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Organic acids of low molecular weight in Palmita-type cheese

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Palmita-type cheese is composed principally of volatile acetic and propionic acids and non-volatile lactic and succinic acids. The average concentrations of these acids in mg per 100 g of cheese decreased in the order: 140 mg of lactic acid, 80 mg of propionic acid, 14 mg of succinic acid, and 7 mg of acetic acid. However, variation coefficients for the concentrations of the acids oscillated between 34·4% and 106%, demonstrating the wide variability in the composition of Palmita cheese, which results from lack of standardization in the processing technology.

INTRODUCTION

Palmita-type cheese is a fresh cheese manufactured without heat treatment of the milk. The milk used for making the cheese comes from dairy-type, crossbred cows. The main breeds are Holstein, Brown Swiss, Zebu and native, this last being called 'Mosaico Perijanero'. The milk is coagulated with rennet at 35–37°C. Subsequently, the gel is finely cut to separate the whey and the curd. The firm curd is then cut into cubes of 3 cm each, which are salted in brine for 15–30 min at room temperature. The salted curd is then placed in 10–18 kg cheese moulds, pressed for several hours and then left for 2–3 days for the formation of the eyes and development of the acid. The cheeses are then removed from the moulds and kept refrigerated until such time as they are sold. Average values for the physicochemical parameters of the cheeses are: pH 5·3, 0·55% lactic acid, 2·79% chlorides (wet basis), 45% moisture content and 22% fat (Ferrer *et al.*, 1987).

The studies carried out for this cheese (Ferrer *et al.*, 1987, 1991) show the need for standardizing the processing technology, the lack of which currently causes a great variation in the physicochemical characteristics of the cheese. The studies also demonstrate the need to improve sanitary control, given the high count of opportunistic and potentially toxigenic bacteria found, such as *E. coli* ranging between 2×10^6 and 2×10^7 per gram of cheese, and *S. aureus* between 6×10^3 and 3×10^8 cfu/g. An alternative solution to both problems would be the pasteurization of the milk, subsequently adding selected starting cultures.

Many of the world's cheeses, at one time produced essentially as a cottage industry, are currently made with pasteurized milk, using starting cultures isolated from the earlier cheeses. In this way, through a controlled process of fermentation, it has been possible to produce a cheese of high organoleptic quality, similar to homemade cheeses and of a higher hygienic quality (Langsrud & Reinbold, 1973). In the case of Palmita-type cheese, the necessary bacteria were isolated (Ferrer, 1989) and a cheese was made using various combinations of these bacteria as starting cultures in pasteurized milk (Cabrera & Ferrer, 1989). Some of these combinations produced cheeses with organoleptic characteristics similar to those of Palmita-type cheese, especially with regard to appearance, eyes, texture, aroma and taste. However, the sensory parameter 'flavour' was considered to be of an inferior quality. It is thought that the principal components of the flavour of Palmita-type cheese may result from lactose and citrate fermentation, that is, the production of organic acids of low molecular weight, since the degree of lipolysis and proteolysis in the cheese must be quite low, given that it is a relatively unaged cheese. Accordingly, Palmita-type cheese does not have a strong flavour, a characteristic of the fatty acids produced by lipolysis, nor a bitter flavour, which is the product of certain peptides released during proteolysis (Aston & Dulley, 1982), but a rather slight acidic flavour.

Acids of low molecular weight produced by certain specific lactic cultures have been correlated with flavour characteristics in cheeses such as Cheddar, Swiss, Emmental, Tilsit and Edam (Langsrud & Reinbold, 1973), each of which possesses a characteristic profile. In these cheeses, due to the aging process, certain components exist that influence the flavour, such as fatty acids, carbonylic compounds, amino acids, etc. (Aston & Dulley, 1982). These compounds arise, not only from glycolysis, but also from the use of citrate, proteolysis and lipolysis, as well as from certain chemical reactions (Franklin & Sharpe, 1963; Jensen *et al.*, 1975; Paulsen *et al.*, 1980; El-Gendy *et al.*, 1983; Green & Manning, 1982).

The objective of the present study was to determine and quantify the relative concentrations of the volatile and non-volatile organic acids of low molecular weight, that are present in commercial Palmita-type cheese, in order to characterize the acid flavour, thus serving as a model for producing a similar cheese from pasteurized milk.

MATERIALS AND METHODS

Sampling of Palmita cheese

Twenty-six samples of 500 g each were purchased, from a single retailer of Palmita cheese, produced by the principal factory making this cheese in Zulia State. Each sample was purchased at 3–4 day intervals, following the delivery schedule of the plant. The samples were taken to the laboratory under refrigeration and immediately subjected to extraction of organic acids.

Preparation of the samples for acid extraction

A 40 g specimen was taken from each sample of cheese, and liquefied at high speed for 5 min with 60 ml of distilled water, in order to obtain a 40% w/w emulsion, which, in turn, was filtered through Whatman No. 4 paper. Subsequently, the filtrate was centrifuged in order to separate the remains of insoluble fatty matter from the liquid portion. The resulting liquid portion was subjected to extraction of organic acids in order to determine the characteristic profile of Palmita cheese.

Extraction of volatile acids

The technique of Lombard and Dowell (1982) was used to determine the short-chain volatile acids: formic, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids. A standard solution was prepared using 1 mmole of each acid per 100 ml of solution. In order to determine the percentage of recovery for each acid during the separation and extraction processes, a known volume of a standard solution of volatile acids was added to an emulsion of cheese, and the resulting solution was subjected to the same process as the emulsion that did not contain the standard solution.

The extraction process consisted in transferring 2 ml of the sample to a clean tube with a screw-on top. 0·2 ml of a 50% solution (v/v) of sulphuric acid was added to the sample in order to acidify it to pH < 2, thus guaranteeing the absence of ionic forms of the acid. Subsequently 1 ml of ethyl ether was added and lightly mixed in by inverting the tube. This was centrifuged for 5 min at 1500–2000 rev/min in order to break down the water–ether emulsion, and then put into a tube inside the freezer or in a solution of alcohol and ice until the water layer was frozen. Subsequently 10 ml of the ether phase was extracted with a syringe and injected into the chromatograph. The volatile acids were identified by comparison of the elution time of the chromatographic compounds with that of the acids present in the standard solution of volatile acids chromatographed the same day of the run.

Extraction of non-volatile acids

The technique of Lombard & Dowell (1982) was used to determine the short-chain, non-volatile acids: lactic, pyruvic, oxalacetic, oxalic, malonic, fumaric and succinic acids. A standard solution was prepared by adding 1 mmole of each acid to 100 ml of solution. The percentage of acid recovery in the emulsion of cheese was determined in a similar way to that used for volatile acids.

The extraction process consisted in transferring 1 ml of the sample to be analysed to a clean tube with a screw-on lid. 0·4 ml of 50% (v/v) solution of aqueous sulphuric acid and 2 ml of methanol were added to the sample and mixed by inverting the tube. Then the tube was placed in a water bath at 55 ± 0·2°C and left overnight in order to obtain the methyl esters of the acids. After the specified amount of time, 1 ml of distilled water was added to the tube, which was mixed by inverting. Subsequently 0·5 µl of chloroform were added and mixed by inverting. The resulting solution was centrifuged briefly at 1500–2000 rev/min in order to break down the emulsion and separate the chloroformic and aqueous phases. Then 10 ml of the chloroformic phase were extracted by syringe, carefully placing the end of the needle below the aqueous phase. The end of the needle was cleaned, the volume was adjusted and then injected into the chromatograph. The methylated acids were identified by comparing the time of elution of the products in the chloroform extract with that of the standard solution of non-volatile acids chromatographed the same day of the run. In this procedure, it was assumed that the reaction of esterification would occur approximately in the same proportion in all the acids, since the number of carbons was low (3–4) and the time of the reaction was sufficient to permit the esterification of all carboxyl groups.

Chromatographic analysis

The chromatograph used in this study for analysing volatile and non-volatile acids was a HP 5720A with 6 ft by ¼ in stainless steel columns, and a thermal conductivity detector. The chromatograph was equipped with a column packed with 15% SP-1220/1% H3PO4 in 100/120 Chromosorb W/AW (Supelco, 1979), for the analysis of volatile acids, with a second column packed with 10% SP-1000/1% H3PO4 in 100/120 Chromosorb W/AW (Supelco, 1979) for the analysis of non-volatile acids. Helium was used as the carrier gas, with a flow of 100–120 ml/min. The temperatures of the column, of the injection port for non-volatile acids, of the injection port for the volatile acids, and of the detector were 150°C, 225°C, 150°C and 300°C, respectively.

Estimation of acid concentrations

The concentration was estimated by calculating the area of the peak of each acid, and then converting it to a unit of concentration (mg acid/ml of sample), using an area/concentration quotient of the standard solution, obtained the same day as the run of the sample.

The concentration of each acid in mg of acid per 100 g of cheese was given by the following equation:

$$\% \text{ mg acid} = \frac{V_f C_a}{40} \times 100 = 2{\cdot}5 V_f C_a$$

in which C_a is the concentration of the acid in the aqueous portion in mg of acid/ml and V_f is the volume of the aqueous portion (approximately equal to the volume of the filtrate) in ml.

Determination of the recovery of acids in cheese

Tests were made of both volatile and non-volatile acids, adding a known volume of standard acid solution to a sample of liquefied cheese. At the same time, a sample of the same cheese was prepared without the addition of the standard solution, and both samples were subjected to separation and extraction processes and to chromatographic analysis. This process was repeated with samples from the same cheese batch. The concentration of each acid in both types of samples was determined by the procedure mentioned in the section on Estimation of Acids, and was carried out for two aliquots of each aqueous portion, performing duplicate chromatographic analyses. The concentrations found were C_{ai}, concentration of the acid i in the sample, and C_{ait}, concentration of the acid i in the sample with the standard solution included, both of these in mg of acid per ml of filtrate. The percentage of recovery is obtained from the following equations (Woo & Lindsay, 1982):

$$\% \text{ of recovery} = \frac{\text{mg of total acids measured}}{\text{total mg calculated}} \times 100$$

in which mg of total acids measured = $V_{fit} \times C_{ait}$.

total mg calculated = mg of endogenous acid + mg of added acids

mg of endogenous acid = $V_{fi} \times C_{ai}$

mg of added acids = $V_s \times C_s$

in which V_{fit} and V_{fi} are the average volumes of filtrate (ml) obtained from the samples with and without standard solution, respectively. In the same way, the corresponding concentrations represent average values. V_s and C_s are the volume of standard solution (ml) and the concentration of the acid under study in the standard solution (mg/ml), respectively.

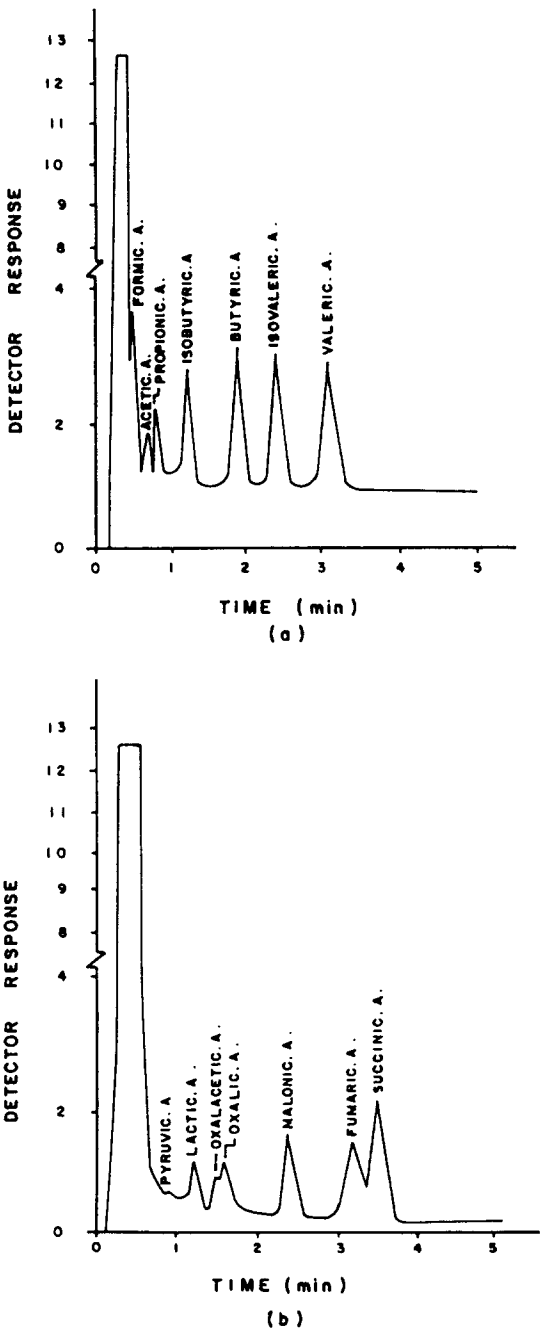

Fig. 1. Gas chromatograms of standard solutions of low molecular weight organic acids: (a) volatile, (b) non-volatile.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms obtained for the standard solutions of volatile and non-volatile acids, which were injected into the chromatograph before injecting the samples that contained the volatile and non-volatile acids of the Palmita cheese. In these, it can be observed that there is a good resolution for oxalacetic, oxalic, fumaric and succinic acids. However, the first three of this group rarely appear in cheeses. Also, formic and pyruvic acids appear as 'shoulders' on the solvent line, causing an appreciable error in the determination of the concentrations, if these are below 20 mg/100 g of cheese. Concentrations equal to or less than 5 mg/100 g of cheese are considered to be traces. Due to the low concentration of some acids in the samples, and to the relatively low sensitivity of the thermal conductivity detector used, as compared to that of the flame ionization detector (Supelco, 1975), some peaks were wide and low, therefore it was considered more appropriate to measure the areas of the peaks rather than their heights in order to determine concentrations.

Table 1 shows the results of the study of organic acid recovery in the samples of cheese. In essence, quantitative recoveries were made of the volatile acids —acetic, propionic, isobutyric, butyric, isovaleric and valeric—and of the non-volatile acids—lactic, malonic and succinic. The relatively high volatility of formic acid is probably the cause of its low recovery, although acetic acid is also very volatile but showed a high recovery. With respect to non-volatile acids, the method seems to be inadequate for pyruvic acid. The recoveries of oxalacetic and oxalic acids were not determined, due to their overlapping. Biede and Hammond (1979) and Harvey *et al.* (1981) have also reported high recoveries for acetic, propionic, lactic and succinic acids. They also reported that more than 96% of these acids were retained in the aqueous portion of the cheese. Harvey *et al.* (1981) recommend deproteinizing the aqueous portion in order to improve recovery, and Keen and Walker (1974) recommend the elimination of amino acids with a cationic resin but these processes were not considered necessary for the purposes of the present study. Although the rate of recovery was high for acids typical of Palmita cheese (Table 1), the results are considered to be semiquantitative, due to the fact that some peaks of the cheese samples were very low (Fig. 2).

Figure 2 represents characteristic chromatograms obtained from a sample of Palmita for volatile and non-volatile acids, respectively. In Fig. 2(a), the clearly defined presence of acetic and propionic acids can be observed, while in Fig. 2(b) lactic and succinic acids can be seen. These acids were found to be characteristic of Palmita cheese, as can be observed in Fig. 3, where the acids appear as a function of the incidence (percentage of cheeses in which their presence was detected). Lactic, propionic and succinic acids occurred in all samples, although 26·9% of the samples contained only traces of succinic acid. Acetic acid occurred in 76·9% of the samples, with 30·8% corresponding to traces only. Isovaleric, valeric, oxalacetic, oxalic, malonic and fumaric acids were not detected, while formic, pyruvic, butyric and isobutyric acids occurred in few samples. Only one cheese sample showed the presence of butyric and isobutyric acids, and had a disagreeable flavour, which coincides with that reported for other cheeses (Krett & Stine, 1951; Hintz *et al.*, 1956).

In Venezuela, no studies have been made of the acids present in cheeses. Elsewhere, various types of cheeses have been studied, including Cheddar and Swiss. In Cheddar cheese, the volatile acids, acetic and propionic, have been found in concentrations of 70 and 181 mg%, respectively (Patton, 1963; Marsili, 1985),

Table 1. Recovery of organic acids added to Palmita cheese

Acid	Measured	Endogenous	Amount added	Calculated total	Measured total		Recovery
	$\bar{X}^a$	SD		$\bar{X}$	$\bar{X}$	SD	(%)
Volatile							
C_1	—	—	46	46	38	3	82·6
C_2	5	1	60	65	63	3	95·4
C_3	25	2	74	99	97	3	98·0
iC_4	—	—	88	88	82	3	93·2
C_4	—	—	88	88	80	3	90·9
iC_5	—	—	102	102	90	2	88·2
C_5	—	—	102	102	85	3	83·3
Non-volatile							
Pyruvic	—	—	88	88	70	13	79·5
Lactic	76	2	90	166	163	3	98·2
Malonic	—	—	104	104	105	3	101.0
Fumaric	—	—	116	116	134	8	115·2
Succinic	6	1	118	124	131	6	105·6

[a] mg/100 g cheese.

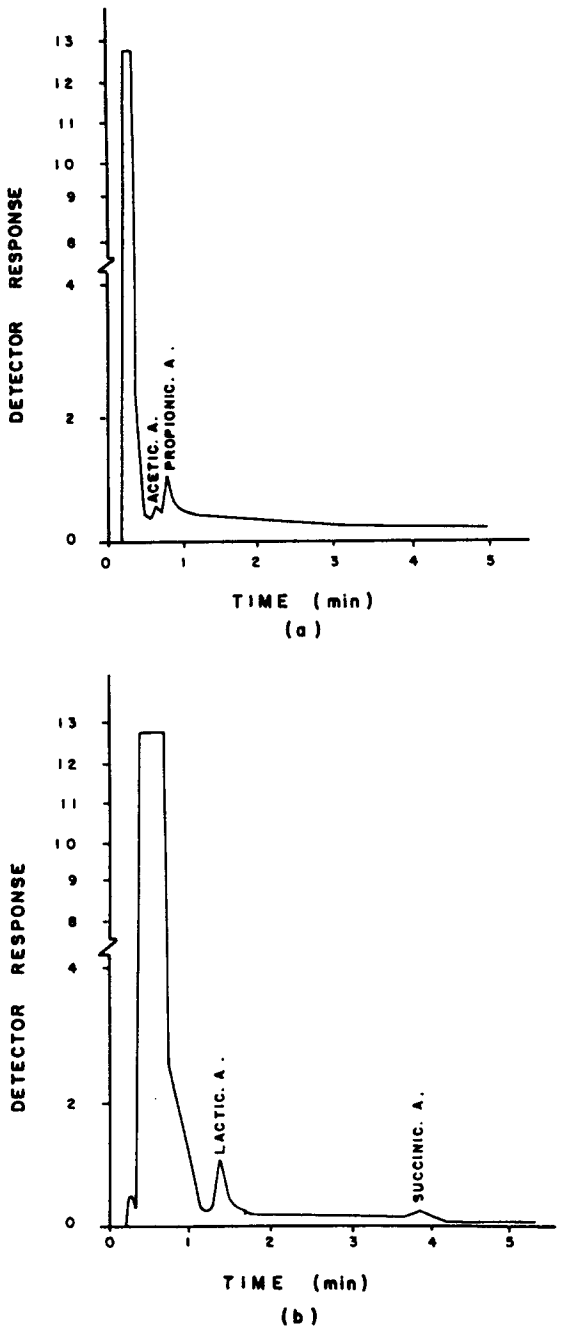

Fig. 2. Gas chromatograms of low molecular weight organic acids isolated from Palmita-type cheese: (a) volatile; (b) non-volatile.

although other investigators have detected propionic acid only in traces (Reiter *et al.*, 1967). Harvey *et al.* (1981) studied non-volatile acids and reported lactic acid (1300 mg %) and succinic acid (6·4 mg %). As can be observed, the acids typical of those cheeses are the same as those of Palmita cheese. Table 2 shows the values of the absolute and relative acid concentrations present in Palmita cheese, which are quite different from those of Cheddar. The average acid concentrations in mg per 100 g of cheese (mg%) were of a decreasing order: 140 of lactic acid, 80 of propionic acid, 14 of succinic acid and 7 of acetic acid, contributing to the characteristic

Table 2. Mean, standard deviation, coefficient of variation and relative concentration of acids in Palmita cheese

	Acetic	Propionic	Lactic	Succinic
$\bar{X}^a$	7	80	140	14
SD	6·45	84·54	49·10	11·28
CV%	92·54	106·22	34·40	82·04
Relative concentration (%)	2·9	33·2	58·1	5·8

[a] mg/100 g cheese.

flavour of Palmita cheese. From these concentrations an acid profile of approximately 20 : 11 : 2:1 with respect to lactic, propionic, succinic and acetic acids can be deduced. The profile of Cheddar cheese obtained from the data of Harvey *et al.* (1981) and Marsili (1985) is 192 : 28 : 1 : 11 for the same sequence. The acid concentrations are given in mg%: 1230 for lactic acid, 181 for propionic, 6·4 for succinic, and 70 for acetic. These data correspond to average values for 10 cheeses aged 10 months. The highest levels are observed for the three most abundant acids, as compared to Palmita cheese. Determining the profile by eliminating succinic acid, since it occurs in negligible quantities with respect to the others, a relationship is obtained of 18 : 3 : 1 for lactic, propionic and acetic acids, showing a marked deficiency of propionic acid with respect to Palmita (20 : 10 : 1). In the case of Cheddar cheese, other compounds are produced during the aging process that contribute to its characteristic flavour and aroma, such as hydrogen sulphide, methanethiol, dimethyl sulphide, diacetyl (Manning & Robinson, 1973; Green & Manning, 1982). However, it is generally recognized that the major component of the flavour of Cheddar cheese comes from the aqueous portion of the cheese, both from the volatile acids (quality of the flavour) and from the non-volatiles, as well as from salt, and the amino acids and peptides (intensity of the flavour) that are produced by proteolysis (McGugan *et al.*, 1979). Also playing a part are the bacterial enzymes liberated during the pasteurization process, and by autolysis during the aging process, by direct action of the enzymes produced by the starting cultures used, and by chemical reactions of precursors produced by enzymatic action (Franklin & Sharpe, 1963; Sharpe, 1979). These actions include glycolysis, citrate metabolism, lipolysis and proteolysis. In Palmita cheese, the contributing factor seems to be related to glycolysis and citrate metabolism, given that it is an unaged cheese of only 2 days maturation. However, as this process takes place at room temperature (30°C) and the curd shows a low salt content, it is very possible that processes of proteolysis and lypolysis take place. Montoya & Ferrer (1989) report a certain degree of proteolysis and lipolysis in Palmita cheese, probably produced by

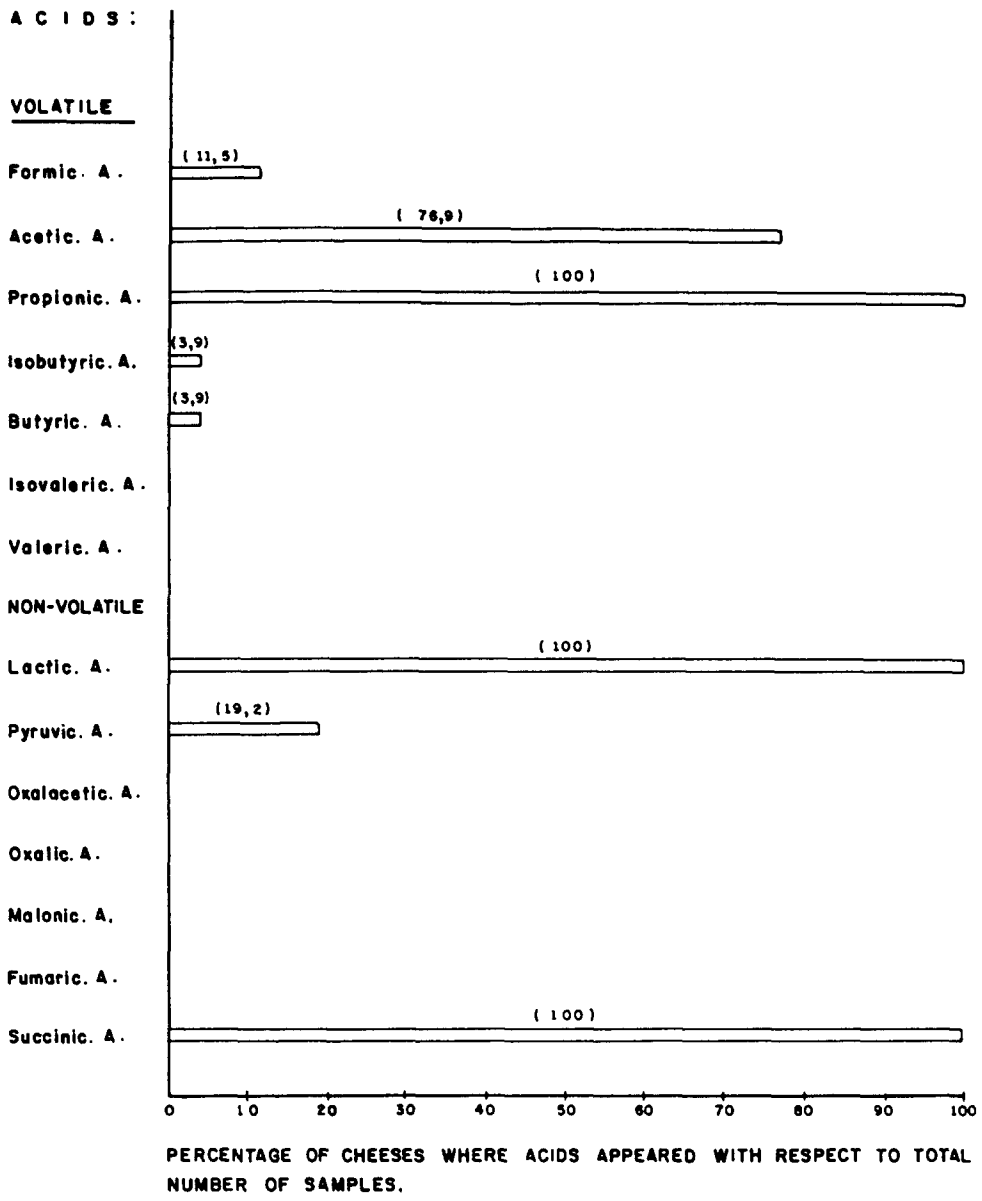

Fig. 3. Incidence of low molecular weight organic acids in Palmita-type cheese samples.

enterococci. Swiss cheese, a cheese with the strong flavour that characterizes long aging processes, shows the presence of the volatile acids acetic (167 mg%), propionic (344 mg%) and butyric (18 mg%) in high concentrations (Krett & Stine, 1951; Hintz *et al.*, 1956; Langsrud & Reinbold, 1973; Biede & Hammond, 1979; Paulsen *et al.*, 1980). In this cheese, the high concentrations of acetic and propionic acids are due to the presence of propionic bacteria that produce these acids from lactate and citrate. Succinic acid is also a by-product of lactate metabolism. Diacetyl has also been reported for Swiss cheese (Biede & Hammond, 1979). Non-volatile acids in this cheese have not received great attention. Valeric acid has been reported in concentrations of 20 mg%, in Stilton and Roquefort cheeses (Hintz *et al.*, 1956) and formic, acetic, propionic, lactic (560 mg%), malic (25 mg%), succinic (124 mg%), pyruvic (10 mg%) and fumaric (traces) acids in Emmental cheese (Langsrud & Reinbold, 1973). When analysing the acid concentrations in Palmita cheese, the relatively high concentration of propionic acid draws our attention, since propionic bacteria have not been detected in the product. It is also noted that Palmita cheese is a cheese that varies widely in its physicochemical parameters (Ferrer *et al.*, 1987) and in its organic acid composition, as can be observed in Table 2, which shows the high coefficients of variation in acid content, with the lowest being lactic acid (34·4%). A cheese such as Cheddar, that is standardized, shows a variation coefficient for lactic acid of 9%, and for succinic acid, 25% (Harvey *et al.*, 1981). Cheeses in which formic acid occurred were not considered acceptable from the organoleptic point of view, as was the case for those showing butyric or isobutyric acids, as mentioned previously.

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