Effect of Moisture Content of Freeze-Dried Peaches and Bananas on Changes during Storage Related to Oxidative and Carbonyl-Amine Browning

H. N. DRAUDT[1] and I-YIH HUANG[2]
Department of Biochemistry, Purdue University, Lafayette, Ind.

In both freeze-dried peaches and bananas during long-term storage in air at 28° C., little change was observed at storage moisture levels below 10% in total phenolic substances, free amino nitrogen, reducing sugar, and sucrose content. In both freeze-dried peaches and bananas, peroxidase activity decreased rapidly at all moisture levels during storage at 28° C. In both freeze-dried peaches and bananas, polyphenol oxidase activity was most stable at the lowest moisture levels and became relatively unstable at 8% moisture and above. Treatment with sulfur dioxide protected ascorbic acid, but had only a limited effect on browning. Loss of ascorbic acid was minimum at minimum moisture levels in both products. After 190 days' storage, the Amadori compounds, "fructose-γ-amino butyric acid" and "fructose-valine," were found in untreated, freeze-dried bananas at moisture levels above 0.70% and in sulfur-dioxide–treated samples above 3.75% moisture. A level of 5% moisture or lower is effective in preventing brown discoloration of peaches and bananas stored at 28° C. for at least 6 months.

The high cost of removal of water in the 1-to-10% range (*11*) makes it economically highly desirable to freeze-dry foods only to moisture levels sufficient to prevent product deterioration during storage. Storage moisture limits could be determined with increased confidence if such moisture limits are based on a knowledge of the effect of moisture on specific chemical changes associated with browning rather than only on subjective examination of the stored product, as has often been the case in the past.

While there has been much work carried out on the chemistry of carbonyl-amine browning (*5, 7, 14, 29*), little information is available on the effect of moisture on carbonyl-amine browning in freeze-dried fruits.

Harper and Tappel (*11*) note that the main deteriorative reaction in dried fruits is believed to be the carbonyl-amine reaction and observed that the amino-nitrogen content decreases in darkening dried apricots. Nichols and Reed (*28*) found that drying apples and apricots to below 10% moisture improved the color stability. They found little deterioration below a moisture range of 5 to 7% for stored, air-dried bananas.

Haas and Stadtman (*10*) used ion exchangers to identify the types of compounds involved in the various types of browning. Anet and Reynolds and Ingles and Reynolds (*17, 18*), using freeze-dried apricot and peach pureé, demonstrated the presence of several intermediates of sugar-amine and sugar-organic acid browning reactions in their samples which were stored at a moisture level of 21%. Huang and Draudt (*15*) found several browning intermediates in freeze-dried peaches stored at 1.68% moisture and above, but not in those containing 0.55% moisture.

The present work was undertaken primarily to determine the effect of moisture on chemical changes associated with various types of browning in peaches and bananas and, secondarily, to determine to what extent sulfur dioxide effects some of these changes.

Experimental

Fresh Georgia cling peaches (50 pounds) were peeled, sliced, and divided into two lots. One lot was untreated, and the smaller lot was blanched in boiling water for 30 seconds. Preliminary tests showed this treatment to be adequate for inactivation of all enzymes studied. Both lots were frozen in 250-ml. bottles surrounded by dry ice and freeze-dried. Freeze drying was carried out for 24 hours at 0.05 to 0.08 mm. of Hg pressure without thawing. After freeze-drying, the untreated lot contained 1.29% moisture, and the blanched lot contained 1.49% moisture.

After 15 days' storage over 69% sulfuric acid at 28° C., 220 grams of untreated, freeze-dried peaches were placed in another desiccator. It was evacuated, and approximately 80 ml. of sulfur dioxide gas was introduced. The samples were held in the presence of the sulfur dioxide gas for 24 hours. Analysis by the Monier-Williams method (*19*) found 697 p.p.m. of sulfur dioxide on the dry basis.

Each of the lots of freeze-dried peaches (untreated, blanched, and sulfur dioxide–treated) was divided into sublots and stored at 28° C. at different humidities over sulfuric acid solutions of different concentrations.

Samples were removed periodically over 240 days for analysis. Time required to bring samples to the desired moisture level is included in the storage times given here.

Fresh ripe bananas (20 pounds) were peeled, sliced ($^1/_4$ inch thick), frozen in 250-ml. bottles surrounded by dry ice, and freeze-dried. Freeze drying was carried out for 24 hours at 0.05 to 0.08 mm. of Hg pressure without thawing. The initial moisture content was 1.93% as determined by vacuum-oven drying. Part of the bananas were treated with sulfur dioxide essentially as described for peaches.

Each of the lots of freeze-dried bananas (untreated and sulfur dioxide–treated) was divided into sublots and stored at 28° C. at different humidities over sulfuric acid solutions of different concentrations.

Samples were removed for analysis periodically up to 180 days. Volatiles lost by drying at 70° C. at 80 mm. for 24 hours were considered to be "moisture" for both freeze-dried peaches and bananas.

The extract for the assay of all enzyme activities in each case was prepared by grinding the dry sample in a mortar and by dispersing the powder in 40 ml. of ice-cold water. After filtration through cheesecloth, the filtrate was made up to 50 ml. with ice-cold water.

Polyphenol oxidase activity was determined by measuring the rate of oxygen uptake by 1 ml. of a 0.075*M* catechol solution buffered to pH 5.5 (0.2*M*

[1] Present address, Peter Eckrich and Sons, Inc., Fort Wayne, Ind.
[2] Present address, Food Research Institute, University of Chicago, Chicago, Ill.

acetate) in the presence of 1 ml. of enzyme extract (*21*).

Results were calculated in terms of Q_{O_2}

$$Q_{O_2} = \frac{\mu l.\ O_2}{mg.\ dry\ wt. \times hr.}$$

Peroxidase activity was determined by the colorimetric guaiacol procedure (*20*) on 5 ml. of enzyme extract. Optical absorbance was determined at 480 mμ with a Bausch & Lomb Spectronic-20 spectrophotometer. Peroxidase activity was calculated as change in optical absorbance per minute per gram of dry sample.

Invertase present in the sample was estimated by measuring the total reducing power of sugars released in a sucrose substrate (*13*). Measurement of the maltose released by the action of amylase in the sample on soluble starch substrate was used as an index of β-amylase activity (*4*).

Guadagni's method (*9*) was employed for determining total phenolic compounds (as tannic acid). Two grams of freeze-dried peaches or bananas were blended with 60 ml. of 70% ethanol in a Waring Blendor for 3 minutes. The resultant slurry was made up to 100 ml. with 70% ethanol and filtered. The filtrate, 2 ml., was mixed with 25 ml. of water, 1 ml. of Folin-Dennis reagent, and 10 ml. of 10% sodium carbonate solution. The resultant solution was made up to 50 ml. and mixed thoroughly. After the solution stood at room temperature for 2 hours, the color was measured at 600 mμ using a Beckman Model DU spectrophotometer.

Total reducing sugars were determined by the Somogyi-Nelson method (*26, 32*). Free amino nitrogen was determined by the formol titration method with slight modification (*12*).

For estimation of sucrose content, an aliquot of the clarified filtrate was hydrolyzed with invertase. A 5-ml. aliquot of the clarified filtrate described above was pipeted into a graduated blood sugar tube. Two drops of the sodium acetate buffer (0.2*M*, pH 4.8) and 5 drops of the invertase solution, prepared by dissolving 20 mg. of invertase (Nutritional Biochemicals Corp., melibiase free) in 10 ml. of ice-cold water, were added before holding overnight at 35° C. The contents of the test tube were diluted to 25 ml. with water before a 2-ml. aliquot was used for color development. The sucrose content was estimated from the difference between the reducing sugar values before and after hydrolysis.

The pH of the original filtrate (not clarified) described above was determined by using a Beckman Model G pH meter. All peach samples exhibited pH values of 3.55 to 3.80 during 240 days' storage.

The indophenol dye method (*19*) was employed for the determination of ascorbic acid. The sample was mixed with 60 ml. of 3% metaphosphoric acid solution in a Waring Blendor. The resultant slurry was made up to 100 ml. with 3% metaphosphoric acid and then filtered. In the case of the SO_2-treated, freeze-dried peaches, the solution was evacuated for 10 minutes before making up to 100 ml. with 3% metaphosphoric acid. A 20-ml. aliquot of the filtrate was titrated by standardized 2,6-dichlorophenolindophenol solution.

Ascorbic acid oxidase activity in stored, freeze-dried bananas was determined by observing the rate of oxidation of added ascorbic acid (*21*). Sulfur dioxide was determined by the Monier-Williams method. Free L-tyrosine in freeze-dried bananas was determined at the end of storage (180 days) by use of L-tyrosine decarboxylase (*4*). A slurry was prepared by blending 3 grams of the dry sample with 66 ml. of 70% ethanol. The resultant slurry was washed into a funnel, and the filtrate was concentrated under reduced pressure. To the dried residue, 0.2*M* phosphate-citrate buffer (pH 5.5) was added to make up the final volume of 6 ml. Two milliliters of this solution was used for determination of L-tyrosine by the manometric method (*4*).

Optical absorption of alcoholic extracts was measured at completion of the storage period by Stadtman's method (*34*) with some modifications. The sample was blended with 60 ml. of 50% ethanol in a Waring Blendor for 3 minutes. The resultant slurry was made up to 100 ml. with 50% ethanol and then filtered. Color was measured at 300 and 430 mμ using a Beckman Model DU spectrophotometer. Color index is expressed as optical absorbance units per gram of dry sample per 100 ml. of solution.

Freeze-dried bananas were removed periodically up to 180 days' storage and examined for browning intermediates by paper chromatography in a manner similar to that used in a study of browning intermediates in stored, freeze-dried peaches (*15*).

Two-gram samples were blended with 60 ml. of 70% ethanol in a Waring Blendor, made up to 100 ml., and filtered. Cationic constituents were collected by placing the alcoholic extracts on a Dowex 50-X8W (20–50 mesh, H-form, 1.0 × 10 cm. column). The column was washed with 100 ml. of 40% ethanol and 100 ml. of water. Finally, cationic constituents were eluted with 100 ml. of 0.5*N* ammonium hydroxide solution and concentrated at 60° C. under reduced pressure.

Browning intermediates were separated on Whatman No. 1 filter paper for 42 to 96 hours (descending) at 20° C. using a 1-butanol–acetic acid–water mixture (4:1:1 v./v.). Three spray reagents, 0.2% ninhydrin, 5% ammoniacal silver nitrate, and *p*-dimethylaminobenzaldehyde (*p*-DAB), were employed after pretreatment with acetylacetone (*8*).

The R_g (relative to migration of D-glucose) values of spots were compared with those of several reference compounds, prepared by the method of Anet and Reynolds (*2, 15*).

Areas corresponding to ninhydrin and

Table I. Analysis of Fresh Bananas Used for Freeze Drying

Moisture (Volatiles at 70°, 80 mm.)	74.5% of fresh weight
Reducing sugar	17.9% of dry weight as glucose
Sucrose	55.1% of dry weight
Amino nitrogen	1.58 mg. of N per gram dry weight
Total phenolic compounds	2.34 mg. of tannic acid per gram dry weight
Ascorbic acid	46.0 mg. of ascorbic acid per 100 gram dry weight
Polyphenol oxidase	22.6 μl. O_2 absorbed per hr. × mg. dry weight
Peroxidase	49.5 (Δ O.D. per minute per gram dry weight) × 100

Table II. Polyphenol Oxidase Activity in Freeze-Dried, Untreated and SO_2-Treated Cling Peaches Stored at 28° C. at Different Moisture Levels

	Freeze-Dried, Untreated, Moisture, %					
	0.72	*2.04*	*3.40*	*5.36*	*8.43*	*12.67*
Storage Time, Days	*Polyphenol Oxidase Activity (μl. O_2 Absorbed/Hr. × Mg. Sample)*					
5	2.7					
20	3.4					
32	4.1	5.2	3.9	3.0	0.7	0.8
53	1.8	2.0	1.2	1.2	0.8	0.3
102	1.2	1.6	1.6	1.0	0.0	0.0
242	2.0	1.7	2.2	1.1	0.0	0.0
	Freeze-Dried, SO_2-Treated, Moisture, %					
Storage Time, Days	*0.90*	*2.06*	*3.42*	*5.35*	*8.52*	*11.50*
	Polyphenol Oxidase Activity					
5	2.9					
20	0.3					
43, 135, 244	0	0	0	0	0	0

Table III. Polyphenol Oxidase Activity in Freeze-Dried, Untreated and SO_2-Treated Bananas Stored at Different Moisture Levels

	Freeze-Dried, Untreated, Moisture, %					
	0.70	3.43	5.44	8.04	10.02	19.14
Storage Time, Days	Polyphenol Oxidase Activity ($\mu l.$ O_2 Absorbed/Hr. × Mg. Sample)					
5	21.7					
47	46.9	37.3	16.5	12.3	7.9	6.5
80	21.9	20.1	12.8	7.7	2.2	2.2
136	19.2	15.9	14.7	6.4	2.4	0
176	24.1	22.0	21.3	10.3	4.1	0
	Freeze-Dried, SO_2-Treated, Moisture, %					
Storage Time, Days	0.93	3.74	5.99	7.91	9.40	16.16
	Polyphenol Oxidase Activity					
5	21.7					
83	Sl. P[a]	Sl. P	Sl. P[b]	V. Sl. P	0	0
139, 177	V. Sl. P	V. Sl. P	0	0	0	0

[a] Sl. P: Slightly positive.
[b] V. Sl. P: Very slightly positive.

Table IV. Total Phenolic Content of Untreated, Freeze-Dried Peaches Stored at 28° C. at Different Moisture Levels

	Moisture, %					
	0.55	1.68	3.09	5.31	7.97	12.75
Storage Time, Days	Total Phenolic Compounds (Mg. Tannic Acid per Gram Dry Sample)					
0	7.91					
90	8.29	8.10	8.00	8.00	7.91	7.23
138	7.95	8.26	8.25	8.84	8.71	7.56

Table V. Peroxidase Activity of Freeze-Dried, Untreated and SO_2-Treated Cling Peaches Stored at 28° C. at Different Moisture Levels

	Freeze-Dried, Untreated, Moisture, %					
	0.72	2.04	3.40	5.36	8.43	12.67
Storage Time, Days	Peroxidase Activity[a] (O.A./Min./Gram Dry Sample) × 100					
5	Sl. P[b]					
32	7.8	10.2	10.7	0	0	0
53	8.0	8.4	3.9	0	0	0
102	Sl. P	Sl. P	V. Sl. P[c]	0	0	0
123, 242	Sl. P	Sl. P	V. Sl. P	0	0	0
	Freeze-Dried, SO_2-Treated, Moisture, %					
	0.90	2.06	3.42	5.35	8.52	11.50
Storage Time, Days	Peroxidase Activity					
5						
20	Sl. P.	V. Sl. P.	V. Sl. P.	V. Sl. P.	V. Sl. P.	0
43, 60, 105, 135, 244	V. Sl. P.	V. Sl. P.	V. Sl. P.	V. Sl. P.	V. Sl. P.	0

[a] O. A.: Optical absorbance units.
[b] Sl. P.: Slightly positive.
[c] V. Sl. P.: Very slightly positive.

ammoniacal silver nitrate positive areas were cut from the paper, eluted with 50% ethanol, and hydrolyzed for 2 hours in 1*N* HCl. The hydrolyzates were neutralized with 10*N* NaOH and chromatographed on Whatman No. 1 paper using 1-butanol–acetic acid–water (4:1:5 v./v. upper layer) as solvent and 0.2% ninhydrin for amino acids and 0.1% resorcinol in 2*N* HCl for sugars as spraying reagents. Also employed as chromatographic solvents for amino acids were 1-butanol–acetic acid–water (4:1:1 v./v.) and phenol-water (4:1 w./w.).

Results and Discussion

Analytical results for the fresh bananas employed for freeze drying are given in Table I.

In untreated, freeze-dried peaches with moisture contents below 3.4%, polyphenol oxidase activity decreased slowly during storage (Table II). At higher moisture contents, its activity decreased rapidly. Sulfur dioxide (697 p.p.m.) inhibited polyphenol oxidase completely in freeze-dried peaches.

As noted in Table III, in untreated, freeze-dried bananas with moisture contents of 5.44% and below, polyphenol oxidase activity remained at a high level during 176 days' storage at 28° C. At higher moisture contents, its activity decreased rapidly. The apparent increase in activity during the initial stage of storage of freeze-dried bananas with moisture contents below about 4% is unexplained, although Maier and Schiller (*24*) suggested that the increase in phenolase activity in storage of domestic dates is due to an increased extractability of the enzyme resulting from tissue and cellular disorganization. In freeze-dried bananas, sulfur dioxide, largely but not completely, inhibited polyphenol oxidase.

Total phenolic compound content in untreated, freeze-dried peaches calculated as milligrams of tannic acid per gram of dry sample is shown in Table IV. Polyphenol oxidase substrates remained at a fairly constant level during 100 days' storage except for the 12% moisture level sample. While this suggested that oxidative browning either by polyphenol oxidase or by nonenzymic oxidative processes may not be significant below an 8% storage moisture level in freeze-dried peaches, it is clearly possible that the amount of change involved in browning may be smaller than sampling and analytical errors occurring here.

In another series of duplicate experiments not shown here, there was little difference in total tannic acid content after one year's storage at 28° C. in blanched and untreated freeze-dried Georgia cling peaches containing 1 to 8% moisture. In freeze-dried bananas, little change in tannic acids, from the initial value of 2.4 mg. per gram of dry sample, was exhibited up to 183 days' storage in samples containing 8.04% and lower moisture levels. The 10.02 and 19.4% moisture samples increased to 3.26 and 3.08 mg. of tannic acid per gram of dry sample, respectively, after 183 days' storage at 28° C.

Free L-tyrosine was essentially the same (0.06 mg. of L-tyrosine per gram of dry sample) for samples of all storage moisture levels after 183 days' storage. This indicates that release of L-tyrosine was not responsible for the increase in phenolic compounds observed at high moisture levels in freeze-dried bananas.

Peroxidase-catalyzed reactions in freeze-dried peaches can be responsible for only a small portion, if any, of the total enzymic darkening since in the freeze-dried peaches containing 5.36% and higher moisture contents, peroxidase activity was no longer detectable in untreated samples after 32 days' storage (Table V). Peroxidase inactivation during storage was much slower at 3.40% than at 5.36% moisture.

Sulfur-dioxide treatment largely, but not completely, inhibited peroxidase. Activity was observed throughout 244 days' storage in the sulfur dioxide–

treated peaches with moisture contents of 8.5% and below (Table V).

Destruction of peroxidase was very rapid during storage of freeze-dried bananas. After 5 days' storage, the 0.70% moisture samples exhibited an activity of 10.0 expressed as optical absorbance units (O.A.) per minute per gram of dry sample × 100. At 47 days, the activity was zero for all samples except one containing 0.70% moisture which gave a very slightly positive test. Because of rapid loss, it appears that peroxidase-catalyzed reactions in freeze-dried bananas can be responsible for only a small portion, if any, of total browning. Sulfur dioxide-treated, freeze-dried bananas exhibited no peroxidase activity in any samples.

Determination of free amino nitrogen, reducing sugar, and sucrose content during storage of untreated and SO_2-treated, freeze-dried peaches and bananas was of little value for following changes during storage. For samples in the practical moisture range of 1 to 10%, all differences noted during storage were within the range of expected sampling and analytical error. In freeze-dried bananas containing 19.1% moisture, free amino nitrogen decreased from 1.70 to 1.12 mg. of N per gram of dry sample.

Joslyn and Marsh (*22*) did not observe a loss in free amino nitrogen in orange juice, although the juice had become very dark brown after 136 days' storage. They suggested that the rate of amino-nitrogen formation by hydrolysis of soluble polypeptides might exactly parallel the loss of amino acids through darkening. Loss of free amino nitrogen during storage of dried apricots had been observed (*33*, *36*).

Changes in reducing sugar content (as D-glucose) was observed only in the case of high moisture level samples, including freeze-dried, untreated, and sulfur dioxide-treated bananas with high moisture levels. The initial 5-day reducing sugar content was 13.2% as D-glucose. After 175 days' storage, reducing sugar contents were 20.1, 22.0, and 55.9%, respectively, for 8.04, 10.1, and 19.1% moisture samples. This increase is probably due to the hydrolysis of sucrose.

Invertase activity was found in all samples of untreated freeze-dried peaches throughout the storage period, but a substantial rate of loss of sucrose was observed only at 12.67% moisture and not at 8.43% and lower levels. At 12.67% moisture, sucrose decreased from 47.6 to 37.8% during 226 days' storage period.

Although β-amylase activity was found in the initial stage of storage of the freeze-dried peaches, its activity decreased to zero after 20 days' storage. Results were somewhat erratic since the high concentration of reducing sugars in samples caused difficulty in the measurement of β-amylase activity in the crude enzyme preparation that was employed.

Table VI. Ascorbic Acid Content of Freeze-Dried, Untreated and SO_2-Treated Bananas Stored at 28° C. at Different Moisture Levels

	Freeze-Dried, Untreated, Moisture, %					
	0.70	3.43	5.44	8.04	10.02	19.14
	Ascorbic Acid, Mg. per 100 Grams Dry Sample					
Initial	48.2					
After 178 days' storage	31.8	27.5	17.8	10.5	9.1	4.1
Retention, %	66.0	57.1	37.0	21.8	18.8	8.5
	Freeze-Dried, SO_2-Treated, Moisture, %					
	0.93	3.74	5.99	7.91	9.40	16.16
	Ascorbic Acid, Mg. per 100 Grams Dry Sample					
Initial	48.2					
After 178 days' storage	40.9	42.3	42.7	32.1	10.5	7.1
Retention, %	85.0	87.8	88.6	66.6	21.8	14.7

Table VII. Ascorbic Acid Content of Freeze-Dried, Untreated, Blanched, and SO_2-Treated Georgia Cling Peaches Stored at 28° C. at Different Moisture Levels

	Freeze-Dried Untreoted, Moisture, %					
	0.72	2.04	3.40	5.36	8.43	12.67
	Ascorbic Acid, Mg. per 100 Grams Dry Sample					
Initial	37.6					
After 228 days' storage	36.7	32.1	31.3	23.3	13.3	10.1
Retention, %	97.6	85.3	83.3	62.0	35.4	26.9
	Blanched, Freeze-Dried, Moisture, %					
	1.00	2.17	3.32	5.05	7.98	12.00
	Ascorbic Acid, Mg. per 100 Grams Dry Sample					
Initial	35.7					
After 229 days' storage	35.8					11.4
Retention, %	100.3					31.9
	Freeze-Dried, SO_2-Treated, Moisture, %					
	0.90	2.06	3.42	5.35	8.52	11.50
	Ascorbic Acid, Mg. per 100 Grams Dry Weight					
Initial	39.5					
After 229 days' storage	37.8	39.0	31.1	24.5	16.0	11.4
Retention, %	95.6	98.7	78.7	62.0	40.5	28.9

Sucrose content of freeze-dried bananas (initial value 52.5%) remained constant within analytical and sampling error during 175 days' storage for all untreated, freeze-dried samples except one containing 19.1% moisture. In this case sucrose decreased to 11.4%. Results were similar for SO_2-treated samples. The 16.2% moisture sample contained 15.8% sucrose after 177 days. In both cases, these high moisture levels are above those considered practical for freeze-dried foods.

Ascorbic acid oxidase activity in freeze-dried bananas was such that 2.0 mg. of ascorbic acid was destroyed per 15 minutes per gram dry weight at the beginning of storage of untreated, freeze-dried bananas and after 176 days' storage only two samples with moisture contents of 0.70 and 3.43% showed slightly positive activity (less than 0.2 mg. of ascorbic acid destroyed per 15 minutes per gram dry weight). Other samples exhibited no detectable activity. Thus, loss of ascorbic acid as noted in Table VI is not primarily due to ascorbic acid oxidase. Ascorbic acid oxidase was not determined in SO_2-treated samples. Stone (*35*) showed that ascorbic acid oxidase in bananas catalyzed the reversible oxidation of ascorbic acid. Armentano and Bartok (*3*) noted that bananas contain considerable ascorbic acid but little ascorbic acid oxidase.

Ascorbic acid content expressed as mg. per 100 grams of dry sample is shown in Table VI. Sulfur dioxide gave substantial protection to the ascorbic acid present but the mechanism of action of this protection is not clear from the present work. All samples of the untreated lot had pH values of 4.90 to 5.00 during 180 days' storage. Freeze-dried, SO_2-treated bananas exhibited pH values of 4.75 to 4.80 during 180 days' storage.

Ascorbic acid content of freeze-dried peaches expressed as mg. per 100 grams of dry sample is shown in Table VII. Its destruction is moisture dependent in respect to the treatments before storage and roughly parallels the change in color (Figure 1,*A*). Browning in citrus fruit is associated with destruction of

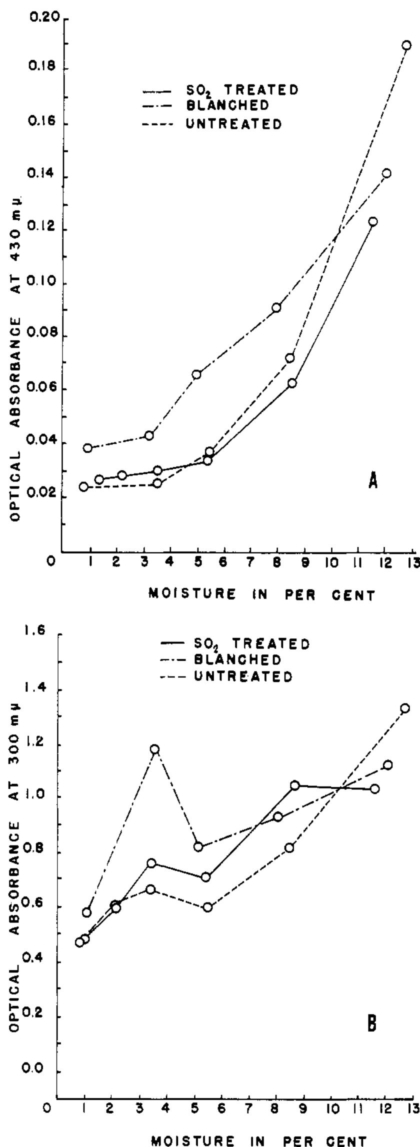

Figure 1. Optical absorbance vs. storage moisture content for alcohol extracts of freeze-dried peaches stored 241 days at 28°C.

Initial values immediately after freeze drying were 0.024, 0.040, and 0.024, respectively, for untreated, blanched, and SO_2-treated samples
A. At 430 mμ
B. At 300 mμ

ascorbic acid (*33*). Wong *et al.* (*37*) found that in vacuum-dried tomato powder stored at 37.7° C. or below, ascorbic acid loss was very small if moisture content was below 2.5%. The results given here suggest that oxidative browning processes occur at levels as low as about 3% moisture.

The results in Table VII suggest that ascorbic acid may be destroyed by non-enzymic rather than by enzymatic processes, since the pattern of ascorbic acid loss was similar for untreated, SO_2-treated, and blanched, freeze-dried peaches. Koppanyi, Vivino, and Veitch (*23*) observed that ascorbic acid, after conversion to dehydroascorbic acid, can react with β-amino acids to produce strongly colored complexes. Dulkin and Friedemann (*6*) showed that both reductic and ascorbic acids undergo a type of browning reaction analogous to that occurring in heat-processed and stored food. They further showed that oxidation of these compounds to dehydroform is a prerequisite to this type of browning.

Table VIII. Residual SO_2 Content of Freeze-Dried Peaches after 238 Days' Storage at 28° C. at Different Moisture Levels

Moisture, %					
0.90	2.06	3.42	5.35	8.52	11.50
SO_2 Content, p.p.m.					
365	297	244	329	324	335

The initial SO_2 content was 697 p.p.m. on dry basis.

Only those peaches held at 8% moisture or higher exhibited substantial darkening after 100 and 242 days' storage at 28° C. Others remained normal at 242 days. Untreated, freeze-dried peaches held 540 days at 5.36% moisture were slightly brown in color.

Figure 1 gives the optical absorption at 430 and 300 mμ for 50% ethanol-water (v./v.) extracts of freeze-dried peaches stored 241 days at 28° C.

Figure 1*A* shows that, below 5% moisture, all curves follow approximately the same pattern. Above 5% moisture the extent of browning increases rapidly for all treatments with increasing moisture content up to approximately 12% moisture. At 3% moisture, substantial formation of browning reaction intermediates as judged by ultraviolet absorption at 300 mμ had occurred in 241 days (Figure 1*B*). At between 5 and 6% moisture, there was less accumulated ultraviolet absorbing material than in samples containing 3.5% moisture. At 3.5% moisture and below, formation of final-stage browning products absorbing at 430 mμ is very low, and this may be the factor limiting discoloration. In the range of 5 to 6% moisture, ultraviolet absorbing browning intermediates may be used up rapidly in forming final products. In this range, the accumulation of intermediates is lower. About 5% moisture appears to be needed for relatively rapid formation of appreciable amount of final products. Additional work is needed in this area since only suggestions can be made from the limited work reported here.

Sulfur dioxide treatment had little effect on the formation of materials absorbing at 300 mμ, except possibly at the highest moisture level of 11.5%. This result is in line with Anet's work (*1*) on model browning systems in which he found that bisulfite had only a small effect on formation of the browning intermediates absorbing at 295 mμ.

The absorption at 430 mμ for both blanched and SO_2-treated peaches was lower than for untreated peaches at the highest moisture level. In general, SO_2 treatment provided only partial protection against browning. Mohammad, Fraenkel-Conrat, and Olcott (*25*) noted that SO_2 did not have much effect on carbonyl-amine browning.

Observations on the initial and residual SO_2 content of the freeze-dried peaches are given in Table VIII. Approximately 50 to 60% of the SO_2 originally present disappeared during storage. Disappearance of SO_2 during storage of dried fruits is commonly observed (*27, 33*). Freeze-dried peaches initially containing 697 p.p.m. sulfur dioxide became dark during long-term storage at a moisture level of 8% or above, even though they still contained SO_2 (335 p.p.m.).

Optical absorption of alcoholic extracts of freeze-dried bananas expressed as optical absorbance units per gram of dry sample per 100 ml. of solution are shown in Figure 2. Only those bananas held at 8% moisture or higher exhibited substantial changes in extractable color after 180 days' storage at 28° C. (Figure 2*A*). Heavy SO_2 treatment provided limited color protection.

In the case of the highest moisture level sample (19.14%), considerable color formation was observed at 50 days' storage (Figure 2*A*). This could be due to enzyme-catalyzed browning. The sample containing 0.7% moisture decreased in color at a nearly constant rate. Such a result would arise if some pigments absorbing at 430 mμ, which are present originally, are destroyed or changed to other 70% ethanol-unextractable forms during storage. In the case of 3.43% and 5.44% samples, the rate of actual color formation may have balanced the rate of decrease in 70% ethanol-extractable color to yield little net change.

Figure 2*C* indicates the optical absorption of 70% ethanol solution of freeze-dried, SO_2-treated bananas *vs.* storage time in days at 430 mμ. Up to 80 days' storage, none of the samples exhibited much change in actual color; however, after 80 days, extractable color increased in samples with moisture levels of 9.40 and 16.16%. Other samples with moisture levels below 7.91% exhibited a distinct color bleaching. Bleaching of color by SO_2 is commonly observed and has been discussed by Richert (*30*). Increased rate of oxidation has been observed in foods at very low moisture levels (*31*), and

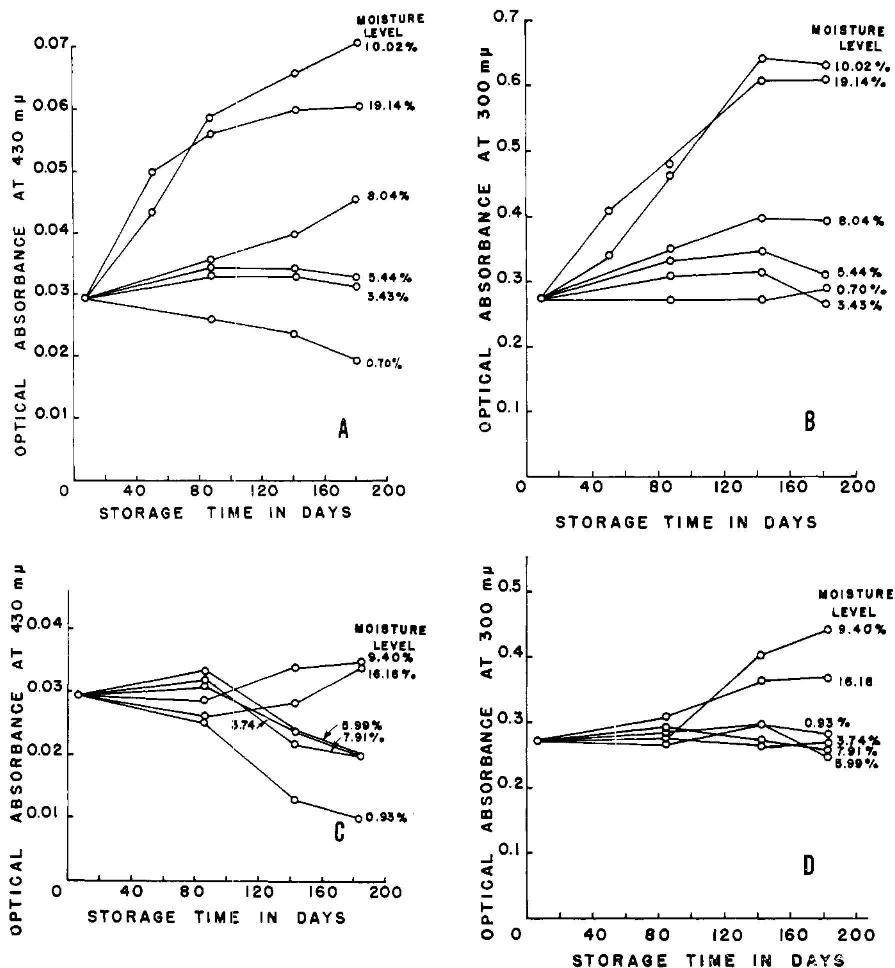

Figure 2. Optical absorbance vs. storage time for alcohol extracts of freeze-dried treated and untreated bananas at different moisture levels at 28° C.

A. 430 mμ, untreated
B. 300 mμ, untreated
C. 430 mμ, SO_2 treated
D. 300 mμ, SO_2 treated

Table IX. Paper Chromatography of Parent Amino Acids and Carbonyl Part of Browning Intermediates (Compounds 7 and 8) after Hydrolysis

Compounds	R_f of Parent Amino Acids: n-BuOH–AcOH–H_2O (4:1:5, v./v.)[a]	n-BuOH–AcOH–H_2O (4:1:1, v./v.)[b]	Phenol–H_2O (4:1, w./w.)	R_f of Carbonyl Part: n-BuOH–AcOH–H_2O (4:1:5, v./v.)[a]
Compound 7	0.33	0.51	0.75	0.81
Compound 8	0.43	0.67	0.74	0.81
Authentic γ-aminobutyric Acid	0.33	0.51	0.75	. . .
DL-Valine	0.43	0.67	0.74	. . .
5-Hydroxymethyl-2-furfural	. . .	. . .	. . .	0.81

[a] Paper: Whatman No. 1 filter paper. Time: 25 hours, descending. Temperature: 20° C.
[b] Paper: Whatman No. 1 filter paper. Time: 5 hours, ascending. Temperature: 28 ± 2° C.

bleaching may be due to destruction of carotenoids.

Figure 2*D* shows the optical absorbance of 70% ethanol solutions of freeze-dried, SO_2-treated bananas *vs.* storage time in days at 300 mμ. Up to 80 days, all curves followed a similar pattern and, after 80 days, the samples with higher moisture levels (9.40 and 16.16%) increased in browning intermediates which absorb at 300 mμ. The initial SO_2 level was 3900 p.p.m. After 179 days' storage, residual SO_2 contents were 544, 649, 592, 624, 571, and 641 p.p.m. at 0.93, 3.74, 5.99, 7.91, 9.40, and 16.16% moisture contents, respectively. Thus, approximately 85% of the SO_2 originally present disappeared during storage. The fate of this SO_2 is not known. Ingles (*16*) demonstrated that SO_2 can change to sulfate and sulfur and also can combine with sugars to form sugar sulfonates. In commercial practice, SO_2 is ordinarily applied before drying; also, the levels of SO_2 used here are considerably above those commercially employed. Although heavy SO_2 treatment decreased the extent of browning, it did not appear to raise substantially the moisture level for browning color formation.

Paper chromatographic techniques were applied to the detection of carbonyl-amine browning intermediates, as well as to the hydrolysis products of these intermediates in the freeze-dried bananas. Eight major intermediates were observed on the paper chromatograms at the end of storage (190 days) in the samples with moisture content of 3.43% and above in untreated lots and 5.99% and above in SO_2-treated lots. Their R_g values were 0.07, 0.19, 0.23, 0.40, 0.69, 0.91, 1.28, and 1.68 (1-butanol–acetic acid–water, 4:1:1, v./v.). All spots reacted with ninhydrin, ammoniacal silver nitrate, and *p*-dimethylaminobenzaldehyde.

Among these eight major intermediates, compounds with R_g values of 1.28 and 1.68 yielded γ-aminobutyric acid and valine, respectively, and 5-hydroxymethylfurfural as the carbonyl part after hydrolysis with 1*N* HCl. Their chromatographic behaviors, color reactions, and hydrolysis products are identical with those of authentic reference compounds prepared from D-glucose and γ-aminobutyric acid, and D-glucose and valine (Table IX). Therefore, compounds with R_g values of 1.28 and 1.68 are identified as "fructose-γ-aminobutyric acid" with "fructose-valine," respectively.

Six other spots seen on the chromatograms could not be obtained in chromatographically pure states by the solvent used herein and were not further investigated. No intermediates of this type were found in freeze-dried, untreated bananas stored at 0.70% moisture and in freeze-dried, SO_2-treated bananas stored at 0.83 and 3.74% moisture levels for 180 days. This suggests that the rate of carbonyl-amine browning in stored, freeze-dried bananas is slow at very low moisture levels.

Literature Cited

(1) Anet, E. F. L. J., *Australian J. Chem.* **12,** 491 (1959).
(2) Anet, E. F. L. J., Reynolds, T. M., *Ibid.,* **10,** 182 (1957).
(3) Armentano, L., Bartok, H. A., *Biochem. Z.* **311,** 418 (1942).
(4) Bernfeld, P., "Methods in Enzymology I," S. P. Colowick and N. O. Kaplan, Eds., p. 149, Academic Press, New York, 1955.
(5) Danehy, J. P., Pigman, W. W., *Advan. Food Res.* **3,** 241 (1951).
(6) Dulkin, S. I., Friedemann, T. E., *Food Res.* **21,** 519 (1956).
(7) Ellis, G. P., *Advan. Carbohydrate Chem.* **14,** 63 (1959).
(8) Gale, E. F., "Methods of Biochemical Analysis," D. Glick, ed., Vol. **4,**

p. 285, Interscience, New York, 1957.
(9) Guadagni, D. G., Sorber, D. G., Wilbur, J. S., *Food Technol.* **3,** 359 (1949).
(10) Haas, V. A., Stadtman, E. R., *Ind. Eng. Chem.* **41,** 983 (1949).
(11) Harper, J. S., Tappel, A. L., *Advan. Food Res.* **7,** 171 (1957).
(12) Henriques, V., Sörensen, S. P. L., *Z. Physiol. Chem.* **64,** 120 (1909).
(13) Hestrin, S., Feingold, D. S., Schramm, M., "Methods in Enzymology I," S. P. Colowick and N. O. Kaplan, eds., p. 251, Academic Press, New York, 1955.
(14) Hodge, J. E., J. AGR. FOOD CHEM. **1,** 928 (1953).
(15) Huang, I-Yih, Draudt, H. N., *Food Technol.* **18,** 124 (1964).
(16) Ingles, D. L., *Australian J. Chem.* **13,** 404 (1960).
(17) Ingles, D. L., Reynolds, T. M., *Ibid.*, **11,** 575 (1958).
(18) *Ibid.*, **12,** 483 (1959).
(19) Jacobs, M. B., "The Chemical Analysis of Foods and Food Products," pp. 156, 728, Van Nostrand, Princeton, N. J., 1958.
(20) Joslyn, M. A., *J. Assoc. Offic. Agr. Chemists* **33,** 504 (1950).
(21) Joslyn, M. A., Bedford, C. L., Marsh, G. L., *Ind. Eng. Chem.* **30,** 1068 (1938).
(22) Joslyn, M. A., Marsh, G. L., *Ibid.*, **27,** 186 (1935).
(23) Koppanyi, T., Vivino, A. E., Veitch, R. P., Jr., *Science* **101,** 541 (1945).
(24) Maier, V. P., Schiller, F. H., *J. Food Sci.* **26,** 322 (1961).
(25) Mohammad, A., Fraenkel-Conrat, H., Olcott, H. S., *Arch. Biochem. Biophys.* **24,** 157 (1949).
(26) Nelson, N., *J. Biol. Chem.* **153,** 375 (1944).
(27) Nichols, P. F., Mrak, E. M., Bethel, R., *Food Res.* **4,** 67 (1939).
(28) Nichols, P. F., Reed, H. M., *Western Canner Packer* **23,** 11 (1931).
(29) Reynolds, T. M., *Advan. Food Res.* **12,** 1 (1963).
(30) Richert, P. H., J. AGR. FOOD CHEM. **1,** 610 (1953).
(31) Salwin, H., "Freeze Drying of Foods," F. R. Fisher, ed., p. 58, National Academy of Sciences–National Research Council, 1962.
(32) Somogyi, M., *J. Biol. Chem.* **195,** 19 (1952).
(33) Stadtman, E. R., *Advan. Food Res.* **1,** 325 (1948).
(34) Stadtman, E. R., Barker, A. H., Mrak, E. M., Mackinney, G., *Ind. Eng. Chem.* **38,** 99 (1946).
(35) Stone, W., *Biochem. J.* **31,** 508 (1937).
(36) Weast, C. A., Mackinney, G., *Ind. Eng. Chem.* **33,** 1408 (1941).
(37) Wong, F. F., Dietrich, W. C., Harris, J. G., Lindquist, F. E., *Food Technol.* **10,** 96 (1956).

Received for review August 6, 1964. Accepted January 17, 1966. This paper reports in part research undertaken in cooperation with the Armed Forces Food and Container Institute, U. S. Army Quartermaster Research and Engineering Center, and has been assigned Number 2261 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author(s) and do not necessarily have the endorsement of the Department of Defense. Journal Paper No. 2079 of the Agricultural Experiment Station, Purdue University, Lafayette, Ind.

NUCLEOTIDE MEASUREMENT

Rapid Measurement of Inosine Monophosphate and Total Adenosine Nucleotides in Fish Tissue

JOHN SPINELLI and BARBARA KEMP

Bureau of Commercial Fisheries Technological Laboratory, Seattle, Wash.

A method is described for the rapid determination of inosine monophosphate (IMP) and total adenosine nucleotides in fish tissue. Perchloric acid extracts of the tissue are made, and the nucleotides are absorbed and separated from nucleosides and purine bases on a Dowex 1-X4-(Cl) resin. After elution from the resin with H_2SO_4, total adenosine and inosine nucleotides are determined by measuring the absorbancy of the effluent at 250 mμ. Adenosine nucleotides in the effluent are then determined chemically and subtracted from the total nucleotides to yield the IMP content of the extract. The method is useful over varying concentrations of adenosine and inosine nucleotides, and values are in close agreement with those obtained by using classical ion exchange systems.

THE measurement of inosine monophosphate (IMP) and related nucleotides in fish tissue is important because it furnishes information regarding the freshness of the fish (*7, 10*) and also because IMP is an important flavor constituent of the tissue (*4, 11*). IMP is derived as the hydrolytic deamination product of adenosine triphosphate (ATP), and amounts generally ranging from 4 to 7 μmoles per gram (*7, 10, 15*) accumulate in fish tissue soon after the death of the fish by the following pathway: ATP → ADP → AMP → IMP. Although the rate of total nucleotide breakdown varies between species (*6, 10*), nearly all of the nucleotides found post mortem in commercially important fish are those of adenosine and inosine. Small amounts of guanosine, cytidine, and uridine-based nucleotides have been reported in rested freshly killed cod (*8*). In some species, the breakdown of IMP is almost complete after 8 to 10 days of storage (*10*), whereas in other species, such as halibut, there are still substantial quantities of both adenosine nucleotides and IMP even after 20 days of storage (*14*).

Although the extent of nucleotide dephosphorylation can be rapidly estimated by several methods (*9, 13, 15*), accurate measurements of IMP are generally made by chromatographic ion-exchange separations (*5, 16*).

The work presented here shows that IMP and the adenosine nucleotides in fish tissue can be rapidly and accurately measured after a simple separation of the total nucleotides from their corresponding nucleoside and purine bases. The method should be applicable to the determination of total adenosine nucleotides in animal tissue other than fish.

Method

Preparation of Tissue Extracts. Extracts were prepared by grinding 40 grams of fish tissue with 80 ml. of chilled 3% $HClO_4$ for 1 minute in a blender. The homogenate was filtered, and 20 ml. were immediately neutralized with 10% KOH to pH 6.5 to 6.8. The neutralized extract was stored for approximately 30 minutes at 0° C. to permit crystallization of the $KClO_4$.

Separation of Nucleotides. Dowex 1-X4-(Cl) was recycled with 10% NH_4OH and 4*N* HCl. Twenty milliliters of the neutralized extract were passed over a 1 × 2 cm. resin bed to separate the nucleotides from nucleosides, purines, and free sugars (*13*). Distilled water (30 to 35 cc.) was passed over the column until the effluent was free from ultraviolet-absorbing material. The column was then eluted with 25 ml. of 1*N* H_2SO_4, followed by 5 ml. of 6*N* H_2SO_4. This removed 98 to 100%