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Non-Volatile Organic Acids, pH and Titratable Acidity Changes in Pineapple Fruit Slices During Frozen Storage

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Abstract: Effects of freezing and frozen storage on pH, titratable acidity and non-volatile organic acids of two pineapple fruit cultivars, Smooth Cayenne and Red Spanish, were studied. Pineapple fruit was frozen as slices in a cold room at −18°C and stored at this temperature for a 12 month period. A negative correlation was found between pH and titratable acidity in the two cultivars throughout frozen storage ($r = -0{\cdot}67$ for Smooth Cayenne and $r = -0{\cdot}71$ for Red Spanish). Non-volatile organic acids were determined by high performance liquid chromatography. The major components were citric and L-malic acids. A high correlation was found between these two acids during frozen storage ($r = 0{\cdot}75$ in Smooth Cayenne and $r = 0{\cdot}78$ in Red Spanish). There were significant differences ($P \leqslant 0{\cdot}01$) in pH and titratable acidity between the two studied varieties after a year of frozen storage. Significant differences ($P \leqslant 0{\cdot}05$) were found in pH values during frozen storage in cv Smooth Cayenne and in citric and L-malic acids in cv Red Spanish. Freezing preservation of pineapple fruit slices led to minimal chemical changes after a year of frozen storage.

Key words: pineapple fruit, freezing, storage, pH, titratable acidity, non-volatile organic acids.

INTRODUCTION

Pineapple fruit (*Ananas comosus* L Merr) is widely used as an important constituent in a nutritious diet. This fruit, referred as 'The King of the Fruit' is grown extensively in Hawaii, the Philippines, Ivory Coast, Caribbean area, Malaysia, Taiwan, Thailand, Australia, Mexico, Kenya and the Republic of South Africa. Successful pineapple production requires a frost-free climate, making the pineapple a tropical crop. The pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruit, largely because of its attractive flavour and refreshing sugar–acid balance. Among the principal varieties are Smooth Cayenne and Red Spanish.

The range of chemical constituents of ripe pineapple depending upon stage of fruit ripeness, and agronomic and environmental factors, has been reported by Dull (1971) and Kermasha *et al* (1987). The quality and morphological characteristics of cv Smooth Cayenne and Red Spanish were studied by Bonnasieux (1988).

The pineapple fruit sold at the local markets in Spain is cv Smooth Cayenne of tropical origin (Ivory Coast mainly) and Red Spanish from the Canary Islands (Hierro, Spain). We have found very little data on cv Red Spanish from Canary Islands (Galán *et al* 1988).

Pineapple fruit is preserved by freezing to a limited extent. Smooth Cayenne cultivar is the principal cultivar used for freezing (Luh *et al* 1986). Non-volatile organic acids and sugars are the major chemical entities in fruits and processed fruit products. Fruit flavour is based mainly on the balance between sugars and organic acids, and on numerous compounds providing the aroma associated with fruit. Although citric acid was identified as a major non-volatile acid in pineapple fruit as early as 1904, it was 20 years later when malic acid was reported in pineapple. Gas chromatography (GC) of the methyl esters of the acids, detected succinic acid and confirmed the presence of citric, malic and malonic acids (Chan *et al* 1973).

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TABLE 1
Characteristics of pineapple fruit before processing (means of *n* determinations)[a]

Characteristic	*Cultivar*	
	Red Spanish	*Smooth Cayenne*
Fruit weight ($n = 20$) (g) (without crown)	927·0 b	2060·0 a
Crown weight ($n = 20$) (g)	60·0 b	290·0 a
Fruit length ($n = 20$) (cm)	11·6 b	17·9 a
Maximum fruit diameter ($n = 20$) (cm)	11·1 a	13·3 a
Shape	Round	Elongated
Skin colour	Reddish	Brownish
Leaf colour	Reddish green	Green
Flesh colour	Pale yellow	Intense yellow
Taste	Sweet acid	Sweet
pH ($n = 4$)	3·49 a	3·54 a
Titratable acidity ($n = 4$) (g citric acid per 100 g FW)	1·17 a	0·93 b
Soluble solids ($n = 4$) (°Brix at 20°C)	10·33 b	12·48 a

[a] Different letters in the same row indicate significant differences, $P \leqslant 0{\cdot}05$.

Pineapple juice has a composition similar to that of the fruit from which it is obtained. A summary of the major non-volatile acids reported in pineapple juice and their corresponding concentrations is given by Hodgson and Hodgson (1993). These values also show that pineapple variety influences the amount of total acids in the juice. Cano *et al* (1994) identified and quantified by high-performance liquid chromatography (HPLC) in pineapple fruits, the following non-volatile organic acids: citric, L-malic, oxalic, quinic and succinic.

The non-volatile organic acids found naturally in pineapple fruit may be used to identify different cultivars. It was therefore considered important to determine the changes in organic acids by the freezing process and during frozen storage, since these acids could modify the flavour and aroma. The aim of this study was to evaluate the influence of the freezing process and frozen storage on the pH, titratable acidity and nonvolatile organic acids in two pineapple fruit cultivars (Smooth Cayenne and Red Spanish).

MATERIALS AND METHODS

Plant material

Pineapple fruit (*Ananas comosus* L Merr) Red Spanish and Smooth Cayenne cultivars, from the Canary Islands and Ivory Coast respectively, were obtained from commercial sources. Fruits were stored at 8 ± 1°C and 80–90% relative humidity (Cancel 1974; Bartolomew and Paull 1986) until they reached the proper maturity level for processing (12·5° Brix for cv Smooth Cayenne and 10·3° Brix for cv Red Spanish).

Processing

Fruits with similar ripening characteristics: skin colour, flat eyes and soluble solids (Smith 1988) were selected for freezing. The fruits were hand-peeled, cored, sliced (1·0 ± 0·2 cm) and cut into small pieces. Pineapple fruit slices were packed in plastic bags (Polyskin X, 12 μm thick), containing 390 ± 10 g fruit, sealed, frozen in a cold room at − 18°C and stored at this temperature for 12 months. The temperature of − 18°C was specified for the frozen food chain (Institut International du Froid 1986) and is now incorporated in EU regulations.

Analytical methods

Samples were analysed for pH, total titratable acidity and non-volatile organic acids. Analyses were performed on fresh fruit immediately after freezing (time 0 represents fruit slices frozen and immediately thawed after freezing) and during frozen storage (at 1, 2, 4, 6, 8 and 12 months). Two bags of pineapple fruit slices were removed from frozen storage and thawed under controlled conditions (placed at room temperature, about 20°C, for 3 h prior to analysis). Values reported for each cultivar at each sampling interval reflect the mean of all measurements (at least three).

Soluble solids of the slices were measured with a digital refractometer (Atago dbx-30, Tokyo). Results were reported as °Brix at 20°C.

The pH was measured with a Crison pH meter on a mixture, cooled at 20°C, prepared by mincing 10 g pineapple fruit and blending it with 40 ml deionised water in a Sorwall Omnimixer. Total titratable acidity was determined on this same mixture by titration with 0·1 M NaOH up to 8·1 pH, according to the AOAC (1980) method. The results were expressed as percentage of citric acid (g citric acid per 100 g fresh weight).

The analysis of non-volatile organic acids in pineapple fruit was carried out by HPLC as described by Cano *et al* (1991) for papaya and with slight modifications by Bartolomé (1992) for pineapple fruit. Minced pineapple (10 g) was homogenised with 40 ml methanol in a Sorvall Omnimixer at 2°C. The homogenised sample was refluxed for 30 min at 50°C in a water bath. The extract was vacuum filtered through a Whatman no 1 filter paper on a Büchner funnel and the residue washed with methanol. The filtrate was concentrated to dryness under vacuum at 50°C in a Büchi rotatory evaporator. The residue was dissolved in deionised water, sonicated and its volume made up to 50 ml with distilled water. This solution was filtered through glass wool, cleaned up through a Sep-Pak C18 cartridge (Waters Associates) and filtered through a 0·45 μm Millipore AH filter. For HPLC analysis of organic acids an Ion-300 interaction stainless-steel column (30 cm × 6·5 mm) at 42°C and a diode array detector (214 nm) were used. Samples (20 μl) were introduced through a Hewlett Packard 1050 injector and eluted isocratically with aqueous 8·5 mN sulphuric acid at a flow rate of 0·4 ml min^{-1}. Organic acids in the samples were identified and quantified by comparison with known standards. Chromatographic standards were (μg ml^{-1}) 18·56 oxalic, 0·94 citric, 0·61 L-malic, 0·07 quinic and 0·07 succinic acid. Results were reported as g acid per 100 g fresh weight.

Statistical analysis

Data were analysed using the Statgraphics Statistical Graphics System, Version 5.0, Copyright 1985–1991. The software was provided on an 'AS-IS' basis. All data were subjected to an analysis of variance (ANOVA) and mean separation was by Duncan's multiple range test at $P \leqslant 0·05$. Significant differences were indicated by different letters in the same row. The method of least square was used to determine linear regression equations.

RESULTS AND DISCUSSION

Initial characteristics of the two pineapple fruit cultivars (Red Spanish and Smooth Cayenne) before processing were summarized in Table 1. Significant differences ($P \leqslant 0·05$) between cultivars were found in fruit weight, crown weight, fruit length, titratable acidity and soluble solids content. No significant differences were found in the pH values. The difference in acidity was related to the amount of citric acid (Marchal and Soler 1991). The citric acid content in the two cultivars was 66% total non-volatile organic acids for cv Smooth Cayenne and 85% for cv Red Spanish. The obtained values for total acidity as citric acid were in agreement with those of Iglesias (1981): 0·83–1·09% for cv Red Spanish, and Chan *et al* (1973): 0·80–0·98% for cv Smooth Cayenne.

The pH and total titratable acidity of pineapple fruit slices in the fresh product, after freezing and during frozen storage are shown in Table 2. No significant changes were found in the pH and titratable acidity

TABLE 2
Statistical analysis of pH and titratable acidity in fresh and frozen pineapple fruit slices[a]

	Variety	*Fresh product*	*Storage months at −18°C*						
			0	*1*	*2*	*4*	*6*	*8*	*12*
pH	Smooth Cayenne	3·54	3·65	3·54	3·42	3·56	3·55	3·69	3·73
		c	b	c	d	c	c	ab	a
	Red Spanish	3·49	3·36	3·33	3·23	3·30	3·57	3·25	3·25
		ab	bc	bc	c	c	a	c	c
Titratable acidity	Smooth Cayenne	0·93	0·85	0·94	1·09	0·97	0·96	0·76	0·93
(g citric acid per		b	c	b	a	b	b	d	b
100 g product)									
	Red Spanish	1·17	1·24	1·45	1·47	1·16	1·20	1·34	1·35
		d	cd	ab	a	d	d	bc	bc

[a] Duncan's multiple range test. Different letters in the same row indicate significant differences, $P \leqslant 0·05$.
F values (pH): variety, 127·49**; time, 7·27**; variety × time, 8·54**; ** $P \leqslant 0·01$. *F* values (titratable acidity): variety, 678·85**; time, 17·55**; variety × time, 16·24**; ** $P \leqslant 0·01$.

TABLE 3
Statistical analysis of organic acids in fresh and frozen pineapple fruit slices[a]

Organic acid (g per 100 g product)	*Variety*	*Fresh product*	*Storage months at −18°C*							
			0	*1*	*2*	*4*	*6*	*8*	*12*
Citric	Smooth	0·80	0·67	0·67	0·51	0·63	0·63	0·73	0·72
	Cayenne	a	ab	ab	bc	ab	ab	a	ab
	Red	1·27	0·71	0·84	0·89	0·87	1·13	1·03	1·37
	Spanish	ab	d	cd	bcd	bcd	abc	abcd	a
L-Malic	Smooth	0·38	0·38	0·34	0·31	0·37	0·35	0·38	0·42
	Cayenne	a	a	ab	ab	a	a	a	a
	Red	0·22	0·16	0·22	0·23	0·22	0·32	0·24	0·39
	Spanish	bc	c	bc	bc	bc	a	b	a
Total acids	Smooth	1·22	1·07	1·03	0·82	1·11	0·98	1·12	1·18
	Cayene	a	ab	ab	bc	ab	ab	ab	ab
	Red	1·49	0·88	1·08	1·12	1·10	1·47	1·28	1·77
	Spanish	ab	c	bc	bc	bc	ab	bc	a

[a] Duncan's multiple range test. Different letters in the same row indicate significant differences, $P \leqslant 0.05$. F values (citric): variety, 72·52**; time, 4·63**; variety × time, 2·42 NS; ** $P \leqslant 0.01$. F values (L-malic): variety, 39·65**; time, 4·76**; variety × time, 3·24*; * $P \leqslant 0.05$; NS, no significant difference. F values (total acids): variety, 22·37**; time, 5·25**; variety × time, 3·02*.

values during frozen storage (1 year) in cv Red Spanish but in cv Smooth Cayenne a small increase (2%) in pH value, related to the slight increase (9%) in titratable acidity. It is important to consider that the pH determination in a highly acid fruit cannot be sufficiently accurate to study any slight modifications due to the freezing and storage. A negative correlation was found between pH and total acidity (g per 100 g) citric acid ($r = -0.67$ in cv Smooth Cayenne and $r = 0.71$ in cv Red Spanish).

Chromatograms of the fresh product are given in Fig 1. HPLC made possible the separation and identification of five organic acids: oxalic, citric, L-malic, quinic and succinic.

The main organic acids of ripe pineapple fruit are citric and malic acid (Singleton and Gortner 1965; Dull 1971). Our results for these two acids and total non-volatile organic acids of fresh and frozen pineapple fruit are shown in Table 3. The amount of the main organic acids determined in both cultivars fell within the range reported by Dull (1971), Wills *et al* (1986) and Cano *et al* (1994).

In the cv Red Spanish, the amount of citric acid (1·27%) is higher than in the cv Smooth Cayenne (0·80%). The amounts are also different for the L-malic (0·22% for cv Red Spanish and 0·38% for cv Smooth Cayenne). A different citric/L-malic ratio was found in the two cultivars (5·78 for cv Red Spanish and 2·14 for

TABLE 4
Organic acids (mg per 100 g product) in fresh and frozen pineapple fruit slices

Organic acid	*Variety*	*Fresh Product*	*Storage months at −18°C*							
			0	*1*	*2*	*4*	*6*	*8*	*12*
Oxalic	Smooth Cayenne	3·83	4·06	3·56	2·97	3·78	Tr[a]	3·79	Tr
	Red Spanish	1·87	Tr	Tr	2·68	2·57	2·19	2·40	2·06
Quinic	Smooth Cayenne	26·2	26·0	Tr	Tr	22·9	Tr	18·4	24·6
	Red Spanish	Tr	15·1	18·0	Tr	Tr	Tr	Tr	Tr
Succinic	Smooth Cayenne	19·9	Tr	26·0	Tr	35·2	Tr	Tr	40·8
	Red Spanish	Tr	Tr	Tr	Tr	Tr	20·8	Tr	26·4

[a] Tr, trace.

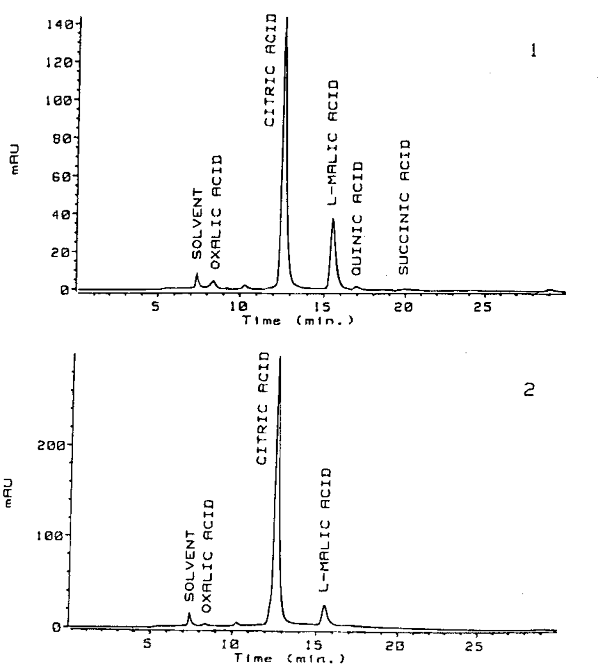

Fig 1. Non-volatile organic acids in fresh pineapple fruit detected by HPLC. (1) Cultivar Smooth Cayenne; (2) cultivar Red Spanish.

cv Smooth Cayenne). Braddock and Marcy (1985) found that citric acid content remained at about twice the concentration of malic acid in cv Smooth Cayenne. This is in agreement with our results for this cultivar.

The freezing process causes an important decrease in the amount of citric acid, greater for cv Red Spanish (44%) than for cv Smooth Cayenne (16%). This decrease can be due to the precipitation of citrates (Marín 1991).

The increase in the amount of citric and L-malic acids during frozen storage can be due to the changes in the permeability of the cell membrane resulting in water leaving the cells and thus increasing the solute concentrations.

A high correlation was found between citric acid content and total organic acids ($r = 0{\cdot}92$ in cv Smooth Cayenne and $r = 0{\cdot}99$ in cv Red Spanish), and between citric and malic acids ($r = 0{\cdot}75$ in cv Smooth Cayenne and $r = 0{\cdot}78$ in cv Red Spanish).

There were no significant differences in total non-volatile organic acids after 1 year of frozen storage in the two cultivars.

Minority non-volatile organic acids (oxalic, quinic and succinic) of the fresh and frozen product are shown in Table 4. Small differences in the concentrations of these acids can go beyond the detection threshold, which differs from acid to acid, concentrations near the threshold of detection are imprecise. It is possible to detect smaller amounts of oxalic acid (1·87 mg per 100 g fresh weight) than of quinic (15·1 mg per 100 g fresh weight) and succinic (13·4 mg per 100 g fresh weight) acids. Oxalic, quinic and succinic acid values in the fresh product were 3·8, 26·0 and 19·9 mg per 100 g fresh weight, respectively, in the cv Smooth Cayennne, and 1·9 and trace amounts in the other cultivar. After 1 year of frozen storage, the levels of oxalic and succinic acids were virtually the same as in the fresh product for cv Smooth Cayenne (3·8 and 24·6 g per 100 g fresh weight, respectively), while a higher level was observed for succinic acid (40·8 g per 100 g fresh weight). The changes in these acids for cv Red Spanish during frozen storage (1 year) were the following: the amount of oxalic acid did not change (2·0 mg per 100 g fresh weight), quinic acid was detected (15·1 mg per 100 g fresh weight) immediately after freezing and succinic acid (20·8 mg per 100 g fresh weight) after 6 months of frozen storage.

There were no significant differences in total non-volatile organic acids during 1 year of frozen storage in either cultivar.

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