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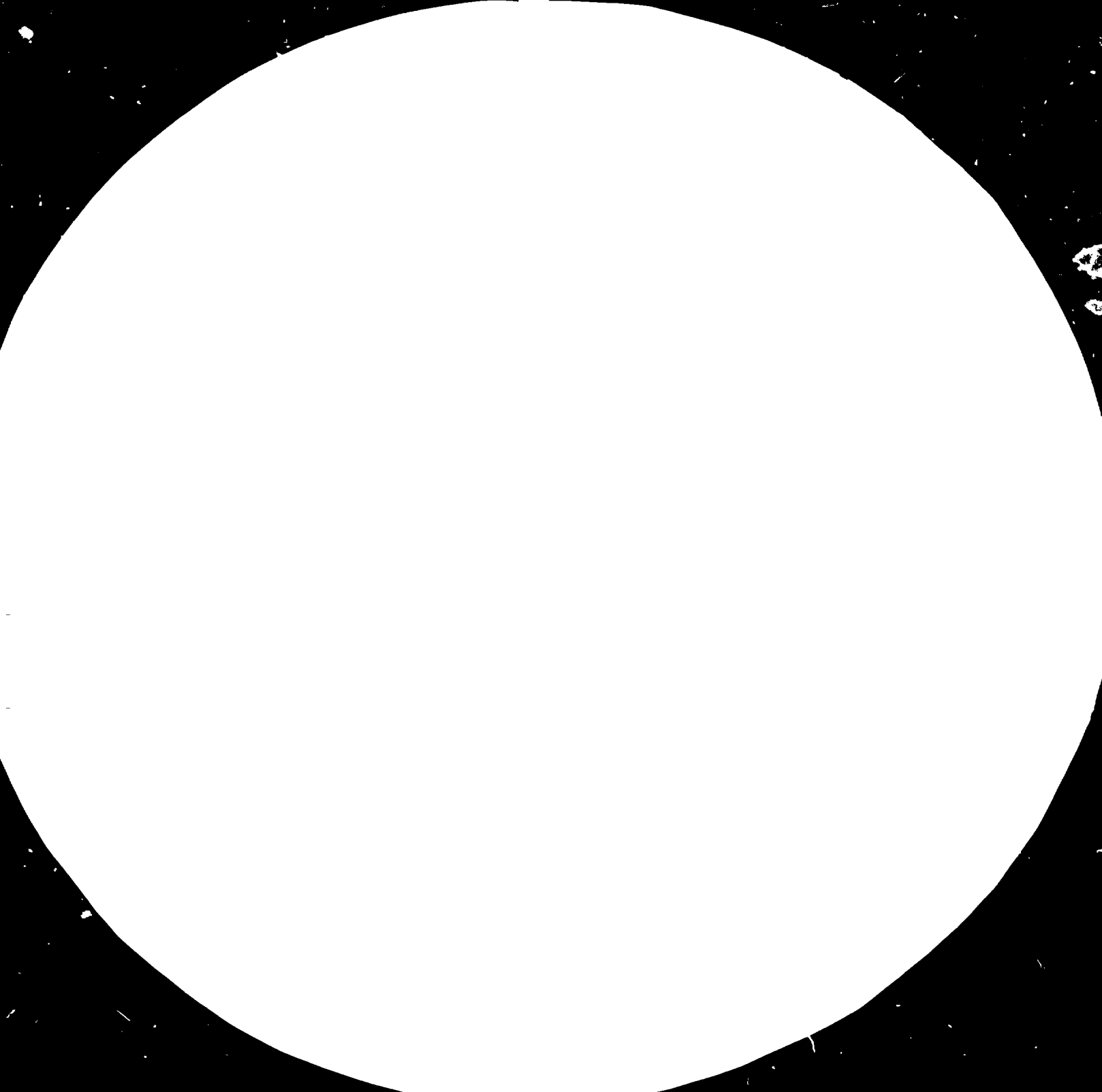
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LACTIC ACID FERMENTATION OF BANANA PUREE\*

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## I. BACKGROUND

To utilize the significant amounts of rejected fresh banana available in Central America in excess from the requirements for the international market commercialization, a fermentation process to preserve the banana puree using lactic acid producing bacteria, that could be applied to cooperatives in rural areas, was developed. To prevent enzymatic browning, the optimum ripened and peeled fruit was immersed in boiling water for 7 minutes; it was made into a puree and 1% milk solids and 1% inoculum (V/W) were added. From lactic bacteria studied, 4 were selected, based on its ability to ferment the puree and reduce the pH below 4.5 in an adequate amount of time. *L. plantarum* ATCC E 8014, *L. casei* TISTR 390, *L. fermentum* TISTR 391 and *L. cellobiosus* TISTR 398 reduced the pH from 4.8 to 4.00, 3.95, 4.10 and 4.00 respectively in 24 h at 37°C. At this time, the aroma flavor and color of the resulting products were pleasant. After an additional 24 h the desirable organoleptic characteristics decreased. Fermentation must be stopped by refrigeration or pasteurization (boiling water) for 34 min and cooling for 30 min. *L. plantarum* ATCC E 8014 was the final selected strain after a preferential response in the sensory evaluation. The fermented puree is suitable for use in many banana flour specialty food products like "bocadillo" product chosen as the one with greatest commercial possibilities in the Central American region.

## II. INTRODUCTION

The natural pH of banana puree is high (4.8-5.0), requiring a drastic heat sterilization process for preservation, that is only practical with aseptic canning. For a small operation in a banana-producing cooperative, acidification plus a moderate heat treatment (pasteurization) is more feasible if the final product will be also acid, like a jelly, because acid-flavored banana puree may have a low acceptability by people familiar with the natural product.

An alternative will be the preparation of a puree fermented by lactic bacteria, which could be associated more with yoghurt than with natural banana puree.

Lactic bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*) have been used industrially since about a hundred years to produce a variety of fermented food products. Lactic acid produced by these bacteria enhance food preservation by inhibiting the growth of pathogenic microorganisms (Gilliland and Speck, 1977).

Its use in food processing has been widely described in the literature (Speck, 1978; Teply et al, 1979; Mital and Steinkraus, 1979; Patel et al, 1980; Tiwari and Pandey, 1978; Ngata and Lee, 1979; Beuchat and Nail, 1978; Bucker et al, 1979; Field et al, 1981). However, there are few papers on lactic fermentation of fruit products such as fruit yoghurt (Kriel, 1979), and brined mango slices (Maitra et al, 1979).

In relation with fermentation of banana puree by lactic acid bacteria, a german company (Eden Waren, GmbH, 1972, 1972a) obtained a patent for a pasteurized banana puree fermented by a mixed culture of *L. salivarius*, *L. casei*, *S. lactis*

and *S. cremoris* during 12-20 h, starting at 38°C with a gradual temperature reduction of 1-3°C. Final pH was from 4.1 to 3.7 according to fermentation time.

Wheeler and Gillies (1973), prepared a yoghurt from a 1:5 by wt banana puree/milk mixture with acceptable flavor. Aegerter and Dunlap (1980), tested *L. bulgaricus*, *S. thermophilus*, *S. faecalis*, *L. fermentum* and *L. mesenteroides* in banana puree from ripe and green fruits with a fermentation time of 7 days at 37°C. The fermented products became spongy, with soft solids floating in a clear liquid and of medium to light creamy brown color. The inocula were not standardized. Matamoros (1981) used *L. casei* to ferment banana puree during 10 to 19.5 h at 38°C. The product had a shelf life of 32 days at 7°C. Dilution of the puree with water and addition of pectinolytic enzymes was also tried.

In the present work, the objective was to select an appropriate lactic acid bacteria to produce a fermented banana puree with a pH below 4.5, acceptable flavor, texture and light color; and with a simple enough process that could be implemented in small plants located in a rural areas. This process required a simple procedure and low cost equipment.

### III. MATERIALS AND METHODS, EXPERIMENTAL

Bananas (*Musa Sapientum* variety Grande Naine) in the mature-green stage of ripening were procured from Bananera, Izabal, Guatemala and San Manuel, Cortes, Honduras. The fruit was stored at 28°C and 85-90% relative humidity and treated with ethylene to obtain homogeneous ripening.

Of 34 lactic acid producing bacteria obtained from different sources, 16 were selected on the basis of good growth in MRS liquid medium (Man, Rogosa Sharpe; Oxoid Ltd. Basingstoke, England). B-12 Inoculum Culture Broth (DIFCO) was substituted for MRS after several months of experimental work because some strains began to show poor growth.

The 16 strains selected are listed in Table 1. These strains were obtained from the American Type Culture Collection (ATCC), the Thailand Institute of Scientific and Technological Research (TISTR), the Northern Utilization Research and Development Division (NRRL), the National Collection of Dairy Organisms (NCDO) and Christian Hansen's Laboratory, Inc.

Each of the assayed strains was rehydrated with liquid medium, with successive transfers until good growth was obtained.

The same standardized cell concentration (71-77% transmittance at 540 nm, Bausch and Lomb Spectronic 20 spectrophotometry was used in all inocula. The number of microorganisms present in the inocula was determined by standard plate count (Speck, 1976).

Optimum growth temperatures recommended by suppliers were used and time for maximum growth registered.

TABLE 1

OPTIMUM GROWTH TIME AND TEMPERATURE OF THE LACTOBACILLUS STRAINS ASSAYED

<u>MICROORGANISM</u>	<u>Optimum Growth Temperature (°C)</u>	<u>Optimum Growth Time (h)</u>
L. plantarum ATCC E 8014	35	18-20
L. casei TISTR 390	35	20
L. acidophilus NRRL B 1910	35	20
L. acidophilus NCDO 1	35	20
P. pentosaceus NCDO 1859	35	18-20
S. lactis NCDO 1966	32	26
S. thermophilus Cristian Hansen's GH	43	N
L. bulgaricus NRRL B 1909	43	N
L. bulgaricus NCDO 1489	43	24
L. delbrueckii ATCC 9649	43	18-20
L. delbrueckii NRRL B 763	43	18-20
S. lactis NCDO 1967	32	26
L. brevis NRRL B 4527	32	24
L. fermentum ATCC 9338	43	24
L. fermentum TISTR 391	35	24
L. cellobiosus TISTR 398	32	24

N = Inadequate growth which prevented its determination

Before processing, the ripe fruit was washed with a 20 p.p.m chlorine in water solution, peeled by hand and blanched for 7 min in boiling water. The puree was prepared in a Langsenkamp pilot plant pulper Model 18-1227 with a 3.18 mm screen. 1% milk solids (skimmed milk powder) was added to the puree before 1% V/W bacterial inoculation. 150 ml portions of inoculated puree were poured into glass jars (237 ml) and transparent polyethylene bags (12.6 x 21.3 cm). After sealing, the jars and bags were incubated at 37°C for 24 h. The fermentation process was then stopped either by refrigerating at 4°C or by immersion in hot (85°C) water for 34 min followed by cooling with water to ambient temperature. A process diagram is presented in Figure 1.

Total acidity of the puree was determined according to AOAC (1975), total sugars were analyzed following the methods reported by Browne and Zerban (1955) The pH meter used was a Corning Model 10. Strains were selected for their ability to reduce the pH of the puree and generate a pleasant flavor.

A sensory evaluation was performed on 4 selected fermented banana puree samples following the hedonic scale method (Larmond, 1970) by a trained panel of 14 persons. Two samples of fermented banana puree were compared with a sample of commercial banana yoghurt, with natural banana puree and with banana puree acidified with lactic acid to the same pH of the fermented samples on each sensory



Figure 1

Processing scheme for Fermented Banana Puree

Banana Grande Maine Variety  
(optimum ripeness for processing)

Washing (enough water and 20 ppm chlorine solution)

PEELING

Enzyme inactivation  
(Boiling water for 7 min)

PULPING

Langsenkamp Pulper  
(screen 1/8" (3.18 cm)  
(skimmed milk powder)

1% milk solids

Control 1 1% Inoculum (V/P) Mix to ensure a homogeneous  
distribution

Control 2

Sampling 0 h FILLING Glass jars (8 oz polyethylene  
bags 0.025" (12.6x21.3 cm)

Closing and sealing

Incubation at 37°C for 24 h

Sampling 16, 24, 42 h STORING

Room temperature (23°C) Refrigeration (4°C)

Sensory evaluation  
at 24 h fermentation

evaluation session. A statistical analysis was performed to determine significant differences in preference among samples. The best fermented banana puree was then mixed with commercial milk yoghurt in 40:60, 20:80 and 10:90 fermented puree/yoghurt proportions by weight and compared in a hedonic scale sensory test using a sample of banana puree acidified with lactic acid as standard.

IV. RESULTS AND DISCUSSION

In the fermentation assays, an increase of total acidity (expressed as g malic acid/100 g puree) with time was observed (Figure 2) while pH and sugar decreased

(Figures 3 and 4). There was some gas production that increased slowly with time. During the first 24 h of fermentation, most of the samples had an aroma and flavor which were pleasant and the color changed slightly to a beige-yellow.

FIGURE 2

FERMENTED BANANA PUREE ACIDITY vrs TIME

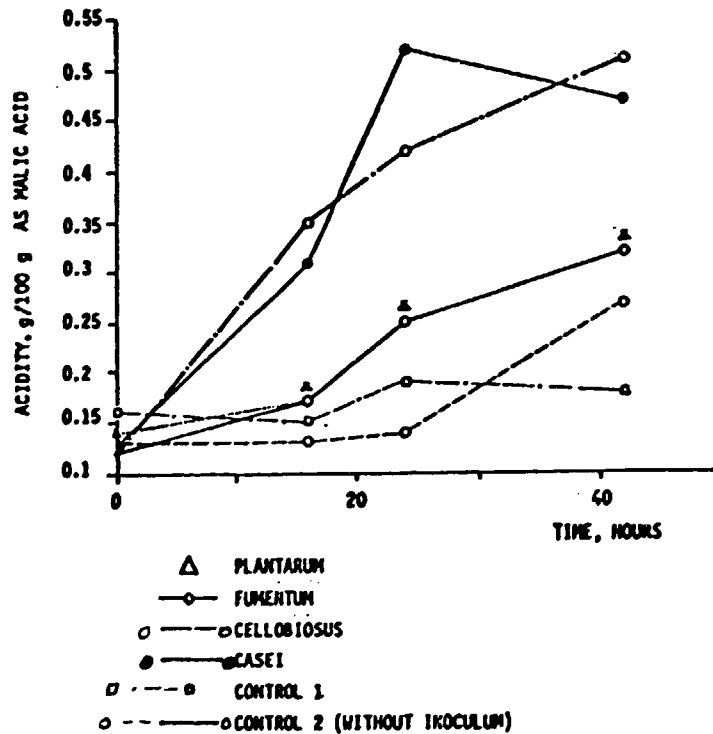


FIGURE 3

FERMENTED BANANA PUREE ACIDITY vrs TIME

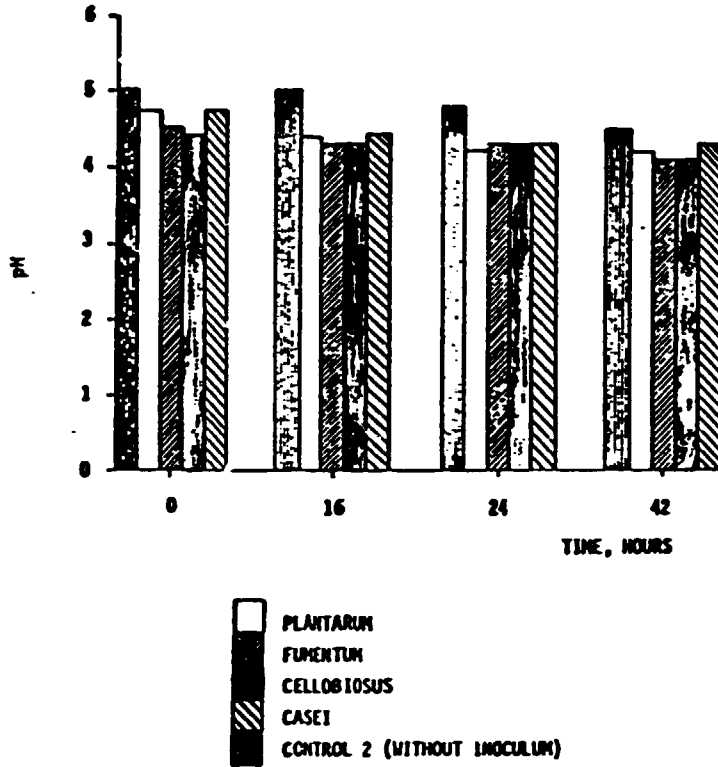
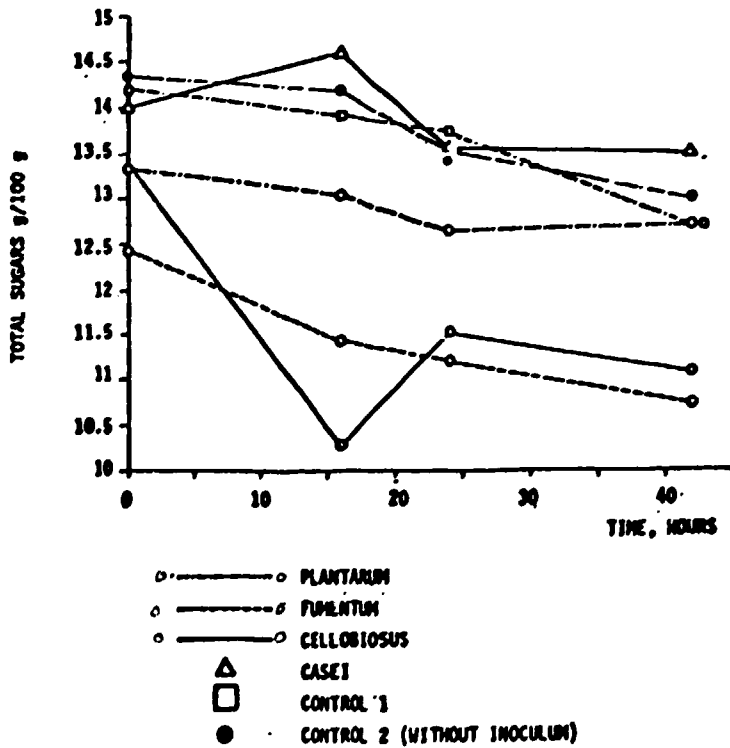


FIGURE 4

FERMENTED BANANA PUREE SUGARS vrs TIME



Some fermentation experiments were carried on at room temperature (23°C), but the pH did not decrease below 4.5.

Table 2 presents the pH values obtained with the 16 strains studied (see Table 1) at 24, 42 and 48 h of incubation. Those strains which were presented in duplicate from two different sources in Table 1, were merged in Table 2

TABLE 2

pH INTERVALS AT 24, 42 AND 48 HOURS FOR THE LACTIC ACID PRODUCING BACTERIA IN THE FERMENTATION EXPERIMENTS.

MICROORGANISM	pH		
	24 h	42 h	48 h
<i>L. acidophilus</i>	4.40 - 4.80	4.40	4.03 - 4.32
<i>P. pentosaceus</i>	4.30 - 4.60	4.05	4.03 - 4.20
<i>L. casei</i>	3.95 - 4.44	3.84 - 4.30	3.80 - 4.24
<i>S. lactis</i>	4.60 - 4.70	4.40	4.2 - 4.40
<i>L. plantarum</i>	4.00 - 4.40	3.83	3.92 - 4.02
<i>L. delbrueckii</i>	4.80	4.50	4.40
<i>L. brevis</i>	4.70	-	4.30
<i>L. fumentum</i>	4.10 - 4.30	4.10	3.90
<i>L. bulgaricus</i>	4.80	-	4.60
<i>L. cellobiosus</i>	4.00 - 4.30	4.10	3.90
<i>L. fermentum</i>	4.30	-	4.00
Control sin inóculo	4.57 - 5.11	4.48 - 5.00	4.20 - 4.70
Control con 1% de leche, sin inóculo	4.65 - 4.80	4.50 - 4.60	4.60

- = Not determined

Four strains were selected on the basis of their ability to reduce pH in a 24 hours period while producing an acceptable flavor: *L. plantarum* ATCC E 8014, *L. casei* TISTR 390, *L. fumentum* TISTR 391 and *L. cellobiosus* TISTR 398.

It was found that after 24 h of incubation the flavor began to deteriorate.

The sensory evaluation by the hedonic scale method compared samples of fermented banana puree with the 4 selected strains, a commercial banana yoghurt, natural and acidified banana purees. The fermented puree sample with the highest preference was that prepared with *L. plantarum* ATCC E 8014; although the commercial yoghurt scored highest overall. There was no significant difference in preference among the sample incubated with *L. plantarum* ATCC E 8014

and the natural and acidified banana puree controls. This is important because it means that the fermented product was considered at least as good as the natural banana pulp.

It can be seen from Figure 2 that *L. plantarum* and *L. casei* had the lower acidity among the fermented samples, (after 16 h of incubation both had the same acidity values, but as shown in Figure 3, the pH of *L. plantarum* was the lowest after 24 h of incubation. The total sugar content of the banana puree fermented with *L. plantarum* was the second highest among the fermented samples (Figure 4). A combination of low acidity with high sugar at a pH of 4.2 explains why this sample had the better acceptability within the fermented banana purees.

Another sensory test by hedonic scale compared 40:60, 20:80 and 10:90 fermented puree (*L. plantarum*)/commercial yoghurt mixtures with banana puree acidified with lactic acid to the same pH of the fermented samples. No significant difference in preference could be detected among these samples. Hence, it can be concluded that the fermented puree can be blended with commercial yoghurt in order to produce an excellent food product.

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