Table 3.   Total nitrogen content of commercially produced 42° Brix frozen concentrated orange juice.

| Code | Nitrogen content Replicates | | |
	1	2	Average
	(grams per 100 grams)		
U51	.432	.417	.425
U53	.432	.442	.437
S52	.447	.443	.445
P52	.403	--	.403
R52	.446	.446	.446
051	.422	.425	.424
D54	.453	.450	.452
R54	.464	.464	.464
P53	.403	.412	.408
N53	.450	.439	.445
M54	.446	.447	.446
053	.462	.457	.460

either the soluble solids or nitrogen content in the samples taken in October. The fruit picked in this early month were undoubtedly those with the highest solids of the prevailing crop, although the nitrogen content may still be low. Also rootstock, soil characteristics, and cultural practices which increase the nitrogen content of the juice have an opposite effect on the soluble solids of the same fruit. Negative correlation between these 2 factors in the early fruit could be possible.

Total Nitrogen Content of Frozen Orange Concentrate: Duplicate determinations of 12 samples of 42° Brix frozen orange juice concentrate showed good reproductibility (Table 3).

The nitrogen content of these samples ranged from 0.403 to 0.464 g/100 g. When calculated on a reconstituted juice basis, the nitrogen content ranged from 0.118 to .137 g/100 ml which is comparable to the fresh juice of late season fruit. The range was within 20% of the mean and the coefficient of variation was about 4%.

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CHANGES IN ORGANIC NITROGEN INCLUDING FREE AMINO ACIDS DURING PROCESSING OF FLORIDA ORANGE CONCENTRATE[1]

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ABSTRACT

Total nitrogen, soluble nitrogen, and total free amino acids were determined in 'Valencia' fresh and reconstituted juices of 65° Brix concentrates. Significantly higher amounts of soluble nitrogen and accompanying lower amounts of insoluble nitrogen were found in reconstituted than in

fresh juices. Quantitative differences in total free amino acids between the fresh and reconstituted juices were small. Thin layer chromatographic fractionations showed good resolution of amino acids from fresh juice extracts. A slightly different qualitative amino acid pattern was obtained for extracts of reconstituted juices while the resolution was not as good as with the fresh juice extracts. Non-enzymatic reactions between amino acids and glucose in a model system at pH 4.5 were demonstrated. Volatile carbonyls evolved were trapped in dinitrophenylhydrazine and were tentatively identified by gas chromatography.

INTRODUCTION

Orange juice is known to have a relatively high content of nitrogenous compounds. Up to 10% of the soluble solids in the juice contain nitrogen (14). Free amino acids constitute a major portion of the nitrogen-containing substances (14, 16, 17).

Browning reactions between free sugars and amino acids, induced by heat during food processing have been reported (1, 5, 13, 15). During processing and storage of orange juice, Rockland (14) found a 60% increase in amino nitrogen within 60 seconds when the juice was heated at 93°C. These values were decreased to a level slightly higher than that of fresh juice when the heated juice was stored for 5 months at 38°C. Also, heating of orange juice at 100° C for 15 minutes was reported to result in a 20-60% reduction in the level of gamma-aminobutyric acid, aspartic acid, serine, glutamic acid, and alanine. Joslyn (8) found that part of the lysine and glutamic acid contents is destroyed during browning of orange juice stored at ambient temperature for 4 years.

This investigation was designed to study the effect of processing of Florida orange concentrate on the major forms of organic nitrogen including total free amino acids. The possibility of reactions between glucose and each of four amino acids in a model system was also studied.

MATERIALS AND METHODS

Five samples each of 'Valencia' orange evaporator feed juice and 65° Brix orange concentrate pumpout from a high temperature, short-time evaporator were quickly frozen and stored at −22.2°C for 38 days before analyses were made.

The concentrates were reconstituted just prior to analyses to Brix values equal to that of the single-strength juices.

Determination of total and soluble nitrogen.—A 10 ml juice sample was used for determination of total nitrogen by a semi-micro Kjeldahl method (18). Difficulties encountered due to foaming during the digestion process were alleviated by the addition of 3 ml of antifoaming agent to the mixture. For the determination of soluble nitrogen, a 10 ml juice sample was extracted with 200 ml 80% ethanol for 2 hours after which the sample was filtered. The residue was washed with 40 ml 80% ethanol and both the wash and filtrate were combined and made to a volume in a 250 ml volumetric flask. A 150 ml aliquant (equivalent to 6 ml signle-strength juice) was then reduced to 10 ml volume *in vacuo* for the analysis. The rest of the filtrate was used for the separation and purification of amino acids.

Amino acids analysis.—The remaining 100 ml of the combined filtrate from the soluble nitrogen determination was acidified to pH2 with 1 N HCl and passed through a 2 cm X 11 cm column of Dowex 50W−8X (H+ form) according to the method described by Freeman and Woodbridge (4). Amino acids were eluted with 30 ml solution of 95% ethanol and ammonium hydroxide (1:1 v/v). The eluate was reduced to 10 ml volume under a stream of air. Total amino acids were determined on a Technicon Auto-analyzer using a method based on that described by Moore and Stein (9). A mixture of 10 amino acids was used as a standard for the quantitative determination of these compounds in the juices. The standard mixture contained 0.005 mole each of alanine, aspartic acid, gamma-aminobutyric acid, glutamic acid, glutamine, asparagine, glycine, arginine, proline and serine.

Thin layer chromatography of amino acids.—Amino acids extracted from orange juice were separated by two-dimensional thin layer chromatography on 300 μ thick cellulose layers coated on 20 cm X 20 cm glass plates using the same solvent system in the first direction as that of Jones and Heathcote (6). The solvent system utilized in the second direction of chromatography was slightly modified as shown below since it resulted in better separation.

First dimension: isopropanol: formic acid: water (40 : 2 : 10 v/v).

Second dimension: tert-butanol: 2-butanone: ammonium hydroxide: water (45 : 27 : 10 : 18 v/v).

Table 1. Effect of processing of Florida 'Valencia' orange concentrate on various forms of nitrogen.

| Sample No. | Nitrogen content (mg/ml) | | | | | |
| | Total-N | | Soluble-N | | Insoluble-N | |
	Fresh juice	Reconst. juice	Fresh juice	Reconst. juice	Fresh juice	Reconst. juice
1	1.248	1.279	0.894	1.063	0.355	0.216
2	1.248	1.288	0.869	1.039	0.379	0.249
3	1.219	1.229	0.857	1.014	0.362	0.215
4	1.219	1.232	0.869	1.039	0.350	0.193
5	1.207	1.231	0.845	1.014	0.362	0.217

The amino acid eluate from the Dowex column was reduced to 1 ml volume and 30 μl were applied to the cellulose layer. Chromatography in both directions took about 5.5 hours.

Reactions between glucose and amino acids.— These reactions were conducted as described by El' Ode' et al. (3). Volatiles produced were trapped in an acidic solution of dinitrophenyl-hydrazine and were regenerated as described by Dornseifer and Powers (2). The regenerated volatiles were injected as a gas sample in the Perkin Elmer 226 Gas Chromatograph equipped with a flame ionization detector and a 300 ft X 0.01" capillary column coated with Apiezon L, 95%: Igepal C0880, 4%; DOPC (antioxidant), 1%. Helium was used as carrier at 42 psig.

RESULTS AND DISCUSSION

Higher values for soluble nitrogen were observed in reconstituted juices of 65° Brix 'Valencia' orange concentrates than in fresh juices (Table 1). Values for insoluble nitrogen, which include protein nitrogen, were higher in the fresh than in the reconstituted juices (Table 1). These values were obtained by subtracting soluble nitrogen from total nitrogen. Table 2 shows the values for total free amino acids in fresh and reconstituted juice samples. It is evident that a slight change took place in the total free amino acids during juice processing. This agrees with results reported by Rockland (14) on fresh 'Valencia' juice and juices processed for 1, 2 and 3 minutes at 93° C. After 5 months of storage at 38°C, juice samples initially heated for 1

minute showed about 70% decrease in their insoluble nitrogen (14).

Qualitative changes in amino acids during pasteurization and concentration of juices were studied by separating the free amino acids extracted on thin layers of cellulose powder. The chromatographic separation of 20 amino acids in a standard mixture and those in extracts from fresh and reconstituted juices are shown in Fig. 1. The corresponding R_f values are presented in Table 3. Thus, there were no major qualitative differences in composition of the amino acids found in the fresh and reconstituted juice samples (Fig. 1). However, spot 23 Fig. 1B, R_f 0.22 and 0.50) was consistently found in the fresh juices, although in a small quantity, but not in the reconstituted juices. Also, the resolution of amino acids in the center of the chromatogram, where alanine and proline (spots 8 and 9, Fig. 1-B, 1-C) were found, was better with extracts obtained from fresh juices than those from the reconstituted juices. Two large, irregularily-shaped ninhydrin sensitive components were de-

Table 2. Total free amino acids in fresh and reconstituted Florida 'Valencia' orange juices.

| Sample No. | Total free amino acids (mg/ml) | |
	Fresh juice	Reconstituted juice
6	1.60	1.34
7	2.08	2.09
8	2.02	1.91
9	2.04	1.83
10	2.31	2.04

tected only in extracts from concentrates (Fig. 1-C). The identity of these components was not determined. This may indicate that the heat treatment and concentration of orange juice results in the destruction of some ninhydrin sensitive components and in the formation of others. Joslyn (7) reported the formation of ninhydrin positive compounds, other than amino acids, in mixtures containing amino acids, sugars and ascorbic acid. These compounds are believed to be sugar amines (14).

The increase in soluble nitrogen (Table 1) and the detection of two ninhydrin sensitive components (Fig. 1-C) in the amino acid extracts of concentrates indicate indirectly that some destruction in the insoluble nitrogen fraction, including protein, may have taken place during processing. Degradation of protein in orange juice was found to result in the release of amino acids that are not normally found in the free amino acid fraction (17). The reason that these changes were not substantially reflected, either
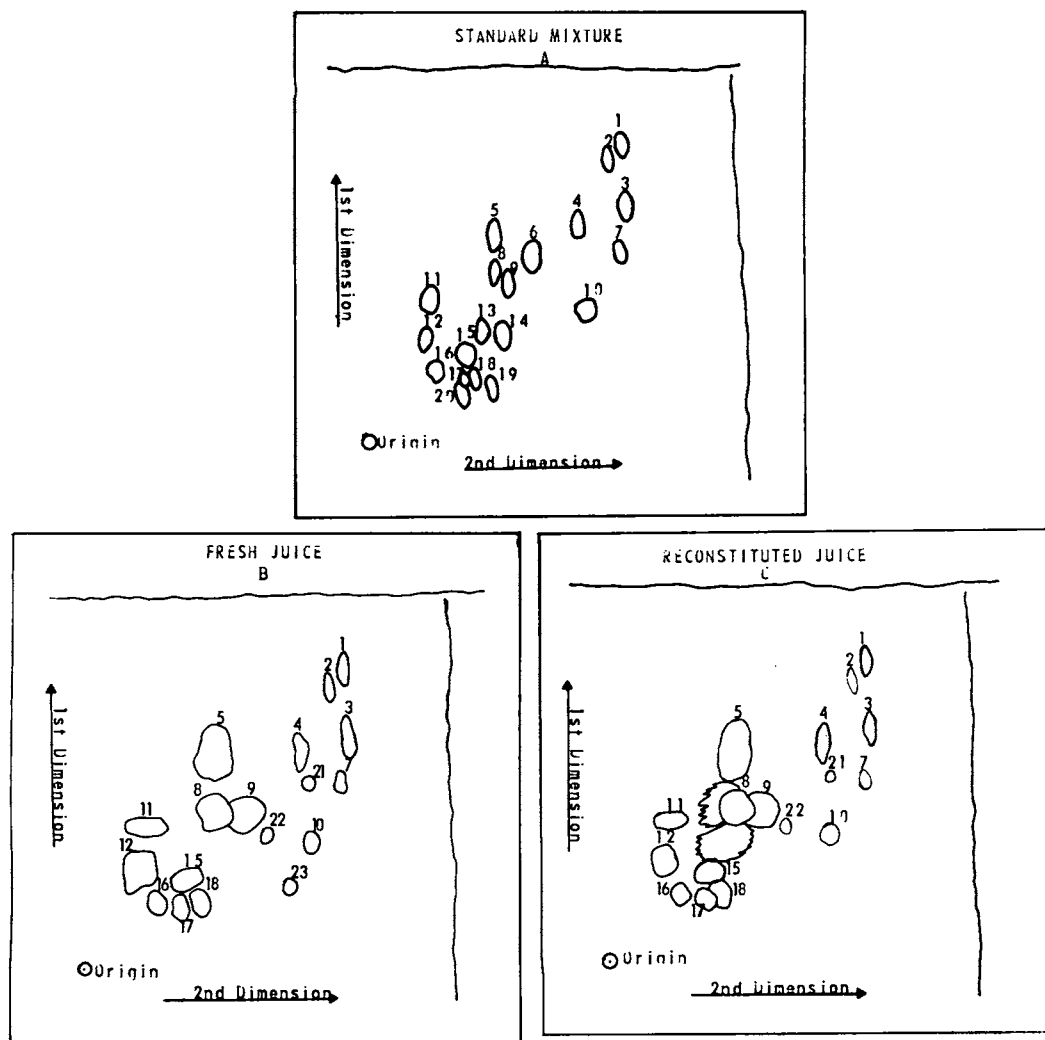

Fig. 1.—Thin layer chromatographic separation of amino acids in a (A) standard mixture, (B) fresh juice, and (C) reconstituted juice from 65° Brix concentrate.

Table 3. Rf values of 20 amino acids in a standard mixture and those separated from extracts of fresh and reconstituted 'Valencia' orange juice by two-dimensional thin layer chromatography.

Spot No.	Amino acid	Standard mixture Dimension		Fresh juice Dimension		Reconstituted juice Dimension	
		1st	2nd	1st	2nd	1st	2nd
1	Leucine	.79	.70	.81	.70	.79	.71
2	Isoleucine	.75	.66	.75	.67	.74	.67
3	DL-Phenylalanine	.62	.71	.63	.72	.62	.72
4	Methionine	.57	.58	.58	.58	.60	.59
5	Gamma-aminobutyric	.55	.33	.58	.35	.56	.35
6	Tyrosine	.49	.45	-	-	-	-
7	Tryptophan	.49	.67	.50	.70	.48	.70
8	Alanine	.45	.33	.42	.35	.44	.35
9	Proline	.42	.38	.42	.42	.39	.42
10	Threonine	.34	.59	.34	.60	.33	.62
11	Glutamic acid	.38	.17	.38	.16	.38	.16
12	Aspartic acid	.27	.15	.26	.15	.27	.50
13	Glycine	.29	.31	-	-	-	-
14	Serine	.28	.36	-	-	-	-
15	Glutamine	.23	.26	.24	.27	.24	.27
16	Arginine	.19	.18	.17	.16	.18	.20
17	Ornithine	.16	.26	.16	.26	.16	.26
18	Histidine	.16	.28	.17	.31	.18	.30
19	Cysteine	.13	.33	-	-	-	-
20	Cystine	.12	.25	-	-	-	-
21	Unknown	-	-	.50	.61	.49	.61
22	"	-	-	.36	.50	.36	.48
23	"	-	-	.22	.50	-	-

qualitatively or quantitatively in the results of amino acid analyses, may be due to the solubilization of nitrogen containing compounds that are not proteinaceous in nature. It could also be due to the participation of amino acids in non-enzymatic reactions with non-nitrogeneous constituents, e.g., reducing sugars. Reactions of this type have been reported. For example, Pearson et al. (10) reported that browning of pork meat during heating was a result of reactions between sugars and free amino acids. Rohan and Stewart (12, 13) also found that reactions of this nature were involved in the browning and in the production of volatile chocolate aroma during roasting of cocoa beans. These reactions have also been reported to occur in model systems at various pH values ranging between a pH of 5 and 8 (3). Various carbonyl compounds have been isolated from these systems and Pinto and Chichester (11) suggested that the majority of these compounds produced during the roasting of cocoa beans were the result of oxidative deamination of free amino acids.

The involvement of amino acids in non-enzymatic reactions with glucose has been demonstrated in a model system at pH 4.5 This pH value was selected to approximate that of the natural orange juice. The various gas chromatographic profiles shown in Fig. 2 were obtained from dinitrophenylhydrazone derivatives of products of reactions between glucose and each of 4 amino acids. The amino acids used were alanine, glycine, proline, and gamma-aminobutyric acid. The tentative identifications of some of the volatile compounds isolated are shown in Table 4. In every case, darkening of color was noticed in the reaction mixture and it was more severe with glycine and gamma-aminobutyric acid. It is possible that some of these reactions could take place during processing of orange juice, resulting in the production of carbonyl compounds. Such compounds may participate in the primary processes believed to be involved in the browning reaction (7). They could also participate in reactions with amino compounds which are believed to be intermediate products of browning.

It was concluded that processing of orange juice into concentrate results in a major change in the insoluble nitrogen fraction which may be related to reactions responsible for browning. Reactions between free amino acids and glucose
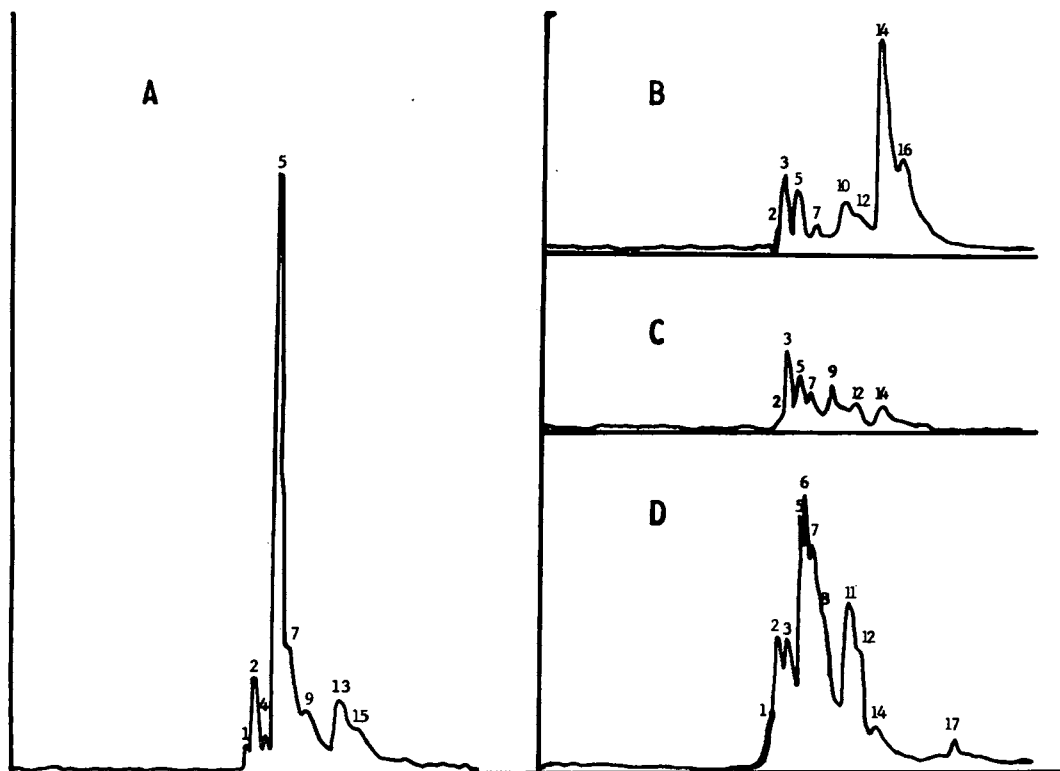
Fig. 2.—Gas chromatographic elution profiles of dinitrophenylhydrazine derivatives of volatile carbonyls generated by reacting glucose with each of 4 amino acids in acetate buffer (pH 4.5) at 100°C. A = alanine; B = glycine; C = proline; and D = gamma-aminobutyric acid.

Table 4. Identified volatile carbonyls generated by the reaction of glucose and each of 4 amino acids at 100°C and pH 4.5.

Peak* No.	Compound	A Alanine	B Glycine	C Proline	D Gamma-aminobutyric acid
1	Ethanal	+	-	-	+
2	Propanal	+	+	+	+
3	Acetone	-	+	+	+
5	2,3-Butanedione	+	+	+	+
6	Butanal	-	-	-	+
11	Crotonal	-	-	-	+
14	Valeraldehyde	-	+	+	+

* Peaks number 4, 7, 8, 9, 10, 12, 13, 15, 16, and 17 shown in Fig. 1 were not identified.

at pH 4.5 are possible and could take place during processing of orange juice resulting in the production of volatile carbonyls.

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RELATIONSHIP OF BACTERIAL CONTAMINATION IN ORANGE OIL RECOVERY SYSTEM TO QUALITY OF FINISHED PRODUCT

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Recent introduction by the beverage industry of drinks using citrus oils as one of their flavor components has created a large demand for some of the oils, particularly oil of grapefruit. Increased usage of these oils has attracted more processors not only to recover their oil, but also to seek refinements in their operation to increase yields.

To the best of the authors' knowledge, approximately 65% of the oils currently produced in Florida employ the FMC (Food Machinery Corp.) In-line extractor procedure. The remainder use the screw press method. A typical FMC In-line installation is shown in Fig. 1. This method consists briefly of a special oil cup assembly on the extractor which is used to recover the peel oil expressed during juice extraction. The oil is released by a shredding action. The shredded peel, generally referred to as peel grit slurry, along with a small amount of water is collected on the baffles on one side of the extractors. It is then conveyed, usually by means of a small screw conveyor, to one or more finishers where the solids are removed. The aqueous phase or oil finisher emulsion is then pumped to a desludger where most of the water is removed by centrifugal action. The oil emulsion (oil-rich fraction) from the desludger may now contain from 30 to 70% oil. It is polished and then winterized to remove the waxy materials. Orange peel oil is referred to as cold-pressed (*c.p.*) when