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Agronomie

KESTER, J.J. and FENNEMA, O. 1989a. An edible film of lipids and cellulose ethers: Barrier properties to moisture vapor transmission and structural evaluation. J. Food Sci. *54*(6), 1383.

KESTER, J.J. and FENNEMA, O. 1989b. An edible film of lipids and cellulose ethers: Performance in a model frozen-food system. J. Food Sci. *54*(6), 1390.

KOELSCH, C.M. 1992. The functional, physical and morphological properties of edible fatty acid and methyl cellulose based barriers. Ph.D. Dissertation, University of Minnesota.

KOELSCH, C.M. and LABUZA, T.P. 1992. The functional, physical and morphological properties of methyl cellulose and fatty acid-based edible barriers. Lebensm. Wiss. Technol. *25*, 404.

McHUGH, T.H., AVENA-BUSTILLOS, R.J. and KROCHTA, J.M. 1993. Hydrophilic edible films. Modified procedure for water vapor permeability and explanation of related thickness effects. J. Food Sci. *58*(4), 899.

MOHNEY, S.M., HERNANDEZ, R.J., GIACIN, J.R., HARTE, B.R, and MILTZ, J. 1988. Permeability and solubility of d-limonene vapor in cereal package liners. J. Food Sci. *53*, 253.

PRESS, W.H, FLANNERY, B.P, TEULKOSKY, S.A. and VETTERLING, W.T. 1986. *Numerical Recipes*, Cambridge University Press, Cambridge.

SCHONHERR, J. and MERIDA, T. 1981. Water permeability of plant cuticular membranes: the effect of humidity and temperature on the permeability of non-isolated cuticles of onion bulb scales. Plant Cell Envir. *4*, 349.

VAGENAS, G.K and KARATHANOS, V.T. 1991. Prediction of moisture diffusivity in granular materials, with special applications to foods. Biotechnol. Prog. *7*, 419.

QUALITY LOSS OF DOUBLE CONCENTRATED TOMATO PASTE: EVOLUTION OF THE MICROBIAL FLORA AND MAIN ANALYTICAL PARAMETERS DURING STORAGE AT DIFFERENT TEMPERATURES

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ABSTRACT

This study deals with nonsterile canning of double concentrate tomatoes (≈30° Brix) stored for 210 days at three different temperatures (4, 10 and 25C) in 200 kg drums. The evolving analytical composition (soluble solids, total solids, glucose, fructose, pH, total acidity, volatile acidity, citric acid, malic acid, succinic acid, hydroxymethylfurfural (HMF) and color parameters) that the product underwent during storage was dependent both on storage temperature and on different aerobic levels within the drum (top and bottom sections). The microbial profile (yeast, lactic acid bacilli and molds) was correlated with many important metabolites (D- and L-lactic acids, ethanol, acetic acid and diacetyl). The results indicate that the increase of these substances is dependent both on storage temperature as well as the oxygen tension within the drums.

Taken all together, the analytical findings offer a great help in evaluating the quality of semifinished tomatoes. We also found that lactic bacteria grow rapidly at 25C and after 15 days their number from both sampling areas in the drums (i.e., 10 cm below the sample surface and 15 cm above the bottom of each drum) is already greater than 10^5 cfu/ml. At 10C, 30 days were needed to reach such a cell concentration, and after 45 days the level reaches 10^7–10^8 cfu/ml. By contrast, at 4C there were differences between top and bottom sampling areas. In the top area, 10^5 cfu/ml was reached after 60 days, while for the bottom area this was reached after 120 days. Regarding yeast at 25C, the

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cfu/ml values were 10^5–10^6 in both areas of the drum after the 60th storage day. At 10C the behavior was the same: about 10^5 cfu/ml had been found after 30 days. Finally, at 4C the yeast reached 10^5 cfu/ml after 45 days in both sampling areas. Regarding mold, no growth in the sampling areas was seen.

INTRODUCTION

The temporary storage of semifinished tomatoes is usually brought about by sterile packaging of pulp, diced, double and triple tomato concentrates. Among the numerous advantages of this technology, we cannot fail to mention that during the canning season the preparation of semifinished and reworked product can be kept separate. The reworked product is essentially used to make sauce for canning. In the case of double and triple tomato concentrates, however, because of the high costs for sterile production it is still the practice in some regions of Italy and other Mediterranean countries to store partially refined tomatoes in 180–220 L plastic drums under nonaseptic conditions.

This semifinished product has found a good market above all because of the low cost, and it is often blended with other costlier semifinished tomatoes of good quality (Leoni and Bellucci 1980).

The technology behind packaging a semifinished product requires that the fruit be canned in nonsterile drums at relatively low temperature (65–70C). In addition, since the drums cannot be closed immediately because their walls undergo mechanical stress during cooling these products are subject to easy contamination. What is more, the superficial storage with salt (under widespread use) quickly alters the product. Moreover it is a more or less diffuse practice to store this product on truck loading grounds at the processing plant where, in absence of properly sealed cover, the product is exposed to the high temperatures usually occurring in these regions during summer (the season coinciding with tomato production and refinement).

Technical-scientific studies regarding these nonsterile products are scarce, especially those concerning variations of composition correlated with taste and smell changes. There is also scarce analytic data for better quality blend and for technological data about the best storage time and temperature to limit compositional changes.

Based on these considerations, this first study attempts to correlate the fermentation profile (microbial growth) with the composition (metabolite production) found in these semifinished products and to evaluate this change at three different storage temperatures. Finally, we evaluated the compositional difference between the same product at the bottom of a drum with the product at the top of a drum, where the oxygen tension is presumably higher.

MATERIALS AND METHODS

Tomato Samples

Six drums of tomato concentrate (≈30° Brix), produced and packaged by CPC. S.P.A., Castel S. Giorgio (SA), Italy, were used in the present study. The drums were first washed with detergents and then rinsed with tap water. They were finally treated with saturated steam for 1 min. The product was previously mixed in a tank to guarantee homogeneity and then filled at 68–70C.

Two drum lots each were stored at three different temperatures (4, 10 and 25C) for a total of 210 days. Two samples of tomato concentrate were drawn from each drum at various storage times. These were taken 10 cm from the top surface, and 15 cm from the bottom by a 4 cm cylindrical probe (Fig. 1). A 3 cylinder probe, 4 cm in diameter, was used to guarantee that the samples were evenly represented. The probe was sterilized by immersion in an alcohol solution of Iosan (Ciba-Geigy) and passed through a flame immediately before sampling.

Microbiological Analysis (Lactic Acid Bacteria, Molds and Yeasts)

Lactic acid bacteria cell counts were carried out on Rogosa Agar (Oxoid) by pour plate technique; petri plates were then incubated at 30C for 24–48 h (Rogosa *et al.* 1951). Then catalase tests and microscopic observations were carried out. The catalase-negative bacterial colonies were counted and considered as lactic acid bacteria. Yeast and mold counts were carried out in Malt Extract Agar (Oxoid) acidified to pH 4.0 with 50% citric acid solution sterilized by filtration; petri plates were then incubated at 30C for 48–72 h (Galloway and Burgess 1952); colonies were finally observed by microscope.

The cellular growth velocity is defined by $r_x = dX/dt$ where X is the cellular concentration of the microorganism (expressed as cfu/ml) and t is the time. From the ratio between r_x and X at various sampling time, one calculates the specific growth velocity, expressed as $\mu(time^{-1}) = (dX/dt) \cdot (1/X)$.

Analytical Determinations

For proximate analyses, the soluble solids, expressed as °Brix, were determined by measuring the refractive index at 20C. The total solids were determined by oven drying 10 g of sample, under vacuum at 70C, to constant weight. Titratable acidity (total acidity), expressed as citric acid monohydrate, was determined by titrating a 10 g sample to end-point pH 8.1 with 0.1 N

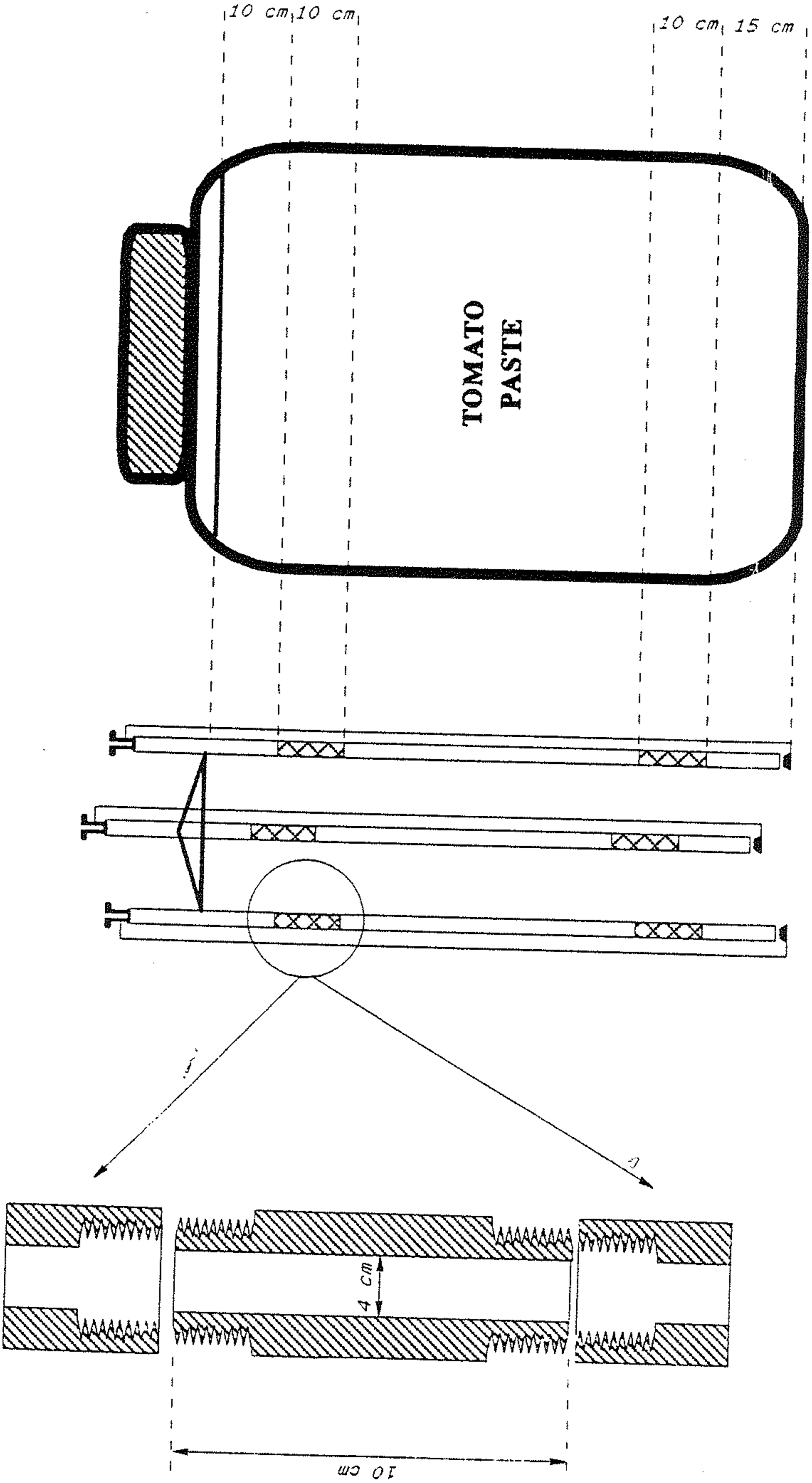

FIG. 1. SCHEMATIC DIAGRAM OF CYLINDRICAL PROBE

NaOH, according to Metodi Ufficiali di Analisi delle Conserve Vegetali (MUACV 1961). The volatile acidity was determined according to International Federation of Fruit Juice Producers method 5 (IFFJP 1962) calculated as acetic acid and expressed in g/100. The pH was determined potentiometrically at 20C by a Crison pH meter TT2050. Total sugars were determined by the MUACV (1961) procedure. The hydroxymethylfurfural (HMF) content was determined by reference to a standard curve according to IFFJP (1972) method 12. Briefly, the determination principle is that HMF reacts, like various other aldehydes, with barbituric acid and p-toluidine forming a red-colored compound. The intensity of the red color (read in a spectrophotometer at 550 nm) depends on the amount of HMF and can therefore be used as the basis of a quantitative colorimetric determination. D- and L-lactic acids, ethanol and acetic acid were determined by enzymatic methods (Boehringer Mannheim Biochemicals 1980). Luminance (L* value), redness (a* value) and yellowness (b* value) were measured using a tristimulus Hunter colorimeter model D25A. Standard plate N° C20-2105 with Hunter L* value of 25.8, a*value of 28.6 and b* value of 12.9 was used as a reference. The Hunter color values were measured at a soluble solids level of 8.2%.

Sample Preparation for Diacetyl Determination

The diacetyl was determined spectrophotometrically. Tomato paste (10 g) was diluted with 200 ml of H_2O. The prepared sample was distilled at 100C and the first 25 ml of distilate was collected. α-Naftol (5 ml) in isopropyl alcohol (obtained by dissolving 5 g of α-naftol in 100 ml of 95% isopropyl alcohol) and 2 ml of creatine solution (from 20 g of KOH and 0.15 g of creatine in 50 ml of H_2O) were added to 10 ml of distillate and shaken for 15 min. The red color produced was measured photometrically against air at 545 nm and compared to a standard curve obtained using diacetyl as a standard (standard curve ranged 0–3 mg/kg).

Sample Preparation for Sugar Determination (Glucose and Fructose):

HPLC Analysis

Tomato paste (10 g) was diluted to 50 g with distilled H_2O and clarified by centrifugation at 12000 × g for 15 min. The clarified extract was filtered through 0.45-μm Millipore filters. A sample of 20 μl was used for analysis. A Merck Lichrosorb NH_2 (10 μm) cartridge was used. The eluent consisted of acetonitrile-water (80:20), which was degassed and filtered through a 0.22 μm Millipore filter. A Waters-Millipore 600E liquid chromatograph was employed

and a differential refractometer (Waters-Model 410) was utilized as a detector for sugar analysis.

Sample Preparation for Acid Determination (Citric, Malic and Succinic): HPLC Analysis

Tomato paste (30 g) was diluted to 50 g by H_2O and clarified by centrifugation at 12000 × g for 15 min. The clarified extract was filtered through a 0.45 μm Millipore filter; 10 ml were chromatographed through a cation-exchange column [AG-I-X8, (HCOO−) Bio-Rad] and washed with water to a total volume of 100 ml.

The organic acids were eluted with 6M formic acid (about 130 ml), collected, and evaporated. The dry samples were recovered with water (10 ml) and filtered through a 0.45 μm Millipore filter before HPLC analysis.

A Merck Lichrosorb RP-18 (10 μm) cartridge was used for HPLC analysis. The eluent consisted of water adjusted to pH 2.4 with H_3PO_4 at a flow rate of 2.0 ml/min. A Waters 410 differential refractometer was used as detector.

Statistical Analysis of Results

Analysis of variance (Snedecor and Cochran 1980) was used to determine differences in mean values obtained from results of six determinations in duplicate for each sample. Significance was determined at $P = 0.05$.

RESULTS AND DISCUSSION

Microbial Growth During Storage

The number of lactic bacteria and yeast at different storage temperatures is shown in Table 1. At the starting time, the numbers of yeasts and lactic bacteria both fell within the range of 0–10 cfu/ml.

Influence of Storage Conditions on Lactic Bacteria Levels. At 4C (Table 1), for storage time exceeding 120 days, the concentration of lactic acid bacteria in both sampling areas (top and bottom drum) was greater than 10^5 cfu/ml. At shorter storage time, the lactic acid bacterial growth-curve was different. In fact, for top level samples, 10^5 cfu/ml were already present at about 60 days and remained almost constant until the end of storage. In contrast, in the bottom

TABLE 1.
LACTIC ACID BACTERIA AND YEASTS COUNTS FROM TOMATO PASTE STORED AT 4, 10 AND 25C

Storage Time (Day)	Sampling Zone	Lactic Acid Bacteria[c]			Yeast[c]		
		4 C	10 C	25 C	4 C	10 C	25 C
0	TOP[a]	< 10	< 10	< 10	< 10	< 10	< 10
15		2.4×10^1	2.6×10^3	1.9×10^6	1.8×10^1	1.3×10^3	6.4×10^3
30		4.0×10^1	1.3×10^3	2.0×10^4	5.8×10^2	4.0×10^4	6.4×10^3
45		1.4×10^4	4.0×10^7	6.0×10^5	3.0×10^5	5.0×10^4	2.6×10^4
60		1.0×10^5	2.5×10^8	1.2×10^5	2.2×10^6	1.2×10^7	3.5×10^5
75		1.0×10^5	2.0×10^8	2.0×10^4	2.6×10^6	1.6×10^6	2.8×10^5
90		1.3×10^5	1.2×10^8	6.0×10^4	1.2×10^6	1.4×10^7	5.6×10^5
105		3.6×10^5	4.4×10^7	8.0×10^3	8.0×10^4	2.6×10^6	7.3×10^5
120		7.0×10^5	3.0×10^7	8.0×10^3	8.0×10^5	4.0×10^6	2.2×10^5
180		2.6×10^6	1.4×10^7	1.1×10^5	1.4×10^6	1.2×10^6	1.7×10^5
210		5.4×10^6	9.3×10^6	1.2×10^3	3.0×10^6	1.9×10^6	3.9×10^6
0	BOTTOM[b]	<10	<10	<10	<10	<10	<10
15		3.6×10^1	5.2×10^2	4.4×10^5	2.2×10^2	4.8×10^2	8.8×10^3
30		1.0×10^2	1.2×10^4	8.0×10^3	6.6×10^2	1.4×10^5	1.2×10^3
45		1.2×10^4	4.0×10^7	8.0×10^6	1.0×10^5	2.2×10^5	5.4×10^4
60		3.0×10^4	1.2×10^8	7.0×10^7	1.8×10^4	1.0×10^5	4.4×10^6
75		2.0×10^4	4.0×10^8	4.0×10^5	4.0×10^4	2.6×10^7	7.0×10^5
90		1.3×10^4	4.0×10^7	3.0×10^4	6.8×10^4	6.0×10^6	8.0×10^5
105		1.0×10^4	3.2×10^7	1.4×10^4	2.2×10^4	8.0×10^6	2.6×10^5
120		2.8×10^5	9.0×10^7	3.4×10^4	9.0×10^4	3.0×10^6	5.4×10^4
180		1.3×10^6	1.0×10^8	1.0×10^4	2.6×10^6	1.0×10^6	1.1×10^5
210		4.8×10^6	2.1×10^7	3.9×10^4	1.2×10^6	1.8×10^6	1.1×10^6

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom.
[c] The counts are expressed as cfu/mL

level samples, lactic acid bacteria concentrations remained constant between 45 days ($1.2\ 10^4$ cfu/ml) and 105 days ($1.0\ 10^4$ cfu/ml) and rose to 10^5 cfu/ml at 120 days and thereafter.

This trend was probably due to composition changes in the substrate occurring during storage. In these conditions the growth and inactivation of various strains are influenced by the type and concentration of metabolites produced. Since some strains are rapidly inactivated, others may also develop because of decreased microbial competition.

At 10C (Table 1) there appears to be no substantial difference between growth curves of lactic acid bacteria in the top storage samples compared with the bottom ones. 10^3–10^4 cfu/ml are present at about 30 days. As seen in Table 1, the bacteria growth curve continues to rise until 45 days and then levels off (10^7–10^8 cfu/ml) until the end of storage.

At 25C (Table 1), the growth of lactic acid bacteria was rapid. At 15 days the concentration reached 10^5 cfu/ml for both samples (top and bottom sites of the drum). The findings in Table 1 also show that at storage time exceeding 60 days the concentration of lactic acid bacteria in the top and bottom samples tended to decrease with storage temperature until leveling off within a range of 10^3–10^4 cfu/ml. These findings show that the lactic acid bacteria growth-curve, as predicted (Kandler and Weiss 1986), is not only dependent on storage time, but also and primarily on more aerobic conditions that probably occur in the top of the drum compared to those occurring at the bottom. These findings are in agreement with those reported by Lucey and Condon (1986) who found a higher rate of growth under aerobic conditions using some strains of *Leuconostoc* and with a greater production of acetate compared to anaerobic conditions.

Influence of Storage Conditions on Yeast Levels. At 4C (Table 1) the yeast growth-curves were identical (approximately 10^5 cfu/ml) after 45 storage days in the top and bottom areas of the drum. Instead, at higher storage temperature, the difference between top and bottom drum areas become relevant. In fact, at the top area of the drum, the number of yeasts continued to rise after 45 days until reaching approximately 10^6 cfu/ml, and then remained constant until the end of storage. By contrast, at bottom area of the drum, the yeast numbers remained almost constant, at about the value found on the 45th storage day, and only at the end of the storage reached 10^6 cfu/ml.

At 10C (Table 1), the difference in the number of yeasts is still higher than before. In fact, the content of yeasts after 30 storage days was about 10^4 cfu/ml in the upper area of the drum, and this is substantially lower than observed at the same storage time in the bottom sampling area (about 10^5 cfu/ml). The concentration of yeasts in both sampling areas became constant at 10^6 and 10^7 cfu/ml after 75 days of storage and remains so for the duration of the storage period. Finally, at 25C, the data reported in Table 1 show immediate growth in both the upper and lower areas of the drum with values reaching 10^3 cfu/ml at the 15th day and remaining between 10^5 and 10^6 cfu/ml after 60 days of storage.

Metabolite Production and Specific Growth Rate. To determine the influence of different metabolites produced during storage on the growth of yeasts and lactic acid bacteria, Fig. 2–7 report the specific growth rates (day^{-1}) as influenced by ethyl alcohol, acetic acid, diacetyl and lactic acids D-L.

The first finding to emerge from examination of the data reported in Fig. 2–4 is the varying level of ethanol produced during the 210 days of storage. There was a lower production at 4C (maximum concentration 9 g/kg) compared to the product stored at 10C (maximum concentration 32 g/kg) and at 25C (maximum concentration 38 g/kg).

It is noteworthy that the inhibition of yeast growth seen at different temperatures occurred almost always at ethanol levels reaching 4g/kg. The only exception is the product stored at 4C, which presents inhibition of the lower area sample (Fig. 2) with ethanol levels nearing 2g/kg. Generally speaking, the accumulation of ethanol from the fermentation of sugars by yeast does not appear to have the same inhibitory effect on lactic acid bacteria. As shown in Fig. 5–7, these latter had a lowered specific growth rate at different times, which seems to be coupled more with the ethanol than sugar level.

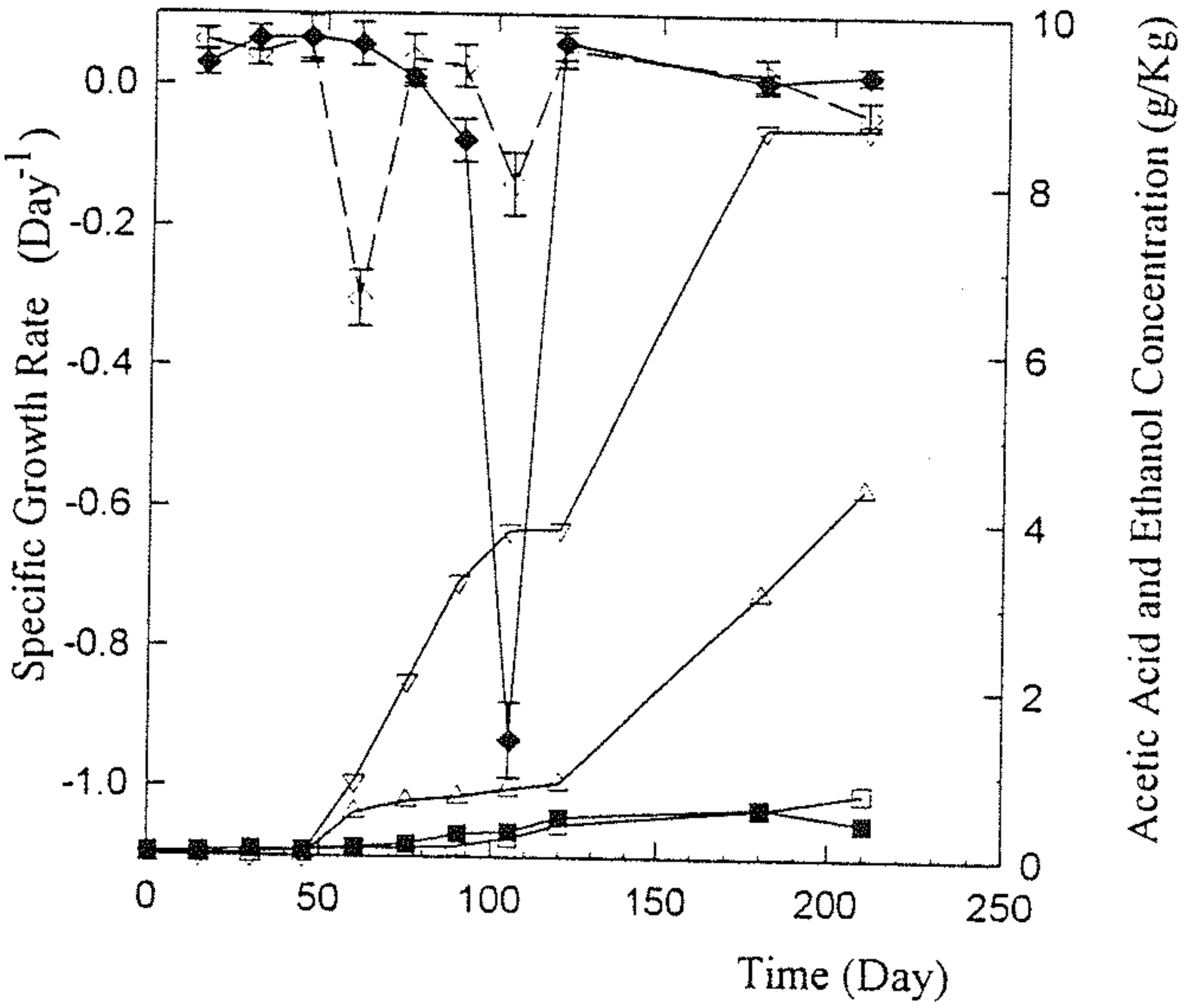

FIG. 2. EVOLUTION OF YEAST SPECIFIC GROWTH RATE, OF THE ACETIC ACID AND THE ETHANOL PRODUCT ON TOMATO PASTE STORED AT 4C
(◆) yeast specific growth rate (top), (◇) yeast specific growth rate (bottom), (∇) ethanol production (top), (Δ) ethanol production (bottom), (■) acetic acid production (top), (□) acetic acid production (bottom).

At 25C, inhibition of lactic acid bacteria in the top and bottom areas was almost immediate, the maximum occurring at about 25–30 days (Fig 7). At 10C the maximum inhibition in the top area of the drum occurred at approximately 90 days, while in the bottom area it occurs at about 75 days (Fig 6). At 4C (Fig. 5), the specific growth velocity in the top and bottom areas was almost always greater than zero; indeed, there appears to be no inhibition during storage.

Examination of the analytical data in Tables 2,3 and 4 clearly reveals that the fermentation of all samples stored at different temperatures features an increased Fru/Glu ratio and a lowered sugar quotient (SQ% = ((Fru + Glu)/total solids)) × 100). The latter parameter (SQ%) appears to be linked to a slowed

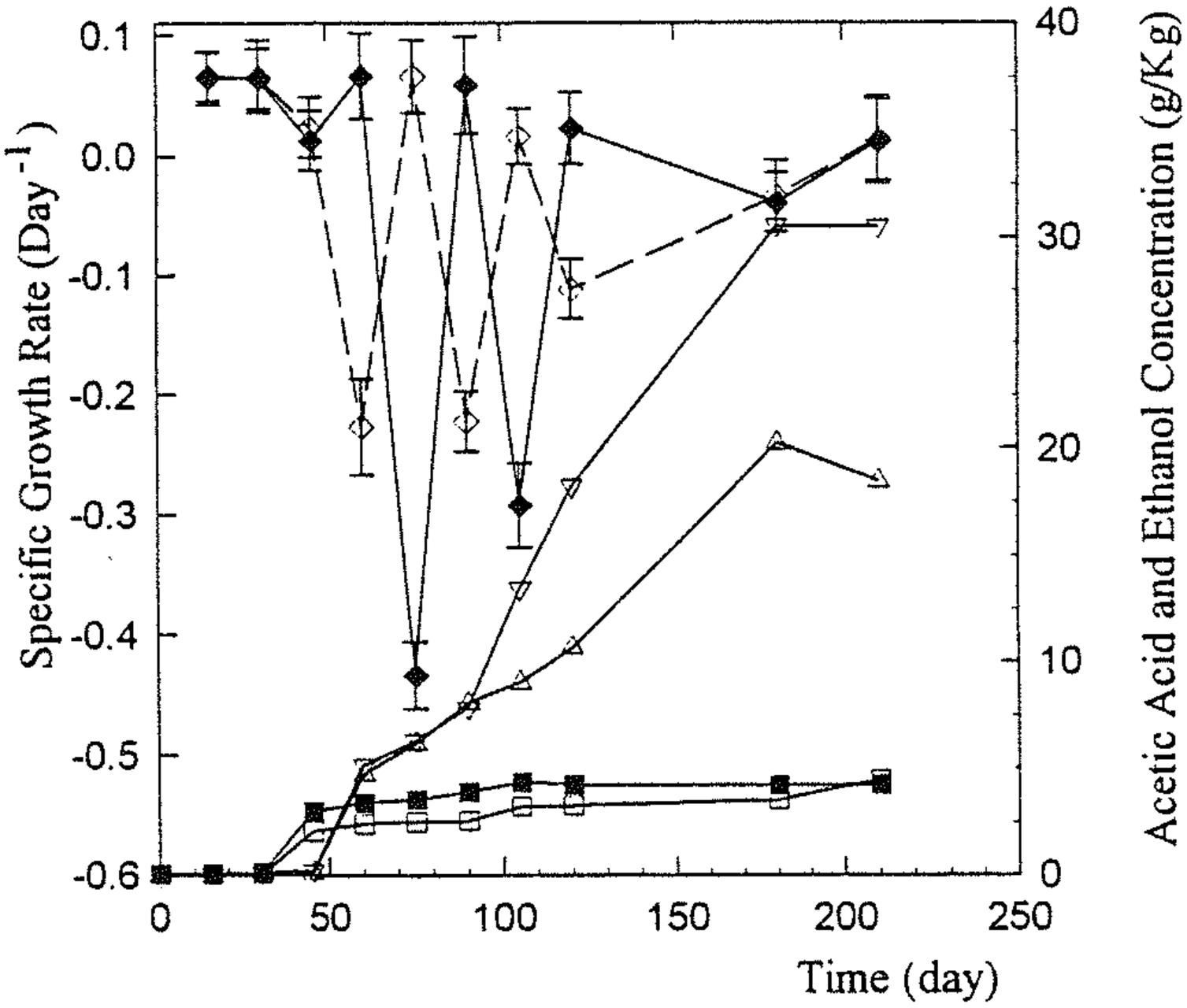

FIG. 3. EVOLUTION OF YEAST SPECIFIC GROWTH RATE, OF THE ACETIC ACID AND THE ETHANOL PRODUCT ON TOMATO PASTE STORED AT 10C

(◆) yeast specific growth rate (top), (◇) yeast specific growth rate (bottom), (▽) ethanol production (top), (△) ethanol production (bottom), (■) acetic acid production (top), (□) acetic acid production (bottom).

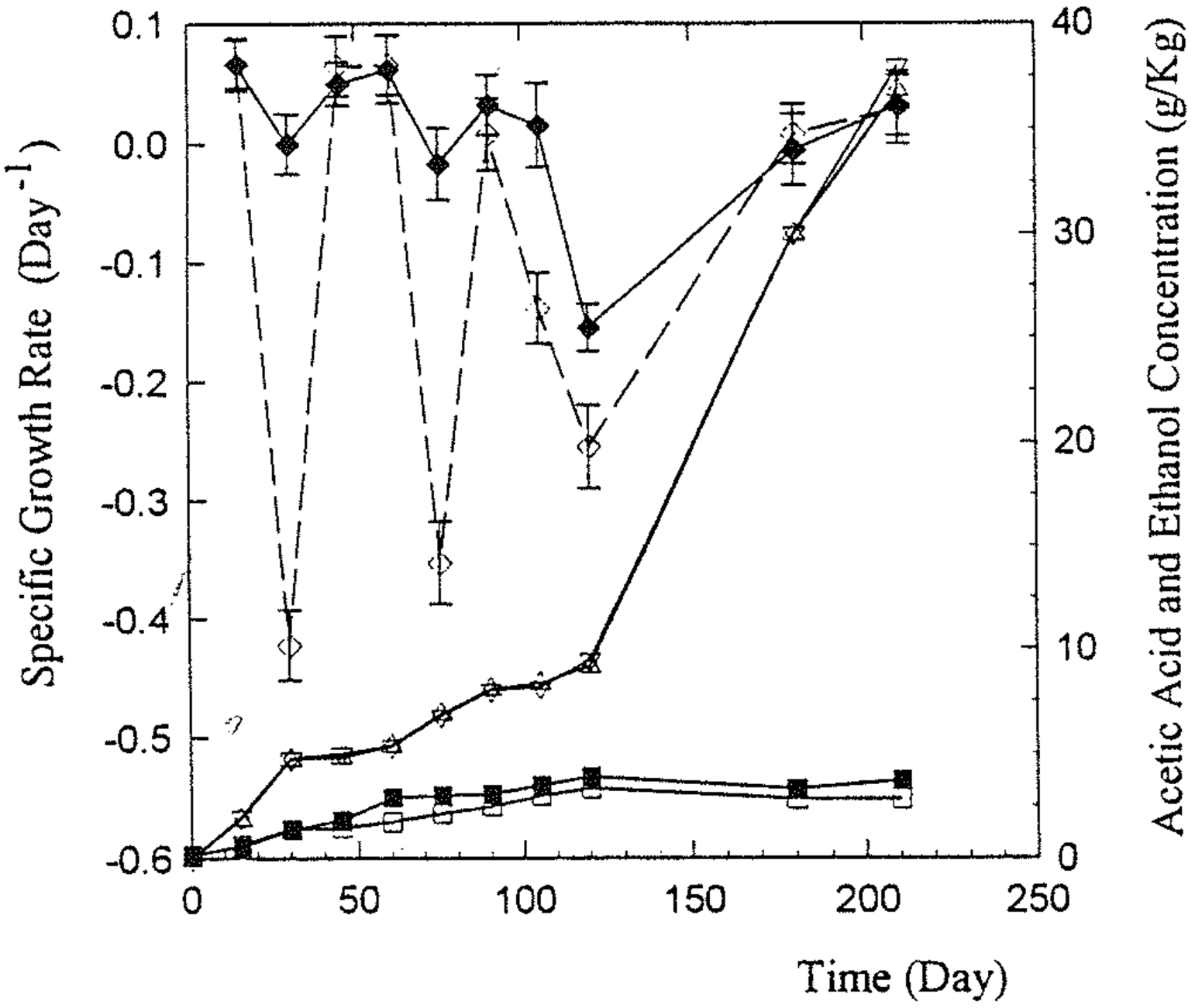

FIG. 4. EVOLUTION OF YEAST SPECIFIC GROWTH RATE, OF THE ACETIC ACID AND THE ETHANOL PRODUCT ON TOMATO PASTE STORED AT 25C

(◆) yeast specific growth rate (top), (◇) yeast specific growth rate (bottom), (▽) ethanol production (top), (△) ethanol production (bottom), (■) acetic acid production (top), (□) acetic acid production (bottom).

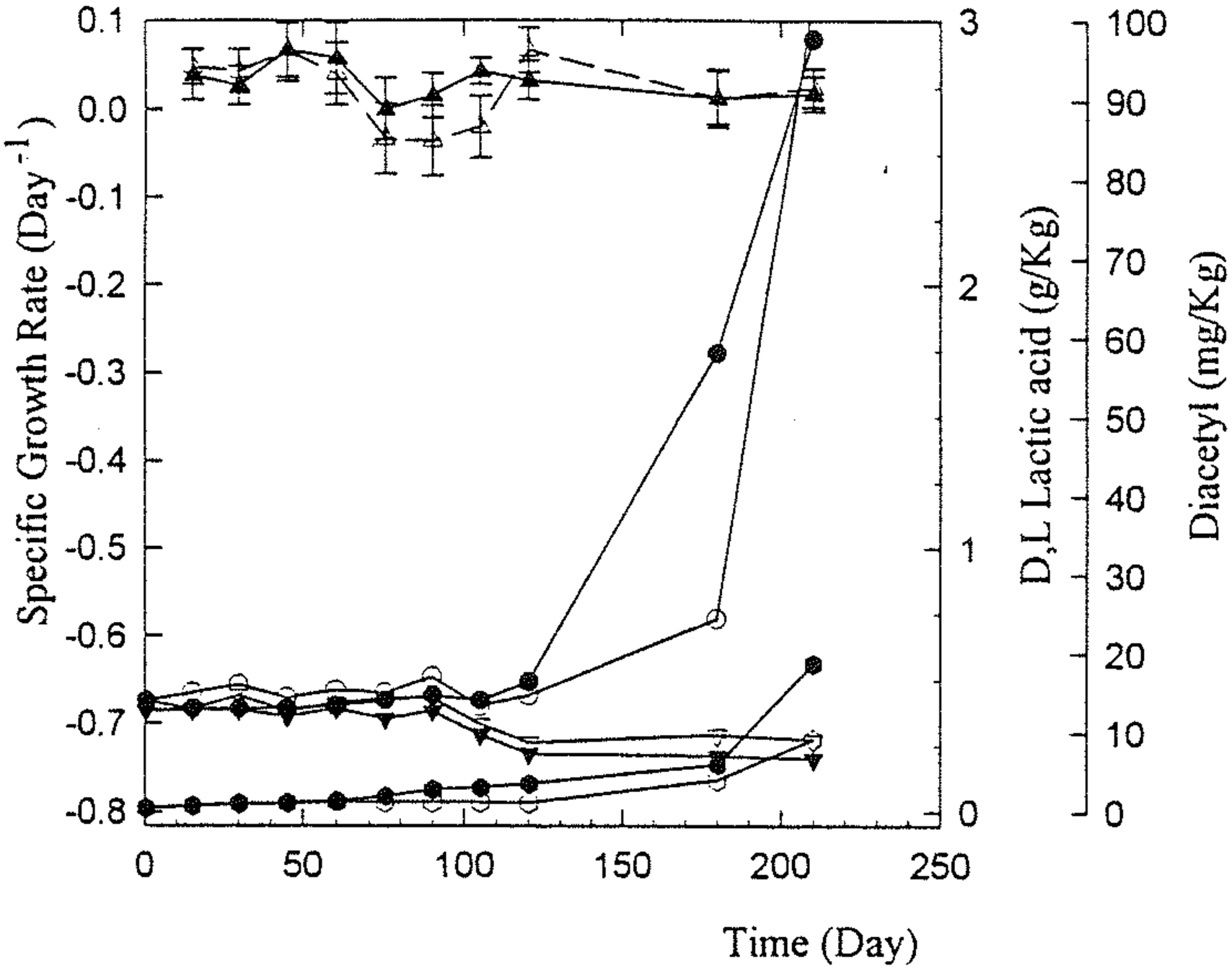

FIG. 5. EVOLUTION OF LACTIC ACID BACTERIA SPECIFIC GROWTH RATE, OF THE D-L LACTIC ACIDS AND DIACETYL PRODUCT ON TOMATO PASTE STORED AT 4C

(▲) lactic acid bacteria (top), (Δ) lactic acid bacteria (bottom), (●) L-lactic acid (top), (○) L-lactic acid (bottom), (▼) D-lactic acid (top), (∇) D-lactic acid (bottom), (◉) diacetyl (top), (◌) diacetyl (bottom).

growth velocity specific for lactic acid bacteria found in stored samples at different temperatures and seems to be correlated with the different storage temperature conditions. In fact, with the sample stored at 25C, the inhibition (Fig. 7) was present in the top and bottom zones at about 30 days with the sugars quotient at 30% and about 40%, respectively. At 10C the inhibition (Fig. 6) occurred in the top area at about 90 days. This corresponds to a sugars quotient which was the same as that found in the upper product at 25C (26.2% in Table 3). The same behavior was found in the lower area of the drum where the sugars quotient was 40.9 (in Table 3). For the product stored at 4C (Fig. 5) the absence of a marked inhibition of lactic acid bacterial growth seems to be linked to the finding that the sugars quotient was always above 40% during storage. This suggests different lactic acid bacteria behavior linked both to the different aerobic conditions probably occurring in the product and to the sugar availability within the product. Since the sugar content remained high (sugars quotient $>$ 40%) growth inhibition did not occur. Conversely, when this value neared 40%, the growth of lactic acid bacteria subjected to low oxygen levels (bottom samples) was inhibited, whereas the same occurred for the upper samples with much lower sugars quotient (sugars quotient $<$ 30%). This last finding is in agreement with data reported by Kandler and Weiss (1986). They

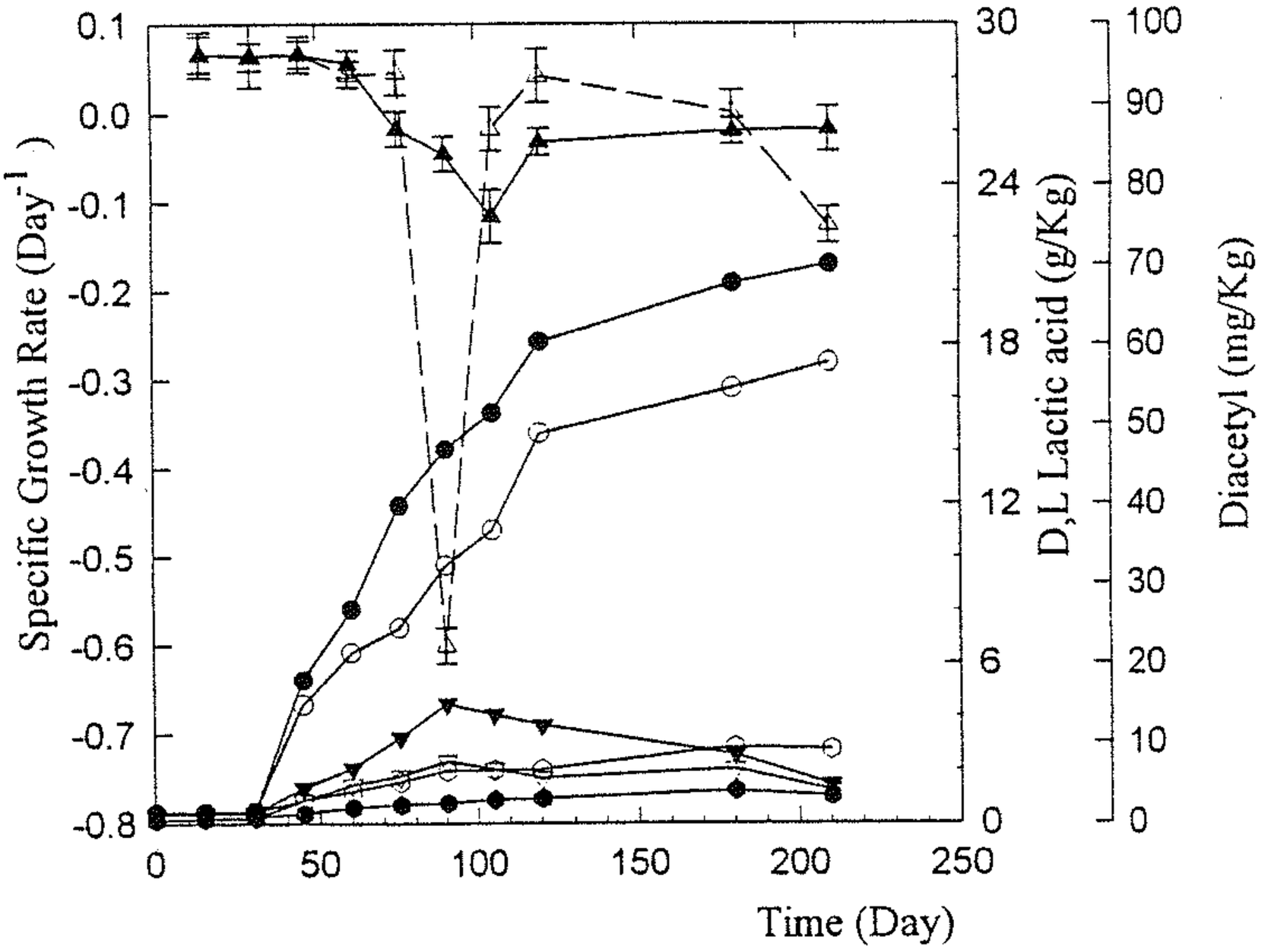

FIG. 6. EVOLUTION OF LACTIC ACID BACTERIA SPECIFIC GROWTH RATE, OF THE D-L LACTIC ACIDS AND DIACETYL PRODUCT ON TOMATO PASTE STORED AT 10C
(▲) lactic acid bacteria (top), (Δ) lactic acid bacteria (bottom), (●) L-lactic acid (top), (○) L-lactic acid (bottom), (▼) D-lactic acid (top), (∇) D-lactic acid (bottom), (◉) diacetyl (top), (⊃) diacetyl (bottom).

found that in aerobic conditions many microbes can reoxidize $NADH_2$, using oxygen as an electron acceptor, so that acetyl-CoA is not completely reduced to EtOH. Under these conditions more ATP is produced and may be used by microbes for growth. Regarding the production of different metabolites, the data concerning acetic acid (Figs. 2–4), D-lactic acid, L-lactic acid and diacetyl (Fig. 5–7) are reported. The analysis of variance revealed that for the samples taken at the two heights (top and bottom) the analytical compositional findings were significantly different, while no significant difference was seen in the samples taken at the same height (horizontal variance) but at different sites.

There was an upward trend in acetic acid growth at all storage temperatures. However, as to be expected the content of acetic acid differed notably with the different storage temperatures. At 4C (Fig. 2) there was almost the same acetic acid production at the top and at the bottom areas, although it was slightly higher in the top area of the drum.

At 10C (Fig. 3) the greatest production of acetic acid occurred in the top part of the drum. At 25C we saw (Fig. 4) an acetic acid production that was very fast. After 15 days it reached 0.6–0.7 g/kg in both the top and bottom

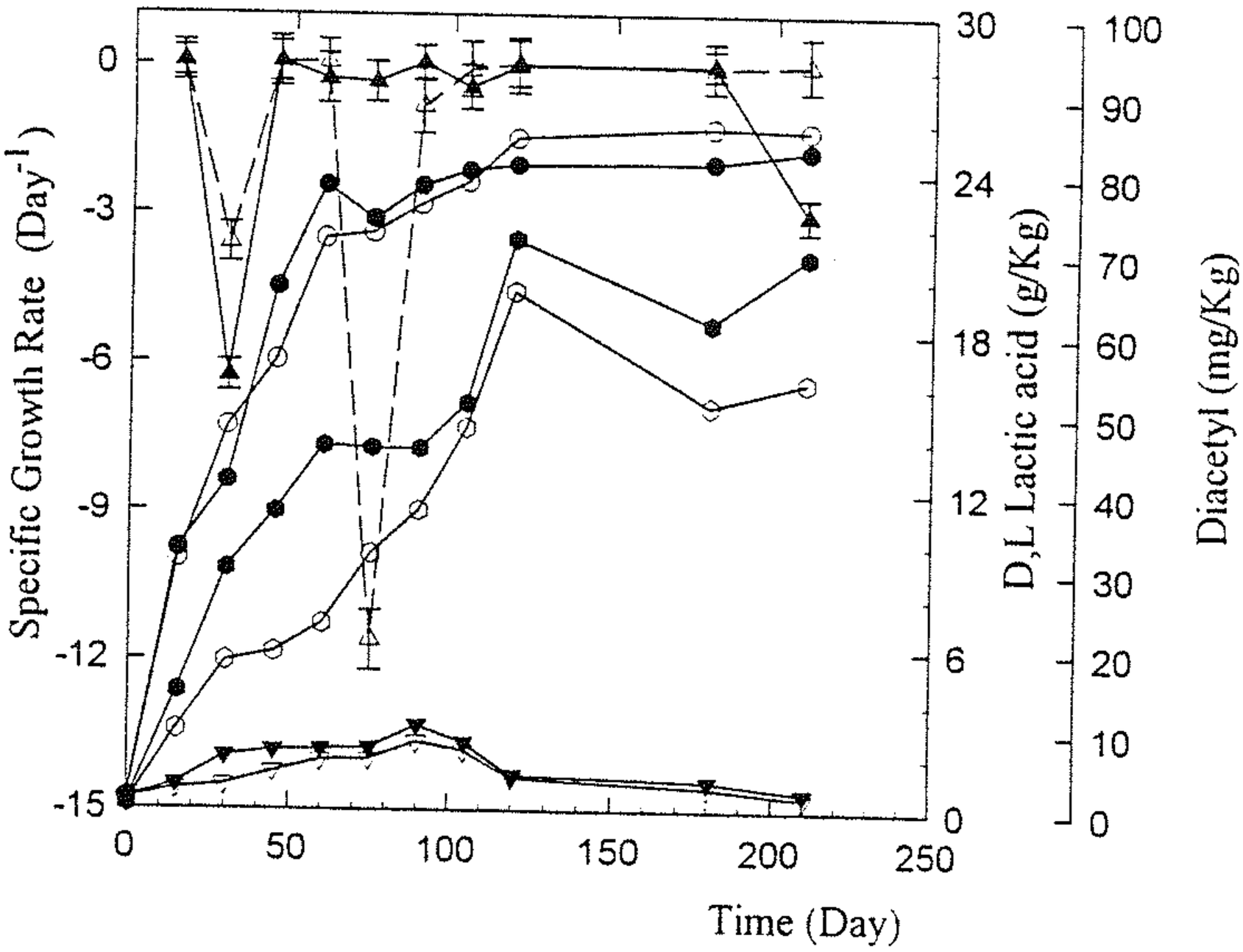

FIG. 7. EVOLUTION OF LACTIC ACID BACTERIA SPECIFIC GROWTH RATE, OF THE D-L LACTIC ACIDS AND DIACETYL PRODUCT ON TOMATO PASTE STORED AT 25C (▲) lactic acid bacteria (top), (Δ) lactic acid bacteria (bottom), (●) L-lactic acid (top), (○) L-lactic acid (bottom), (▼) D-lactic acid (top), (∇) D-lactic acid (bottom), (◉) diacetyl (top), (◌) diacetyl (bottom).

samples. At this temperature, as well, the production of acetic acid was greater in the top area of the drum.

Regarding the diacetyl content, our findings may be summarized as follows: at storage temperatures of 4C (Fig. 5) and 25C (Fig. 7) there was a higher production of diacetyl in the top part of the drum, while at 10C (Fig. 6) there was a greater production of this metabolite in the bottom sampling zone (about 9.5 mg/kg produced after 210 days versus 3.5 mg/kg produced in the top area). It could be that the high level of acetic acid produced at 10C in anaerobic conditions (about 3.60 g/kg), which was considerably higher than what was produced at 4C (about 0.77 g/kg, see Fig. 2) and at 25C (about 2.80 g/kg, see Fig. 4), determines negative feed back on Pyruvic acid -- > -- > -- Acetate pathway, forcing D,L-lactic acid and diacetyl production. This may be confirmed (a) experimentally, by the greater production of D-lactic acid produced at 10C compared to that at 4C and at 25C, (b) by reports of Kandler (1983), showing that the diacetyl production is low when hexoses only are present in the medium but it increases when pyruvate is produced. At 4C L-lactic acid content (Fig. 5) is at the same concentration in the top and bottom areas of the drum (mean value about 0.4g/kg for the first 120 storage days).

At 10C the L-lactic acid production was decisively greater, going from about 0.4 g/kg to 18 g/kg (in the top part) and to about 15 g/kg (in the bottom part) after 120 days. The lowest lactic acid content in the bottom area of the drum may be correlated with the data in Fig. 6, showing an inhibition time of lactic acid bacteria activity briefer than in the top part of the drum, thus conditioning the final L-lactic acid level. In addition, at 25C, where the inhibition of lactic acid bacteria was the same in both sampling areas, the L-lactic acid production is the same at all storage times. As far as D-Lactic acid is concerned, at 4C its levels were found constant during the storage at about 0.3 g/kg (Fig. 5). At 10C, in the top part of the drum, there was a maximum production at about 90 days (4.5 g/kg) which then declined to 1.5 g/kg at 210 days. Finally, at 25C (Fig.7) a maximum was reached at about 90 days (3.18 g/kg) and then declined to 0.71 g/kg at 210 days. The same behavior is seen in the bottom area of the drum as reported in Fig. 5–7. These findings appear to be consistent with the presence of an enzyme able to break down D-lactic acid alone and seem to be in agreement with the literature (Kandler 1983) regarding NAD-independent lactate dehydrogenase (LDH) responsible for lactate oxidation.

The overall picture shows, however, that it is the L-lactic acid metabolite that has the greatest effect on the total lactic acid content, as widely reported in the literature (Luster 1978; Juven and Weisslowicz 1981; Vicini *et al.* 1988; Gherardi *et al.* 1988).

Metabolites in double tomato concentrate at different storage temperatures influenced taste and smell: fermented and sour taste was dependent on storage temperature and on the contamination level as well as on the different conditions occurring inside the drum. In fact, the sample stored at 4C (top section) had a sour taste and slight acid aroma after 75 days, while a sample taken from the bottom of the same drum had the same characteristics after about 120 storage days.

At 10C a persistent acid and/or fermented taste was found after about 45 days in both the top and bottom sections. Finally, in those samples preserved at 25C, taste and smell was greatly altered (high acidity) already after 15 days of storage.

Analytical-Compositional Aspects During Storage

The influence of storage temperature on analytical parameters is shown in Tables 2, 3 and 4.

Soluble Solids, Total Acidity and pH Evolution. It is interesting to note that the soluble solids in the bottom region of the drum remained stable for the whole storage period at 4C, while in the top section of the drum the soluble

TABLE 2.
CHANGES IN ANALYTICAL PARAMETERS OF TOMATO PASTE (≈ 30° Brix)
STORED AT 4C

Storage Time (Day)	Sampling Zone	Soluble solids %	Total solids g %	pH	Volatile Acidity [c] g%	Total Acidity [d] g%	Fru/Glu ratio	Fru+Glu sum [e]	Total Sugar [f] g%	Sugar Quotient [g] %
0	TOP [a]	30.5	31.8	4.36	0.013	2.17	1.08	15.44	16.7	48.6
15		30.4	31.8	4.37	0.017	2.16	1.08	15.33	16.7	48.3
30		30.1	31.8	4.39	0.019	2.18	1.09	15.04	16.6	47.3
45		30.1	31.8	4.33	0.022	2.23	1.11	14.89	16.6	46.7
60		30.1	31.4	4.31	0.024	2.22	1.16	15.01	16.6	47.9
75		30.0	31.4	4.30	0.025	2.27	1.14	14.59	15.7	46.5
90		29.9	31.3	4.29	0.031	2.31	1.15	13.86	14.5	44.3
105		29.2	30.8	4.33	0.031	2.27	1.18	13.15	14.3	42.7
120		28.9	30.3	4.32	0.031	2.24	1.25	12.90	14.1	42.6
180		27.1	28.5	4.33	0.052	2.11	1.29	12.69	13.1	44.6
210		25.0	26.3	4.34	0.075	2.10	1.20	11.66	11.9	44.4
0	BOTTOM [b]	30.5	31.8	4.37	0.015	2.17	1.08	15.48	16.6	48.7
15		30.5	31.6	4.37	0.018	2.19	1.13	15.03	16.6	47.5
30		30.2	31.5	4.38	0.024	2.21	1.10	14.54	16.5	46.1
45		30.1	31.5	4.29	0.024	2.25	1.10	14.49	16.5	46.1
60		30.0	31.9	4.30	0.023	2.23	1.10	14.44	16.4	45.3
75		30.0	31.8	4.30	0.023	2.26	1.14	14.58	16.3	45.8
90		29.7	31.8	4.30	0.026	2.29	1.11	14.49	16.1	45.5
105		30.4	31.8	4.29	0.025	2.10	1.18	14.07	15.9	43.9
120		30.5	32.1	4.30	0.024	2.10	1.29	13.94	15.9	43.4
180		30.4	31.8	4.34	0.024	2.10	1.11	14.43	15.8	45.4
210		29.8	30.9	4.34	0.024	2.10	1.08	14.07	15.3	45.5

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom.
[c] Volatile acidity expressed as acetic acid. [d] Total acidity (pH 8.1) expressed as citric acid monohydrate.
[e] Sum of fructose and glucose by HPLC. [f] Total sugars determined by Fehling method. [g] Sugar quotient = ((Fru+Glu)/total solids) x 100.

solids remained stable only for the first 90 days and then declined until 25° Brix at the end of storage. At 10C the same thing occurred (Table 3). The soluble solids stayed stable in the bottom of the drum for about 60 days while in the top area for 30 days.

Finally, at 25C the soluble solids in both areas of the drum stayed the same for only 15 days, to confirm that at this temperature the compositional changes are very fast. This is even more apparent given the data in Tables 2, 3 and 4 regarding the total acidity trends at the different storage times. In fact, at 4C for both region samples, the total acidity remained substantially the same while at 10C it increased from 2.17 to 3.85 (top sample) and from 2.17 to 3.51 g% (bottom sample) at the end of the storage. This effect was even greater at 25C where after only 15 days a mean total acidity exceeding 3.0 g% was reached.

TABLE 3.
CHANGES IN ANALYTICAL PARAMETERS OF TOMATO PASTE
(≈ 30° Brix) STORED AT 10C

Storage Time (Day)	Sampling Zone	Soluble solids %	Total solids g %	pH	Volatile Acidity [c] g%	Total Acidity [d] g%	Fru/Glu ratio	Fru+Glu sum [e]	Total Sugar [f] g%	Sugar Quotient [g] %
0	TOP [a]	30.5	32.6	4.36	0.013	2.17	1.08	15.66	16.6	48.0
15		30.3	32.2	4.39	0.015	2.17	1.09	15.28	16.6	47.4
30		30.2	32.1	4.45	0.016	2.17	1.05	15.02	16.3	46.9
45		26.5	28.8	4.32	0.062	2.52	0.99	11.31	12.0	39.3
60		24.8	26.0	4.03	0.180	3.04	1.16	5.99	7.0	23.1
75		22.5	23.8	4.00	0.213	3.12	1.28	5.06	5.8	21.3
90		20.1	21.4	3.90	0.270	3.35	1.34	5.62	5.7	26.2
105		20.3	21.8	3.96	0.321	3.43	1.25	3.00	3.4	13.8
120		20.5	21.3	3.96	0.340	3.67	1.24	3.02	3.0	14.2
180		19.8	21.1	3.99	0.296	3.85	1.07	2.20	2.7	10.5
210		19.7	20.7	3.99	0.257	3.85	1.04	1.92	1.8	9.3
0	BOTTOM [b]	30.5	32.6	4.33	0.015	2.17	1.08	15.31	16.9	48.3
15		30.1	31.8	4.34	0.021	2.29	1.11	14.90	16.7	46.8
30		30.0	31.8	4.38	0.027	2.29	1.12	14.40	16.6	45.2
45		29.5	31.1	4.23	0.039	2.29	1.11	13.86	15.2	44.5
60		28.9	30.5	4.11	0.062	2.34	1.16	13.10	14.5	43.0
75		27.1	28.1	4.08	0.079	2.55	1.23	11.49	12.1	40.9
90		26.3	27.7	4.07	0.091	2.73	1.27	10.25	10.7	37.1
105		25.9	27.1	4.04	0.115	3.03	1.26	8.57	8.4	31.6
120		25.8	27.8	4.01	0.122	3.11	1.27	8.40	8.6	30.2
180		25.6	27.6	4.02	0.235	3.32	1.19	8.28	8.5	30.0
210		25.5	27.5	4.01	0.241	3.51	1.12	8.01	8.4	29.1

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom. [c] Volatile acidity expressed as acetic acid. [d] Total acidity (pH 8.1) expressed as citric acid monohydrate. [e] Sum of fructose and glucose by HPLC. [f] Total sugars determined by Fehling method. [g] Sugar quotient = ((Fru+Glu)/total solids) x 100.

Finally, as shown in Tables (2–4), pH decreased notably at 25C and 10C while it remained stable in the product stored at 4C.

Sugars and Organic Acids Evolution. The finding about sugar and organic acid use by the microbial flora may be summarized as follows. The FRU/GLU ratio increases considerably with the storage time of 25C both in top and bottom areas of the drum. Instead, this behavior is less evident in the storage samples at 4 and 10C. It is interesting to note that the FRU/GLU ratio at the higher storage temperatures increased during the first phase and thereafter decreased. These findings therefore show a differentiated use of fructose and glucose during the storage period, characterizing a preferred glucose use in the early storage period (increased FRU/GLU ratio) followed by a greater fructose use, compared to glucose in the late storage period (decrease in the FRU/GLU ratio).

TABLE 4.
CHANGES IN ANALYTICAL PARAMETERS OF TOMATO PASTE
(≈ 30° Brix) STORED AT 25C

Storage Time (Day)	Sampling Zone	Soluble solids %	Total solids g %	pH	Volatile Acidity [c] g%	Total Acidity [d] g%	Fru/Glu ratio	Fru+Glu sum [e]	Total Sugar [f] g%	Sugar Quotient [g] %
0	TOP [a]	30.5	31.5	4.38	0.018	2.17	1.05	15.21	15.4	48.6
15		29.3	31.1	4.12	0.093	3.02	1.16	11.37	14.0	36.6
30		25.3	26.4	4.02	0.141	3.67	1.27	7.94	8.7	30.1
45		25.5	26.1	4.02	0.227	3.71	1.49	5.59	6.7	21.5
60		24.0	25.6	4.02	0.301	4.02	1.57	4.11	4.9	16.1
75		22.9	24.1	4.00	0.345	4.12	1.47	3.71	4.1	15.4
90		22.2	23.7	3.96	0.379	4.21	1.37	3.49	4.4	14.8
105		22.4	23.5	3.94	0.378	4.21	1.30	3.46	4.3	14.7
120		22.0	23.2	3.95	0.375	4.16	1.23	3.08	4.1	13.3
180		21.1	22.3	3.96	0.280	4.02	0.72	1.95	2.8	8.8
210		21.0	22.2	3.97	0.199	4.01	0.70	1.91	2.7	8.6
0	BOTTOM [b]	30.4	31.5	4.38	0.018	2.17	1.03	15.49	16.1	48.5
15		29.0	30.2	4.22	0.049	2.67	1.05	13.63	15.2	45.2
30		27.3	29.2	4.12	0.078	2.81	1.13	11.45	11.7	39.3
45		25.8	27.2	4.11	0.176	3.57	1.29	7.34	7.8	27.0
60		24.0	24.8	4.09	0.270	4.11	1.98	4.09	5.8	16.5
75		23.1	24.7	3.97	0.273	4.11	1.82	3.79	4.9	15.4
90		23.5	24.6	3.96	0.290	4.13	1.57	3.63	4.4	14.8
105		23.2	24.5	3.97	0.288	4.15	1.55	3.67	4.4	15.0
120		23.3	24.8	3.99	0.294	4.09	1.56	3.93	4.7	15.9
180		24.0	24.4	3.99	0.191	4.06	1.45	4.09	4.4	16.7
210		23.0	24.1	3.99	0.182	4.02	1.41	3.44	3.7	14.3

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom.
[c] Volatile acidity expressed as acetic acid. [d] Total acidity (pH 8.1) expressed as citric acid monohydrate.
[e] Sum of fructose and glucose by HPLC. [f] Total sugars determined by Fehling method. [g] Sugar quotient = ((Fru+Glu)/total solids) x 100.

Citric acid showed an increased percentage breakdown with storage temperature. Its concentration was 19.1 g/kg at beginning of the storage but after 210 days it became 10% lower at 4C, 28% lower at 10C and 37% lower at 25C.

Malic acid behaved like citric acid, from a starting concentration of 1.95 g/kg, a percentage drop of about 14% at 4C and about 52% at 10C and about 60% at 25C was observed after 210 days of storage.

D-isocitric acid did not seem to be very much degraded at 4C, but at 10C and at 25C, after 210 storage days, the breakdown was about 26% and 28%, respectively, from a starting concentration of 0.45 g/kg.

Finally, for succinic acid, we found substantial unexplainable stability at 25C, against a degradation of about 25% both at 4C and at 10C after 210 storage days.

Color and HMF Evolution. The last aspect of this study regards the influence of storage temperature on product color (unreported data) and on the evolution of hydroxymethylfufurol (HMF) content. The findings show a substantial color stability (the a/b ratio ranged from 2.32–2.15 while the L parameter ranged from 24.2–25.0). The HMF content was almost constant at about 5 mg/kg at all temperatures.

CONCLUSION

The analytical compositional variance of double tomato concentrate stored in nonsterile drums was determined. Our findings show that a practical recommendation would be to store the product at temperatures below or equal to 4C. At that temperature the product remained relatively stable from an organoleptic and compositional standpoint for at least three months. The analytical data have in fact confirmed at this temperature a substantial consistency of main analytical parameters. Soluble solids, the parameter usually used in the trade of this semifinished product, stayed constant for at least 90 days in both the top and bottom of the drum. Citric acid, the main tomato acid also remained stable at 4C. The decay was in fact about 10% in 210 days of storage. From a purely technological outlook, a slower product decay may be obtained by preventively cooling the semifinished tomato to less than 10C immediately after concentration, instead of placing the product in drums at 65–70C and then letting it cool. In such a way prolonged storage at temperatures ideal for lactic acid bacteria growth is avoided before the ideal storage temperature is reached. In fact, our data show that samples slowly cooled and stored at 10C and 25C had a remarkable production of D,L-lactic acid exceeding 1 g/kg of product in the first days of storage. These values are decisively high compared to the generally accepted quality standard (D,L-lactic acid < 0.5 g/kg) for a good quality product.

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REFERENCES

Boehringer Mannheim Biochemicals. 1989. Methods of Enzymatic Analysis.

GALLOWAY, L.D. and BURGESS, R. 1952. *Applied Micology and Bacteriology*, 3rd Ed. Leonard Hill, London.

GHERARDI, S., VICINI, E., ZANOTTI, A., BAZZARINI, R. and TRIFIRO, A. 1988. Sviluppo di metaboliti e alterazioni chimiche causate da alcuni tipi di microrganismi in derivati vegetali. Ind. Conserve *63*, 364–370.

IFFJP. 1962. Analysis, Method 5, International Federation of Fruit Juice Producers, Paris.

IFFJP. 1972. Analysis, Method 12, International Federation of Fruit Juice, Paris.

KANDLER, O. 1983. Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek; J. Microbiol. Serol. *49*, 209–224.

KANDLER, O. and WEISS, N. 1986. *Bergey's Manual of Systematic Bacteriology,* Vol. 2.

LEONI, C. and BELLUCCI, G. 1980. Le conserve di pomodoro. (ed.) Stazione Sperimentale Industria Conserve Alimentari In Parma.

LUCEY, C.A. and CONDON, S. 1986. Active role of oxygen and NADH oxidase in growth and energy metabolism of *Leuconostoc*. J. Gen. Microbiol. *132*, 1789–1796.

LUSTER, C. 1978.A rapid and sensitive sterility monitoring technique for aseptically processed bulk tomato paste. J. Food Sci. *43*, 1046–1052.

JUVEN, B.J. and WEISSLOWICZ, H. 1981. Chemical changes in tomato juices caused by lactic acid bacteria. J. Food Sci. *46*, 154–156.

MUACV. 1961. Metodi ufficiali di analisi delle conserve vegetali. (ed.) Ministero dell'agricoltura e delle foreste, Istituto poligrafico dello stato, Roma.

ROGOSA, M., MITCHELL, J.A. and WISEMANN, R.F. 1951. A selective medium for isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. *62*, 132–133.

SNEDECOR, G.W. and COCHRAN, W.G. 1980. *Statistical Methods*, 7th Ed., The Iowa State University Press.

VICINI, E., GHERARDI, S., BONICI, A., ZONI, C. and TRIFIRO, A. 1988. Sviluppo di metaboliti e alterazioni chimiche causate da alcuni tipi di microorganismi in derivati vegetali. Ind. Conserve. *63*, 39–44.