicates

studies on production of cells. The procedure reported here, however, has produced reasonable cell yields, and in a mixed yeast-propionibacteria culture, a cell mass is obtained that compares favorably with other single-cell proteins in nutritive value. The higher methionine levels in this product is a distinct advantage since many SCP products need supplemental methionine (Lipinsky and Litchfield 1974).

The high nucleic acid content of microorganisms is a limiting factor in the acceptability of SCP, particularly from bacteria. Their metabolic products e.g., purines, lead to uric acid formation which in excess amounts can lead to gout or kidney stone formation in humans (Sinskey and Tannenbaum 1975). This would not be a problem in animal feeds, however. The mixed fermentation cells have nucleic acid concentrations similar to those from yeast cells alone.

Thus, when coupled with the utilization of a waste product as the substrate, the mixed yeast-propionibacteria fermentation reported here is another source of SCP that could have application in areas where whey is not being utilized.

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COLOR STABILITY OF BEET POWDERS

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Received for Publication September 7, 1977

ABSTRACT

Color thermal stability kinetics tests of the major beet pigments, betanine and vulgaxanthin (I), were conducted in beet powder during storage (25, 31, 35, 40 and 45°C) and in its reconstituted solution (40 and 86°C heat treatment at pH 4.0, 5.7 and 6.8).

The powders evaluated were produced by two different drying methods — drum drying and the conventional cube dehydration. Pigment degradation rates generally followed a first order reaction with an energy of activation of 7.0 and 8.0 Kcal/mole for betanine and 6.2 and 6.4 Kcal/mole for vulgaxanthin for drum and air dehydrated powders, respectively. Optimal pigment stability in the reconstituted juice was obtained at pH 5.7 for both the high and low tested temperatures (40 and 86°C).

Freeze dried powder (used as a control), although having a higher initial pigment concentration, showed a markedly higher degradation rate in its reconstituted juice at the 40°C temperature, thus suggesting an enzymatic pigment degradation activity in the native beet.

INTRODUCTION

As a consequence of heavier restrictions on the use of synthetic coloring compounds in foodstuffs, considerable attention has been focused in recent years on natural food pigments as substitutes.

Red beet is one of the most logical sources of water-soluble colorants, and has found extensive use in this context (von Elbe and Maing 1973; von Elbe *et al.* 1974a; Pasch *et al.* 1975; von Elbe 1975). The principal manner in which beet color is being used is in the form of powder or reconstituted beet juice, added to various food products with different pH's, and which might undergo further thermal processing.

The color of beet consists of two major pigments — the betanine (red) and vulgaxanthin (yellow). Stability and kinetic parameters (of

Journal of Food Process and Preservation 1 (1977) 217—224.

interest to the food industry) are available only for model solutions of betanine or for beet juice systems (Austad and Dahle 1973; von Elbe *et al.* 1974b; von Elbe 1975; Pasch and von Elbe 1975). Similar information on the other main pigment, vulgaxanthin, or for beet powders or their reconstituted form is scarce.

The purpose of this investigation was to determine the stability of the major beet pigments, betanine and vulgaxanthin, in beet powder and in reconstituted juice. The powders were manufactured by two different methods, drum drying (Kopelman & Saguy 1977) and the conventional cube dehydration method.

MATERIALS AND METHODS

Beet Powders

Beet powders were produced by (a) the common cube dehydration; (b) a proposed drum drying technique; (c) freeze dehydration (used as a reference).

Drum Drying. Drum dried beet powder was prepared according to the procedure outlined by Kopelman & Saguy (1977), whereby 0.5 cm cubes of Detroit beet root were ground down to $\sim$ 5 μ particles slurry. The slurry was dried down to 4.0% moisture powder, on a 35 ϕ $\times$ 60 cm drum drier (Escher Wyss), with 18 sec retention time, 123°C drying temperature and 0.137 mm gap.

Cube Dehydration — Drying was carried out according to the procedure outlined by Kopelman & Saguy (1977), whereby beet cubes (0.5 cm) were dried in a controlled (±0.5°C) cabinet with 250 fpm air velocity, to a final 4.0% moisture.

Freeze Dehydration. Beet cubes (0.5 cm) were freeze dried on a 2.5m^2 Grenco Rl-25 pilot plant unit for 20 hr at 0.01–0.2 Torr with the heating plate maintained at 40°C. The heat was supplied by radiation from both sides of the product tray. Condenser temperature was kept at 45 to 50°C below zero. Final moisture was 1.5%.

All beet cubes dehydrated by the various methods were ground down to pass through a U.S. # 60 sieve.

Analytical Methods

Color measurements for betanine and vulgazanthin (I) were determined simultaneously by the method described by Saguy *et al.* (1978).

The procedure is based on computer-aided nonlinear curve fitting of the visible spectrum of the beet solution with a predicted function of the individual beet pigments, thus dispensing completely with the separation stage of the pigments.

Storage studies were conducted for beet powders placed in 25, 31, 35, 40 and 45°C thermostatically controlled (±0.25°C) cabinets for up to 200 days period. Samples were withdrawn periodically and were analyzed for color content. The powders were placed at the final moisture content in sealed glass vials and no change of moisture occurred during storage.

Beet powder reconstituted aqueous solutions (0.1% w/v) were adjusted to the desired pH levels (4.0, 5.7 and 6.8) with 0.1N HCl or 0.1N NaOH as required. Ten ml of the reconstituted solution were filled up with zero headspace into vials. The vials were sealed and placed in a thermostatically controlled (±0.05°C) shaking bath for the thermal treatment. The vials were withdrawn periodically and cooled down immediately in an ice bath and analyzed for color content.

Data were processed and analyzed on the Technion IBM 370/168 computer. Regression lines and related statistical coefficients were obtained by BMDO2R (Dixon 1971).

RESULTS AND DISCUSSION

Color stability studies of the drum and the conventional air dehydration beet powder, conducted at various storage temperatures (Fig. 1 and 2), showed that both products followed a first order reaction, i.e.

$$C/C_o = \exp(-kt) \quad (1)$$

where:

- C — pigment concentration at time, t
- C_o — initial pigment concentration
- k — rate constant (k_1-betanine; k_2-vulgaxanthin)
- t — time.

The degradation rate constant, k_1 and k_2, or their alternate format, the half life time, $T_{1/2}$, are listed in Tables 1 and 2 for betanine and vulgaxanthin, respectively. The rate constants and the half life values indicate that betanine and vulgaxanthin retention during storage is slightly better in the drum dried powder as compared to the conventional air dehydration product. The degradation rate constants (Tables 1 and 2) can be fitted into an Arrehenius temperature coefficient pattern (Fig. 3) yielding an energy of activation of 7.0 and 8.0

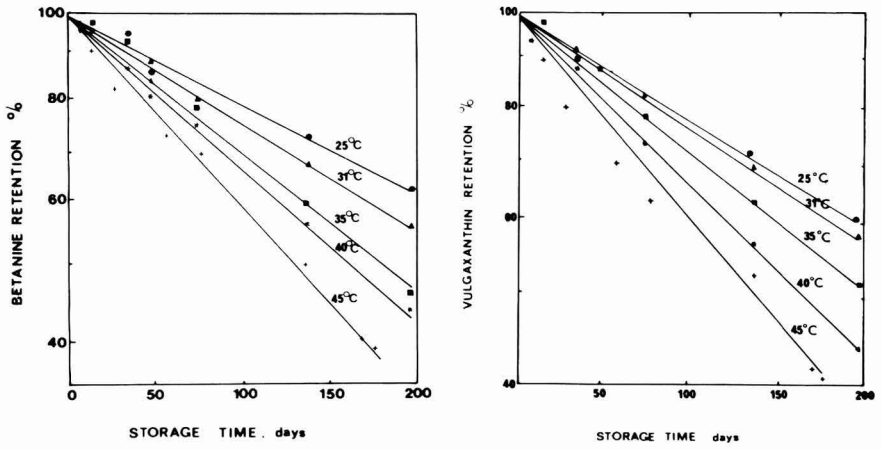

FIG. 1. PIGMENT RETENTION DURING STORAGE OF DRUM DRIED BEET POWDER

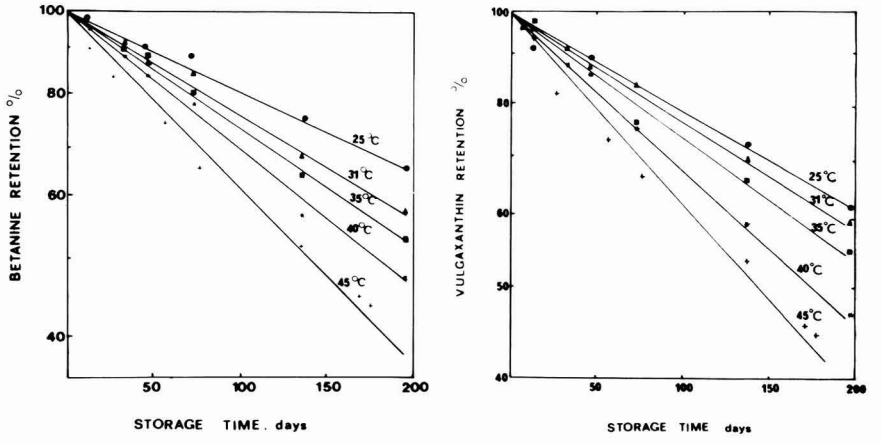

FIG. 2. PIGMENT RETENTION DURING STORAGE OF CONVENTIONAL AIR DEHYDRATED BEET POWDER

Table 1. Betanine degradation rate constants of beet powders

Temperature (°C)	DRUM		CABINET	
	$k_1 \times 10^3$ [a] (day^{-1})	$T_{1/2}$ [b] (day)	$k_1 \times 10^3$ (day^{-1})	$T_{1/2}$ (day)
25.0	2.34	296.9	2.65	261.5
31.0	2.86	242.4	3.02	229.5
35.0	3.41	203.3	4.02	172.4
40.0	3.81	181.9	4.72	146.8
45.0	4.37	159.7	5.02	138.1

[a] Rate constant
[b] Half life

Table 2. Vulgaxanthin degradation rate constants of beet powders

Temperature (° C)	DRUM $k_1 \times 10^3$ [a] (day^{-1})	DRUM $T_{1/2}$ [b] (day)	CABINET $k_1 \times 10^3$ (day^{-1})	CABINET $T_{1/2}$ (day)
25.0	2.52	275.0	2.40	288.8
31.0	2.59	267.6	2.82	245.7
35.0	3.15	220.0	3.42	202.6
40.0	3.86	179.5	4.17	166.2
45.0	4.33	160.0	4.65	149.0

[a] Rate constant
[b] Half life

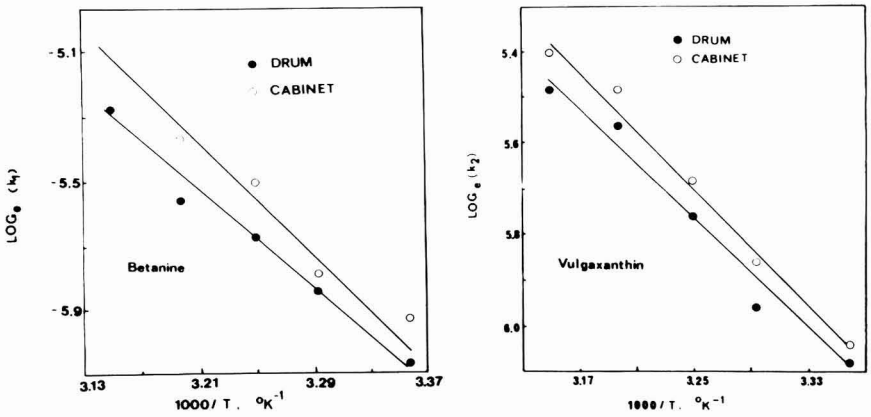

FIG. 3. EFFECT OF TEMPERATURE UPON PIGMENT DEGRADATION RATE CONSTANT OF BEET POWDERS

Kcal/mole for betanine and 6.2 and 6.4 Kcal/mole for vulgaxanthin for drum and air dehydrated powders, respectively. Table 3 indicates that the initial pigment concentration of the freeze dried powder was higher as compared to that dried by either the drum or the cabinet methods. The above can be expected due to pigment loss from the thermal effect of the drying process. In a similar manner, but to a much lesser extent, the reconstituted drum dried powder showed better pigment retention compared to that of the cabinet dried powder.

At the high temperature evaluated (86° C) the degradation rate of the aqueous solutions reconstituted from all three types of powders showed a similar pattern, i.e. a first order reaction with similar degradation rates as seen in Table 3.

On the other hand, compared to either the drum or the cabinet pow-

Table 3. Effect of temperature on pigments degradation in aqueous solution reconstituted from beet powders

Temperature (°C)	Time	Betanine (%)									Vulgaxanthin (%)								
		Drum[a]			Cabinet[b]			Freeze Dried[c]			Drum[d]			Cabinet[e]			Freeze Dried[f]		
		ph 4.0	5.7	6.8	4.0	5.7	6.8	4.0	5.7	6.8	4.0	5.7	6.8	4.0	5.7	6.8	4.0	5.7	6.8
86.0	0 min	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	5	78.4	71.9	70.2	80.4	86.3	79.2	73.7	78.9	76.2	53.5	74.0	70.7	72.1	87.9	78.5	68.4	79.0	74.0
	10	48.0	67.7	61.4	62.0	69.9	65.9	59.0	66.5	61.6	41.5	69.7	59.3	53.1	72.8	65.9	50.1	68.0	60.3
	15	34.7	54.4	52.3	39.0	58.2	51.0	44.1	57.5	44.5	31.0	59.4	53.5	37.1	63.1	54.6	37.2	59.3	52.0
	25	21.7	38.6	34.6	25.3	39.8	34.5	26.5	41.8	32.9	17.7	45.9	36.1	25.3	47.6	39.2	29.0	43.5	38.0
40.0	0 hr	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	3	87.1	84.2	87.7	18.3	77.4	82.3	10.2	73.3	77.6	87.5	94.4	86.4	49.7	89.3	80.1	46.3	73.0	74.7
	24	41.3	44.5	50.8	4.4	35.7	44.2	1.5	3.5	17.8	37.3	63.8	42.8	12.0	40.9	40.5	7.1	9.4	25.3
	48	16.7	18.2	26.1	—	14.6	16.4	—	—	7.6	11.7	44.9	28.5	6.1	28.6	12.9	—	—	16.5

Betanine initial concentration (mg/g powder): a — 6.8; b — 6.80; c — 8.21
Vulgaxanthin initial concentration (mg/g powder): d — 5.78; e — 5.47; f — 6.60

ders the reconstituted freeze dreid powder showed a much higher degradation rate at the lower (40°C) temperature evaluated. This strongly suggests a possible enzymatic activity in pigment degradation. Such a potential enzyme activity is preserved in the freeze dried method and becomes quite effective in the 40°C temperature evaluated.

Table 3 also shows that minimal degradation of betanine and vulgaxanthin were obtained at pH 5.7 for the high and low temperature evaluated, thus indicating the greatest stability of the pigments tested at pH 5.7. This optimal stability at pH 5.7 was also indicated by Saguy (1977) for other beet systems, such as beet juice and beet slices. However, the reconstituted freeze dried powder at the low temperature evaluated (40°C) showed a shift from pH 5.7 to 6.8 for the minimal degradation, indicating the influence of the pH on the enzymatic activity.

The above data indicate the great importance of adequate thermal inactivation in minimizing color loss. Since diced beet cubes do not undergo blanching (as to avoid leaching of the water soluble pigments), more attention should be given to the time-temperature air dehydration program as to assure sufficient deactivation. In this context the drum drying process for beet powder production seems to offer more effective enzyme inactivation during the course of drying.

Further study as to the specific enzymatic color degradation in various beet systems is being presently carried out.

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MOISTURE TRANSFER IN A PACKAGED PRODUCT IN ISOTHERMAL STORAGE: EXTRAPOLATING DATA TO ANY PACKAGE-HUMIDITY COMBINATION AND EVALUATING WATER SORPTION ISOTHERMS

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Received for Publication September 13, 1977

ABSTRACT

Moisture increase data of packaged food samples stored at constant temperature were used to calculate the sorption isotherm of the food. This method uses either the derivative of time versus moisture content curve or an integrated form of an appropriate isotherm equation. A technique was also developed for extrapolating the above data to any given package-storage humidity combination. This is accomplished by utilizing the previously determined isotherm or by transformation of polynomials. These methods were successfully tested in dehydrated cabbage and in cottonseed flour.

INTRODUCTION

Data on moisture content of packaged food stored under continuously changing isothermal conditions were used in a method for accelerated stability tests reported by us earlier (Mizrahi and Karel 1977a) and in calculating parameters for a kinetic model of deteriorative reactions (Mizrahi and Karel 1977b). These methods are applicable to moisture-sensitive products in which the moisture content-time history is known. Since this history depends on sorption isotherms, it should be possible to develop a method based on this data for the evaluation of the isotherm. Such a method enables calculation of moisture changes in other container-storage humidity combinations without need for an independent determination of the sorption isotherm. We report here the use of a recorded moisture change in a product stored under controlled conditions to evaluate the isotherm and to calculate moisture content as a function of time for any isothermal package-storage combination.

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EXPERIMENTAL PROCEDURE

The equation for the sorption isotherm of dehydrated cabbage at 37°C was taken from Mizrahi *et al.* (1970) (Equation 1)

$$a = \frac{m - 0.579}{m + 9.583} \tag{1}$$

where a = water activity
m = moisture content (g H_2O/100 g solids)

Moisture gain data were obtained by simulation of storage according to the procedure outlined by Mizrahi *et al.* (1970) using the Wang Model 380 programmable calculator. Similar data were obtained experimentally for extracted, deglanded cottonseed flour (PRO-FAM C-650, Grain Processing Corporation, Muscatine, Iowa). Prior to the following analyses the flour was kept at room temperature in a desiccator over drierite (anhydrous calcium sulfate) for five days.

The sorption isotherm of the cottonseed flour at 37° C (±0.2° C) was determined by placing 5 g of the flour in an evacuated desiccator over saturated salt solutions maintaining a constant water activity of 0.11 (LiCl), 0.22 (CH_3COOK), 0.32 ($MgCl_2$), 0.43 (K_2CO_3), and 0.53 ($Na_2Cr_2O_7$). The moisture content was calculated from the weight of the sample after 48 hours. Moisture gain data were obtained by monitoring weight changes in 10 g flour samples packaged in 1 mil thick 7 × 5.5 cm, polyvinyl chloride pouches. These pouches were obtained through the courtesy of the U.S. Army Natick Research and Development Command. The samples were placed in a desiccator over saturated NaCl solution (a=0.75) and kept in an incubator maintaining 37 ± 0.2° C. Permeability of the package to water vapor was determined from the initial moisture gain. Curve fitting was performed by an IBM 360 system at the MIT Information Processing Center. Programs BMD P5R and BMD P3R (Dixon 1975) were used for polynomial and nonlinear regression respectively.

RESULTS AND DISCUSSION

A convenient mathematical expression for moisture changes in isothermal storage of a sample packaged in a permeable container is:

$$t = F(m) \tag{2}$$

where t = time (days)

The rate of moisture transfer can be calculated from:

$$\frac{dm}{dt} = K(a_e - a) = K[a_e - f(m)] \tag{3}$$

where K = permeance constant g H_2O/(100 g solids) (day)
a_e = water activity in the atmosphere
f(m) = sorption isotherm

The isotherm f(m) can be evaluated by two techniques: (1) by the derivative of Equation 2; and (2) by integrating Equation 3. The first technique is applicable to any isothermal storage, even to those cases where a_e changes with time. Differentiating Equation 2 and equating to Equation 3:

$$\frac{dm}{dt} = \frac{1}{F'(m)} = K[a_e - f(m)] \tag{4}$$

thus

$$f(m) = a_e - \frac{1}{KF'(m)} \tag{5}$$

If a more appropriate model is not available, Equation 2 can be fitted by a polynomial. The moisture gain data (Fig. 1) obtained for cottonseed flour was fitted, for example, by:

$$t = -0.033 + 0.213m + 0.055m^2 + 0.0017m^3 \tag{6}$$

The derivative is:

$$F'(m) = 0.213 + 0.01m + 0.0051m^2 \tag{7}$$

Thus the sorption isotherm can be calculated from Equation 5 using the values of K = 5.18 and a_e = 0.75.

$$f(m) = 0.75 - \frac{1}{5.18\,F'(m)} \tag{8}$$

Data in Fig. 2 indicate a very good agreement between the sorption isotherm calculated from Equation 8 and the experimentally deter-

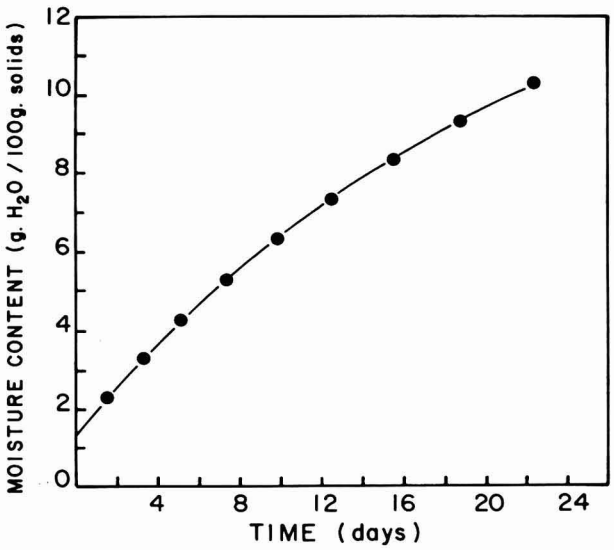

FIG. 1. MOISTURE CHANGES IN COTTONSEED MEAL PACKAGED IN POLYVINYL CHLORIDE POUCHES

(K=5.18) and stored at 37° C and a_e =0.75

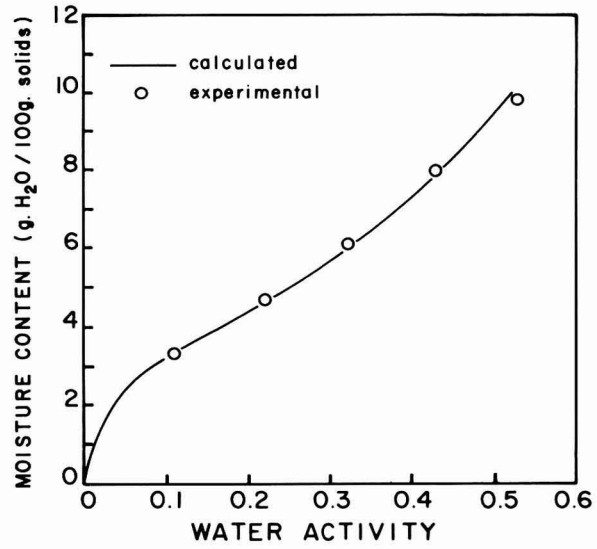

FIG. 2. COMPARISON OF CALCULATED AND OBSERVED SORPTION ISOTHERM FOR COTTONSEED MEAL

mined isotherm. It should be noted that when a large number of data points are available, accuracy can be improved by fitting a low order polynomial (e.g., second order) to different fractions of the whole moisture content range.

The second technique is applicable only if an analytical solution to the integral of Equation 3 is available. This can be done for constant a_e (or also theoretically for a_e given as a function of m) by using an appropriate isotherm equation. The simplest, although the least accurate, is the linear isotherm which can be used to approximate a narrow range of water activity. When a broader range is considered, a better equation is the one suggested by Mizrahi *et al.* (1970);

$$a = \frac{c_1 + m}{c_2 + m} \tag{9}$$

where c_1 and c_2 are constants

This is an empirical equation which has been found to fit the concave part of the isotherm. Although not accurate enough for water activity close to zero, this isotherm is satisfactory for moisture transfer calculation especially when $a_e \gg O$. Using Equation 9, the integrated Equation 3 reads:

$$t = \frac{1}{K}\left[\left(\frac{c_2}{a_e - 1} - \frac{a_e c_2 - c_1}{(a_e - 1)^2}\right) \ln\left(\frac{a_e c_2 - c_1 + (a_e - 1)m}{a_e c_2 - c_1 + (a_e - 1)m_o}\right) + \frac{m - m_o}{a_e - 1}\right] \tag{10}$$

where m_o is the initial moisture content

If the value of K is known, the parameters of the isotherm (Equation 9) can be evaluated by fitting Equation 10 to actual data as shown in Fig. 3 for moisture uptake by dehydrated cabbage ($K=1.0$, $a_e = 0.75$, and $m_o = 1.32$). The calculated values of c_1 and c_2 (Table 1) are -0.584 and 9.568 respectively, practically the same as the actual -0.579 and 9.584. Moreover, Equation 10 offers the possibility of simultaneous evaluation of K, if unknown, in addition to c_1 and c_2. The third column in Table 1 shows an accurate estimation of these parameters compared to the actual ones. Therefore, in cases where data are obtained at constant temperature and humidity, no independent determination of package water vapor permeability is necessary. Once the

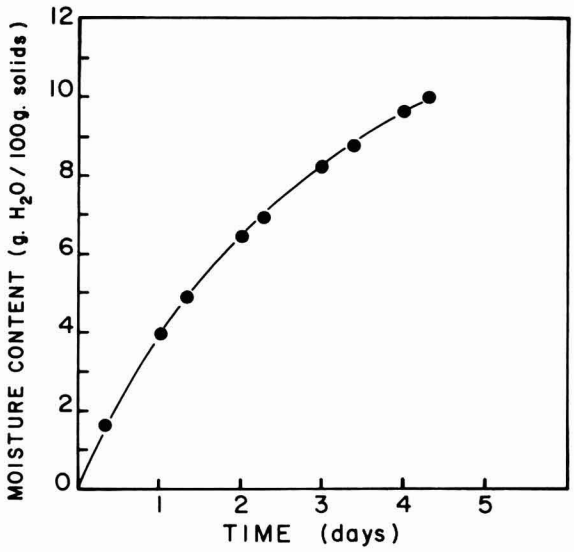

FIG. 3. MOISTURE CHANGES IN DEHYDRATED CABBAGE

Stored at 37° C and $a_e = 0.75$ in pouches with a K value of 1.0

Table 1. Calculated and actual values of K and the isotherm parameters

	Actual Values	Calculated Values for Actual K	Calculated Values
c_1	−0.579	−0.584	−0.550
c_2	9.584	9.568	9.684
K	1.0	1.0[a]	0.999

[a]Actual value

parameters in Equation 10 are evaluated, moisture uptake or loss can be evaluated for any package-humidity combination as long as a_e is constant during storage. Moisture gain calculated for dehydrated cabbage ($K = 0.7$, $a_e = 0.5$) from the parameters in the middle column of Table 1 are practically the same as the actual ones (Fig. 4).

If storage humidity is not constant or product isotherms deviate considerably from Equation 9, Equation 10 is no longer applicable, and a different approach should be used. Evaluation of the isotherm by

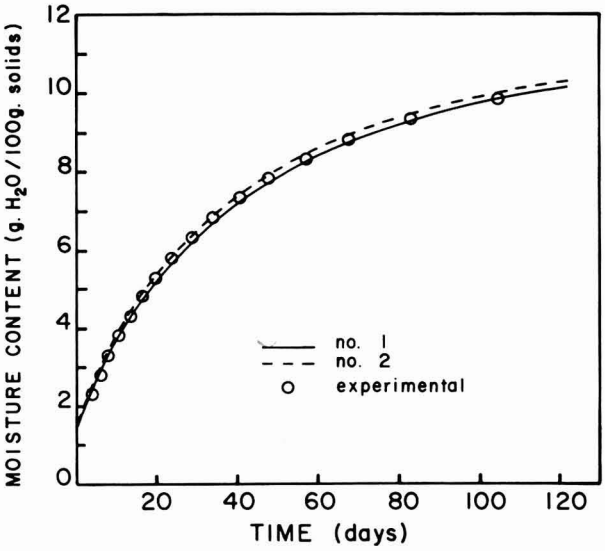

FIG. 4. COMPARISON OF PREDICTED AND OBSERVED CHANGES IN MOISTURE CONTENT OF DEHYDRATED CABBAGE

Stored at 37° C and $a_e = 0.5$ pouches with a K value of 0.7.

Curve 1 Predicted from integrated isotherm equation;
Curve 2 Predicted using a polynomial.

Equation 5 and using Equation 5 for moisture uptake calculation is a possible two-step procedure that can be undertaken. A one-step technique, however, is also available as detailed below.

Under the new storage conditions (e.g., when k changes to $\overline{K}$ and a_e to $\overline{a_e}$) the moisture gain or loss function reads:

$$t = \overline{F}\,(m) = \overline{c_o} + \overline{c_1}\,m + \overline{c_2}\,m^2 + \cdots\cdots \qquad (11)$$

The common basis for Equation 2 and Equation 11 is the same isotherm since it does not change because of rate of moisture transfer. Therefore,

$$a_e - \frac{1}{KF'\,(m)} = \overline{a_e} - \frac{1}{\overline{K}\,\overline{F'}\,(m)} \qquad (12)$$

If F(m) and $\overline{F}(m)$ are expressed by a polynomial, then:

$$a_e - \frac{1}{K(c_1 + 2c_2 m + \cdots)} = \overline{a}_e - \frac{1}{\overline{K}(\overline{c}_1 + 2\overline{c}_2 m + \cdots)} \tag{13}$$

To evaluate Equation 11, the values of the constants $\overline{c}_1$, $\overline{c}_2$, $\cdots$ are calculated from the corresponding c_1, c_2, etc. as follows:

for $m = 0$

$$a_e - \frac{1}{\overline{Kc_1}} = \overline{a}_e - \frac{1}{\overline{Kc_1}} \tag{14}$$

thus

$$\overline{c_1} = \frac{Kc_1}{\overline{K}[Kc_1(\overline{a}_e - a_e) + 1]} \tag{15}$$

By differentiating Equation 13 and Equating $m = 0$, one can show that:

$$\overline{c}_2 = \frac{\overline{K}}{K}\left(\frac{\overline{c_1}}{c_1}\right)^2 c_2 \tag{16}$$

In a similar manner one can evaluate any number of the polynomial constants. However, since in this method the constants of the higher terms are dependent on the value of the lower term, the accuracy is greatly impaired with every additional calculated constant. Therefore, as already suggested before, a polynomial no higher than second order is to be fitted to parts of the moisture content range rather than a higher order polynomial to be fitted to the whole range. For example, such a procedure based on data obtained for dehydrated cabbage at $a_e = 0.75$ and $K = 2.5$ (Fig. 5) is shown in Table 2. The constants in Table 2 were calculated by fitting a polynomial of the following form to any given moisture content range:

$$t = c_o + c_1 (m - m_o) + c_2 (m - m_o)^2 \tag{17}$$

where m_o is the initial value of the moisture content range.

Using Equations 15 and 16, $\overline{c}_1$ and $\overline{c}_2$ were calculated for a case where $\overline{a}_e = 0.5$ and $\overline{K} = 0.7$. The value of $\overline{c}_o$ is that of t at the top of

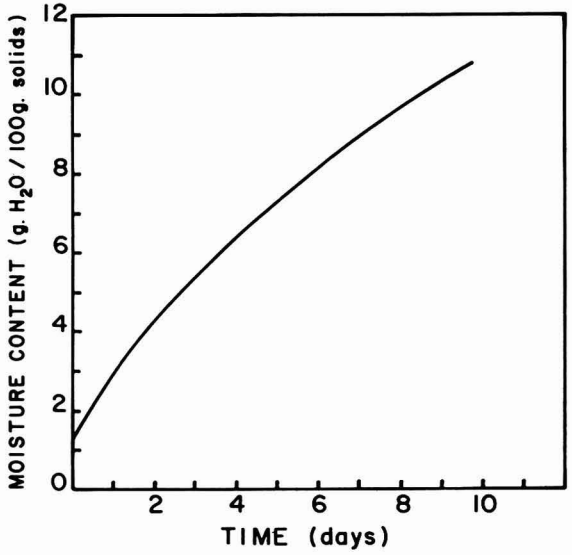

FIG. 5. MOISTURE GAIN OF DEHYDRATED CABBAGE

Stored at 37° C and a_e=0.75 in pouches with a K value of 2.5

Table 2. Parameters of the moisture gain for two different storage conditions

Moisture Content Range (g H_2O/100 g solids)	Parameters of F (m-m_o)			Parameters of $\overline{F}$ (m-m_o)		
	c_o	c_1	c_2	$\overline{c}_o$	c_1	c_2
1.32 — 2.82	0.0	0.579	0.040	0.0	3.24	0.35
2.82 — 4.82	0.958	0.696	0.044	5.6	4.4	0.49
4.82 — 6.82	2.526	0.870	0.051	16.4	6.8	0.87
6.82 — 8.82	4.471	1.073	0.060	33.4	11.6	2.0
8.82 —10.82	6.857	1.312	0.072	64.5	26	7.8

the preceding range except for the first range where $\overline{c}_o = 0$. In the second moisture content range (2.82–4.82), $\overline{c}_o$ is calculated from the first polynomial

$$\overline{c}_o = 3.24(2.82 - 1.32) + 0.35(2.82 - 1.32)^2 = 5.6$$

Similarly, for the third range (4.82—6.82)

$$\overline{c}_o = 5.6 + 4.4(4.82 - 2.82) + 0.49(4.82 - 2.82)^2 = 16.4$$

etc.

Data in Fig. 4 show that the moisture gain values calculated from the polynomials in Table 2 are practically the same as the actual ones. In conclusion, data on changes of a packaged sample under isothermal storage conditions can be used to evaluate the product sorption or desorption isotherm. In addition, moisture gain or loss can be calculated for any given package-storage humidity combination.

ACKNOWLEDGMENTS

This research was supported in part by Contract No. DAAK03-75-C0038 and Contract No. DAAK60-77-C-0049 with the U.S. Army Research and Development Command, Natick, Massachusetts. Dr. William Porter served as the project officer, and his criticism is gratefully acknowledged. This paper represents the position of the authors and not necessarily that of the Department of the Army or of other sponsors.

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POTATO PROTEIN CONCENTRATES: THE INFLUENCE OF VARIOUS METHODS OF RECOVERY UPON YIELD, COMPOSITIONAL AND FUNCTIONAL CHARACTERISTICS

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Received for Publication October 4, 1977

ABSTRACT

Potato processing effluents represent a potential source of valuable protein as well as a major waste disposal problem. Potato protein is commonly recovered by heat (in excess of 90°C) with pH adjustment between 3.5 and 5.5. The present study compared yield, and some compositional, and functional characteristics of potato protein concentrate (PPC) recovered with either HCl or $FeCl_3$ (pH 3.0, 20–22°C), or HCl/heat (pH 4.8, 98–99°C). Under pilot plant conditions, recoveries of 22.7, 36.7, and 37.5% of the crude protein (N × 6.25) were obtained with HCl, $FeCl_3$, and HCl/heat, respectively. Crude protein content of the PPC precipitated by HCl, $FeCl_3$, and HCl/heat were 65.6, 57.5, and 78.2% respectively. Ash and vitamin C values were higher in those PPC recovered at room temperature, with Fe content being highest in the PPC recovered with $FeCl_3$. The nitrogen solubility of the $FeCl_3$ precipitate, at pH 7.0, was 1.5 and more than 7 times that of the HCl, and HCl/heat precipitates, respectively. Whipping capacity of PPC was not influenced by precipitation method. The most favorable fat absorption and water absorption capacities were exhibited by the HCl and HCl/heat precipitates, respectively.

INTRODUCTION

Potato tuber(s), henceforth termed potato(es), contain an average of 2.1% crude protein on a fresh weight basis. Annual, world-wide production of potato protein is ≃ 6 million metric tons (Markakis 1975). In the U.S., approximately 268,000 metric tons of crude potato protein

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Journal of Food Processing and Preservation 1 (1977) 235—247.

(N × 6.25) are available annually (U.S. Dept. of Agr. 1976). A portion of this potato protein is in the form of processing waste effluents resulting from the manufacture of potato starch, flakes, granules, chips and french fries. Approximately one-third of the crude protein in the waste effluent (potato juice) may be recovered with heat or a trichloracetic acid/heat treatment.

Potato protein is recovered from the effluents of potato starch manufacture in various European countries. Quantities such as 2,000 and 25,000 metric tons of potato protein are potentially available annually in Austria and The Netherlands, respectively (De Noord 1975; Huchette and Fleche 1976; Vlasblom and Peters 1958; and Wohlmeyer 1974). The waste effluent from potato starch plants contains 2–5% solids, and accounts for ≃ 55% of the BOD leaving the plant. A typical composition of the soluble solids is: 35% crude protein, 35% total sugars, 20% minerals, 4% organic acids, and 6% others.

There has been interest in the recovery of potato protein during the past 60 years, and several methods have been reported. Generally, these methods consist of heat coagulation, heat coagulation with pH adjustment, pH adjustment alone with HCl, H_3PO_4, $FeCl_3$, or H_2SO_4, ion exchange chromatography, and reverse osmosis. Proponents of heat coagulation (Strolle *et al.* 1973; Vlasblom and Peters 1957; and Xander and Hoover 1959) most commonly use temperatures in excess of 90°C. When pH adjustment is used, it is usually between 3.5 and 5.5. Meister and Thompson (1976) demonstrated, in laboratory experiments, that $FeCl_3$ compared favorably with HCl as a precipitant of potato protein. Ion exchange chromatography has been used to recover protein, amino acids and potassium from potato waste streams (Heisler *et al.* 1972), and Porter *et al.* (1970) studied the use of reverse osmosis for potato protein recovery.

Heat coagulation is the most commonly used method to commercially recover potato protein. The energy costs of concentrating and heating the dilute waste effluent are a disadvantage of this method. In addition, heat coagulated protein is, generally, quite insoluble, which could limit some potential food applications. Of those acids used for pH adjustment, HCl is preferred in terms of cost and potential hazards to the public water supply. With the use of $FeCl_3$ as the precipitant, any Fe recovered with the protein could add to the nutritional value of the final product.

The amino acid balance of potato protein is quite favorable. Nitrogen balance studies with human adults have shown potato protein to be superior to most major plant protein, with its nutritive value approaching that of whole egg (Kofranyi and Jekat 1965; Jekat and Kofranyi 1970; and Meister and Thompson 1967).

An economic analysis of alternative methods for processing potato starch effluents was conducted by Stabile *et al.* (1970). At that time, the authors concluded that concentration of effluents by evaporation appeared to be the only economically feasible method. An up-dated economic analysis may be warranted in light of increased energy costs.

The purpose of this study was to compare the effectiveness of HCl, $FeCl_3$, and HCl combined with heat, as precipitants of potato protein in the laboratory, as well as under pilot plant conditions, and to evaluate some compositional, nutritional and functional characteristics of the protein concentrates recovered by these three methods.

MATERIALS AND METHODS

Preparation of Potato Protein Concentrate

Potato processing water was simulated in the pilot plant. Washed Russet Burbank potatoes (1.1 metric tons), containing 2.5% crude protein (N × 6.25) and 23% total solids, were used. The potatoes were loaded into a modified drag chain feeder (Model A 632-44, Arnold Dryer Co., Milwaukee, Wisc.) where 0.2% (w/w) $NaHSO_3$ was added to inhibit darkening of the potatoes. The potatoes were then metered into a 98 cm diameter vertical hammermill with swinging hammer (Owens Mfg. Co., Verdon, Neb.) followed by a Morehouse Mill (Model 350, Morehouse Ind. Inc., Los Angeles, CA). The slurry was diluted with water ($\simeq$ 1:1 v/v) and insoluble solids were removed by centrifugation at 3,200 G in a horizontal flow, decanter type centrifuge (Type P-3000 S, Sharples Co., Philadelphia, Penn.). The resulting supernatant, pH 5.6 contained 1.2 and 2.2% crude protein and total solid, respectively.

The aqueous solution containing the soluble protein (protein water) was equally divided into three portions and processed as outlined in Fig. 1. Two batches were adjusted to pH 3.0 and 4.8, respectively, with 2N HCl. The third was adjusted to pH 3.0 with a 28% (w/w) aqueous solution of $FeCl_3 \cdot 6H_2O$. The two batches at pH 3.0 were stirred (Model Ag 100, Mixing Equipment Co. Inc., Rochester, N.Y.) in a holding tank for 1 hr at 20–22°C. The precipitates were recovered by using a high speed, disk-type solids discharging centrifuge, with 31 cm bowl diameter and a RCF_{max} of 14,500 G (Model BRPX-207 S, De Laval Separator Co., Poughkeepsie, N.J.). The batch adjusted to pH 4.8 was stirred for 15 min and then heated by steam injection to 98–99°C (McDaniel Suction Tec, Dairy Industries Inc., Foster City, CA) as described by Edwards *et al.* (1975) The heated coagulum was then pumped

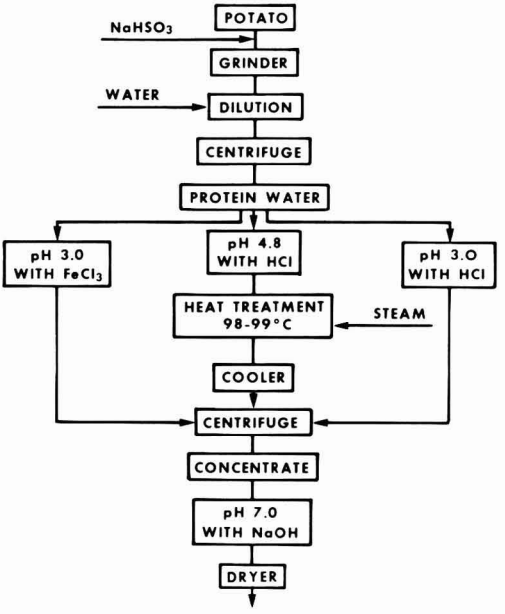

FIG. 1. SIMPLIFIED FLOW DIAGRAM FOR THE RECOVERY OF POTATO PROTEIN CONCENTRATES IN THE PILOT PLANT PROCESS

(Moyno pump type 380, Robbins & Myers Inc., Springfield, Ohio) through a plate type heat exchanger (Model Sc-3196, Creamery Package Co., Chicago, Ill.) where it was cooled to 24–26°C. The solids discharging centrifuge was used for the collection of this precipitate.

After collection by centrifugation the total weight of the concentrates was determined and samples were taken for Kjeldahl analysis. The yield was calculated as the amount of crude protein in the concentrates as a percent of the total amount in the protein water (see Table 1). The pH of each of the precipitated protein concentrates was adjusted to pH 7 with 2N NaOH. The concentrates were then spray dried at an air inlet temperature of 200–210°C and an outlet temperature of 105–110°C (Conical-type, laboratory model, Bowen Engineering Inc., North Branch, N.J.).

One experiment was conducted in the pilot plant on "potato cut water" obtained from a commercial potato chip processing plant. The effectiveness of the three precipitation methods, i.e. HCl, $FeCl_3$ and HCl/heat, was evaluated.

Table 1. Recovery of potato protein concentrates in the pilot plant by various methods

	Precipitation Method		
	HCl at RT pH 3.0	$FeCl_3$ at RT pH 3.0	HCl/Heat pH 4.8
	--	-- % --	--
Recovery of Crude Protein	22.7	36.7	37.5
Recovery of TCA/heat Insoluble Protein	61.5	99.4	102.0

Methods for Analysis and Functional Properties

The standard AOAC methods (AOAC 1975) were used for the determination of total solids, nitrogen, crude fat, ash and vitamin C. Total sugars were determined by the method of Potter *et al.* (1968) and total carbohydrates (in terms of glucose) were assayed according to the procedure of Dubois *et al.* (1956). The method of Kohler and Palter (1967) was followed for determining amino acid composition. Procedures outlined in Analytical Methods for Atomic Absorption Spectroscopy (Analytical Methods 1973) were used for the determination of calcium, iron, magnesium and sodium. Trichloracetic acid (TCA)/heat treatment, as reported by Finley and Hautala (1976), was employed to determine TCA coagulable protein of the protein water. For the determination of coagulable protein of the protein water at different pH levels (see Fig. 2) the pH was adjusted with 2N HCl and 28% (w/w) $FeCl_3 \cdot 6H_2O$ solution at room temperature and filtered after 60 minutes through an S & S 576 filter paper. The nitrogen content of the filtrate was determined by Kjeldahl analysis. In the case of HCl/heat treatment the pH was adjusted and then the protein water was heated to 95°C for 10 min, cooled to room temperature and filtered after 50 min. A previously described method was used to evaluate nitrogen solubility (Betschart 1974). Water absorption capacity, fat absorption capacity, and whipping capacity were determined using minor modifications of the methods of Sosulski (1962), Lin *et al.* (1974) and Lawhon *et al.* (1972), respectively, as described by Betschart and Kohler (1975).

All experiments, with the exception of nitrogen solubility, were conducted at the initial pH attained. The means of laboratory experiments are the result of from 2 to 5 replications. Pilot plant data are based upon a single run with analyses of these samples carried out in from 2 to 5 replications.

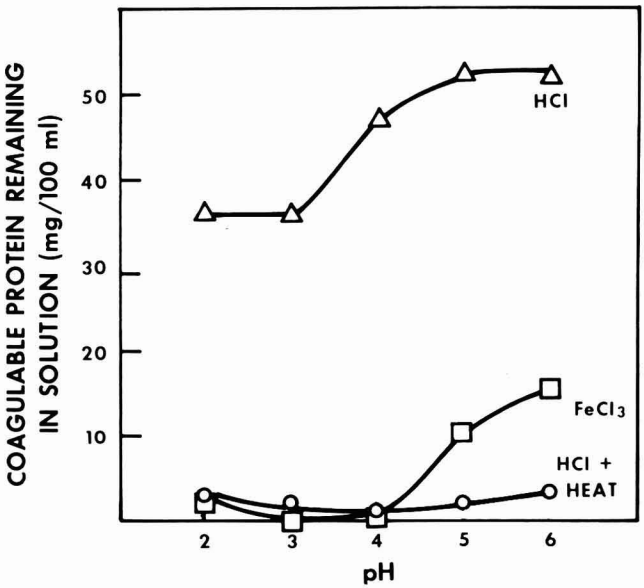

FIG. 2. RELATIONSHIP BETWEEN TCA HEAT INSOLUBLE PROTEIN REMAINING IN SOLUTION AND PH FOR THE DIFFERENT PRECIPITATION METHODS

RESULTS AND DISCUSSION

Protein Recovery

Laboratory experiments showed that $FeCl_3$ compared favorably with HCl/heat treatment at pH 2–4 with respect to the amount of coagulable protein recovered from the protein water (Fig. 2). At pH 5 and 6 HCl/heat was the most effective precipitation method studied.

By the use of TCA/heat, in combination, 37 ± 2% of the crude protein in the protein water was recovered (termed coagulable protein). Recoveries of crude protein by HCl, $FeCl_3$, and HCl/heat precipitation methods were 23 ± 1, 40 ± 1, and 35 ± 2%, respectively. These recoveries represented 62, 108, and 95% of the coagulable protein by HCl, $FeCl_3$, and HCl/heat precipitation, respectively. Thus, at pH 3.0 $FeCl_3$ was more effective than HCl in recovering potato protein concentrate (PPC). Meister and Thompson (1976) also found $FeCl_3$ to be more effective than HCl as a precipitant of potato protein. They reported that, at pH 3.0, 31 and 36% of the crude protein were recovered by HCl and $FeCl_3$ precipitation, respectively. From these data it is also apparent that Meister and Thompson achieved more effective results

with HCl and somewhat less effective results with $FeCl_3$ when compared with results reported in the present study.

Results obtained in the pilot plant with simulated waste effluent indicated that $FeCl_3$ and HCl/heat were equally effective in recovering protein (Table 1), with HCl recovering significantly less crude protein. Quantities of protein recovered by HCl and $FeCl_3$ were 62 and 99%, respectively, of the TCA/heat coagulable protein. The recovery of PPC by various methods was also studied in the pilot plant with commercial "potato cut water." Thus, results were similar to those obtained with simulated potato processing water. By using $FeCl_3$ as a coagulant, 97% of the TCA/heat coagulable protein could be recovered.

The quantity of $FeCl_3 \cdot 6H_2O$ required to precipitate PPC was 1.6 kg per kg protein (dm). By raising the precipitation pH to 4.0, 0.9 kg $FeCl_3 \cdot H_2O$ would be needed per kg protein. Although laboratory experiments by the authors indicated that slightly less protein would be recovered at pH 4.0 (Fig. 2), Meister and Thompson (1976) showed that $FeCl_3$ precipitation produced maximum recovery at pH 4.0.

Composition

Proximate analyses of the spray dried PPC revealed that the crude protein contents of the HCl, $FeCl_3$, and HCl/heat precipitates were 65.6, 57.5, and 78.2%, respectively. In addition to the differences in protein content of the PPC recovered by various methods, major compositional differences were observed for ash, vitamin C, iron and sodium (Table 2). The increased ash content associated with HCl precipitation at room temperature was also observed by Meister and Thompson (1976), who noted that HCl recovered more total solids from the effluent than does precipitation by HCl/heat. The PPC precipitated at ambient temperatures with HCl and $FeCl_3$ would be more appropriate for human consumption if ash values were reduced. Vitamin C content (15–18 mg/100 g) was significantly higher in those PPC recovered at room temperature, whereas the $FeCl_3$ precipitate was markedly higher in iron than the other two precipitates.

Functional Properties

With the exception of whipping capacity, those functional properties of PPC evaluated were markedly influenced by method of precipitation. Nitrogen solubility of PPC recovered at room temperature was much higher than that of the HCl/heat precipitate (Fig. 3). At pH 6 and above, the nitrogen solubility of $FeCl_3$ precipitate was superior to that precipitated by HCl; at pH 7, it was >7 times that of the HCl/heat

Table 2. Analyses and select functional properties of potato protein concentrates[a]

Composition/Property	Precipitation method HCl at RT	$FeCl_3$ at RT	HCl/heat
		% Dry matter[b]	
Total solids (%)	93.7	94.7	95.4
Nitrogen	10.5	9.2	12.5
Crude fat	2.3	1.3	2.4
Ash	24.5	25.1	7.2
Total sugars	3.6	2.6	1.3
Total Carbohydrates	7.2	7.1	7.1
Total Vitamin C (mg/100 g)	18.1	14.9	0.01
Calcium	0.14	0.04	0.14
Iron	0.10	4.32	0.12
Magnesium	0.20	0.11	0.10
Sodium	4.25	3.85	1.53
Functional Properties		Percent	
Nitrogen solubility (pH 7)	56.0 ± 0.1[c]	87.5 ± 2.1	11.5 ± 0.7
Water absorption capacity (pH 7)	214 ± 3	86 ± 5	273 ±6
Fat absorption capacity (pH 7)	234 ± 16	188 ± 10	110 ± 10
Whipping Capacity Foam (% Volume increase pH 7)	568 ± 40	524 ± 9	523 ± 9

[a]Means of 2 to 5 replications
[b]% dry matter unless otherwise indicated
[c]Means ± standard deviation

precipitated PPC (Table 2). Increased nitrogen solubility indicates that the PPC were less serverely denatured during processing, and would more likely be functionally active in food systems in which protein solubility was a prerequisite.

Water absorption capacity was highest in the HCl/heat precipitate which had been most severely heated during precipitation, whereas fat absorption capcity was the lowest in this precipitate (Table 2). The high water absorption of heat precipitated plant proteins vs those recovered with HCl at room temperature has also been reported for alfalfa leaf protein concentrate (Betschart and Kohler 1975). HCl precipitation produced the PPC with the most favorable fat absorption capacity. The spray dried PPC precipitated by HCl were light and fluffy, with greyish beige overtones. That precipitated at room temperature was the lightest in color, whereas the $FeCl_3$ precipitate had a light green cast.

These data on functionality within simple, model systems provide an indication of potential functionality in food systems.

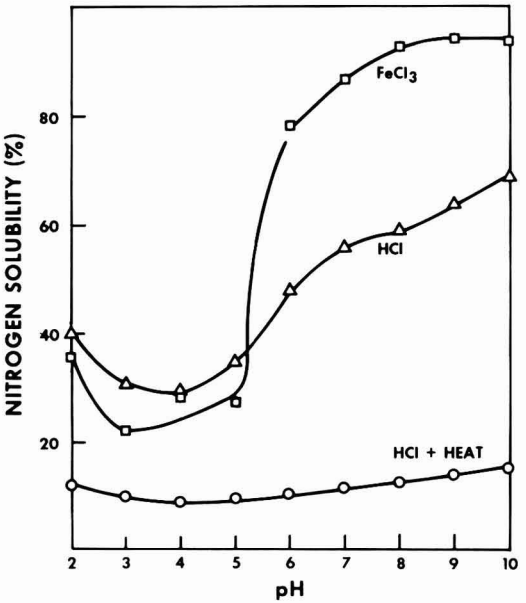

FIG. 3. RELATIONSHIP BETWEEN NITROGEN SOLUBILITY AND PH OF DIFFERENT POTATO PROTEIN CONCENTRATES

Amino Acid Analyses

With the exception of arginine, aspartic and glutamic acids, the amino acid composisition of the HCl/heat treatment PPC was equal or greater than that reported by FAO (1972) for potato protein (Table 3). The higher levels of methionine and cystine in PPC are of interest since these amino acids were previously reported to be low in potato protein (Scrimshaw and Young 1976). When compared with FAO (1973) Provisional Amino Acid Scoring Pattern, the PPC contain quantities of amino acids equal to or greater than the suggested levels for all the essential amino acids (except tryptophan which was not determined).

In summary, $FeCl_3$ and HCl/heat treatment recovered similar quantities of potato protein, whereas HCl at room temperature was the least effective method. Differences between these precipitation methods include the energy input required for steam (HCl/heat) and ingredient costs (HCl, $FeCl_3$). Compositional differences among the PPC precipitated by various methods included higher crude protein in the HCl/heat precipitate, higher vitamin C and ash in those PPC precipi-

Table 3. Amino acid analyses of potato protein concentrates recovered by various methods

Amino Acid	Precipitation Method			Potato Protein FAO (1972)
	HCl	$FeCl_3$	HCl/Heat	
		—	g/16 g N	—
Lysine	6.02	6.46	6.79	5.28
Histidine	2.04	2.03	2.11	1.76
Arginine	4.40	4.76	4.74	5.28
Aspartic Acid	12.83	13.37	11.08	13.12
Threonine	4.27	4.43	4.86	3.84
Serine	4.15	4.49	4.90	3.52
Glutamic Acid	11.67	11.58	10.47	17.60
Proline	3.35	3.70	4.11	3.84
Glycine	3.51	3.86	4.14	3.52
Alanine	4.01	3.91	4.41	4.00
Cystine	1.41	1.74	1.47	0.94
Valine	5.50	5.89	6.24	5.76
Methionine	2.09	1.96	2.70	1.12
Isoleucine	4.53	4.76	5.20	3.84
Leucine	7.20	7.41	8.53	6.24
Tyrosine	4.17	4.30	4.74	2.72
Phenylalanine	4.67	4.82	5.34	3.36

tated at ambient temperatures (HCl, $FeCl_3$) and higher Fe values in the $FeCl_3$ precipitate. In terms of functionality, PPC precipitated by all three methods possessed similar whipping capacity. Differences included greater nitrogen solubility for the $FeCl_3$ precipitate, with the highest water absorption capacity and fat absorption capacity exhibited by the HCl/heat and HCl precipitates, respectively. The ultimate selection of a precipitation method for potato protein will depend upon an analysis of the nutritional and antinutritional, economic, engineering, compositional and functional parameters, within the constraints of the end product use of the PPC.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

ACKNOWLEDGMENTS

The authors wish to thank K. V. Smith, and co-workers, for their expert assistance in preparing protein concentrates in the pilot plant; L. V. Richards for the proximate analyses, R. Fong for the analyses of some of the functional properties, M. E. Allis and A. T. Noma for the amino acid analysis, and R. M. Saunders for guidance and support throughout this study.

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EFFECTS OF WATER AND SPIN BLANCHING ON OXIDATIVE STABILITY OF PEANUTS

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Received for Publication October 27, 1977

ABSTRACT

Several methods of blanching have been developed to remove skins from peanuts — spin-, water-, alkali-, and hydrogen peroxide-blanching. The present investigation compared the effects of wet- and spin-blanching on oxidative stability of raw and roasted peanuts stored for seven months. The water-blanched raw peanuts had the shortest shelf-life, whereas the shelf-life of unblanched raw and spin-blanched peanuts was similar. The opposite results were found for roasted peanuts; the water-blanched peanuts had the longest shelf-life. Blanching induced major differences in the electrophoretic mobility and protein banding patterns of sodium dodecyl sulfate-soluble extracts of raw peanuts. Electron microscopy revealed that there are surface differences between the spin and water blanched peanuts.

INTRODUCTION

Differences in flavor, aroma, and color of peanuts and peanut products may be caused by many factors, e.g., curing, blanching, storage conditions, processing techniques. In order to insure high quality products, each of these factors must be properly controlled.

Blanching is the process of removing the skins, or testae, which contain tannins that may contribute to off-flavors and off-color. Blanching will also remove foreign materials, dust, or mold. Several procedures have been developed for blanching peanuts — i.e. dry, water-, spin-,

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Journal of Food Processing and Preservation 1 (1977) 249—260.

alkali-, and hydrogen peroxide-blanching. Each of these utilizes various temperatures, from a high of 138°C for dry blanching, to a low of 49°C, the temperature sometimes used after water blanching (Woodroof 1973). The different processes can also affect texture. Water-blanched peanuts are crispy, while spin-blanched nuts are softer (Reeve 1962).

It has been reported that the shelf-life of water-blanched peanuts is longer than unblanched nuts, that of spin-blanched is almost as long as water-blanched peanuts, and alkali-blanched peanuts keep longer before becoming stale or rancid than those treated with either hot water or hot air (Woodroof 1973). However, there are no reports that compare the effects of different blanching methods on oxidative stability (shelf-life) of the same peanuts before and after roasting. The purpose of this investigation was to compare the effects of water- and spin-blanching on factors involved in oxidative stability of raw and roasted peanuts.

MATERIALS AND METHODS

For these studies, three batches of Virginia 56-R peanuts were received from a commercial processing plant immediately after two were blanched. The third batch of peanuts was unblanched for the control. The first batch was spin-blanched by passing kernels through a skin cutter, then a dryer to lower the moisture content from 8% to 5% at about 71°C, a temperature considerably lower than that used for roasting. After the skins were loosened, the kernels were passed through a spin-blancher to remove the skins. The nuts were then air-separated and sorted.

The second batch was water-blanched by spraying 86°C water on the slit kernels for 90 sec. The kernels were dried to bring the moisture down to 5%, and the skins were removed mechanically. Each sample of peanuts was divided into two portions. One portion was sealed in glass jars and stored in metal cabinets at 25°C. The remaining portions of the three samples were roasted at 175°C for 20 min in a rotisserie oven, cooled to room temperature, then stored in sealed jars alongside the unroasted nuts.

The degree of staling was tested by measuring the conjugated diene hydroperoxide (CDHP) content, using a modification of the extraction procedure of St. Angelo *et al.* (1972, 1973, 1975). The CDHP spectrophotometric method of analysis was shown to be directly proportional to peroxide values (PV) commonly used (St. Angelo, *et al.* 1975). Every 28 days, 10 g samples of whole peanuts were broken into small pieces with a mortar and pestle to obtain uniform mixing and duplicate 2 g

subsamples were removed, weighed, homogenized to a thick paste, and extracted with 30 ml of hexane. After standing 1 hr in capped centrifuge tubes and stirred twice during the hour, the suspensions were centrifuged at 12,000 g for 15 min at 4°C, then 0.2 ml portions were removed, diluted to 3 ml with hexane, and read at 234 nm against a hexane blank. The concentrations of CDHP were calculated employing an E_{max} of 24,500 (Johnston *et al.* 1961), and are represented as μ-moles of CDHP per g of peanuts.

The dried, hexane-defatted meals were extracted with 0.1 M tris-HCl buffer, pH 7.2. Lipoxygenase was determined by the method of St. Angelo and Ory (1972) and by gel electrophoresis, using a modification of the procedures of Steward *et al.* (1965), and Cherry *et al.* (1970). For electrophoresis, (a) the glass gel tubes were 86 mm × 5 mm i.d.; (b) gels were 10% acrylamide with 0.27% crosslinking; (c) 3 drops of 40% sucrose solution was added to each tube instead of capping the upper gel with a thin strip of lower gel; (d) the reservoir buffer was that of Davis (1964). The stock solution of buffer consisted of 6.0 g of tris, 28.8 g of glycine, and water to 1 liter (pH 8.3) but was diluted to 1/10 its strength for each experiment. Electrophoresis was carried out at 4°C and 3 mA per tube for 1½ hr, or until the bromophenol blue tracking dye had migrated to the end of each tube. After each run, the gels were removed and stained for 16 hr with 0.5% amido black solution. Gels were destained overnight in a gel diffusion destainer containing 7% acetic acid.

The tris-HCl insoluble residues were incubated 16 hr at 37°C in 0.1 M phosphate buffer, pH 7.2, containing 1% sodium dodecyl sulfate (SDS), then centrifuged at 10,000 × g for 5 min. Supernatants were analyzed on SDS-acrylamide gels according to Weber and Osborn (1969), except that electrophoresis was performed at 5 mA per tube, and the time of each run was 5 hr, or until the tracking dye was 1 mm from the bottom of the tube.

For scanning electron microscopy, samples were first sputter-coated with a 200–300 Å thick layer of gold-paladium alloy, then examined in an ISI Super II scanning electron microscope.

RESULTS AND DISCUSSION

Investigations of the effects of wet and dry heat on peanut proteins have shown that the amount of moisture in the peanut can influence the conformation of proteins, including some enzymes. Conformational changes in these proteins were shown to alter antigenicity, electro-

phoretic mobility, and enzymic activity (Neucere 1972; Thomas and Bright 1972; Thomas and Neucere 1974). The effects of lipid peroxides on proteins of peanuts stored under conditons designed to promote oxidation was reported earlier (St. Angelo and Ory 1975a). In the present communication, the effects of blanching methods on the rate of lipid peroxidation and concomitant effects on the electrophoretic protein patterns during storage was investigated.

Disc electrophoresis was used to examine the effects of the two blanching procedures on electrophoretic protein profiles. Fig. 1 shows the electrophoretic patterns of proteins from extracts of defatted raw peanuts. In the Group I profiles (the buffer-soluble protein fractions) there were four major and several minor protein bands found in extracts of the water-blanched and unblanched peanuts. These protein bands were observed at the 35, 39, 48, and 52 mm regions. In extracts of spin-blanched peanuts, there were only three major protein bands observed; the 39 mm band was missing. These differences were confirmed in electrophoretic patterns of diluted protein solutions.

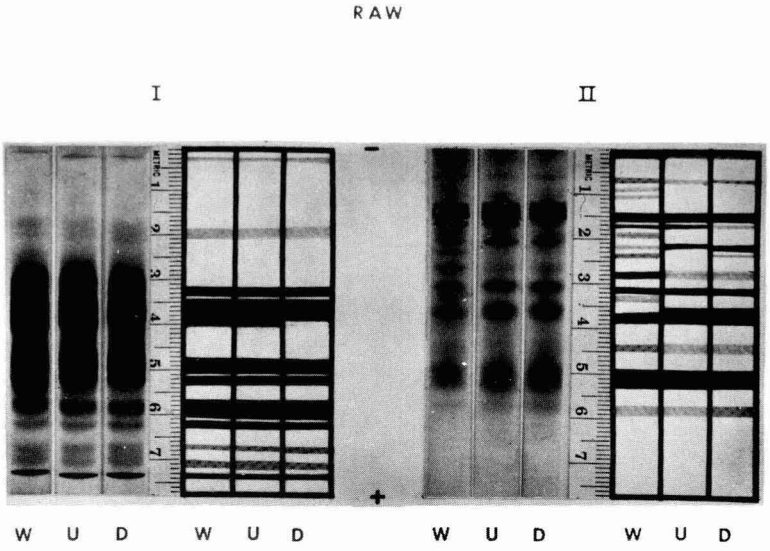

FIG. 1. POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERNS OF TRIS-HCl BUFFER SOLUBLE PROTEINS (GROUP I) AND SDS-SOLUBLE PROTEINS (GROUP II) FROM RAW PEANUTS

W, water-blanched; U, unblanched; D, spin-blanched; migration from top.

In Group II (the SDS-soluble proteins extracted from the buffer-insoluble residues), several differences in protein banding patterns were observed, primarily in the 9 mm to 36 mm range. The molecular weights of proteins in this region vary between 40,000 and 80,000. In this group, extracts of water-blanched peanuts had a larger band at the origin and a smaller band at 16 mm than did either the unblanched or the spin-blanched peanuts. The protein band at 22 mm was missing in the water-blanched extract, and the band at 18 mm was missing in extracts from spin-blanched peanuts. In addition, the water-blanched extract contained a major protein band at 28 mm that was absent in both the unblanched and spin-blanched extracts.

Fig. 2 represents the protein patterns from extracts of defatted roasted peanuts. In Group I of this figure, four major protein fractions and at least five minor constituents were observed in the gel patterns of unblanched and spin-blanched extracts. Major protein bands appeared at 37, 50, 52, and 73 mm. However, in the water-blanched extracts there were only two major protein bands; a very intense band at 73 mm and a smaller protein band at 77 mm. Extracts from the unblanched and spin-blanched peanuts also indicated several minor bands.

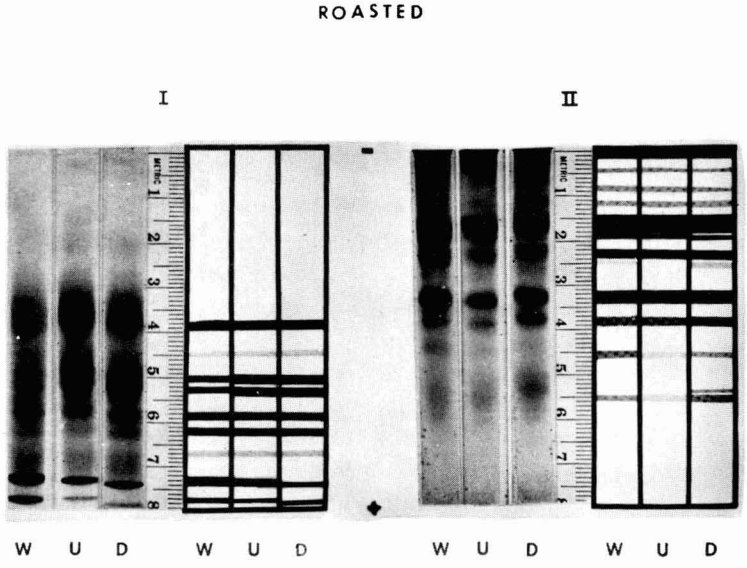

FIG. 2. POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERNS OF TRIS-HCl BUFFER SOLUBLE PROTEINS (GROUP I) AND SDS-SOLUBLE PROTEINS (GROUP II) FROM ROASTED PEANUTS

W, water-blanched; U, unblanched; D, spin-blanched; migration from top

Roasting denatures peanut proteins, therefore most of the proteins should be insoluble in the extraction buffer; this was evident in Fig. 2. Group II clearly showed that most of the roasted peanuts were buffer-insoluble, but were solublized by SDS for electrolytic studies.

The effects of wet- and spin-blanching on the SDS-soluble proteins were less in roasted peanuts than were observed in raw peanuts, as can be seen by comparing Fig. 1, Group II with Fig. 2, Group II. In the roasted samples, only a few differences were observed. The unblanched peanuts had one band at 24 mm, whereas two protein bands were found for both the wet- and spin-blanched peanuts in the 24–26 mm range. Spin-blanched peanuts had a major protein band at 38 mm, whereas this band was a minor component in both of the other two extracts. The spin-blanched peanuts also had two components in the 51–54 mm range that appeared only as a trace in the other two extracts.

The shelf-life stability of the three raw peanut samples (unblanched, spin-blanched, and water-blanched) was examined monthly for about 200 days and is diagrammed in Fig. 3. There was no significant difference in the shelf-life stability of unblanched and spin-blanched peanuts. However, the water-blanched raw peanuts had a shorter shelf-life than the unblanched and spin-blanched samples and peroxidized faster. The water-blanched sample had a ΔCDHP value of 15 after 200 days storage; whereas the unblanched peanuts had a value of only 6. By converting the actual CDHP value to peroxide values (PV) according to St. Angelo, *et al.* (1975), the PV's were 66 and 14, respectively. During water blanching, the moisture content rises to about 12%, then is dried to about 5%. In the spin blanching procedure, the moisture never exceeds the normal 7–8% content for peanuts. In 1958, Cosler stated that peanuts become stale more rapidly with the absorption of moisture. Labuza, *et al.* (1971) have discussed the effect of water upon lipid oxidation in foods in terms of water activity or relative humidity. When water activity is plotted against lipid oxidation, an inverted bell-shaped curve is obtained that indicates, water acts as a prooxidant at very low and very high water activities. In between these two extremes, water acts as an antioxidant. We have recently confirmed these results in peanut butter studies (St. Angelo and Ory 1975c).

In roasted peanuts, a different situation exists because excessive heat denatures proteins. Fig. 4 illustrates the effect of storage on the shelf-life of the three roasted samples. The unblanched control had the shortest shelf-life; the roasted, spin-blanched peanuts had a longer shelf-life. Water-blanched peanuts had the longest shelf-life of all three roasted samples. After 200 days storage, the ΔCDHP value for the unblanched peanuts converted was 16 (PV of 89), whereas the water-

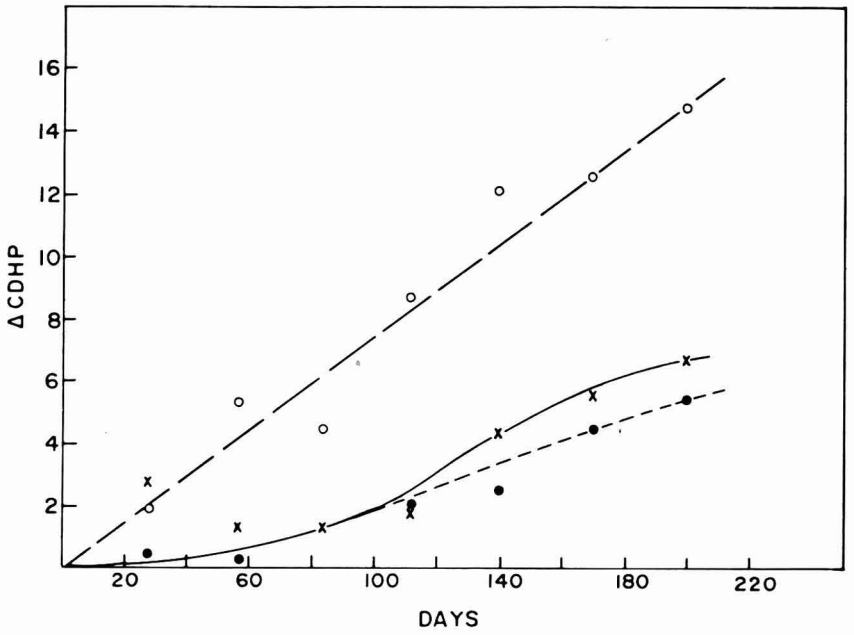

FIG. 3. SHELF-LIFE OF RAW PEANUTS STORED AT 25° C. VALUES REPRESENT THE DIFFERENCE BETWEEN CDHP CONTENT FOUND AT THE TIME OF ANALYSIS AND THE INITIAL VALUE

Unblanched (•---•); spin-blanched (x_____x); water-blanched (0——0).

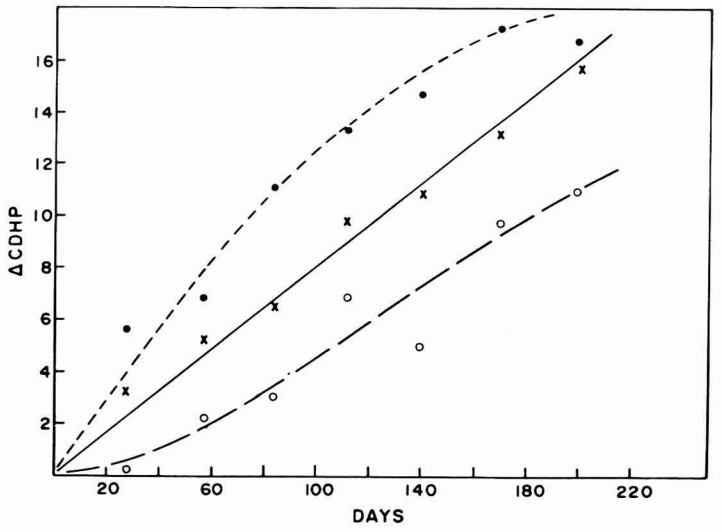

FIG. 4. SHELF-LIFE OF ROASTED PEANUTS STORED AT 25° C. VALUES REPRESENT THE DIFFERENCE BETWEEN CDHP CONTENT FOUND AT THE TIME OF ANALYSIS AND THE INITIAL VALUE

Unblanched (•---•); spin-blanched (x_____x); water-blanched (0——0).

blanched samples had a ΔCDHP value of 11 (PV of 28). These results are in direct contrast to those obtained with raw peanuts. Results of observations from Figs. 3 and 4 are summarized in Table 1.

Table 1. Shelf-life stability of raw and roasted peanuts

Unblanched, roasted	59 days[a]
Spin-blanched, roasted	100
Water-blanched, raw	110
Water-blanched, roasted	153
Spin-blanched, raw	>200
Unblanched, raw	>200
[b]Unblanched, roasted, 4° C storage	56
[b]Unblanched, raw, 4° C storage	>>200

[a]Time to reach a CDHP value of 8
[b]Data calculated from St. Angelo and Ory (1975b)

According to the methodology described herein, peanuts samples are broken and macerated for about 1–2 min prior to extraction for CDHP analysis. During this time, lipoxygenase could be activated to produce hydroperoxides, but this additional oxidation would be insignificant compared to that produced in peanuts over the 200-day storage period. To confirm this supposition, an additional experiment was performed. Two 20-gram samples of raw unblanched peanuts were used. The first sample was treated as described previously (see Methods). From this first sample, triplicate 2-gram portions were weighed, testae removed manually, and the peanuts were extracted with hexane, etc. The average CDHP value for the triplicate samples were 4.87 ∓ 0.29 μmoles/g peanut.

The second 20-gram sample was boiled for five minutes to destroy lipoxygenase activity, dried, then triplicate 2-gram portions (minus testae) were extracted as before. The results with boiled peanuts showed a CDHP value of 4.77 ∓ 0.20 μmoles/g dry peanuts. These results clearly show that the differences between boiled and unboiled peanuts (emanating from enzymic activity) would be insignificant when compared to CDHP contents measured over the 200-day storage period.

In considering the order of shelf-life stability described in Table 1, selection of storage conditions for peanuts should depend on their pretreatment and ultimate use. If the peanuts will be stored without vacuum-pack conditions after roasting, they should be water-blanched. On the other hand, if they will be stored raw, then they should be either unblanched or spin-blanched.

We recently reported that unblanched, roasted peanuts will

peroxidize faster than raw, unblanched peanuts when stored at 4°C (St. Angelo and Ory 1975b). Our current results, demonstrated in Fig. 3 and 4, are similar to those findings even though these data were compiled with peanuts stored at 25°C. Peroxidation of unblanched, roasted peanuts, stored at room temperature and at 4°C proceeded at about the same rate. Raw, unblanched peanuts stored at room temperature had a faster rate of peroxidation than those stored at 4°C, but the rate was less than that of the roasted, unblanched nuts. (See Table 1).

When peanuts are spin- or water-blanched, the skins are first cut on opposite sides with razor blades. In water-blanching, the spray of hot water dissolves some of the surface protein, and upon subsequent drying, a glaze is formed on the surface that may act as a protective shield against oxidation and mechanical injury (Woodroof 1973). Woodroff explains the longer shelf-life of water-blanched peanuts on this basis. In spin-blanching, the sliced nuts bypass the hot water spray and pass directly to the dryers.

Our data in Fig. 3, however, show that water-blanched raw Virginia peanuts are *least* stable; Fig. 4 indicates that roasting the water-blanched peanuts enhances their stability. These differences might possibly be due to the different heat treatments of the raw seeds; e.g., hot-water blanching 1.5 min at 86°C and, as mentioned earlier, drying to remove excess moisture. Hot water used in the wet-blanching process adversely affects the lipids more than the proteins, since their electrophoretic mobilities were not significantly different (Fig. 1 and 2) in stored raw and roasted peanuts. If the water blanched peanuts are immediately roasted, the surface lipids are volatilized; thus, the oxidative process is retarded. The effect of *hot* water on cell components of spin-blanched peanuts is, of course, eliminated because that process uses *dry* heat.

Because lipoxygenase is the enzyme usually associated with lipid peroxidation, extracts from the defatted raw peanut meals were examined for activity. Enzyme activity was similar for both unblanched and spin-blanched samples, but no lipoxygenase activity was found in the water-blanched peanuts. These results were surprising, since the only difference between the peanuts was the hot water treatment for 90 sec during water blanching. It is even more intriguing because the results in Fig. 3 clearly indicate that water-blanched raw peanuts were the least stable over the seven months tested. These results suggest that some of the enzyme may lie near the surface, as well as inside the cotyledon, and that the oxidation may involve a surface phenomenon.

Having observed the glaze on water-blanched peanuts (absent on spin-blanched peanuts), reported by Woodroof (1973), we examined the

surface of a few peanuts and the "slit" marks made by the razor blades during the blanching procedure, using scanning electron microscopy (Fig. 5).

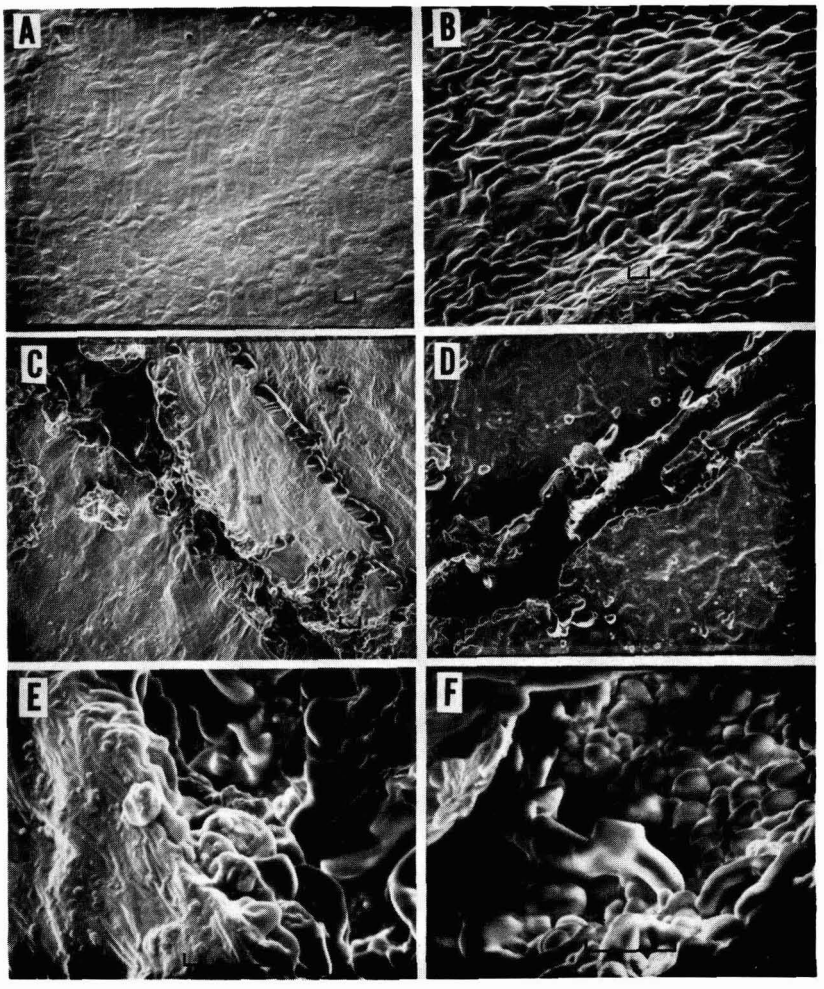

FIG. 5. PHOTOMICROGRAPHS OF WHOLE PEANUT SEEDS

A, C, and E, water-blanched; B, hand-blanched; D, F, spin-blanched; marker is 10 microns.

At low magnification, the surface of the water-blanched peanuts was smooth (5A), while that of hand-blanched control peanuts were very rough (5B). the spin-blanched peanuts were somewhere in between, but more rough than smooth. Slit marks appeared on both the water-

blanched and spin-blanched peanuts (see 5, C and D). At low magnification, they appeared very similar, except for the presence of a subcellular network found in the walls of the spin-blanched peanuts. Slit marks were not observed on the hand-blanched peanuts.

Higher magnification of the walls of the slits made on the spin-blanched peanuts confirmed the presence of subcellular particles, which probably include aleurone grains (protein bodies), spherosomes (fat bodies), and starch granules (5F). Examination of the water-blanched peanuts at high magnification revealed that only a few of these particles remained (5E). This suggests that the fat and protein were washed from the razor-made slits and could possibly be responsible for the glaze observed on the surface of water-blanched peanuts.

To determine if the glazed surface contained oxidized lipid, 10 ml of hexane was added to peanuts from the three samples. After incubating for 60 min, the hexane was removed and the absorbance read at 234 nm. The results showed that extracts from the spin- and hand-blanched peanuts had an absorbance of 0.26 and 0.16 respectively, while extracts from the water-blanched peanuts showed an absorbance of 0.88. These results indicate that there was more peroxidized lipid on the surface (or at least, more readily susceptible to hexane extraction) of water-blanched peanuts than on the other two samples.

In summary, we feel that the glaze is associated with a combination of the washed protein and lipid constituents fixed to the surface, but instead of acting as a protective coating, we feel that it is responsible for the shortened shelf-life of water-blanched peanuts. Together, the protein and lipid fixed to the surface in the presence of oxygen allows for an excellent oxidative reaction medium.

Additonal studies are currently underway to pursue this theory on surface and oxidative phenomena in stored peanuts.

ACKNOWLEDGEMENT

The authors are grateful to J. J. Bergquist for the photography.

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MEETINGS

August 1978. The University of Guelph, in cooperation with the Federal Ministry of Industry, Trade and Commerce, is sponsoring an International Symposium on Protein Utilization to be held in Guelph, August 13–16, 1978. This conference is aimed at bringing together the best possible information on protein utilization and proposing feasible alternatives for expanding the supply of protein materials. Marketing and management techniques for assuring the acceptance of new protein foods will be examined as well as the quality of the scientific and technical programs aimed at improving protein processing.

The Symposium will have a limited enrollment. For further information contact the Office of Continuing Education, University of Guelph, Guelph, Ontario, Canada, N1G 2W1 (519-824-4120, Ext. 3958).

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GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J. T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.) pp. 82–91, Academic Press, New York.

HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. The title of the table should appear as below:

Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) of author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the scientific literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

EDITORIAL OFFICE: Prof. T. P. Labuza, Editor, Journal of Food Processing and Preservation, University of Minnesota, Department of Food Science and Nutrition, Saint Paul, Minnesota 55108 USA

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