Differences in Amino Acid Composition in Commercial Orange Juices

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The amino acid composition of two sets of commercial orange juice was determined, and the mean value and range of values for each amino acid were tabulated. Single-strength juices from concentrates showed lower content of amino acids than straight-processed juices. Statistical comparison by ANOVA revealed statistical significance ($p \leq 0.001$) for aspartic, arginine, asparagine, glutamine, serine, threonine, and isoleucine, between straight-processed orange juices, and those single-strength juices from concentrates. The combination of glutamine, asparagine, and alanine allowed a 100% correct classification.

Keywords: *Commercial orange juices; amino acids; discriminant analysis*

INTRODUCTION

During the last years, orange juice demand has been expanding at a rapid rate, in part due to the constant modernization of the technologies which have had the effect of improving the quality of commercial juices. Since there is a shift in consumer preferences from pasteurized orange juice to totally fresh juice, one of the major efforts underway is to minimize changes during the orange juice processes.

Even under mild processing conditions, changes in the volatile constituents of orange juice take place, and quantitative data have been used to correlate changes in components with processing conditions. Velez et al. (1993) classified orange juice samples which had been stored at different temperatures, based on analysis of volatile constituents, and Shaw et al. (1993, 1994) were able to classify commercial orange juice samples into four types, based on the type of processing.

Although the mechanism responsible for the deterioration of citrus juice during processing and storage is not well understood, nonenzymatic browning reactions between carbohydrates and amino acids are generally believed to be involved (Kimball, 1991; Del Castillo et al., 1998). Since prolonged storage of juice concentrates may cause changes in the amino acid composition, this study was undertaken to determine whether the quantitative determination of individual amino acids would allow the distinction between two types of commercial orange juices: straight-processed and single-strength juices from concentrates.

MATERIALS AND METHODS

Samples. Seven samples of straight-processed orange juices and 17 samples of single-strength orange juices from concentrates were purchased at different local markets.

Determination of Amino Acids. Aliquots (10 mL) of orange juice were centrifuged at 6940 g for 20 min at 20 °C, and free amino acids were determined in the supernatant diluted (1:25) with borate buffer 0.4 M, pH = 10, and were filtered through a 0.22-μm membrane filter (Millipore Ibérica, Madrid, Spain). Analysis, in duplicate, was carried out by HPLC using a Waters (Milford, MA) liquid chromatograph controlled by a Maxima 820 chromatography workstation (Waters). Samples were submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA) (González de Llano et al., 1991) to determine primary amino acids. The quantification of proline was performed by automatic precolumn double derivatization with OPA and 9-fluorenylmethylchloroformate (FMOC) (Fluka Quimica, Madrid, Spain) (Einarsson, 1985). The separation of amino acids was performed on a Novapak C-18 60-Å 4-μm column (3.9 cm × 150 mm) (Waters). Detection was by fluorescence using the wavelengths of excitation and emission at 340 and 425 nm, respectively, for OPA derivatives. For FMOC derivatives the excitation and emission wavelengths were 250 and 335 nm, respectively. All reagents used were HPLC grade.

Statistical Analysis. The BMDP package (Dixon, 1988) was used for variance (BMDP7D program) and discriminant analysis (BMDP7M).

RESULTS AND DISCUSSION

Table 1 shows the mean values, ranges, and relative standard deviations of the amino acids of orange juices marketed as straight-processed and as single-strength from concentrates. In the two sets of samples, γ-aminobutyric acid, arginine, asparagine, aspartic acid, glutamic acid, proline, and serine were present in larger amounts than the rest of amino acids and they accounted for some 89% of total amino acids. In general, single-strength juices from concentrates showed lower mean content of amino acids (2740 mg/L) than straight-processed juices (3835 mg/L). Considerable dispersion in amino acid content between samples of the same set was observed. Except for glutamic acid, histidine, leucine, ornithine, proline, tyrosine, and methionine, higher dispersion was found in single-strength juices from concentrates than in straight-processed juices, mainly for glutamine which showed a relative standard deviation of 65.7% in single-strength juices from concentrates.

Apart from variations due to maturity, cultural practices, and variety of the fruit (Aristoy et al., 1989),

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Table 1. Amino Acid Concentrations (mg/L) of Commercial Orange Juices[a]

amino acid	straight-processed ($n = 7$)				single-strength from concentrates ($n = 17$)			
	mean[a]	rsd[a] (%)	max[a]	min[a]	mean[a]	rsd[a] (%)	max[a]	min[a]
aspartic acid***	260.12	24.37	363.64	159.69	189.37	31.21	309.29	86.95
glutamic acid**	128.99	33.98	194.29	75.94	87.77	27.32	124.88	47.91
asparagine***	419.11	16.03	540.41	328.71	275.04	28.64	431.95	127.29
serine***	148.77	13.91	189.13	116.84	103.47	29.03	182.15	54.51
glutamine***	51.89	38.53	84.60	24.86	17.53	65.68	37.96	0.00
histidine*	31.35	35.31	54.45	20.52	25.33	32.37	49.88	16.05
glycine**	22.14	7.88	24.63	18.28	19.93	15.83	27.39	14.80
threonine***	21.74	11.53	26.26	15.82	17.54	23.07	27.33	11.67
arginine***	785.76	20.33	1141.39	616.73	516.77	36.82	922.98	205.88
alanine**	74.51	12.73	88.69	55.05	61.24	35.17	108.41	33.43
γ-aminobutyric acid*	230.53	34.76	377.34	127.57	170.13	35.00	301.41	97.23
tyrosine	21.16	16.20	29.29	17.68	19.89	9.74	26.02	16.78
methionine	13.11	42.67	16.44	0.00	14.63	32.95	29.40	0.00
valine**	21.27	13.20	26.61	17.03	18.94	14.80	24.24	14.98
tryptophan	27.13	6.98	30.96	25.17	24.95	26.80	28.96	0.00
phenylalanine	29.01	16.72	39.99	22.84	27.77	29.07	39.82	19.58
isoleucine***	16.38	4.48	17.92	15.36	15.14	5.17	16.98	13.69
leucine**	18.62	8.25	22.39	16.89	17.51	4.61	19.39	16.16
ornithine	44.70	39.65	86.34	29.81	41.35	26.87	69.93	27.31
lysine*	45.40	19.46	64.74	36.14	38.51	19.44	52.67	26.55
proline	1423.88	53.15	2392.39	512.50	1037.93	42.85	2289.10	322.50

[a] Means, maximum (max) and minimum (min) values, and relative standard deviation (rsd). [b] Significant differences between straight-processed and single-strength from concentrates juices at $*p \leq 0.05$, $**p \leq 0.005$, and $***p \leq 0.001$.

Table 2. Classification of Spanish Commercial Orange Juices Using as Variables in Discriminant Analysis Glutamine (1), Asparagine (2), and Alanine (3)

classification	variables 1		1 + 2		1 + 2 + 3	
	SP	SSC	SP	SSC	SP	SSC
SP[a]	5	2	7	0	7	0
SSC[b]	2	15	2	15	0	17

[a] SP, straight processed ($n = 7$). [b] SSC, single-strength from concentrates ($n = 17$).

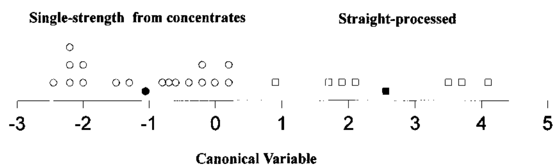

Figure 1. One-dimensional canonical plot of (○) single-strength from concentrates and (□) straight-processed orange juices. ● and ■ group mean.

changes in the amino acid content may have originated during the different steps of juice manufacture. Straight-processed juices are produced from freshly extracted juices which are heat-stabilized under mild conditions so that no significant deterioration of the amino acid fraction is expected. In the case of single-strength juices from concentrates, the concentration process did not alter the amino acid composition of orange juice (Cavazos et al., 1996; Maccarone et al., 1996). However, during the storage period between the concentrates' elaboration and reconstitution processes, nonenzymatic reactions may take place (Ting and Rouseff, 1986; Del Castillo et al., 1998), implying that the amino acid content of concentrated juice may vary according to storage conditions.

Statistical analysis by ANOVA revealed significant differences ($p \leq 0.001$) between straight-processed and single-strength from concentrates juices for aspartic acid, arginine, asparagine, glutamine, serine, threonine, and isoleucine (Table 1).

The results of discriminant analysis are shown in Table 2. No single compound could be use to differentiate the two sets of juices. Glutamine was the variable with higher discriminant power; a 71.4% correct assignment was obtained for straight-processed juices and a 88.2% for single-strength juices from concentrates. With the combination of glutamine, asparagine, and alanine, a 100% correct classification was attained.

Figure 1 shows the monodimensional space canonical variable representation obtained by discriminant analysis. All straight-processed juices showed canonical variable values higher than single-strength juices from concentrates. In the set of straight-processed juice samples, canonical variable values decreased from 4.12 to 0.90 as label declared shelf life of samples increased. Differences of shelf life between samples may be attributed to the severity of the thermal processes to which were submitted.

In the 50% of single-strength from concentrates juices samples with canonical variable value lower than −1, no presence of glutamine was detected. Relation among shelf life and the canonical variable values was not observed. Differences between canonical variable values may be attributed to the processing and storage conditions of concentrates prior to reconstitution.

Although further studies on juice samples processed and stored under controlled conditions are needed, present results show the usefulness of determination of the amino acid composition for the differentiation between straight-processed juices and single-strength juices from concentrates.

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