SELECTION AND METABOLIC CHARACTERIZATION OF MESOPHYLIC STARTER CULTURES FOR OPTIMIZING THE SENSORY ATTRIBUTES OF FRUIT FLAVOURED MAAS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

Garron M. Arendse 13 December 1999

ABSTRACT

Maas is a traditional fermented milk drink of the indigenous people of Southern Africa and can thus be used to uplift the nutritional status of the South African population, especially for the lower income groups. Furthermore, the problem of lactose intolerance among the Black population can also be addressed by the consumption of Maas. The objective of this study was to screen mesophylic lactic acid bacterial strains (25 in total) from the University of Stellenbosch Food Science Culture Collection for suitable metabolite production and then to produce traditional Maas with a starter culture combination that produces a distinctive acid and traditional flavour.

The representative 25 single lactic acid starter strains were identified as *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (12 strains), *L. lactis* subsp. *lactis* (four strains) and *L. lactis* subsp. *cremoris* (nine strains). These strains were inoculated into pasteurised full cream milk and activated for 8 h at 22°C. Pasteurised full cream milk was then inoculated with each of the activated starter strains, incubated at 22°C for 16 h and assessed for acid production abilities (pH = 4.6) under controlled time-temperature conditions. The results of this study showed that nine of the single strains, *L. lactis* subsp. *lactis* biovar *diacetylactis* (S1, S2, S3 and S5), *L. lactis* subsp. *lactis* (S13, S15 and S16) and two *L. lactis* subsp. *cremoris* strains (S17 and S22), produced sufficient acid, rendering them suitable for the use as starters in the production of traditional Maas. A pH range of 4.3 – 5.1 was reached by the nine single strains after 16 h at 22°C.

Two-strain starter combinations were then formed by combining the most suitable single *L. lactis* subsp. *lactis* biovar *diacetylactis*, *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* strains, respectively. From the data, it was concluded that acceptable Maas could be produced with four two-strain combinations (S3S17, S3S22, S5S17 and S5S22). This selection was again based on suitable acid and metabolite production, as well as on sensory evaluation of the final product. These four two-strain combinations produced sufficient acid to reach a pH in the 4.6 – 4.8 range, and showed a high metabolite concentration for the most suitable compounds and formed a thick, smooth and creamy body texture after 16 h at 22°C.

Three-strain combinations formed between the two-strain starter combinations and *L. lactis* subsp. *lactis* strains (S13, S15 and S16), were also evaluated. With these combinations a lack of a pronounced Maas flavour was found. Thus, it was decided to add aroma producing strains of the species *Leuconostoc mesenteroides* subsp. *dextranicum* (strain L1) and *L. mesenteroides* subsp. *citrovorum* (strain L2) to the three-strain combinations. Four culture combinations (A, B, C and D) were then formed by combining the selected *Leuconostoc* strains (L1 and L2) with the most suitable *Lactococcus* strains (S3, S17, S13 and S22). These combinations produced sufficient acid to reach the pH 4.5 – 4.6 range after 14 h at 22°C. Acetaldehyde was the major flavour metabolite formed in the Maas made with these four combinations, with concentrations ranging between 26.6 – 89.3 $mg.l^{-1}$, while other flavour metabolites (ethanol, acetone, diacetyl and 2-butanone) were present at lower concentrations. It was found that three of the four culture combinations (A, C and D) were characterised by a superior, but delicate flavour and a typical characteristic Maas body texture.

Fruit flavoured Maas was subsequently prepared with the three most suitable culture combinations (A, C and D) using 11 flavours and a sensory evaluation performed. The statistically evaluated data showed that the appearance, smoothness, flavour intensity, sweetness and overall acceptability were influenced by the type of fruit flavour and the culture combination. Fruit flavour 4 (banana) was the most preferred flavour. The sensory panellists also indicated that the culture combination C gave the best overall acceptability over a three week study period.

Data on the shelf-life study of natural unflavoured Maas, prepared with the three culture combinations (A, C and D), showed that the Maas still had an acceptable appearance, taste and good microbiological quality after 15 d at refrigerated temperatures.

UITTREKSEL

Maas is 'n tradisionele gefermenteerde melkdrankie onder die inheemse bevolking van Suid-Afrika en kan gebruik word om die voedingstatus van die Suid-Afrikaanse bevolking te verhoog, veral vir die laer inkomste groepe. Bowendien, kan die probleem van laktose intoleransie onder die Swart gemeenskap ook aangespreek word deur die verbruik van Maas.

Die doel van hierdie studie was om enkelstam mesofiliese melksuur bakterieë (25 in totaal) van die Universiteit van Stellenbosch Voedselwetenskap Kultuur Versameling te ondersoek vir geskikte metaboliet produksie en tradisionele Maas met 'n kenmerkende suurheid en tradisionele geur met 'n geskikte kultuur kombinasie te produseer.

Die toonaangewende 25 enkelstamme is *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (12 stamme), *L. lactis* subsp. *lactis* (vier stamme) en *L. lactis* subsp. *cremoris* (nege stamme). Hierdie stamme was in gepasteuriseerde volroom melk geïnokuleer en geaktiveer vir 8 h teen 22°C. 'n Inokulum van die onderskeie geaktiveerde stamme is hierna in gepasteuriseerde volroom melk geplaas, vir 16 h teen 22°C geïnkubeer en hul vermoë om suur te produseer (pH = 4.6) onder beheerde tyd-temperatuur kondisies is bepaal. Die resultaat van die studie het aangedui dat nege enkelstamme, naamlik *L. lactis* subsp. *lactis* biovar *diacetylactis* (S1, S2, S3 en S5), *L. lactis* subsp. *lactis* (S13, S15 en S16) en twee *L. lactis* subsp. *cremoris* (S17 en S22), geskikte suurheidsvlakke vir die produksie van Maas bereik het. 'n pH vlak van 4.3 – 5.1 is na 16 h teen 22°C deur hierdie nege enkelstamme bereik.

Twee-stam kombinasies is onderskeidelik gevorm tussen die geskikte enkel *L. lactis* subsp *lactis* biovar *diacetylactis*, *L. lactis* subsp. *lactis* en *L. lactis* subsp. *cremoris* stamme. Die gevolgtrekking gemaak uit die data, is dat aanvaarbare Maas voorberei kan word met vier van die twee-stam kombinasies (S3S17, S3S22, S5S17 en S5S22) op grond van suurvorming, metaboliet produksie en sensoriese evaluasie. Hierdie vier kombinasies het genoegsame suur geproduseer om 'n pH vlak van 4.6 - 4.8 beriek, hoë metaboliet konsentrasies geproduseer en 'n dik, gladde en romerige tekstuur aangeneem na 16 h teen 22°C.

Drie-stam kombinasies is gevorm tussen die onderskeie twee-stam kombinasies en *L. lactis* subsp. *lactis* stamme (S13, S15 en S16) en ook geëvalueer. Die tekort aan 'n skerp Maas geur in die drie-stam kombinasies het daartoe gelei dat *Leuconostoc mesenteroides* subsp. *dextranicum* (stam L1) en *L. mesenteroides* subsp. *citrovorum* (stam L2) bygevoeg is. Vier kultuur kombinasies (A, B, C en D) is gevorm deur die geselekteerde *Leuconostoc* stamme (L1 en L2) te kombineer met die mees gepaste *Lactococcus* stamme (S3, S17, S13 en S22). Hierdie kombinasies het genoegsame suur geproduseer wat 'n pH vlak van 4.5 – 4.6 na 14 h teen 22°C bereik het. In die Maas wat met bovermelde kombinasies gemaak is, was die asetaldehied die mees geproduseerde geur metaboliet teen konsentrasies van 26.6 – 89.3 $mg.l^{-1}$. Ander geur metaboliete (etanol, asetoon, diasetiel, 2-butanoon) is in laer konsentrasies geproduseer. Daar is gevind dat drie uit die vier kultuur kombinasies (A, C en D) 'n superieur, delikate geur wat 'n tipies karakteristiek van die Maas gehad het.

Vrugte gegeurde Maas geproduseer met die drie kultuur kombinasies (A, C en D) deur 11 geursels te gebruik, is sensories geëvalueer. Die statistiese geëvalueerde data het getoon dat die voorkoms, gladheid, geur intensiteit, soetheid en die algehele aanvaarbaarheid beïnvloed is deur die tipe vrugte geursels en die kultuur kombinasies. Die vrugte geursel 4 (piesang) het voorkeur geniet. Die sensoriese paneellede het ook aangedui dat kultuur kombinasie C die algehele mees aanvaarbare Maas geproduseer het oor die studie periode van drie weke.

Data van die rakleeftyd van die natuurlike ongegeurde Maas wat geproduseer is met die drie kultuur kombinasies (A, C en D) het aangedui dat die Maas na 15 d by yskas temperatuur steeds 'n aanvaarbare voorkoms, smaak en goeie mikrobiologiese kwaliteit gehad het.

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

Fermented milk products were originally developed as a means of preservation, but it is now also used to develop a wide range of products with different flavours, textures and consistencies (Buttriss, 1997). In most ancient manuscripts fermented milks are mentioned at one or other stage which could make fermented milk as old as the human race itself (Keller & Jordaan, 1990). One could argue that the deliberate fermentation of milk was one of the key achievements that nurtured mankind to grow and develop into productive and pre-eminent species (Kroger *et al.*, 1989). Thus, foods that resulted from growth and action of microorganisms under those rather primitive conditions can be considered as the predecessors of the large variety of cultured foods available today (Dellaglio, 1988).

Studies have shown that the regular consumption of fermented milks leads to several health benefits for the host (Dellaglio *et al.*, 1992). These advantages probably come firstly, from the viable, ingested microorganisms which induce changes and positive influences on the intestinal environment and, secondly, from the metabolites present in the fermented milks. An increasing number of positive and secondary effects have also been proved and include desirable influences on the intestinal ecology, anti-carcinogenic and even hypocholesteremic effects (Dellaglio *et al.*, 1992).

Fermented milks by themselves or combined with cereals offer a means of preserving highly nutritious foods at costs far below canning, freezing and artificial dehydration (Steinkrauss, 1996). Also, fermented milks have high biological and dietetic value and are recommended for consumption by healthy people (Keller & Jordaan, 1990). Therapeutic values have also been claimed and some have been scientifically proven (Keller & Jordaan, 1990). Persons that are lactose intolerant are also able to drink fermented milks. This is one reason why the Black people of Africa prefer fermented milk to fresh milk and they allow milk to ferment naturally in order to convert some of the lactose to organic acids and other compounds. The fermented product can subsequently be used without any after-effects common to lactose

intolerance (Keller & Jordaan, 1990). On the other hand cultured dairy products can be of great benefit in the diet of lower socio-economic population groups, owing to their longer shelf-life and significant contribution to improved health and nutrition.

The lactic acid bacteria (LAB) cultures used to initiate fermentations share some features in that they involve mesophilic and thermophilic bacteria (Anon., 1996). These products are attracting considerable consumer interest due to their probiotic properties, i.e. the ability to inhibit the growth of potentially pathogenic microorganisms (Britz, 1997). Microorganisms necessary for food fermentations may be added as pure cultures or as mixed cultures. In some instances no cultures may be added if the desired microorganisms are known to be in sufficient numbers in the original raw material. However, cultures for food fermentations are selected primarily on the basis of their stability and their ability to produce desired products or changes efficiently. These cultures can include established ones obtained from other laboratories or can be selected after the testing of numerous bacterial strains. Stability is an important characteristic, as well as yields and rates of changes (Britz, 1997). Some cultures may be improved by breeding, but selection is the most commonly used for the improvement of bacterial strains.

In South Africa, some of the traditional fermented milks by trademark are Maas and Inkomasi (Keller & Jordaan, 1990). These products were traditionally made in clay pots and calabashes and milk was periodically added to the containers where the bacteria on the surface served as starter. These fermented milks are now mostly produced commercially in stainless steel vats (Keller & Jordaan, 1990) of 64 million litres (IDF, 1996). Maas appeals for its unique and satisfying tang. This dairy product is produced by adding specific cultures to the milk, which in turn ferments lactose into lactic acid and other typical product flavours (diacetyl, acetaldehyde, etc.). Furthermore, Maas posses several nutritional and health qualities which makes valuable contribution to the overall dietary patterns at all stages of life.

The dairy industry is the fourth largest agricultural commodity in South Africa and represents 7% of the gross value of the agricultural section (Liebenberg, 1996). In the last 20 to 30 years fermented milks have became very popular in South Africa and other countries (Keller & Jordaan, 1990) with yoghurt, drinking yoghurt, Maas and buttermilk being the major cultured products found on the South African food market. There is however, a need in the market for the introduction of new types of

fermented milks (Keller & Jordaan, 1990). The per capita consumption of cultured products in South Africa is only 3.6 kg in comparison to a value of 30 kg in certain European countries (Joubert & De Lange, 1992). In light of these statistics, it is obvious that more can be done to encourage local consumption of cultured milk products. Future research should thus be directed at biotechnological development of selected culture strains so as to produce better quality products (Joubert & De Lange, 1992).

The aim of this study was to produce Maas with different mesophylic starter cultures and compare it with Maas made with a commercial mixed culture. The comparison will be based on the texture, consistency and flavour of the Maas. Expert panellists will evaluate the flavoured Maas products of which the data will be statistically analysed. Furthermore, the shelf-life of the natural unflavoured Maas will be evaluated over a 22 d period.

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CHAPTER 2

LITERATURE REVIEW

Background to fermented and cultured milk products

Milk is the most important mammalian foodstuff and has always been the first food of the newborn (Kroger *et al.*, 1989). At some stage in the course of human evolution it was recognised that the milk of other mammals were equally satisfying in meeting most physiological demands for moisture, energy and nutrients. During one phase of human evolution, our early ancestors from the Near East made the transition from gatherers and hunters to domesticators of plants and animals. The human species thus evolved into what might be called *Homo faber* (man the maker). One of the foods man consumed was milk, which when kept in containers, turned sour within hours and is generally referred to as fermented or cultured milk. Fermented milk products thus originated in the Near East and then spread to parts of Southern and Eastern Europe (McGregor, 1991).

The earliest forms of fermented dairy products were developed by accident by nomadic tribes from cow's, sheep, camel's or goat's milk (McGregor, 1991). The best-known fermented product is the thermophylic fermented milk, yoghurt, which has enjoyed increased popularity within the last three decades. Apart from yoghurt, there are numerous other types of mesophylic fermented milks, also called cultured milks, with high consumption patterns in specific parts of the world. Fortunately, the pre-dominant fermenting bacteria were lactic types and therefore helped to preserve the dairy products by suppressing spoilage and pathogenic bacteria (McGregor, 1991). It can therefore be assumed that mesophylic and thermophylic bacteria were the common milk organisms spoiling the milk (Kroger *et al.*, 1989). Man evidently enjoyed the refreshing tart taste of his discovery and began to handle milk so that this preserving action would be encouraged (McGregor, 1991).

World Consumption

Cow milk production is estimated at about one trillion $lb.y^{-1}$ or 450 million metric tons (Kroger *et al.*, 1989). More easily remembered is the fact that for each of the five billion people on earth, there is 200 lb. cow's milk available per year. The amount of milk produced from other species is totally unknown, except possibly for *Homo sapiens*. Human females in 1988, were estimated to have produced about 9 100 million kg of milk.

The estimated annual world milk production is about 700 billion pounds, about 200 lb. per capita (Campbell & Marshall, 1975a). However, milk production is not uniform world-wide. Instead, it is concentrated in the temperate climate areas (United States, Canada, Argentina, Chile, Uruguay, most of Europe, Northern Africa, South and Southwest Africa, Australia, New Zealand and Japan). An estimated 85% of the world's milk supply is produced in the aforementioned locations and regions whose people represent only about one-third of the world population (Campbell & Marshall, 1975a). About 35% of the world milk supply is produced in the Western Hemisphere, 35% in Western Europe, 20% in Eastern Europe and Russia and the balance (10%) in Oceania, Africa and the Middle East. The demand for milk drinks, fermented products and cream for consumption have grown only slightly in the developed countries, but show stronger increases in those countries where the production level is still relatively low (IDF, 1997). Consumption statistics on fermented milk drinks indicate a growth of around 4% per year, driven by a high rate of new development and the presence of brands that are increasingly sold world-wide.

The major producers are not necessarily those countries with the highest per capita consumption figures, but those with the most inhabitants (Kroger *et al.*, 1989). New categories of fermented milks have been created like buttermilk and yoghurt. Unfortunately, only 28 of the 34 International Dairy Federation (IDF) member countries are providing data to the IDF on fermented milks. Several of these countries are significant with regard to production or consumption of these products. The IDF has been collecting data since 1966 on world consumption of fermented milk products (Kroger *et al.*, 1989). Table 1 illustrates data received by the IDF for the year 1993 (IDF, 1995). From these 12 countries in Table 1, nine produce 19 664 700 metric tons of fermented milk products annually, according to the 1987-data

Table 1. Worldwide total consumption statistics (× 1000 tons) for milk and milk products for 1993 (IDF, 1995).

Section		Australia	Switzerland	Germany	Denmark	Finland	UK
A. Milk							
i. Whole (>0.2% fat)	- sold by dairies	1 307.3	549.3	3 219.0	147.0	150.4	3 199.0
	- household consumption		135.0	593.0	75.0	46.4	183.0
ii. With 0.3 - 2.0% fat		324.5		1 616.0	286.0	500.2	2 700
iii. Up to 0.3% fat		115.4	14.3	71.0	58.0	159.9	600
iv. Buttermilk		–	12.0	209.0	29.0	5.7	–
B. Fermented milks							
i. Yoghurt		85.6	119.0	916.0	43.0	67.3	268.0
ii. Others		–	–	77.0	36.0	120.2	9.0
C. Other liquid milk products							
i. Flavoured milks		135.7	10.2	280.0	38.5		60.0
ii. Others		–	–	400.0	–	–	–
D. Cream (butterfat equivalent)			16.3	148.3	15.0	10.2	n.a.*
E. Butter and butterfat products							
i. Butterfat equivalent		46.9	37.5	461.4	22.3	29.7	181.0
ii. Butter (product weight)		58.3	37.5	555.9	21.5	27.0	205.0
F. Cheese							
i. Speisequark, cottage, fresh		30.7	19.4	652.3	4.5	11.4	483.0
ii. Others (including processed)		151.8	95.3	845.9	75.5	60.8	
G. Condensed and evaporated milk		108.7	4.3	419.3	–	–	150.0
H. Whole milk powder		13.5	13.2	138.8	n.a.	0.7	19.1
I. Skim milk powder		42.7	23.9	32.5	n.a.	11.8	73.0
J. Ice cream		204.0	–	232.1	25.3	34.4	n.a.

*n.a. = not available

Table 1. cont./

Section		India	Japan	Netherlands	New Zealand	USA	South Africa
A. Milk							
i. Whole (>0.2% fat)	- sold by dairies	3 020.0	4 792.0	290.3	257.0	8 906.0	1 191.375
	- household consumption	42 560.0	137.0	64.4		202.0	
ii. With 0.3 - 2.0% fat		120.0	291.0	755.2	78.3	11 358	36.829
iii. Up to 0.3% fat		–	43	37.7	11.4	3 123	
iv. Buttermilk		55.0	–	138.1	–	356	
B. Fermented milks							
i. Yoghurt		14 400	601.0	316.3	–	519	58.683
ii. Others		–	461	n.a.*	–	n.a.	56.982
C. Other liquid milk products							
i. Flavoured milks		n.a.	893.0	60.9	–	1 123	162.235
ii. Others		–	–	208.5	–	–	
D. Cream (butterfat equivalent)		–	20.0	11.4	4.36	150.0	8.107
E. Butter and butterfat products							
i. Butterfat equivalent		118.6	75.0	41.5	26.8	426.0	12.290
ii. Butter (product weight)		58.51	92.0	50.4	31.9	533.0	14.748
F. Cheese							
i. Speisequark, cottage, fresh		–	170.0	26.2	28.1	344	2.686
ii. Others (including processed)		3.07		215.2		3 072.0	48.381
G. Condensed and evaporated milk		5.0	57.0	112.7	–	359.0	17.818
H. Whole milk powder		25.27	29.0	10.0	0.6	54.0	13.949
I. Skim milk powder		66.82	216	10.0	3.0	275	9.557
J. Ice cream		73.0	202.0	n.a.	–	1 886	n.a.

*n.a. = not available

received by the IDF (Kroger *et al.*, 1989).

Table 2 illustrates the per capita consumption of fermented milks by several countries (IDF, 1995). Compared to the estimated 450 million metric tons of cows milk produced world-wide (Kroger *et al.*, 1989), 5.4% goes into fermented milks. The future of fermented products depends on continued endorsement by nutritionists and dieticians, dietary fashions and research findings. According to Kroger *et al.* (1989), 13 of the 25 dairy countries reported per capita consumption of 10 kg or less for all fermented milks and yoghurt production combined in 1985. Comparative figures for the United States and Finland were 3.8 kg and 39.3 kg, respectively.

Third World Countries

Countries outside Europe were producers of dairy products like cheese, butter and fermented milks long before the modern industrialised countries (Bachmann, 1984). In this way fermented milk, that is to say the oldest and most natural way of converting milk, came from the Third World countries. Only centuries later did the dairy technology of the industrialised countries become established in these countries. South Africa and Zimbabwe are the only typical Third World countries reasonably self-sufficient in terms of milk production (Hermann, 1997). Approximately 210 million litres of milk are produced in Zimbabwe (Hermann, 1997). The total milk consumption in Third World countries, including imported milk solids, is probably about 4 million tons which, although very low, is a function of the economic development and buying power of consumers in the various countries.

The climate, economical development and available skills in Southern Africa has limited the development of the dairy research in Southern Africa (Hermann, 1997). The industries in these countries standard deteriorated since political independence in the 1960's. The South African industry, and in some respects that of Zimbabwe, has been developed to a reasonable level with small-scale activities in Namibia, Swaziland, Zambia, Malawi and Tanzania. In the other countries production is limited. All other countries, except South Africa and Zimbabwe, are largely dependent on importation of long-life and concentrated products (Hermann, 1997). Consumption is relatively low, due to a lack of money and infrastructure. The market potential is theoretically big, but is totally dependent on economical development in these countries. If South Africa follows the trend of other Southern

Table 2. Worldwide per capita consumption (kg) statistics for milk and milk products for 1993 (IDF, 1995).

Section	Australia	Switzerland	Germany	Denmark	Finland	UK
A. Milk						
i. Whole (>0.2% fat)	73.7	97.50	47.00	42.80	38.80	58.20
ii. With 0.3 - 2.0% fat	18.3	–	19.90	55.10	98.70	46.40
iii. Up to 0.3% fat	6.5	2.00	0.90	11.20	31.60	10.30
iv. Buttermilk	–	1.70	2.60	5.60	1.10	–
B. Fermented milks						
i. Yoghurt	4.8	17.00	11.30	8.30	13.30	4.60
ii. Others	–	–	0.90	6.80	23.70	0.20
C. Other liquid milk products						
i. Flavoured milks	7.6	1.50	3.40	7.40	–	1.00
ii. Others	–	–	4.90	–	–	–
D. Cream (butterfat equivalent)	–	2.30	1.83	2.90	2.01	n.a.*
E. Butter and butterfat products						
i. Butterfat equivalent	2.7	5.30	5.70	4.30	5.90	3.10
ii. Butter (product weight)	3.3	5.30	6.80	4.10	5.30	3.50
F. Cheese						
i. Speisequark, cottage, fresh	0.8	2.80	8.00	0.90	2.30	8.30
ii. Others (including processed)	8.7	13.60	10.50	14.50	12.00	
G. Condensed and evaporated milk	6.1	0.60	5.20	–	–	2.60
H. Whole milk powder	0.8	1.90	1.70	n.a.	0.10	0.30
I. Skim milk powder	2.4	3.40	0.40	n.a.	2.30	1.30
J. Ice cream	11.4	–	2.90	4.90	6.80	n.a.

*n.a. = not available

Table 2. cont./

Section	India	Japan	Netherlands	New Zealand	USA	South Africa
A. Milk						
i. Whole (>0.2% fat)	51.10	39.50	23.2	74.80	35.30	36.56
ii. With 0.3 - 2.0% fat	0.13	2.30	49.4	22.80	44.00	1.13
iii. Up to 0.3% fat	–	0.30	2.50	3.30	12.10	
iv. Buttermilk	0.062	–	9.00	–	1.40	
B. Fermented milks						
i. Yoghurt	16.14	4.80	20.7	–	2.10	1.80
ii. Others	–	3.70	n.a.	–	n.a.	1.75
C. Other liquid milk products						
i. Flavoured milks	n.a.	7.20	4.00	–	4.30	4.98
ii. Others	–	–	13.60	–	–	
D. Cream (butterfat equivalent)	–	0.16	0.70	1.32	0.58	0.25
E. Butter and butterfat products						
i. Butterfat equivalent	0.132	0.60	2.70	7.70	1.60	0.38
ii. Butter (product weight)	0.065	0.70	3.30	9.30	2.10	0.45
F. Cheese						
i. Speisequark, cottage, fresh		1.40	1.70	}8.1	1.30	0.08
ii. Others (including processed)	0.0034		14.1		11.90	1.48
G. Condensed and evaporated milk	0.0056	0.50	7.40	–	1.40	0.55
H. Whole milk powder	0.028	0.20	0.70	0.17	0.20	0.43
I. Skim milk powder	0.075	1.70	0.70	0.87	1.10	0.29
J. Ice cream	0.082	1.60	n.a.	–	7.30	0.60

*n.a. = not available

African countries the dairy industry will deteriorate. A further drastic liberalisation of imports can easily destroy this industry. A down-scaling or destruction of the local industry would be advantageous to the highly developed industries of the Western world both short and medium term. Strong development of the South African industry is necessary for the growth of industries in the whole of Southern Africa. This will, in the long term, also benefit the industries of the developed countries as infrastructure is created (Hermann, 1997).

South Africa is a mixture of several "worlds" (Hermann, 1997). This also applies to the dairy industry and market. On the production side, a large part of the milk is produced by a number of big farmers utilising intensive, zero grazing, production systems, whilst there are also many small producers supplying as little as 100 l or less per day. Similarly, the retail market consists of a number of modern supermarkets and hypermarkets contrasting with street vendors and thousands of home shops (spaza shops) in the residential areas (Hermann, 1997). Consumption per capita varies from 150 $l.y^{-1}$ to 30 $l.y^{-1}$, thus a European consumption level on the one hand and an African consumption level on the other.

Foods, eating patterns and consumption of foods and food products in South Africa are unique (Anonymous, 1997). The nutrients can vary greatly between Southern African countries due to differing cultivars, soils, climates and agricultural practices. So do different technological practices e.g. flour (different extraction rates and fortification) and recipes for combined dishes with the same name. Changes in consumption patterns can also be expected to occur as changes in the social structure take place with regard to financial empowerment (Anonymous, 1997). Each country should therefore have an established programme to manage their own food composition data - the data being regarded as an important national resource.

The dairy industry is the fourth largest agricultural commodity in South Africa (Liebenberg, 1996). It represents about 16% of the animal production and 7% of the gross value of agriculture. It sometimes seems as if the dairy industry was the pioneer forefront in de-regulation and free market principles. The transition that South Africa has been through in recent years has had implications far broader in scope than originally anticipated (Anonymous, 1997). These recent changes in Southern Africa had a great influence on the dairy industry, especially in dairy politics, policy formulation and the overall management part of our industry. Not only

has the world market opened for South Africa but South Africa has opened for the world (Liebenberg, 1996) as new products from all over the world reach us (Anonymous, 1997). The situation can be wonderfully stimulating on the one hand but also devastating on the other, if managed wrongly (Liebenberg, 1996).

According to the primary structure of the South African dairy market, the present annual commercial milk production is estimated at 1.9 million tons of which roughly 95% is supplied to the secondary dairy industry (Liebenberg, 1996). This milk is produced by 7 300 independent milk producers, with a broad variation in size of enterprise, utilising a total national dairy herd of 1.3 million animals. At present South Africa has a comprehensive secondary dairy infrastructure to process milk produced into a wide range of milk products. Similar to other countries in the world South Africa also has a range of small to large dairy enterprises in the secondary dairy industry. It therefore seems as if South Africa has some good things going to participate in the international market. The market for dairy farmers, particularly the smaller enterprises, is virtually untapped and this leaves room for the industry to increase the value of their milk. Maas, an indigenous South African fermented milk, is one of the best and easiest of the products to produce according to Byford-Jones (1995). The equipment is relatively inexpensive and the market is not yet satisfied. A cooling tank, a pasteuriser (or other heating facility), a packaging machine and starter cultures are all that is required. Cottage cheese can also easily be produced from Maas, but the market is somewhat limited (Byford-Jones, 1995).

Nutritional and health aspects

Cultured or fermented dairy products have been consumed for several thousand years and the belief that they are beneficial to health is equally ancient (Buttriss, 1997). Only in recent years has scientific support for these beliefs been published (Buttriss, 1997).

Fermented dairy products are a palatable and economical source of a wide range of nutrients (Gurr, 1987) and, as shown in Table 3, are rich in proteins, minerals and vitamins B, C, E, F and G (Buttriss, 1997). With added fat, the vitamin A and D concentrations can also be increased (Burke, 1938a). The main features of the possible effects of cultured dairy products in the area of the physiology of

Table 3. Nutrient content of cultured dairy products per 100 g portion, according to Shahani & Chandan (1979).

Nutrients	Whole milk	Plain Yoghurt	Skim milk	Buttermilk	Cream	Sour cream
Calories	65	63	36	40	211	211
Protein (g)	3.5	3.0	3.6	3.31	3.0	3.0
Fat (g)	3.5	1.6	0.1	0.9	20.6	20.4
Carbohydrate (g)	4.9	7.0	5.1	4.8	4.3	4.3
Ca (mg)	118	183	121	116	102	102
Fe (mg)	tr.	0.08	tr.	0.05	tr.	0.04
Mg (mg)	13	17	14	11	0.9	11
P (mg)	93	144	95	89	80	77
K (mg)	144	234	145	151	122	56
Zn (mg)	0.38	0.89	0.40	0.42	0.27	0.27
Na (mg)	50	70	52	105	43	40
Vitamin C (mg)	1.0	0.80	1.0	0.98	1.0	1.0
Thiamin (mg)	0.03	0.04	0.04	0.03	0.03	30
Riboflavin (mg)	0.17	0.21	0.18	0.15	0.15	150
Niacin (mg)	0.1	0.11	0.1	0.06	0.1	0.71
Panthothenic acid (mg)	0.3	0.59	0.37	0.28	0.26-0.34	0.32-0.36
Vitamin B_6 (mg)	40	0.05	42	0.03	35	16
Folacin (mcg)	6.0	11	1.2	-	2.0	11
Vitamin B_{12} (mcg)	0.4	0.56	0.4	0.2	0.35	0.3
Vitamin A (IU)	140	66	tr.	33	840	839

tr. = trace

nutrition are summarised in Table 4 (Blanc, 1984).

The value of cultured milks in the treatment of certain diseases has been recognised and in cases of typhoid fever and many gastric disorders, cultured milks have been used with excellent success (Burke, 1938a). Infants suffering from intestinal troubles have often made rapid recovery when given a diet of modified buttermilk. Since skim milk is often the principle product from which cultured milks are prepared, fat is the only absent constituent. However, cream is frequently added and in many instances, whole milk or part skim milk is used, thus adding an increased food value and making the product far more wholesome and delicious than even the much acclaimed farm or home-churned buttermilk.

Nutritional attributes

Nutritionally, plain yoghurt has a similar composition to that of the milk from which it is made and thus is an excellent source of high quality protein, calcium, phosphorus, magnesium, zinc and the B-vitamins riboflavin, B_{12} and niacin (Buttriss, 1997). There are a few subtle differences, particularly with respect to vitamin content of fermented milk products and the milk from which they are made. Inspite of this, the nutritional properties of fermented milk products and the milk remain fairly similar regardless of the method of production. However, the composition can be modified by changes brought about by bacteria in the starter culture during the fermentation process (Buttriss, 1997). Fermented milk products owe their nutritional qualities primarily to milk, but also to the effects of processing, including the heat treatment of milk, product formulation and the fermentation process itself (Saloff-Coste, 1996). The changes that occur augment the nutritional and organoleptic characteristics (Table 5) and result in a product high in calcium, protein, riboflavin, phosphorus, folic acid and vitamin B_{12}. Historically, fermentation processes involved unpredictable and slow souring of milk, caused by microorganisms inherent in the milk (Shahani & Chandan, 1979). Modern technologies now involve specific fermentation under exacting conditions of pH, temperature and water content to produce fermented products of superior nutritional, physical, chemical and sensory qualities.

Lactic fermentations of cultured dairy products by the lactic acid bacteria (LAB) enhance the nutritional qualities, improve digestibility of milk constituents

Table 4. Main physiological advantages and disadvantages of cultured dairy products (Blanc, 1984; Alm, 1991).

Advantages	Disadvantages
• Increase proteolysis, digestion and re-absorption of proteins	• Gastric pH favourable to protein digestion
• Fine flocculation of casein increase	• Allergic reactions to proteins
• Increase lipolysis and release of (volatile) fatty acids	• Cholesterolemia
• Increase digestibility and re-absorption of fats (cholesterol reduction)	• Formation of toxic biogenic amines
• Higher lactic acid concentrations	• Lower lactose levels
• Increase digestive secretions: saliva; bile; gastric and pancreatic juices	• Presence of amines in the product are reduced
• Speed of gastric evacuation increases	
• Improves P, Ca and Fe retention (compared to fresh milk)	
• Stability and preservation of vitamins B during storage and heat treatments	
• Increase resistance to tumors (anti-mutagenic effect)	
• Increase preservation time of product	
• Organoleptic properties: flavour	
• Relief of constipation	

Table 5. Effects of processing on the nutritional value of fermented milk products (Saloff-Coste, 1996).

Nutrients	Principal differences	Consequences
Protein	Milk fortification with non-fat milk powder (0 - 5%). Denaturation of whey protein by heat treatment. Partial hydrolysis. Milk protein precipitation and "gel" formation.	Increases the protein content. Affects stability of the yoghurt gel. Increases the amount of peptides and free amino acids (5.9 mg.100 g^{-1} in cow's milk vs. 23.6 mg.100 g^{-1} yoghurt). Slows gastric emptying rate and may improve protein digestion. Slows absorption because of the homogenization of casein and lactoserum.
Carbohydrate	Milk fortification with non- fat milk powder and hydrolysis of 20 - 30% of lactose into glucose and galactose. Production of polysaccharides.	A decrease of 15% in the final lactose content vs. milk, which give better tolerance of yoghurt in lactose maldigesters vs. milk. Contributes to a viscous, smooth consistency.
Lipid	Partial hydrolysis leading to the production of volatile fatty acids (acetic, butyric, formic, propionic acids)	Contributes to yoghurt's aroma.

Table 5. cont./

Nutrients	Principal differences	Consequences
Minerals	Milk fortification with non-fat milk powder (0 - 5%).	Fortification increases the mineral content vs. milk (up to 180 mg of milk 100 g^{-1} yoghurt). Results in excellent bio-availability due to the presence of lactose and lactic acid (increase the mineral solubilized form).
Vitamins	Bacterial production of folic acid. Reduction of sensitive vitamin content (B_1, B_6, B_{12}) by heat treatment of milk. Bacterial consumption of vitamin B_{12}.	Increase the folic acid content (up to 10 times that of milk). Most vitamin levels are similar to those of milk (strain dependent).
Lactic acid	Production of L(+) and D(-) lactic acid from lactose to a level of approximately 1%. Ratio depends on the bacterial species used and incubation conditions.	Decrease the pH value (4 - 4.5 in yoghurt vs. 6.6 - 6.8 in milk), leading to a better preservation of the product and to a slightly tart, refreshing taste.
Other trace components	Multiplication of LAB.	Leads to 1% by weight of bacterial cell components. Composition includes protein, nucleic acids, organic acids and enzymes (e.g. β-galactosidase).
	Production of flavour components (acetaldehyde, acetoin, diacetyl).	Comprises the main components of yoghurt's flavour.

(lactose, protein and fat) and synthesize certain B-vitamins (Shahani & Chandan, 1979). During the fermentation of lactose to lactic acid, the acidity of the milk rises and the growth conditions for microorganisms other than LAB become increasingly unfavourable (McGregor, 1991). In addition, the lactic cultures produce metabolites such as bacteriocins, anti-carcinogenic and anti-cholesteremic compounds and enzymes with the result that the cultured products may possess not only enhanced nutritional characteristics but therapeutic values as well (Shahani & Chandan, 1979). For example, the nutritive value will be modified as a result of synthesis or release of nutrients or other substances by the starter bacteria (Deeth & Tamime, 1981). Furthermore, the addition of other ingredients during manufacture like skimmed milk powder (Buttriss, 1997), fruit or fruit juice, caseinates (Deeth & Tamime, 1981) and ultrafiltered concentrates, will also enhance the nutritional characteristics of the dairy product. Many of the additives above will modify the nutritive value by increasing the concentration of proteins, sugars and polysaccharides (Deeth & Tamime, 1981). On the other hand, the nutritive value of a food depends not only on its nutrient composition but on the bio-availability of those nutrients, that can be absorbed and utilised by the body (Gurr, 1987).

Energy

The chief sources of energy in milk are fat and lactose (Gurr, 1987) with a proximate energy value of 311 kJ.100 g^{-1} edible portion. The energy value of yoghurt is very similar to that of milk from which it is made. When the non-fat solids are increased in the basic yoghurt mix then, on a weight for weight basis, yoghurt may provide the consumer with a higher intake of protein, carbohydrate, calcium and certain B-group vitamins. According to Gurr (1987), it has frequently been claimed that the fat is more digestible in cultured products than in milk because a certain degree of 'pre-digestion' has taken place. The reason for this is that starter bacteria have only a limited ability to hydrolyse fat (Stadhouders & Veringa, 1973). The presence of free fatty acids in yoghurt should not, theoretically, aid digestion and absorption of fat and could in fact lead to products of poor organoleptic properties (Tuckey & Stadhouders, 1968).

Lactose

During fermentation 20 - 30% of milk's lactose is hydrolysed to glucose and galactose by the starter bacteria (Bourlioux & Pochart, 1988). Consequently the lactose levels in the fermented product are lower than in milk although this is not always the case as skimmed milk powder or non-fat milk solids are sometimes added during manufacture. This decrease is very important with regard to the problems of lactose tolerance (Alm, 1991). The intestinal microorganisms metabolise lactose forming lactic acid and other volatile and non-volatile compounds. In small amounts lactose is beneficial to the human, but in larger amounts it will cause irritation of the intestinal mucosa leading to abdominal cramps and diarrhoea (Alm, 1991). Lactose may be toxic and damage the intestinal mucosa in lactose-intolerant children giving rise to symptoms similar to those of coeliac disease (Alm, 1991).

Lactic acid, a by-product of the fermentation process, may act as a preservative by reducing the pH and contributing a mild sour and refreshing taste (Gurr, 1987). It has also been suggested that it influences the physical properties of the casein curd so as to promote digestibility and hence improve utilization of calcium and other minerals inhibiting the growth of potentially harmful bacteria in the gut (Gurr, 1987; Alm, 1991).

Vitamin content

Milk as well as fermented milk products are superior sources of vitamins and are essential co-factors in various other metabolic processes (Fernandes *et al.*, 1992). The vitamin content of fermented products depend on the type of milk used (particularly the fat content of the milk, which influences the amount of vitamin A and other fat soluble vitamins present), the bacterial strain, the fermentation conditions (Buttriss, 1997), geographical location (Gregory, 1967), stage of lactation and the cow breed used. In addition, whenever milk is processed there is likely to be an effect on the concentration of the more water-soluble vitamins concentrations of which are influenced by heat and/or light. Vitamin C is heat and light sensitive, but it is more stable in the acid conditions of fermented milk than in normal milk (IDF, 1983).

While variations in the B-vitamin content of milk arise from seasonal changes

and stages of lactation differences in the B-vitamin content of milk products have also been attributed to variation in vitamin content of the milk as well as differences in processing procedures (Shahani & Chandan, 1979). The B-vitamin content of cultured milk products is further influenced by the concentration and type of microbial inoculum and subsequent incubation conditions in the product manufactured. Observations that culture medium, temperature and other factors influence growth and end-products are based mainly on the ability of LAB to use and produce B-vitamins (Collins, 1977). Shahani & Chandan (1979) reported that cultured products definitely contain higher folic acid, niacin, biotin, pantothenic acid B_6 and B_{12}, than fresh milk.

The pasteurisation of milk before fermentation may destroy some vitamins such as B_6, B_{12} and folic acid while the level of thermostable vitamins (niacin and pantothenic acid) remains unchanged (Symons, 1993). This reduction in levels of some B-vitamins is due to the growth requirements of some LAB during fermentation (Bourlioux & Pochart, 1988; Gurr, 1987). Losses of up to 90% of vitamin B_{12} have been reported (Bourlioux & Pochart, 1988; Gurr, 1987) with specific bacterial strains, but losses can be reduced considerably by the use of a 'supplementary culture' capable of synthesizing significant amounts of vitamin B_{12}. In Table 6, the results of fermentations using LAB strains, that produce a net increase in B vitamins, notably folates, during fermentation are summarized (Symons, 1993; IDF, 1983). In general, *Lactobacillus bulgaricus* uses folic acid whereas *Streptococcus salivarius* subsp. *thermophilus* produces it (Kneifel *et al.*, 1992). After fermentation, the levels of some vitamins, especially B_{12} and folic acid, decrease during cold storage (Symons, 1993)

Minerals

Fermented milk products are excellent sources of dietary minerals, particularly calcium (Ca), phosphor (P), magnesium (Mg) and zinc (Zn) (Gurr, 1987; Buttriss, 1997). It has been reported by Rusoff (1987) that lactose improves the absorption of calcium and other minerals (Schaafma, 1983) and it is important to ask whether the decrease in lactose concentration that occurs during fermentation can be correlated to a lower mineral bioavailability (Gurr, 1987). Most experiments performed by Smith *et al.* (1985), Recker *et al.* (1988) and Vonk *et al.* (1988) did not confirm improved calcium absorption because of the more acid pH in the intestine following

Table 6. Variation of vitamin content during yoghurt fermentation in three different experiments (Symons, 1993).

Vitamin	Experiment 1 (%)	Experiment 2 (%)	Experiment 3 (%)
B_1	nd	0	-10
B_2	nd	0	-6
B_6	nd	0	-10
Pantothenic acid	-21	-30	-12
Biotin	0	-5	+60
Folic acid	+800 to 900	+120	+240
B_{12}	-17	-20	-23
Niacin	+7 to 18	0	0

nd = not determined; (-) = net loss; (+) = net increase

consumption of fermented milk products (Renner, 1991). For instance, Smith *et al.* (1985) compared the Ca absorption from milk and yoghurt in seven lactase-deficient subjects and five lactose-tolerant controls. Although there was no evidence to indicate that Ca in yoghurt is better absorbed than Ca in milk, yoghurt remains an excellent source of Ca because this fermented product is well tolerated by lactase-deficient subjects. In experiments performed by Recker *et al.* (1988) 10 healthy post-menopausal women received various test meals containing 250 mg of Ca. The results showed no significant differences in Ca absorbability with mean absorption values of 26.7 and 25.4%, respectively.

Vonk *et al.* (1988) reported that rats fed with yoghurt showed a significantly lower Fe absorption and lower blood values for haemoglobin and heaematocrit than those fed with milk. In yoghurt-fed rats, the intestinal transit time was strikingly less than in milk-fed rats. In general it can be concluded that fermented milk products do not have a superior mineral bioavailability (Fernandes & Shahani, 1989). From a nutritional point of view this difference is not very important and fermented milk products remain an excellent source of minerals (Fernandes *et al.*, 1992).

Partial hydrolysis of the milk constituents in cultured products

The nutritional value of a fermented food is dependant upon the availability and digestibility of the nutritive constituents and the changes in these constituents brought about by microbial growth and fermentation processes (Khedkar & Khedkar, 1993). In general the energy value of a fermented dairy product is similar to that of the milk from which it is prepared. It is, however, claimed that the fermented product is more nutritious because some ingredients are partly pre-digested (Khedkar & Khedkar, 1993).

Lactic culture enzymes partially pre-digest the proteins, lipids and lactose in milk (Shahani & Chandan, 1979). The preliminary hydrolysis of the lactose can be brought about by the commercial enzymatic preparation of β-galactosidase, prepared from *Aspergillus niger* or *A. oryzae*, *Klúveromyces lactis* or *K. fragili* and *Esherichia coli* (Blanc, 1984). An increase and faster production of lactic acid takes place when milk with partially pre-hydrolysed lactose is used. The decrease of the lactose level in fermented milks, although only partial, allows people regarded as

"intolerant to lactose" to consume it.

The lipolytic and proteolytic activities of various lactic cultures were also studied by Chandan *et al.* (1969a and 1969b). These studies demonstrated the relative importance of lactobacilli in protein hydrolysis and of leuconostocs and streptococci / lactococci in fat hydrolysis in cultured dairy products (Shahani & Chandan, 1979). The hydrolytic and protein aggregation effects may contribute to the physical and nutritional properties of cultured dairy products (Chandan *et al.*, 1969a). The specific lipase activity (μmoles fatty acids.mg^{-1} DNA) of various cultures ranged from 0.7 to 32.8. *Leuconostoc dextranicum* appeared to be the most lipolytic and *Lactobacillus acidophilus* to be the least active culture (Chandan *et al.*, 1969a). The addition of 1% cream or 0.2% casein to the growth medium resulted in a slight increase in the cellular lipase of *Lactococcus lactis subsp. cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis*, *L. lactis, Streptococcus durans, Leuconostoc citrovorum* and *Propionibacterium shermannii.* Lipase activity of *L. lactis* cells were unaffected by the addition of olive oil or milk fat emulsion to the growth medium.

According to Chandan *et al.* (1969b) cells of *Leuconostoc dextranicum* harvested from Glucose-Yeastral-Lemco broth showed more specific activity than any other organism tested,. Upon the addition of 1% cream or 0.2% casein to the medium, a significant stimulation in protease activity was observed in the case of *Lactococcus lactis*, *L. lactis* subsp. *lactis biovar diacetylactis* and *Streptococcus durans.*

Health properties

In addition to their high nutritional properties, cultured dairy products have been reported to possess considerable therapeutic (Shahani & Chandan, 1979) and prophylactic value (Fernandes *et al.*, 1992). Although definitive information on therapeutic values of fermented products is not yet available, numerous investigators have reported inhibition of pathogenic and spoilage bacteria by fermented products and the lactic cultures used for the fermentation process (Babel, 1976).

Production of antimicrobial agents

The interest in using bacteriocins from LAB to assure the safety and extend

the shelf-life of refrigerated foods has increased dramatically over the last decade (Montville *et al.*, 1995). The LAB, comprising the genera *Lactobacillus*, *Lactococcus (Streptococcus)*, *Leuconostoc* and *Pediococcus*, are not only involved in the preservation of certain foods but are responsible for the unique identity and sensory attributes unattainable by other food processing methods (Daeschel, 1989). Reduction of pH due to the acid production and removal of large amounts of carbohydrate by fermentation are the primary preserving actions provided by these bacteria. However, it has also been recognised that LAB is capable of producing inhibitory substances other than organic acids (lactate and acetate).

The antibacterial principles enhanced by lactic cultures apparently contributed to the increase of shelf-life and inhibition of foodborne pathogens in fermented milks (Shahani & Chandan, 1979). Natural bacteriocins synthesized by LAB have been identified (Table 7) and may be of primary importance in inhibiting growth of enteric pathogens *in vivo*. Evidence has been mounting on the inhibition of spoilage organisms like *Staphylococcus aureus*, *Pseudomonas putrifaciens*, *Escherichia coli*, *Clostridium perfringens*, *Salmonella tennessee* as well as pathogens by *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *lactis biovar diacetylactis*, *Leuconostoc cremoris* and the lactobacilli by lactic cultures (Shahani & Chandan, 1979). Bacteriocin-producing LAB is used as starter cultures whether intentionally or unintentionally (Montville *et al.*, 1995). It thus seems logical that if LAB is used in a fermentation that the use of naturally isolated bacteriocin-producers would add an extra degree of protection without posing any special problems. The use of cultures (the organisms, their substrate and their products) as preservatives have precedence in Microgard™ and Nisaplin™ (Montville *et al.*, 1995)

The mechanism of the antibiosis associated with lactic cultures is attributed to LAB metabolic by-products, e.g., hydrogen peroxide (Fig. 1), organic acids (acetic, glyoxylic, malonic, α-ketoglutaric acids) and other compounds (Sikes & Hilton, 1987). On the other hand we get nisin and diplococcin which are two chemically defined antibiotics produced by selected strains of *Lactococcus*. When the effects of an inhibitory methanol-acetone extract of *Streptococcus salivarius* subsp. *thermophilus* fermented milk was tested on growth of several spoilage and pathogenic bacteria, it was found that *Clostridium perfringens* (type C) was the most sensitive and

Table 7. Natural antimicrobial substances produced by lactic acid bacteria.

Species	Compound	Reference
Lactobacillus acidophilus	Acidolin	Fernandes *et al.*, 1992; Marshall, 1987; Hamdan & Mikolajcik, 1974.
	Acidophilin	Fernandes *et al.*, 1992.
	Lactocidin	Fernandes *et al.*, 1992; Marshall, 1987; Vincent *et al.*, 1959.
	Lactacin F	Muriana & Klaenhammer, 1991.
L. brevis	Lactobacillin	Fernandes *et al.*, 1992.
Pediococcus acidilactici H	Pediocin AcH	Fernandes *et al.*, 1992.
P. acidilactici	Pediocin PA-1	Marugg *et al.*, 1991.
Pediococcus pentosaceus FBB61	Bacteriocin	Fernandes *et al.*, 1992, Fernandes *et al.*, 1987.
L. lactis subsp. *cremoris*	Diplococcin	Marshall, 1987.
L. fermenti 466	Bacteriocin	Fernandes *et al.*, 1992.
L. helveticus LP27	Lactacin 27	Fernandes *et al.*, 1992.
L. helveticus	Helveticin J	Joerger & Klaenhammer, 1990.
Lactococcus lactis	Nisin A, Nisin Z	Rauch & De Vos, 1992.
	Lactococcin A	Holo *et al.*, 1991; Van Belkum *et al.*, 1991.

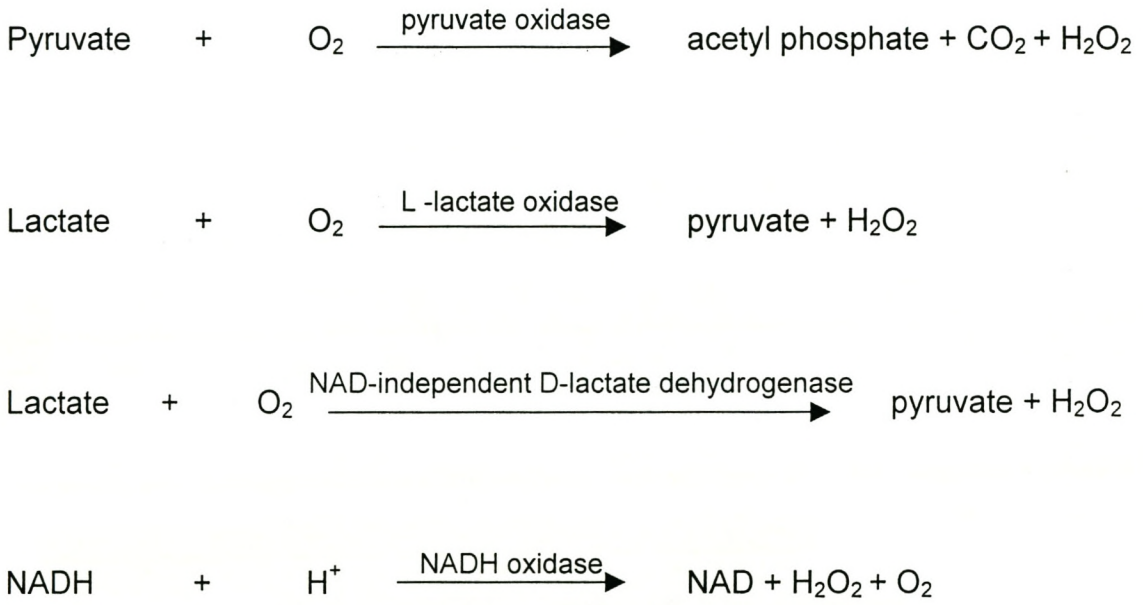

Figure 1. Mechanisms of hydrogen peroxide generation by LAB (Kandler, 1983).

Salmonella enterides was least sensitive (Sikes & Hilton, 1987). Several possible mechanisms have been suggested to explain the observed protective effect obtained by colonization with lactobacilli and streptococci (Reddy *et al.*, 1988) and include: lowering of intestinal pH; adhesion to the intestinal wall preventing colonization by pathogens; competition for nutrients; production of antibacterial substances; and neutralisation of toxins (anti-enterotoxins).

Anti-carcinogenic and anti-mutagenic effect of cultured products

Several studies have revealed that lactic cultures and fermented products possess anti-carcinogenic activity (Table 8) (Farmer *et al.*, 1975; Friend *et al.*, 1982; Kato *et al.*, 1983). According to Table 8, the growth of tumors in mice were reduced and inhibited through a 1 – 2 week yoghurt-feeding scheme (Ayebo *et al.*, 1982). However, long-term feeding of yoghurt to rats (Rao *et al.*, 1984) and mice (Reddy *et al.*, 1983) did not show any significant difference in survival rates following tumor implantation.

A positive correlation between dietary factors such as meat, total fat or animal fat consumption and the incidence of large bowel cancer has been shown to exist (Renner, 1991). Epidemiological studies (Alm, 1991), have shown that the incidence of colon cancer is higher in populations consuming 'western' diets than in those consuming vegetarian diets. In a study involving populations in Copenhagen (high risk), Kuopio and Finland (low risk) a four-fold variation in the incidence of colon cancer was found. Higher intakes of dietary fibre and milk in the 'low incidence' area indicated a protective effect that was unrelated to transit times. Finland, being a nation with a high per capita fat consumption and a relatively low incidence of colon cancer, is therefore an exception. Dairy products, especially yoghurt, are a common component of the Finnish diet, which could explain the high numbers of lactobacilli found in the intestinal tract of the Finns (Goldin & Gorbach, 1984). In a study conducted in the Netherlands, a lower consumption of fermented milk products (yoghurt, buttermilk, and curds) among 133 incident breast cancer cases were found as compared to 238 population controls (Van't Veer *et al.*, 1989). This suggests that the decrease in breast cancer cases found for the population controls could be attributed to the higher or increased consumption of fermented milk products (Van't Veer *et al.*, 1989).

Table 8. Effect of feeding lactic acid, fresh milk, fermented milks, fresh colostrum and fermented colostrum on proliferation of Ehrlich ascites tumor cells (Friend & Shahani, 1984).

Material fed	Tumor cells ($\times 10^6$ mouse^{-1})		Inhibition
	Control	Test	
Lactic acid	28.9	32.3	-
Fresh milk	28.9	32.3	-
Yoghurt from milk	29.5	21.4	27.4
Lactobacillus acidophilus milk	29.2	19.8	32.1
L. delbrueckii subsp. *bulgaricus*	26.4	18.3	30.7
Fresh colostrum	27.2	27.4	-
Yoghurt from colostrum	32.4	23.6	27.2
L. acidophilus colostrum	25.2	18.8	25.3
L. delbrueckii subsp. *bulgaricus* colostrum	26.4	17.3	34.5

The anti-tumor properties of cultured products may be due to inactivation or inhibition of carcinogenic compounds in the gastrointestinal tract (Keating, 1985), improved intestinal motility (Alm, 1991), stimulation of the immune system of the host (Friend & Shahani, 1984) and reduction of the activity of the feacal bacterial enzymes (β-glucuronidase, azoreductase, nitroreductase). The latter activates carcinogens by converting pro-carcinogens into proximal carcinogens (Shackelford *et al.*, 1983).

Hosono *et al.* (1986a and 1986b) reported an anti-mutagenic or dismutagenic effect of cultured dairy products on chemical and feacal mutagens. The anti-mutagenic properties of milk cultured with either *Lactobacillus mesenteroides* subsp. *bulgaricus* or *Streptococcus salivarius* subsp. *thermophilus* were examined using streptomycin-dependent strains of *Salmonella* in an *in vitro* assay system. Two chemical mutagens and feacal mutagen extracts from cats, monkeys, dogs and other mammals were used for testing. Both types of cultured milk exhibited anti-mutagenic activity on all mutagens used. The anti-mutagenic activities of the cultured milks, with the chemical mutagens, increased with incubation time but were destroyed at 55°C for 10 minutes (Hosono *et al.*, 1986b).

Dismutagenic properties of milk cultures with strains of *Lactobacillus mesenteroides* subsp. *bulgaricus*, *Lactococcus lactis* subsp. *lactis* and *Streptococcus faecalis* were examined *in vitro* against two different mutagenic components (Hosono *et al.*, 1986a). Each cultured milk was found to display its dismutagenic effect on the mutagenicity of 4NQO (4-nitroquinolineoxide). Milk cultured with the *S. faecalis* strain showed a significant dismutagenic effect on the mutagenicity of the water extract of dog faeces. Since lactic acid did not exhibit a dismutagenic effect against 4NQO, dismutagenic factors were suggested to be metabolites other than lactic acid or cellular fractions from LAB (Hosono *et al.*, 1986b).

Farmer *et al.* (1975) and Shahani *et al.* (1974) investigated the inhibitory effect of yoghurt on Ehrlich ascites tumor-cell proliferation in the peritoneal cavity of Swiss mice. The lactose and lactic acid in the yoghurt did not show any inhibitory effect, but a direct relationship between the amount of yoghurt consumed and tumor inhibition did exist. The *Lactobacillus mesenteroides* subsp. *bulgaricus* appeared to

be more inhibitory than *Streptococcus salivarius* subsp. *thermophilus* and culture cells killed by heat lost their inhibition activity.

Anti-cholesteremic effect of cultured products

Cholesterol is found in every cell membrane of all animals and plays an important role in normal metabolic processes of humans (Fernandes *et al.*, 1987). Cholesterol is synthesized in accordance to the body's requirements. As recently as 1974, Mann & Spoerry observed that the Masai tribesmen of Africa, in spite of consuming large quantities of saturated fat and cholesterol through fermented milk and meat, had low cholesterol levels in their blood. These studies led to the elucidation of evidence that there existed an "anti-cholesteremic milk factor" (AMF) in fermented milk, which was responsible for the hypocholesteremic effect.

In past years milk has received some adverse publicity in terms of its effect on the consumer's blood cholesterol level (Richardson, 1978). The perception is that milk increases the blood cholesterol level leading to cleaved serum cholesterol. The risk factor in the etiology of atherosclerosis due to elevated serum cholesterol justifies the tendency of many people to avoid whole milk consumption (Richardson, 1978; Hurt, 1972). Hypocholesteremia is considered to be one of the major factors pre-disposing atherosclerotic heart disease and it has been widely accepted that the diet can play a significant role in reducing cholesterol (Fernandes *et al.*, 1987). Indeed, milk fat, when consumed as butter or cream, does exert a hypocholesteremic response in humans. Many workers in animal studies (Fernandes *et al.* 1987) have reported the hypocholesteremic effect of cultured dairy products. Richardson (1978) concluded that a hypocholesteremic effect is observed in human volunteers consuming approximately 2 l of whole milk per day over a two week period. The effect is enhanced if skim milk, fermented whole milk or fermented skim milk is fed at a level of 2 - 4 l per day.

Literature also shows that fermented milk products have an inconsistent effect on serum cholesterol (Jaspers *et al.*, 1984). The variation may be due to differences in levels of hypocholesterolaemic compounds in yoghurt and different bacterial strains used in fermentation (Jaspers *et al.*, 1984). In the yoghurt culture with *Streptococcus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, numerous strains of each species exist. Each strain exhibits different properties including those relating

to hypocholesterolaemic effects.

In a test with human subjects ten males received 681 g of yoghurt daily over three 14 - 21 day periods (Jaspers *et al.*, 1984). Three different starter strains were used for yoghurt production in these three periods. Yoghurt significantly reduced total serum cholesterol 10 - 20% on some days, but the serum cholesterol returned to the control values with continued yoghurt consumption (Jaspers *et al.*, 1984). Rao *et al.* (1981) suggests that metabolites produced during fermentation of milk may be responsible for the hypocholesterolaemic effect of cultured products. Consumption of milk fermented by *Streptococcus salivarius* subsp. *thermophilus* resulted in a significant decrease in the plasma cholesterol levels in rats. The liver cholesterol levels were lower in a group receiving thermophilus milk compared to a group receiving skim milk. The *in vitro* hepatic cholesterogenesis was inhibited by the methanol solubles of milk and thermophilus milk. It was found that the inhibition was enhanced with the methanol solubles from the thermophilus milk. After four weeks of feeding the mean levels of cholesterol in the plasma were lower in the groups fed skim milk or fermented milks than in the control groups. There were however no significant differences between the animals fed skim milk and those fed skim milk fermented by *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus mesenteroides* subsp. *bulgaricus* or *L. acidophilus* (Pulusani & Rao, 1983).

Role of fermented products in lactose intolerance

Mounting evidence indicates that milk contains in its unaltered or unfermented state lactose which is indigestible by a large majority of the world's population (Houts, 1988), as shown in Fig. 2. The reduction of lactose to glucose (Simoons, 1973; Shahani & Chandan, 1979) and galactose (Gurr, 1987) requires the presence of lactase, an enzyme produced in the small intestine. Calcium, a natural ingredient of milk and milk products, however, is required by infants, children and adolescents for proper bone growth and development. A restriction on the intake of milk and milk products would therefore seriously limit the intake of calcium (Shahani & Chandan, 1979).

It is now well established that, in tolerance-deficient subjects, yoghurt (a fermented product) is better tolerated than milk (McDonough *et al.*, 1987). The rationale for using cultured or culture-containing milk relates to the fact that lactose

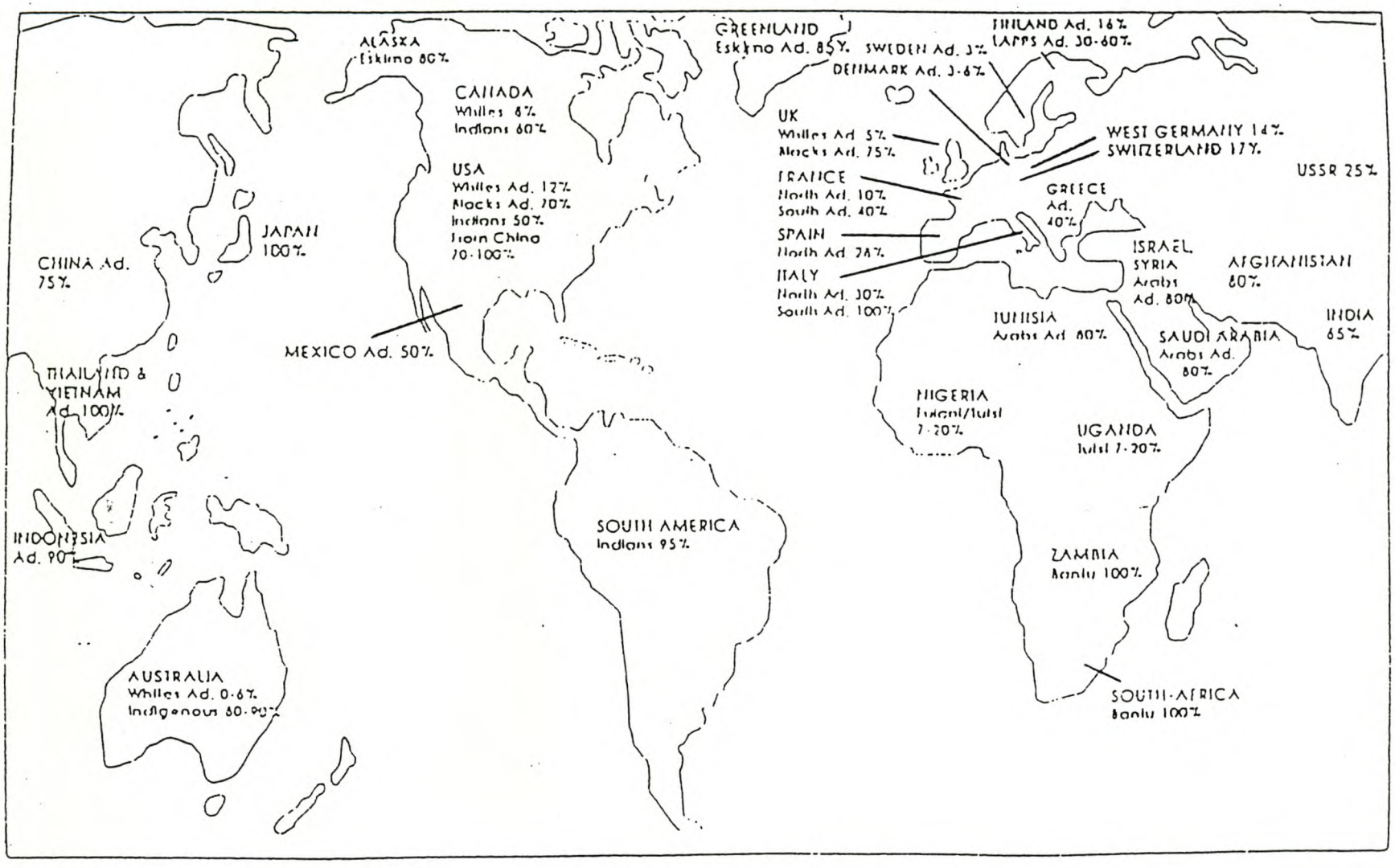

Figure 2. Distribution of lactase non-persistent individuals around the world (Fernandes *et al.*, 1992).

content is reduced during fementation and that the yoghurt organisms - by virtue of being rich in lactase - are able to hydrolyse the ingested lactose (McDonough *et al.*, 1987). However, many commercial products do not have reduced lactose content due to the addition of milk solids (McDonough *et al.*, 1987).

The results of experiments performed by Martini *et al.* (1987) provide a possible explanation for the survival of β-galactosidase activity from yoghurt in the gastrointestinal tract which is related to the buffering capacity of yoghurt and gastric pH. The high buffering capacity of yoghurt seems essential as three times more acid is required to acidify yoghurt than milk. This indicates that β-galactosidase in yoghurt is not denatured during passage through the acid environment of the stomach. Smith & Palumbo (1981) suggest on the other hand that cultured milk products are tolerated by lactose-intolerant individuals because of lactose utilisation in the gastrointestinal tract by ingested lactobacilli.

Fermented dairy products

Fermented milks

The healthiness of fermented milk products have been recognised for centuries (Burke, 1938b). Through the ages man has prepared refreshing and wholesome drinks from fermented milks. In certain foreign countries sour milk, in one form or another, forms a major portion of the diet. Sour milk has become an important ingredient in food in certain tropical lands where the importance of sanitation and cold temperatures in the preservation of milk are not understood or applied. The reason being that sour milk which is in a state of temporary preservation, may be held for several days without any detrimental effect and still be relatively pleasing to the palate (Burke, 1938b).

According to the IDF (1992), fermented dairy products are prepared from milk and/or milk products by the action of specific microorganisms, which result in a reduction of pH and coagulation. These specific microorganisms must be viable, active and abundant in the finished product at the time of sale for consumption. The milk and milk products may or may not be homogenised but must at least be pasteurised. Fermented milks may contain optional additions added to the raw

material with the starter either before (Table 9) or after fermentation. As fresh products the shelf-life is limited (typically up to 30 days) and should be kept refrigerated (4° - 7°C) throughout the distribution period (IDF, 1992).

Milk (cow, buffalo, sheep, goat, horse, camel, yak and zebu) has been used to make traditional fermented milk products throughout the world (Kroger *et al.*, 1989). Each variety of fermented milk product has its own characteristic texture, flavour and composition which depends on the kind of milk used, the type of starter culture (lactic acid bacteria with or without aroma producers and yeasts) and the preparations followed during production procedures (Khedkar & Khedkar, 1993). Fermented milk products, from a biological standpoint, are characterised by the accumulation of microbial metabolic products (Kroger *et al.*, 1989). It was realised very early that such microbial metabolites like lactic acid, ethyl alcohol and dozens of other chemicals, collectively called flavour substances, were not altogether unpleasant and even contributed to the overall preservative action (Kroger *et al.*, 1989).

Classification of fermented milks

Despite the long historic record and world-wide distribution of fermented milks only a few people know more than five or 10 of the several hundred specific products that can be described (Kroger *et al.*, 1989). The following discussion of classification will include: traditional and non-traditional; medium and procedure; further processing; end use; and microbial action.

Traditional and Non-traditional - This is the most fundamental division of fermented milk products. Traditionally fermented milk products have a long history and are known to be made all over the world wherever milk animals were kept (Kroger *et al.*, 1989). For many years their production was a crude art until Pasteur revealed that microbiological activity was responsible for all fermentations. In contrast, non-traditional fermented milk products are those recently developed. They are based on known scientific principles, their microbial cultures are known and their quality can be optimised. This is not the case with traditional products made with an ill-defined empirical culture where you have to take what you get out of the fermentation. Yoghurt is both a traditional and non-traditional product, the latter being represented by ever-changing varieties.

Table 9. Description of essential composition of fermented milk products (IDF, 1992).

Ingredients	Description
Raw material	Any one or combinations of the following: - fresh or reconstituted whole milk; - partially or fully skimmed milk; - buttermilk powder; - concentrated or powdered milk; - cream; - milk proteins (whey proteins, whey protein concentrates, soluble milk proteins, edible casein and caseinates); - butter; and - milkfat. The total solids content may be increased by evaporation or membrane filtration.
Starters	For the fermentation, specific, non-pathogenic, non-toxic microorganisms.
Optional additions	
Sugars	Any carbohydrate sweeteners, other than polyols.
Flavouring	Fruits and vegetables (fresh, frozen, heat-treated, dried) fruit and vegetable juice puree or pulp (concentrated or not) fruit and vegetables preserves nuts coffee spices and other harmless natural flavouring compounds.

Table 9. cont./

Ingredients	Description
Flavours	Natural flavours, nature identical or artificial flavouring substances reported in the Codex Alimentarius (Volume XIV) in the "List of additives for their safety-in-use in foods".
Inorganic salts	NaCl
Food colours[1]	Caramel, carminic acid, others obtained from natural fruit and vegetable sources
Stabilizers[1]	Agar-agar Carrageenan Guar gum Cellulose gum Na, K or Ca alginate Xanthan gum Modified starches Pectins, Gelatin, Starches*
Preservatives[1]	Sorbic acid and Na, K or Ca salts, SO_2, benzoic acid. Only a max. 50 $g.kg^{-1}$ are permitted in the final product, according to Codex Alimentarius Standards
Sweeteners[1]	Acesulfame K (max. 1 $g.kg^{-1}$) Aspartame (max. 1 $g.kg^{-1}$) Cyclamates (max. 500 $g.kg^{-1}$) Saccharins (max. 200 $g.kg^{-1}$)

[1]Listed additives must not exceed 30% per weight of the final product.

*Regarded by Codex Alimentarius Standards as food ingredients and not additives. However, they are included here since the are used to perform a technological "additive" role.

Medium and procedures - Classification by technology differentiates between fermented milks and fermented products not based directly on milk (Kroger *et al.*, 1989). It is obvious that products other than fresh milk can serve as the fermentation medium or substrate and include cream, whey, buttermilk and dry milk solids. It is also possible to further manipulate or change the curd recovered after coagulation. The basic steps necessary in processing are the same regardless of the type of cultured product (Campbell & Marshall, 1975b). These steps are starter culture preparation, treatment (pasteurisation and homogenisation) of milk (skimmed milk, cream or other products), inoculation and incubation (Campbell & Marshall, 1975b). Occasionally, both yeast and moulds and bacteria may be used to produce a desired fermentation (Burke, 1938c).

Further processing - Neither law nor taboo forbids experimentation with fermented milks (Kroger *et al.*, 1989). Numerous products are mixtures of milk and other foodstuffs that have been subjected to fermentation. These include fermented milk-vegetable products, fermented milk-meat extract mixtures and fermented milk-fishmeal hydrolyzate mixtures. Consequently, societies are found that have utilised specific vegetable matters or meat extracts or fishmeal hydrolyzates to enhance the nutritional status and the flavour and variety of their cuisine. Pharmaceutical preparations are unique in that they emphasise microorganisms only instead of milk nutrients or product flavour.

End uses - The earliest type of fermentation was undoubtedly that observed in the natural souring of milk which was brought about by the growth and activity of various organisms commonly present in milk (Burke, 1938c). This led to distinct forms of various fermented milks (Table 10) catering to specific tastes or uses (Britz, 1997). Fermented milk products have traditionally been consumed as beverages, as meal components or as cooking ingredients (Kroger *et al.*, 1989). Food technologists and innovators have created a multitude of new products with most of the developments in the dessert and confectionery category.

Table 10. Examples of fermented milk products (Tamime & Robinson, 1988).

Fermentation	Traditional name	Origin	Starter
Mesophylic	Cultured buttermilk	Ireland	*Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *L. lactis* subsp. *cremoris*.
	Ymer	Denmark	*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar *diacetylactis*.
	Filmyolk	Scandinavia	*Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis biovar diacetylactis*.
	Maziwa lala	Kenya	*Lactococcus* spp., *Leuconostoc* spp.
Thermophilic	Yoghurt	Most countries	*Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*
	Bulgarian buttermilk	Bulgaria	*L. delbrueckii* subsp. *bulgaricus*
	Yakult	Japan	*Lactobacillus casei* subsp. *casei*
Yeasts	Kefir and Koumiss	Russia	*L. lactis* subsp *lactis*, *Lactobacillus brevis*, *Lactobacillus kefir*, *L. acidophilus*, *Candida kefir*, *Saccharomyces cerevisiae*.
	Laban	Lebanon	*L. lactis* subsp. *lactis* biovar *diacetylactis*, *Lactobacillus delbrueckii* subsp. *bulgricus*, *S. cerevisiae*, *Kluyveromyces fragilis*
Mould	Villi	Finland	*Geotrichum candidum*

Microbial actions - Home-made fermented milk products, especially in nomadic or village environments, are still occasionally made by spontaneous fermentation with the use of an empirical culture (Kroger *et al.*, 1989). In other words, the inoculum is obtained from an earlier production of which the microbial identity is unknown. The fermented milk products popularly used in different countries may be broadly divided into three categories (Khedkar & Khedkar, 1993): moderately sour types with a pleasant aroma associated with diacetyl (cultured milk); sour and very sour types (yoghurt, dahi and acidophilus milk); and acid-cum-alcohol in addition to lactic acid (koumiss and kefir) (Khedkar & Khedkar, 1993).

Another classification of fermented milk products was proposed by Kurmann (1984) according to the organisms used for their manufacture (Roginski, 1988). A simplified version of Kurmann's classification is given below with additional information on mixed material plant-milk fermentations:

i. Thermophylic bacteria (incubation temperature 30°/35° - 40°/45°C)
   - Lactic acid fermentation without producing appreciable amounts of gas and alcohol:
     Yoghurt and traditional fermented milks: yoghurt, diluted yoghurt, eyran (Turkey), doogh (Iran),
     Dehydrated yoghurt: kashk (Iran), jub-jub (Lebanon),and Concentrated yoghurt: labneh (Lebanon and other Arab countries), dahi (India), tarho (Hungary).
   - Acid fermentation without producing appreciable amounts of gas and alcohol, using mainly human intestinal bacteria:
     Single-strain fermentation: acidophilus milk, yakult and bifidobacteria milk, and
     Mixed-strain cultures of different formulae (Roginski, 1988);

ii. Mesophylic bacteria (incubation temperature 10°/15° - 20°/30°C)
   - Lactic acid fermentation with simultaneous production of slime:
     Scandinavian fermented milks: viili (Finland) and tykmaelk (Denmark).
   - Lactic acid fermentation using butter cultures:
     Artificial buttermilks - cultured buttermilk and similar products.
   - Concentrated fermented milks:
     Traditional home-made milks, and

Commercial products, and

- Mild lactic acid and ethanol fermentation:
  Kumys (North Central Asia); laban (Lebanon),
  Kefir (Caucasus) made with kefir grains;

iii. Mixed material plant-milk fermentations:

- Products where plant material is a substrate for fermentation, and
- Products where plant material is a carrier of specific microorganisms and/or enzymes.

South African fermented milk products made from mesophylic starters

These cultures usually contain group N streptococci (now called lactococci) and leuconostocs (Keller & Jordaan, 1990). Most of the mesophillic cultures contain *Lactococcos lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *diacetylactis* that are responsible for lactic acid formation. *Leuconostoc mesenteroides* subsp. *cremoris* is in many cases added for flavour production depending on the type of fermented milk.

In South Africa the following types of mesophylic fermented milks are commercially produced (Keller & Jordaan, 1990):

i. Cultured buttermilk
   - made from cultured churned cream
   - made from cultured skimmed milk or partly skimmed milk

ii. Maas

Maas is the local name for mainly cultured whole milk or sometimes partly skimmed cultured milk. In the cultures used, leuconostocs are present to produce fermented milk with flavour and acid. Butter cultures are often used for this product. Maas is the most popular fermented milk in South Africa and is mainly consumed amongst the Black population.

iii. Creamy Maas or Extra Creamy Maas

This product is also known under different trade names like "Inkomasi", "Amasi" and others. The product is basically the same as Maas and in most instances the same cultures are used. The only difference is that cream is added to increase the total fat content from

anything between 4.0 and 6.0%. The added fat gives a full texture and rich creamy taste to the product when fully fermented. The product is also popular amongst the Black population.

Other forms of mesophylic fermented milks are also produced (Keller & Jordaan, 1990). These products are made from raw milk by Black tribes in rural areas. Raw milk is placed in a suitable container like clay pots or a calabash and left to ferment naturally. The same containers are used repeatedly and after a while the microbial population becomes stable in the biofilm that forms on the surfaces of these containers. The fermentation is a mixed fermentation of mainly heterofermentative and homofermentative lactobacilli, streptococci, leuconostoc and yeast (Keller & Jordaan, 1990). In many instances the whey is partly drained to obtain a product with a thick, nearly semi-solid consistency. A certain degree of rancidity is always present due to the lipase enzymes present in raw milk.

South African fermented milks made from thermophylic starters

The main drink produced by thermophylic starter bacteria is yoghurt (Keller & Jordaan, 1990). This drink originated in the Middle East where the summer temperature rises to 40°C or higher. The bacteria responsible for the fermentation are *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These two organisms grow in association in the milk and produce the typical yoghurt flavour and texture. This growth is considered symbiotic as the rate of acid development is greater when the two bacteria are grown together as compared to the growth of single strains (Dellaglio, 1988a). The product will have the distinctive "yoghurt" flavour if the correct ratio is obtained. With *L. delbrueckii* subsp. *bulgaricus* being dominant the yoghurt will tend to be more on the acid side because of the higher lactic acid production ability of these organisms (1.7 - 1.8% lactic acid compared to 0.6 - 0.8% of *S. salivarius* subsp. *thermophilus*). The streptococci should be dominant if a milder flavour is desired (less "yoghurt" flavour is produced) and care should be taken not to make the product atypical for yoghurt due to the lack of the flavour producing lactobacilli.

Flavoured yoghurt and especially fruit yoghurt is very popular in South Africa and more varieties are increasingly produced (Keller & Jordaan, 1990). The total solids of these products are in the region of 18 to 22%, but there is concern over the

relatively low concentration of milk solids in these products (Keller & Jordaan, 1990).

Starter cultures

A starter culture is a controlled bacterial population that is added to milk or milk products to produce acid and aroma substances that characterise cultured milk products (Campbell & Marshall, 1975b). Starter cultures used in producing particular dairy products are listed in Table 11 (Fernandes & Shahani, 1989) and some distinguishing characteristics of different starters involved in production are given in Table 12 (Cogan, 1995). Starters may be a single bacterial strain or a combination of mixed strains (McGregor, 1991). Mixed strain cultures maybe used to enhance the production of specific flavours or characteristics and must therefore be compatible and balanced. Some cultures can be antagonistic while others act in a strong symbiotic relationship.

The ability of LAB to transform food into new products and to exert an antagonistic action towards harmful microorganisms makes them suitable for both domestic and industrial production (Dellaglio, 1988a). Developments in the production of fermented milk types have shown a strong tendency to use natural or selected LAB. The reasons for employing LAB as starters in the production of fermented milks are:

i. their ability to produce lactic and acetic acid, aroma compounds and polysaccharides which give the products their specific taste, structure and keeping quality;
ii. their claimed antibiotic, anti-tumour and anti-leukaemia activities may contribute to human health;
iii. their metabolic activity increases digestibility;
iv. their growth over a wide temperature range and in many types of milk and combinations of LAB leads to the production of a wide variety of products.

For these and many other reasons LAB can be considered as the "motor centre" of all fermented milks.

Table 11. Common cultured dairy products manufactured with lactic acid bacteria (Fernandes & Shahani, 1989).

Cultured product	Starter
Yoghurt	*Streptococcus salivarius* subsp. *thermophilus*
	Lactobacillus delbrueckii subsp. *bulgaricus*
Acidophilus yoghurt	*Lactobacillus acidophilus*
	Lactobacillus delbrueckii subsp. *bulgaricus*
	Streptococcus salivarius subsp. *thermophilus*
Yakult	*Lactobacillus casei* subsp. *casei*
Buttermilk	*Lactococcus lactis* subsp. *cremoris*
	L. lactis subsp. *lactis*
	Leuconostoc mesenteroides subsp. *citrovorum*
Dahi	*Lactococcus lactis* subsp. *lactis*
	L. lactis subsp. *cremoris*
	L. lactis subsp. *diacetylactis*
	Leuconostoc ssp.

Table 12. Examples of starters used in different fermented dairy products (Cogan, 1995).

Product	Starter	Type*	Organism(s)
Lactic butter	Mesophilic	D	Cit^- and Cit^+ lactococci
		L	Cit^- lactococci and Cit^+ leuconostocs
		DL	Cit^- and Cit^+ lactococci and Cit^+ leuconostocs
Cheddar cheese	Mesophilic	O	Cit^- lactococci
		DL	Cit^- and Cit^+ lactococci and Cit^+ leuconostocs
Cultured buttermilk and Gouda cheese	Mesophilic	DL	Cit^- and Cit^+ lactococci and Cit^+ leuconostocs
Yoghurt	Thermophilic		*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*
Beaufort cheese	Thermophilic		*Lactobacillus helveticus*

*D, Mesophilic mixed-strain cultures (MMSC) with Cit^+ lactococci as flavour producers; L, MMSC with *Leuconostoc* sp. as flavour producers; DL, MMSC with Cit^+ lactococci and *Leuconostoc* sp. as flavour producers; O, MMSC with no flavour producers.

Starter structure

Raw milk, even when produced hygienically, is generally contaminated with low numbers of LAB (Cogan, 1995). Incubation at temperatures of 20° - 50°C results in the rapid growth of these contaminants and the eventual coagulation of the milk. This was an important method of producing starter cultures before the advent of controlled dairy microbiology. It was empirically possible to transfer the ability to coagulate milk from one batch of milk to another. In this way soured milks were continuously sub-cultured if they performed well. As these cultures were transferred continuously over the years, their current composition was probably quite different from that of the original coagulum. These cultures are generally referred to as mixed cultures as they contain different genera and species, from unknown origin and undefined cultures, and their exact composition is unknown.

The bacteria of cultured dairy products depend largely on geographical location and are strongly influenced by climatic conditions (Fernandes & Shahani, 1989). Amongst the cultured dairy products (Table 11) yoghurt is the most popular and generally accepted world-wide. There are two types of starter cultures, i.e. undefined (mixed or artisanal) and defined (Cogan, 1995). Each can be sub-divided into mesophylic and thermophylic with optimum growth temperatures of either 30° or 42°C, respectively. The predominant organisms in mesophylic cultures are non-citrate-utilizing (Cit^-) *Lactococcus lactis* subsp. *cremoris* and to a lesser extent *L. lactis* subsp. *lactis*. They contain a small number of citrate-utilizing (Cit^+) *L. lactis* subsp. *lactis* and *Leuconostoc* species and are called the flavour producers because of their ability to produce diacetyl, the flavour compound from citrate.

Mixed mesophylic cultures are assigned to the following types depending on the Cit^+ component (Cogan, 1995): D types with Cit^+ *Lactococcus* sp. as the only flavour producers; L types with Cit^+ *Leuconostoc* spp. as the only flavour producers; DL types with both flavour producers present; and O type cultures which lack flavour producers. Commercially available thermophylic cultures contain *Streptococcus salivarius* subsp *thermophilus* and/or *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactococcus delbrueckii* subsp. *lactis* or *Lactococcus helveticus*. Many artisanal products also contain mesophylic lactobacilli (*L. casei* and *L. plantarum*), thermophylic heterofermentative lactobacilli (*L. fermentum*) and enterococci in large numbers. These organisms are also involved in acid production and flavour

formation.

Fermentation of lactose to lactic acid is the major function of starter cultures in fermented dairy products (Cogan, 1995) and proper production of this acid is critical to the production of suitable fermented products (McGregor, 1991). Lactic acid is not only responsible for the refreshing tart flavour of cultured products but is also responsible for the destabilisation of the milk protein structure that allows milk protein coagulation, which contributes to the products' body and texture characteristics. The cultures also contribute other flavourful compounds (Table 13). While lactic acid is the major end-product of the fermentation other products like diacetyl, acetoin, 2,3-butandiol, acetate, acetaldehyde, acetic acid, ethanol, formate and CO_2 are also produced in varying concentrations (Cogan, 1995). These compounds are responsible for the unique flavour of various products and great care is therefore taken to promote their production in certain characteristic products (McGregor, 1991). The diacetyl and acetate are important in flavour perception and CO_2 is important in the texture of many fermented dairy products (Cogan, 1995).

Occasionally pure cultures may produce what is known as "malty" "caramel" and/or "burnt" flavours (Davis, 1969). This is probably due to a disturbed metabolism or fermentation process as a consequence of unsuitable environmental conditions. The usual reason for the production of off-flavours is contamination.

Starter requirements

The rapid evolution of fermentation dairy technology has led to a demand for suitable starter strains more stringent (Davidson & Hillier, 1995). A satisfactory dairy fermentation requires a stable, predictable rate of acid production. The fermented product must ultimately develop a desirable, long-lasting flavour, preferably after spending the minimum time in storage. The requirements for a starter are (Davis, 1969):

i. it should contain only the LAB of the required type or other selected aroma producing types;
ii. it must be free of any bacterial types capable of causing off-flavours or other defects. The most common contaminants are coliforms, yeast and moulds;
iii. it must be a vigorous grower and produce acid at a brisk and steady

Table 13. Starter cultures and their principal metabolic products (McGregor, 1991; Marshall, 1982).

Starter	Metabolic products
Mesophylic Bacteria	
Lactococcus lactis subsp. *cremoris*	Lactic acid
Lactococcus lactis subsp. *lactis*	Lactic acid
Lactococcus lactis subsp. *lactis diacetylactis*	Lactic acid, diacetyl, acetaldehyde, acetoin, CO_2
Leuconostoc cremoris	Lactic acid, diacetyl
Lactobacillus acidophilus	Lactic acid
Lactobacillus brevis	CO_2, acetic acid, lactic acid, ethanol, acetoin
Lactobacillus casei	Lactic acid
Thermophilic Bacteria	
Streptococcus salivarius subsp. *thermophilus*	Lactic acid, acetaldehyde
Lactobacillus delbrueckii subsp. *bulgaricus*	Lactic acid, acetaldehyde
Lactobacillus delbrueckii subsp. *lactis*	Lactic acid
Bifidobacterium	Lactic acid, acetic acid

rate;

iv. it must be capable of growing under the conditions of the particular process employed; and

v. it should not be influenced appreciably by cultivation in different types of milk.

The development of successful starter strategies to meet these requirements have involved the application of established bacteriological techniques with particular care being devoted to strain characterisation (Davidson & Hillier, 1995).

Incubation profiles

The lactic acid bacteria normally used in milk fermentations are grouped into two wide, but general categories: mesophylic organisms with optimum growth temperature of <30°C and thermophylic ones with optimal growth at >37°C.

Mesophylic starter cultures - Mesophylic starter cultures generally grow in the temperature range of 10° - 40°C with an optimum around 30°C (Pettersson, 1988). Starter cultures composed of mesophylic microorganisms are used in the production of many cheese varieties where the important characteristics are acid producing activity, gas formation and a rapid production of enzymes. These types of starters are also used for the production of ripened cream butter where diacetyl production is an important requirement. Fermented milk production with mesophylic starter cultures include the following:

i. Lactic acid fermentation with ropy consistency (villi - Finland);

ii. Lactic acid fermentation with no ropy consistency (fermented milks prepared with butter cultures);

iii. Buttermilks (conventional buttermilk made from churned sweet cultured cream and new buttermilk made from churned sweet cultured fermented cream);

iv. Lactic acid fermentation / concentrated milk (cellar milk, stored milk, Ymer); and

v. Mesophylic fermented milks with mixed lactic and other fermentations (Kefir, Kumiss).

The important characteristics of fermented milks derived from mesophylic

fermentations are consistency which is due to the lactic acid coagulation of the milk proteins and the aroma and flavour produced by citric acid and lactose fermentation (Pettersson, 1988). The most important activities for dairy fermentation of mesophylic starters, which are often coupled to growth, depend on a number of factors. The most important factors being:

i. genetic properties;
ii. milk factors (nutrients, stimulating and inhibitory compounds);
iii. bacteriophages; and
iv. other growth conditions (pH, oxidation, reduction potential).

These cultures include two genera: *Lactococcus* and *Leuconostoc* (Marshall, 1987). *Lactococcus* is a newly formed genus, which includes the organisms formerly known as the Group N streptococci (Table 14). *Lactococcus lactis* subsp. *lactis* is distinguished from *L. lactis* subsp. *cremoris* by the inability of the latter to produce ammonia from arginine. This inability could be due to a lack of the enzyme arginine deaminase, and in some strains the loss of a second enzyme, ornithine transcarbamylase (Crow & Thomas, 1982). However, the earlier nomenclature differentiated between *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *lactis biovar diacetylactis* because the latter produces diacetyl and utilizes citrate. The two species, however, are very similar in all other respects and the ability to produce diacetyl from citrate is plasmid linked (Kempler & McKay, 1979 and 1981).

In the 1986 edition of Bergey's Manual four species of *Leuconostoc* are listed: *Leuconostoc mesenteroides; L. paramesenteroides; L. lactis*; and *L. oenos*. *Leuconostoc mesenteroides* contains three subspecies, namely: *mesenteroides*; *cremoris;* and *dextranicum* (Garvie, 1986). The important organism for aroma (diacetyl) production in milk fermentations is *Leuconostoc mesenteroides* subsp. *cremoris*. This species ferments only a few sugars such as glucose; galactose; lactose; and maltose but not sucrose, and appears to be specifically adapted from *Leuconostoc mesenteroides* for growth in milk.

Some mixed mesophylic cultures contain strains of *L. lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc lactis*, *L. mesenteroides* subsp. *cremoris*, *L. mesenteriodes* subsp. *dextranicum* (Pettersson, 1988). The first two species, *L. lactis* and *L. lactis* subsp. *cremoris*, produce mainly lactic acid from lactose and are often referred to as acid producers. *L. lactis* subsp.

Table 14. Current nomenclature of the LAB relevant to fermented milk products (Marshall, 1987).

New nomenclature	Old nomenclature
Lactococcus	*Streptococcus* (Group N)
L. lactis subsp. *lactis*	*S. lactis* subsp. *lactis*
L. lactis subsp. *lactis biovar diacetylactis*	*S. lactis* subsp. *diacetylactis*
L. lactis subsp. *cremoris*	*S. lactis* subsp. *cremoris*
Lactobacillus	*Lactobacillus*
L. delbrueckii subsp. *delbrueckii*	*L. delbrueckii*
L. delbrueckii subsp. *leichmannii*	*L. leichmannii*
L. delbrueckii subsp. *bulgaricus*	*L. bulgaricus*
L. delbreuckii subsp. *lactis*	*L. lactis*
L. kefir	*L. caucasicus*
Leuconostoc	*Leuconostoc*
L. mesenteroides subsp. *mesenteroides*	*L. mesenteroides*
L. mesenteroides subsp. *cremoris*	*L. cremoris*
Streptococcus	*Streptococcus*
S. salivarius subsp. *thermophilus*	*S. thermophilus*

lactis biovar diacetylactis and *Leuconostoc* spp., which also ferment citric acid and produce important metabolites such as CO_2, acetylaldehyde and diacetyl, are referred to as flavour producers.

Mesophylic starters are often divided into various types depending on their composition (species and strains) or the identity of the flavour producing bacteria (Pettersson, 1988). Besides *L. lactis* and *L. lactis* subsp. *cremoris,* mesophylic cultures for fermented milks contain either *L. lactis* subsp. *lactis biovar diacetylactis* or *L. meseteroides* subsp. *cremoris* or both. The type of starters is referred to as D-, L- or DL cultures. O type starters contain no aroma bacteria and are mainly used for cheese.

Thermophylic starter cultures - Thermophylic cultures are not known to the same extend as mesophylic ones despite its widespread use. The effective use of these bacteria will be possible only after more information regarding cultural, physiological, biochemical and genetic aspects are acquired (Dellaglio, 1988b). The thermophylic cultures employed in the production of yoghurt and similar fermented milks are composed of obligately homofermentative lactobacilli and streptococci. According to Kurmann's classification of fermented milks, thermophylic cultures have optimum growth temperatures between 30° - 45°C (Roginski, 1988). The thermophylic organisms can be divided into two genera namely *Lactobacillus* and *Streptococcus* (Marshall, 1987). *Lactobacillus* is a large genus containing over 50 species with both homo- and heterolactic fermenters. However, only a few species are involved in milk fermentations with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* being extensively used. Although *Lactobacillus casei* produces diacetyl from citrate, this species is used only by the Japanese for making the fermented milk, Yakult (Speck, 1975).

As is the case with most of the lactobacilli the thermophylic starter cultures are micro-aerophilic (Dellaglio, 1988b). It is therefore important to use freshly heated milk to achieve a better growth of the cultures as the heat treatment reduces the amount of oxygen present in the product. Heating of the milk also produced some protein alterations resulting in a higher availability of peptides, amino acids and stimulatory SH-compounds.

Future trends

Fermented milks have been utilized throughout the world as a means of preserving milk against spoilage (Rasic, 1984). These traditional foods have persisted over the centuries in the developing countries, but their evolution have ranged from home manufacture to large-scale production using selected cultures, automatic processes and modern equipment. The progress in the use and manufacture of cultured dairy products depend on various factors, as shown in Table 15.

In order to develop fermented milks for an increasing and more discerning market it is important to know what is required of a starter organism in terms of its ability to grow in milk and, in so doing, produce flavour, aroma and texture (Marshall, 1987). Some organisms used for milk fermentations are slow growing in milk, or produce little aroma but their growth and activity are stimulated by the presence of (an)other type(s) of organism(s). Knowledge and technology are being accumulated to design starters that will not only improve milk fermentations, but may also be applied to the fermentation of other foodstuffs such as cereals, vegetables and fruit.

Despite our lack of understanding at present, fermented milk products provide a wide range of important nutrients that are generally palatable, widely available at an affordable price and versatile (Buttriss, 1997). Milk is fermented to produce products with good, wholesome and organoleptic qualities (Marshall, 1987). In addition to these qualities evidence of health benefits associated with the presence of specific strains of live bacteria are no longer anecdotal and is gradually gaining established scientific credibility (Buttriss, 1997).

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Table 15. Factors that may lead to growth in the use and production of cultured dairy products (Rasic, 1984).

Possible influencing factors	Methods and effects
Growth in biotechnology and improvements of the product quality	Use of high quality raw material and selected cultures including their correct handling. Adapting organoleptic properties and packaging to the market. Increasing shelf-life by methods other than pasteurization of the finished product. Concentrated starters - technological and economic advance.
Introducing more specialities	Dietetic products, flavoured pastes, beverages, frozen desserts, etc.
Changes in food-consumption patterns	Low-fat fermented milks and reduced sugar solids in flavoured products for people seeking energy reduced diets.
Snack-type foods	Cultured products with various taste sensations and packaging in many sizes and shapes.
Genetic engineering	Methods of gene transfer to the improvement of starter bacteria.
Reduction in the costs of production	Energy savings, increased automation, the utilisation of by-products.
Use in the processing of other foods	Bakery products, confectionery, beverages, desserts, etc.
Quality assurance programs	Ingredient control, manufacturing control and finished product control.

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CHAPTER 3

SCREENING OF MESOPHYLIC STARTER CULTURES TO ENHANCE THE SENSORY PROPERTIES DURING MAAS PRODUCTION

Summary

Twenty five mesophylic single strain starter cultures from the University of Stellenbosch Food Science Culture Collection (USFSCC) were used in the production of Maas. The products were then sensory evaluated, metabolites were determined and a selection, based on acidification activity, was applied.

The ability to produce acid (pH < 5.05) was used to reduce the 25 single strains to nine, which were then combined from different starter culture combinations. Data obtained from the two-strain combinations formed between *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* were used to reduce the number of combinations based on sensory evaluation, metabolites and acid production. Three-strain combinations were then formed that were also sensory evaluated and judged on their ability to produce metabolites and acid. *Leuconostoc mesenteroides* subsp. *dextranicum* and *L. mesenteroides* subsp. *citrovorum* strains were then added to the three-strain combinations to enhance flavour production. The starter culture combinations were finally reduced to four starter types that consisted of four, five and six strain combinations, respectively. pH levels of these products ranged from 4.49 - 4.57 which is acceptable for naturally unsweetened Maas (pH = 4.57). The major flavour metabolite produced in the Maas was acetaldehyde with concentrations ranging between 26.6 – 89.3 $mg.l^{-1}$. The other metabolites (ethanol, acetone, diacetyl, 2-butanone) were also present, but at lower levels. Organoleptic examination was mainly used for evaluating the flavour and texture properties of the Maas. Maas made with three of the four final starter combinations (A, C and D) was characterised by a superior, but delicate flavour, and a typical characteristic Maas body texture.

Introduction

A wide variety of fermented dairy products produced with different species and strains of lactic acid bacteria (LAB) as starter cultures, can be found on the market (Pacher & Kneifel, 1996). This wide selection of products has influenced the public interest especially as a result of the health promoting (Mitsuoka, 1982) and therapeutic (Fernandes *et al.*, 1992; Klaver *et al.*, 1993) benefits of fermented products. Cultured milk, cultured buttermilk and cultured cream, although not as popular as yoghurt, contribute considerably to the per capita consumption of fermented milk products world-wide (Kneifel *et al.*, 1992a).

The production of fermented milk is primarily based on the physical and chemical changes caused in milk by the growth and metabolic activities of the LAB (Khedkar & Khedkar, 1993). The homofermentative LAB types ferment lactose mainly to lactic acid and traces of other organic compounds resulting in the coagulation of milk into a smooth and homogenous curd with a firm texture and a variable lactic acid flavour. The heterofermentative types also produce lactic acid from lactose but in addition, produce other compounds such as acetic, propionic and formic acids, acetylmethylcarbinol, diacetyl, alcohol and carbon dioxide during the fermentation of citric acid present in the milk. These metabolites impart a characteristic pleasant aroma and flavour to the coagulated product. When both these groups of LAB are present they lead to an enhanced acid, aroma and flavour production (Khedkar & Khedkar, 1993).

Quality control of cultured dairy products has traditionally been based on sensory evaluation and on the determination of certain chemical and microbiological characteristics (Kneifel *et al.*, 1992a). However, growing interest in other factors inherent to these products necessitates more in-depth evaluation of the cultured milk technology. Furthermore, the development of advanced analytical methods has promoted the more objective characterization of certain parameters, e.g. the use of chromatographic techniques in aroma analysis (Kneifel *et al.*, 1992a).

The objective of this study was to screen a collection of mesophylic starter cultures (*Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar *diacetylactis*) so as to enhance the sensory properties during the production of Maas. The ability of these cultures to produce typical aroma

compounds was therefore evaluated and compared to Maas produced using a locally available commercial starter culture.

Materials and methods

Culture maintenance

A commercial lyophilised starter culture was obtained from a local supplier and used as the comparative control (Darleon, 1997). The single strain mesophylic cultures evaluated in this study were obtained from the University of Stellenbosch Food Science Culture Collection (USFSCC) and are listed in Table 1. The cultures were isolated from dairy products over a period of nearly 15 years. Stock cultures were propagated in sterile MRS-medium (Biolab) and incubated anaerobically at 30°C for 24 h. The stock cultures were then transferred to fresh MRS slants every 4 - 5 weeks. Long-term storage of the cultures was done by freeze-drying the individual strains according to the method of Joubert & Britz (1987). The MRS-medium consisted of ($g.l^{-1}$): beef extract (5); peptone (10); yeast extract (5); dextrose (20); potassium phosphate (2); Tween 80 (1); tri-ammonium citrate (2); magnesium sulphate (0.1); and sodium acetate (5). The pH was set at 7.10 and the medium sterilised at 121°C and steam pressure of 100 kPa for 15 min.

Growth Studies

The lyophilised single strain cultures were suspended in sterile MRS-medium and incubated at 30°C for 24 h. A 1% (v/v) inoculum of each culture was transferred to 100 ml MRS-medium (> 1×10^8 $cfu.ml^{-1}$) and incubated at 30°C. The optical density (OD_{600}) was then monitored hourly using a Bausch & Lomb Spectronic 20 (USA). At the same time, a serial dilution in quarter-strength Ringers solution was made up and plated on the MRS-medium and incubated at 30°C for 48 h. The data obtained during the growth period was fitted by a linear regression from which cell concentrations could be estimated.

Inoculum preparation

Full cream pasteurised milk was obtained from local supermarkets and given an additional heat treatment in a thermostatically controlled waterbath at 85°C for 20

Table 1. Inoculum concentration of the different mesophylic cultures used as starter bacteria during Maas production.

Strain	Inoculum concentration ($cfu.ml^{-1}$)
Control (commercial starter)	0.20×10^8
L. lactis ss. *lactis* biovar *diacetylactis*	
S1	1.90×10^8
S2	1.53×10^8
S3	1.88×10^8
S4	1.10×10^8
S5	1.86×10^8
S6	1.94×10^8
S7	1.10×10^8
S8	1.10×10^8
S9	1.10×10^8
S10	1.10×10^8
S11	1.38×10^8
S12	1.10×10^8
L. lactis ss. l*actis*	
S13	1.50×10^8
S14	1.03×10^8
S15	1.50×10^8
S16	1.35×10^8
L. lactis ss. *cremoris*	
S17	1.45×10^8
S18	1.50×10^8
S19	1.10×10^8
S20	1.03×10^8
S21	2.20×10^8
S22	1.45×10^8
S23	1.35×10^8
S24	1.35×10^8
S25	1.30×10^8

min and then cooled to 22°C before inoculation. The additional heat treatment led to the whey proteins having a greater water binding activity leading to better structure and texture of the final product (Training Board for the Dairy Industry, 1997). Considerable variations in microbial content of raw milk have been reported by Mutukumira *et al.* (1996a). For this reason the additional heat treatment was also necessary to standardise the microbiological quality. It was of interest to note that the pH, titratable acidity (TA) and microbial content of the full cream milk purchased from local supermarkets differed from litre to litre.

A lyophilised standardised commercial starter culture was used as comparative control. This culture contained *Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* biovar *diacetylactis* (5 – 30%, m/v) and *Leuconostoc mesenteroides* subsp. *cremoris* (1 – 10%, m/v) with a minimum cell concentration of 5×10^{10} cfu.g^{-1} (Darleon, 1997). This starter culture was activated by inoculating 0.4 g of the lyophilised culture into one litre of full cream pasteurised milk and then incubating the container at 22°C for 8 h (Human, 1998). The activated commercial starter culture was then inoculated (1%, v/v) into one litre pasteurised milk and incubated at 22°C for 16 h. The pH and TA were monitored at 2 h intervals during the activation phase and the production of the subsequent Maas.

Identification of the lactic acid bacterial strains

The morphology of the 25 strains (Table 1) grown on MRS-media was determined by bright field microscopy of Gram-stained preparations. The following characteristics were also determined: growth at 40° and 45°C; growth in MRS-media supplemented with 4.0 and 6.5% NaCl; growth at pH 9.20; reaction in litmus milk (Harley & Prescott, 1993); and the utilisation of citrate (Harley & Prescott, 1993).

The biochemical and enzymatic profiles of the 25 strains were determined with the API 20E system (BioMérieux sa, 69280 Marcy l'Etoile, France) according to the manufacturer's instructions. The galleries were incubated at 30°C and changes in colour were observed after 24 h. Final identification was confirmed using the identification systems of Mundt (1986), Garvie & Farrow (1982) and Kotzé (1991).

Analytical procedures

The percentage titratable acidity (%TA) was determined by titrating 10 ml of

each Maas sample against standardised sodium hydroxide (0.1 M) with phenolphthalein as indicator (James, 1995). The pH of each Maas sample was measured using a Knick Portamess 751 Calimatic (West Germany) pH meter.

The metabolites formed during the incubation of the Maas were determined gas chromatographically as follows (De Haast *et al.*, 1978): each Maas sample (9.75 ml) was measured into a 20 ml glass vial and 0.25 ml n-buthanol was added as internal standard to give a final concentration of 25 $mg.l^{-1}$ (De Haast *et al.*, 1978). The vials were then closed with a silicone-PTFE seal and aluminium cap and heated for 30 min in a waterbath at 70°C (Marsili, 1981). A 1.5 ml sample (Xanthopoulus *et al.*, 1994) of the headspace gas was withdrawn using a gas-tight syringe (Dynatech Corporation) without removing the sample vials from the waterbath and split-injected (1:100) onto the GC column. A larger sample of 1.5 ml of the headspace was injected onto the column because 1 ml (Xanthopoulos *et al.*, 1994) did not give satisfactory peaks.

Quantitative determination of each compound was done by integration of the peak areas using internal standard calibration (Mutukumira *et al.*, 1996b). Identification of the unknown compounds was achieved by comparing the retention times (Mutukumira *et al.*, 1996b) to those of the analytical grade standard compounds (Ulberth, 1991). These included: 128 µl acetaldehyde (Merck); 127 µl ethanol (Merck); 127 µl acetone (Reidel-de Haën); 102 µl diacetyl (Aldrich); 124 µl 2-butanone (Merck) and 124 µl n-buthanol (internal standard) (Protea Laboratory Services, Johannesburg) in 100 ml volumetric flask and diluting with distilled water. An aqueous stock solution containing these analytical grade standard compounds was prepared to give a final concentration of 1 000 $mg.l^{-1}$ of each compound. For headspace analysis a 10 ml quantity of the standard stock solution was then pipetted into a 20 ml glass vial which was sealed and heated for 30 min at 70°C. The standard solution was then used for internal calibration before sampling of the Maas and integration of the unknown peaks.

A Fisons GC 8000 series gas chromatograph (Fisons Instruments SpA, Milan, USA) equipped with a flame ionisation detector was used with helium as carrier gas at 1.1 $ml.min^{-1}$. The temperature profile was: 45°C at 10 min, increased by 5°$C.min^{-1}$ to 220°C with a holding time of 10 min. The temperature of the injector and the

detector were 150° and 200°C, respectively. A fused silica capillary column 30 m × 0.25 mm id (Quadrex Corporation, New Haven, CT, USA), coated with chemically bonded methyl 5% phenyl silicone (film thickness, 0.2 μm), was used.

Sensory evaluation

Sensory evaluation was conducted on all the Maas samples made with the 25 single starter cultures at 22°C for 16 h by a two-member expert panel trained in the examination of fermented dairy products. The expert panel evaluated the samples for overall flavour and texture quality, as well as the degree of sourness and sweetness after the samples had been stored at 4°C for 4 h.

Results and discussion

Identification

The 25 *Lactococcus* strains used in this study obtained from the USFSCC were from the three subspecies of *L. lactis*: *L. lactis* subsp. *lactis*, *L. lactis* subp. *cremoris* and *L. lactis* subsp. *lactis* biovar *diacetylactis*. Additional characterization methods were used to differentiate the strains by their ability to grow under various growth conditions. The principal physiological and biochemical characteristics of the strains obtained from the tests are summarised in Tables 2 and 3.

The data in Table 2 shows that of the four *L. lactis* subsp. *lactis* strains (S13, S14, S15, S16) used, some displayed unconventional biochemical profiles: one produced acid from sucrose; three produced acid from melibiose; three produced acid from amygdalin and all four produced acid from arabinose. However, all four strains hydrolysed arginine. Typical *L. lactis* subsp. *lactis* characteristics were also displayed by these four strains (Table 3): no growth at 45°C; growth in the presence of 4% NaCl, but not 6.5% NaCl; reduction, acidification and coagulation of litmus milk; and growth at pH 9.20 (Mostert, 1975; Schleifer *et al.*, 1985; Dellaglio, 1988; Dellaglio *et al.*, 1992; Centeno *et al.*, 1996; Mutukumira *et al.*, 1996b). These four strains were therefore, inspite of some variability in the profiles, assigned to *L. lactis* subsp. l*actis*.

Strains of *L. lactis* subsp. *lactis* biovar *diacetylactis* have been reported

Table 2. Biochemical profiles of the 25 mesophylic starter bacteria used in the study for the production of Maas.

Strain	Biochemical profiles*																
	ONPG	ADH	ODC	CIT	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
S1	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+	+	+
S2	+	+	-	-	+	-	+	-	+	-	-	-	+	-	+	+	+
S3	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+	+	+
S4	+	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+
S5	-	+	-	-	+	-	+	-	+	-	-	-	+	-	-	+	+
S6	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+	+	+
S7	-	+	-	-	+	-	+	-	+	-	-	-	+	-	-	+	+
S8	-	+	-	-	+	-	+	-	+	-	-	-	+	-	-	+	+
S9	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+	+	+
S10	-	+	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+
S11	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+
S12	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+
S13	-	+	-	-	+	-	+	-	+	+	-	-	+	-	-	+	+
S14	-	+	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+
S15	-	+	-	-	+	-	+	-	+	+	-	-	+	-	+	+	+
S16	-	+	-	-	+	-	+	-	+	+	-	-	+	-	+	-	+
S17	-	+	-	-	+	-	+	-	+	-	-	-	+	-	-	+	-
S18	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+
S19	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+
S20	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+
S21	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	-	-
S22	-	+	-	-	+	-	+	-	+	-	-	-	+	-	-	+	+
S23	+	+	-	-	+	-	+	-	+	-	-	-	+	+	-	+	+
S24	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+
S25	+	+	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+

*ONPG = Ortho-nitro-phenyl-galactoside; ADH = Arginine; ODC = Ornithine; CIT = Sodium citrate; TDA = Tryptophane; IND = Tryptophane (indole production); VP = Sodium pyruvate; GEL = Kohn's gelatin; GLU = Glucose; MAN = Mannitol; INO = Inositol; SOR = Sorbitol; RHA = Rhamnose; SAC = Sucrose; MEL = Melibiose; AMY = Amygdalin; ARA = Arabinose

Table 3. Principal physiological characteristics of the 25 mesophylic starter bacteria used in the study for the production of Maas.

Strain	Growth at 45°C	Litmus milk reaction	Growth in 4% NaCl	Growth in 6.5% NaCl	Growth at pH 9.2
S1	-	RAC	+	+	+
S2	-	AC	+	-	+
S3	-	RAC	+	-	+
S4	-	RAC	+	-	-
S5	-	AC	+	-	+
S6	-	RAC	-	-	+
S7	-	AC	+	+	+
S8	+	RAC	+	-	+
S9	-	AC	+	-	+
S10	-	RAC	+	-	+
S11	+	RAC	+	-	+
S12	-	RAC	+	+	+
S13	+	RAC	+	+	+
S14	-	dRAC	+	-	-
S15	-	RAC	+	-	+
S16	-	RAC	+	-	+
S17	-	RAC	+	+	-
S18	-	RAC	-	-	-
S19	-	RAC	-	-	-
S20	-	RAC	+	-	-
S21	-	RAC	-	-	-
S22	+	RAC	+	-	+
S23	+	RAC	-	+	+
S24	-	RAC	-	-	-
S25	+	RAC	-	-	+

R = reduction; A = acidification; C = coagulation; d = delayed reaction

taxonomic entities similar to the two autonomous subspecies *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Mostert, 1975). However, according to Schleifer *et al.* (1985), *L. lactis* subsp. *lactis* biovar *diacetylactis* has a greater affinity and phenotypically very similar to *L. lactis* subsp. *lactis* than *L. lactis* subsp. *cremoris*. The strains of *L. lactis* subsp. *lactis* biovar *diacetylactis* examined in this study possess (Schleifer *et al.*, 1985) the same biochemical and physiological characteristics as the *L. lactis* subsp. *lactis* strains (Tables 2 and 3), but differ in the utilisation of citrate (Schleifer *et al.*, 1985; Centeno *et al.*, 1996). However, in this study citrate utilisation (Table 2) by the latter obtained with the API 20E system method, as well as the techniques of Cappuccino & Sherman (1992) was found to be negative.

In most respects the description of *L. lactis* subsp. *cremoris* corresponds to that of *L. lactis* subsp. *lactis* (Yawger & Sherman, 1937; Schleifer *et al.*, 1985; Dellaglio, 1988). However, the major criterion for distinguishing these two subspecies is the inability of the former to hydrolyse arginine (Pettersson, 1988; Mostert, 1975; Yawger & Sherman, 1937). This criterion is based on the fact that the specific metabolism involves three enzymes, namely ornithine transcarbamylase, carbamate kinase and arginine deaminase (Pettersson, 1988). Most strains of *L. lactis* subsp. *cremoris* contain only two of these enzymes (Crow & Thomas, 1982), namely carbamate kinase and ornithine transcarbamylase.

The nine strains of *L. lactis* subsp. *cremoris* included in this study (Table 1) displayed unconventional biochemical profiles (Table 2): five produced ammonia from arginine; three produced acid from sucrose; six produced acid from melibiose; four produced acid from amygdalin; seven produced acid from arabinose; and all nine strains produced acid from rhamnose (Yawger & Sherman, 1937; Schleifer *et al.*, 1985; Dellaglio, 1988; Pettersson, 1988; Holt *et al.*, 1994).

The most important differential characteristics (Yawger & Sherman, 1937; Schleifer *et al.*, 1985; Holt *et al.*, 1994) of *L. lactis* subsp. *cremoris* are: no growth occurs at 45°C; reduction, acidification and coagulation of litmus milk; no growth in broth containing 4% NaCl; and no growth in alkaline broth at a pH of 9.20. According to the data obtained in this study (Table 3), six or more strains were negative for the above parameters, but all were positive for the litmus milk test. Therefore, it was concluded that these strains are members of *L. lactis* subsp.

cremoris despite some variations.

Based on the strain characteristics obtained in this study, it is concluded that since some of the 25 *Lactococcus* strains showed variable reactions under different growth conditions, a more extended description of these strains is needed to verify their identity to the specific subspecies level.

Growth Studies

Growth studies were performed on all 25 bacterial strains (Table 1), but only the growth and standard curves of *Lactococcus lactis* subsp. *cremoris* strain S21 will be discussed. The growth of strain S21 was followed in MRS-medium for 15 h at 30°C (Fig. 1 and 2). After inoculating (1%, v/v) the starter culture into the MRS-medium, a typical lag phase was found that continued for 4 h before the logarithmic growth phase was reached. The logarithmic phase continued and a maximum optical density (OD) level of 2.4 was reached after 9 h, which continued into the stationary phase. This data shows that *L. lactis* subsp. *cremoris* strain S21 has the best growth activity over the 4 to 9 h period in the MRS-medium. The cell concentration (1×10^8 cfu.ml^{-1}) that corresponded to an OD of 1.0, which is recommended for activation of the strains in the milk, was then calculated (Fig. 2). This minimum cell concentration (1×10^8 cfu.ml^{-1}) used corresponded to the cell concentration of the inoculum of other fermented milk products (Hunger & Peitersen, 1992) where a typical inoculum rate of about 1.0 – 1.5% was used.

Commercial starter culture (control)

The lyophilised commercial culture was used as the comparative control and was inoculated (0.4 g.l^{-1}) into one litre pasteurised milk to activate the culture. The pH and %TA were monitored every 2 h over a period of 8 h at 22°C (Human, 1998) (Fig. 3). The 22°C incubation temperature used was recommended by Darleon (1997) and confirmed by Human (1998) as being the optimal temperature for the activation of the commercial starter culture resulting in the best flavour during the production of Maas. The control reached a pH and TA of approximately 4.70 and 0.72% after 6 h of incubation and a final pH and TA of 4.50 and 0.87% after 8 h of activation, respectively. It was previously recommended that a pH of 5.70 – 5.40

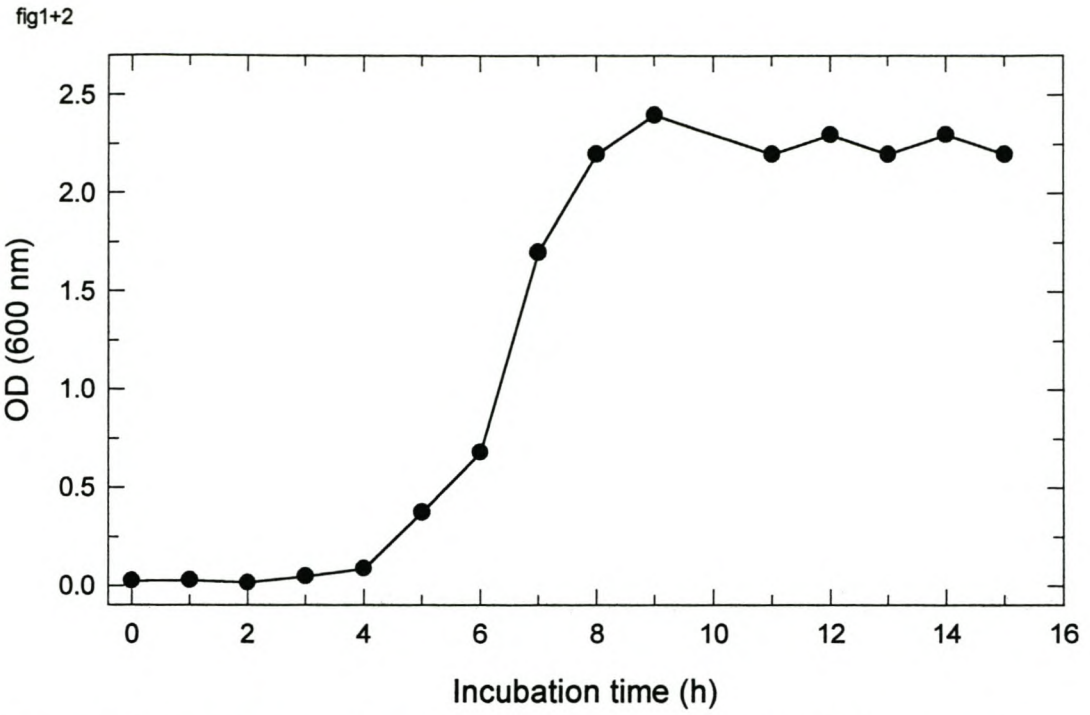

Figure 1. An example of a growth curve of the mesophylic single strain cultures (strain S21) activated in MRS-medium and incubated at 22°C for 15 h.

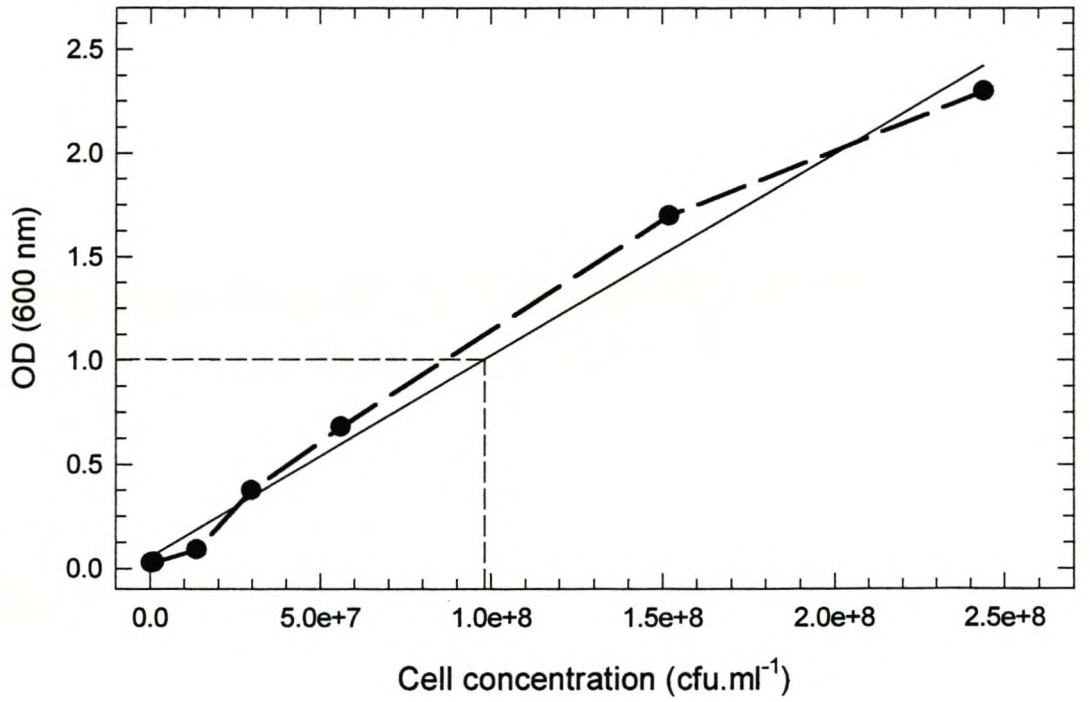

Figure 2. Standard curve of the mesophylic single strain cultures (strain S21) in MRS-medium and incubated at 22°C for 15 h. The dashed line represents the cell concentration in the MRS-medium used to inoculate the milk. The long dashed line represents the actual optical densities of the culture.

fig3&4

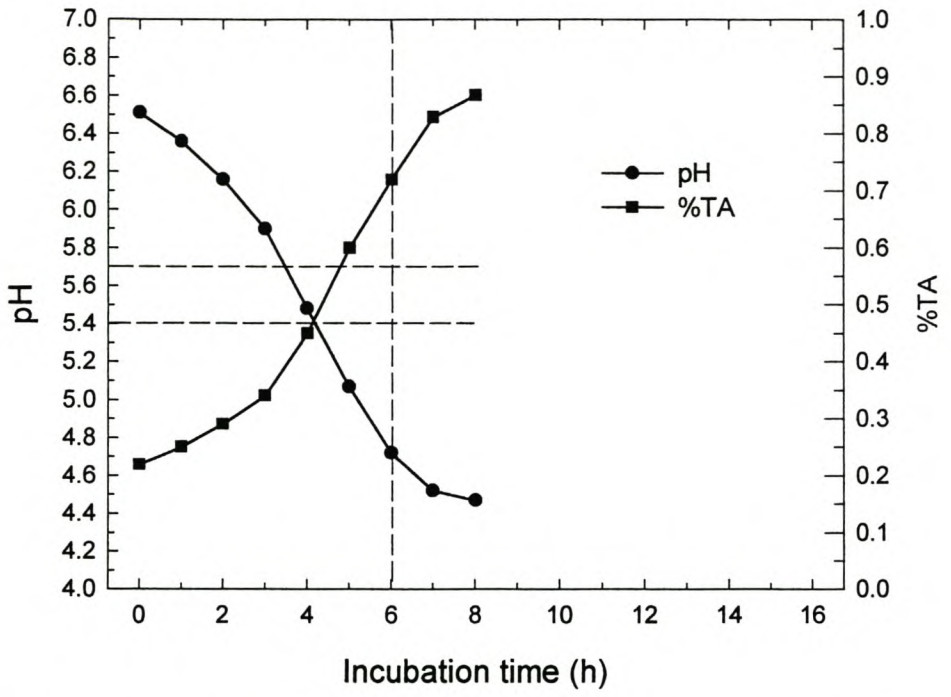

Figure 3. Changes in pH and %TA of the control Maas made with a 1% (m/v) activated inoculum during the activation of the commercial starter culture and incubated at 22°C for 8 h. The dashed lines represent the recommended pH range that must be reached by 6 h.

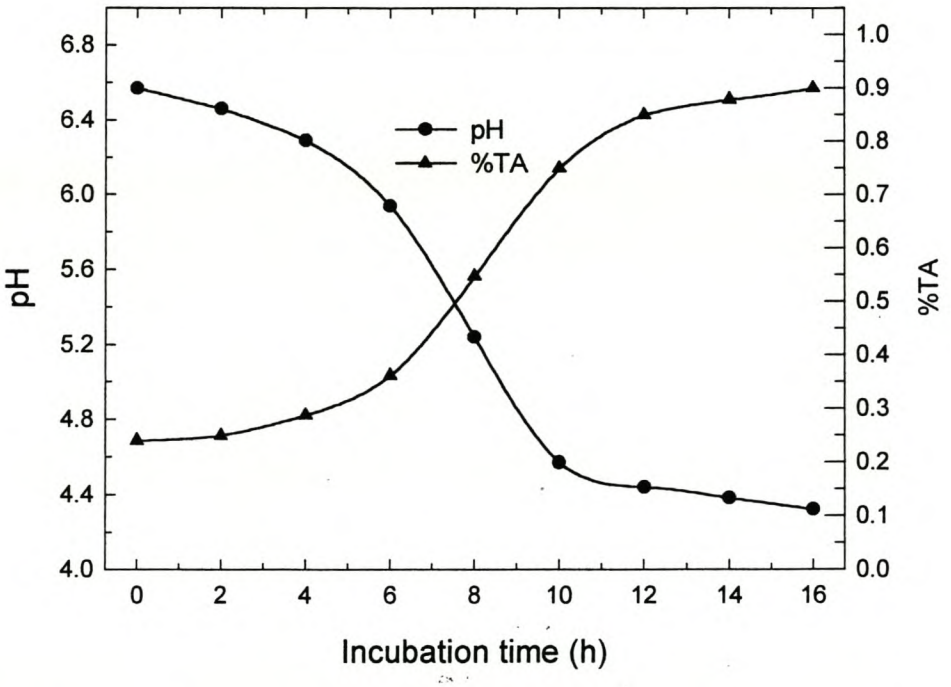

Figure 4. Changes in pH and %TA of the control Maas made with the 1% (v/v) activated commercial starter culture and incubated at 22°C for 16 h.

must be reached within 6 h during activation (Human, 1998) to produce favourable Maas characteristics.

The data in Table 1 shows that the cell concentration of the control starter culture was lower than the cell concentration of each of the single strain cultures used during the activation period. However, it was found that the control produced acid faster over a shorter period probably because it consisted of a selected combination of mixed cultures compared to the single strain cultures used in this study.

A 1% (v/v) inoculum of the activated starter culture after 8 h (from Fig. 3) was inoculated into one litre of pasteurised milk, incubated at 22°C for 16 h and the pH and TA monitored at 2 h intervals (Fig. 4). A final pH and TA of 4.30 and 0.90%, respectively, were reached after 16 h incubation. The pH of the control Maas thus decreased more slowly over the 16 h than during the 8 h activation of the lyophilised starter culture but at the same time produced more acid. The reason for the extended time is probably that the culture had to adapt to the new pH conditions and lactose concentration of the fresh milk.

Single strain starter culture – Activation step

The 25 single strain starter cultures (Table 1) were all propagated in MRS-medium and incubated at 30°C for 8 h and the optical density (OD) monitored. The starter cultures were only inoculated into the milk after an OD > 1.0 (equivalent to a cell concentration > 1×10^8 cfu.ml^{-1}) had been reached (Fig. 2). After reaching this cell concentration, a 1% (v/v) inoculum of each of the single strain starter cultures from the MRS-medium was transferred to one litre of pasteurised milk and incubated at 22°C for 8 h (Human, 1998). This was taken as the activation phase and the pH and %TA of the inoculated milk were then monitored at 2 h intervals.

The recommended pH range of 5.70 – 5.40 (Darleon, 1997; Human, 1998) for activation was not reached by the 12 *L. lactis* subsp. *lactis* biovar *diacetylactis* strains within the 6 h period at 22°C (Fig. 5A and B). Furthermore, the strains did not produce enough lactic acid to reach the 0.55 – 0.45% range (Fig. 5C and D) as recommended. The fermentations using strains S1 and S3, however, required 7 – 8 h of activation at 22°C to reach the upper limit of the pH range (Fig. 5B). The

figure5

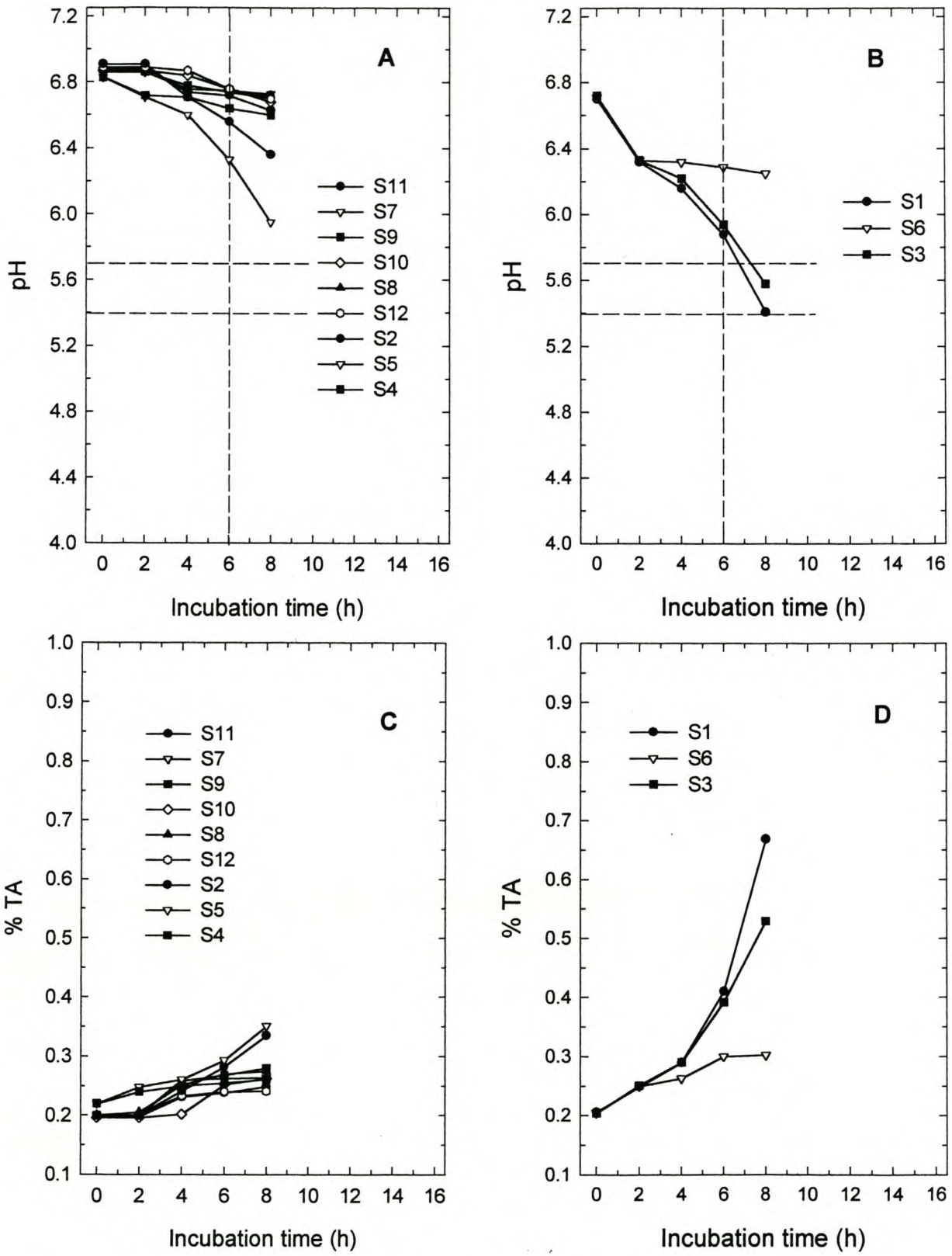

Figure 5. Changes in pH and %TA values during the activation phase of Maas incubated at 22°C for 8 h and produced with different *L. lactis* subsp. *lactis* biovar *diacetylactis* strains. The dashed lines represent the recommended pH range that must be reached by 6 h.

cultures reached pH values of 5.41 and 5.58 and corresponding TA values of 0.67 and 0.53% after 7 – 8 h activation, respectively (Fig. 5B and D). This indicates that these *L. lactis* subsp. *lactis* biovar *diacetylactis* strains, also flavour producers, could probably also be used as acid producers (Daly, 1983; Cogan, 1995). Although strains S2 and S5 (Fig. 5A) did not reach the recommended pH range after 6 h, they could possibly, after a longer time period, reach a pH nearer to the recommended range. This possibility is suggested by the data given in Fig. 5A and C where strains S2 and S5 reached a pH of 6.36 and 5.95 and TA values of 0.34 and 0.35% after 8 h, respectively. It therefore appears that certain *L. lactis* subsp. *lactis* biovar *diacetylactis* strains could, after an extended activation period, produce enough acid to be used as acid producing starters. The more general mesophylic acid-producing starters are considered to be strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Daly, 1983; Cogan, 1995). In this study the other *L. lactis* subsp. *lactis diacetylactis* strains, however, showed only minor acidification and were therefore excluded as major acid producers from this study on the basis of fermentation activity alone.

The data in Fig. 6A and B show the changes in pH and TA values of the four *L. lactis* subsp. *lactis* strains during their 8 h activation at 22°C. A clear distinction can be made between the fast-acid and slow-acid producing *L. lactis* subsp. *lactis* strains (Fig. 6A and B). Only strains S15 and S13 (Fig. 6A) of the four *L. lactis* subsp. *lactis* strains, with a pH of 5.69 and 5.62, respectively, reached the recommended pH range (Human, 1998; Darleon, 1997) by 6 h at 22°C. The fermentation with strain S16, however, needed 7 h to reach the upper recommended pH limit of 5.70. The results presented in Fig. 6A also show that strain S14 only lowered the milk pH to 6.46 after 8 h of activation, which was insufficient according to the pH recommendations (Darleon, 1997; Human, 1998). The data in Fig. 6B shows the titratable acid produced by these strains after 8 h at 22°C. The fermentation with strain S13 resulted in a 0.70% TA value and with S15, S16 and S14 in a 0.60, 0.49 and 0.29% TA, respectively, after 8 h activation (Fig. 6B). The amount of lactic acid (0.29%) produced by strain S14 clearly shows that it is a weak acid producer.

The results presented in Fig. 7A and B show that the nine *L. lactis* subsp. *cremoris* strains used in this study were unable to acidify the milk to the recommended pH range (Darleon, 1997; Human, 1998). The pH after 6 h at 22°C

figure6

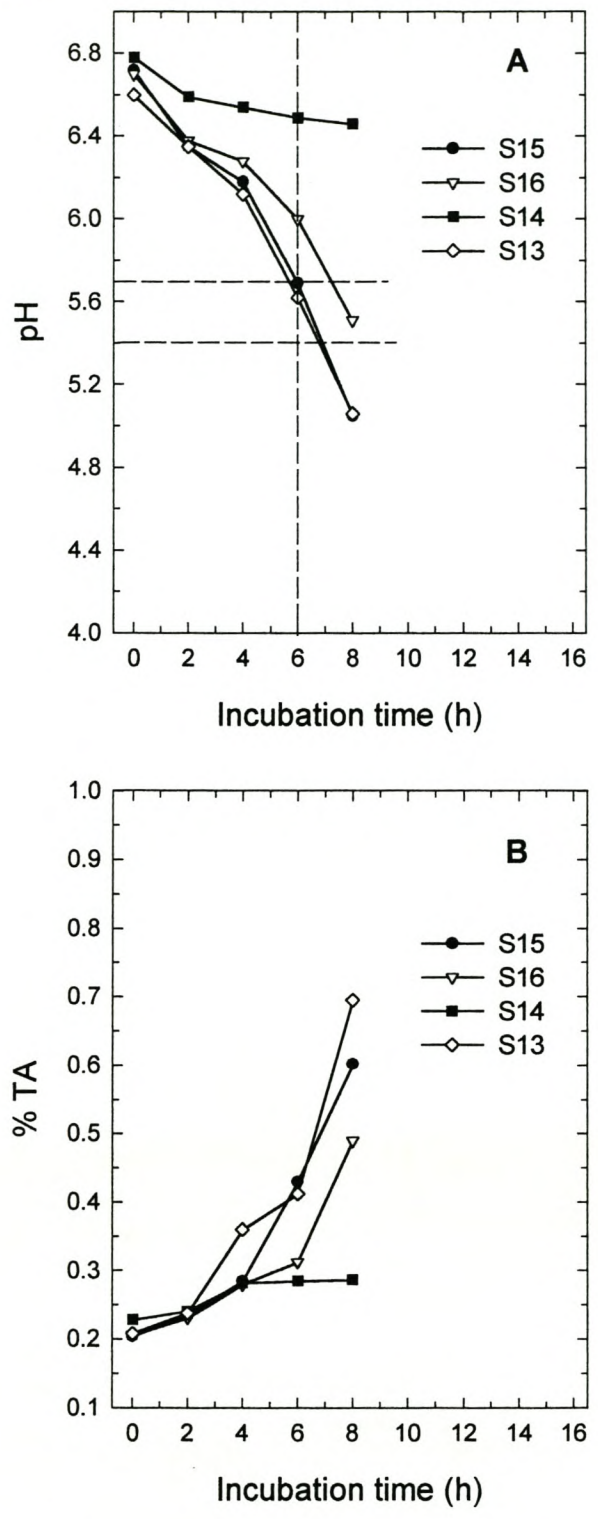

Figure 6. Changes in pH and %TA values during the activation phase of Maas incubated at 22°C for 8 h and produced with different *L. lactis* subsp. *lactis* strains. The dashed lines represent the recommended pH range that must be reached by 6 h.

figure7

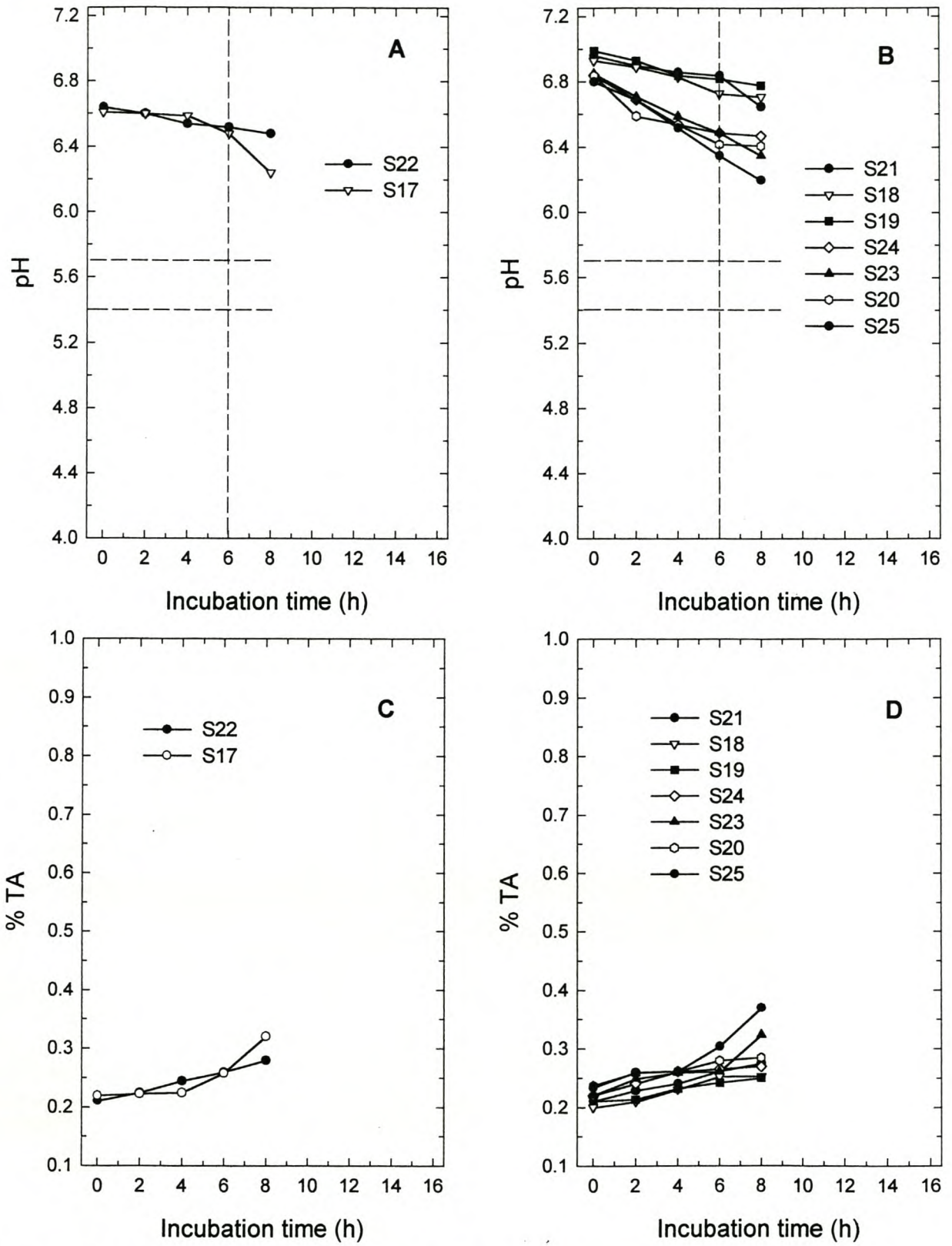

Figure 7. Changes in pH and %TA values during the activation phase of Maas incubated at 22°C for 8 h and produced with different *Lactococcus lactis* subsp. *cremoris* strains. The dashed lines represent the recommended pH range that must be reach by 6 h activation.

ranged between 6.35 and 6.85 after 6 h activation. Only three strains (S17, S23 and S25) could, after a longer time period, reach a lower pH, which was nearer to the recommended range. The milk pH was, however, lowered (6.20 – 6.80) after 8 h activation with corresponding TA values ranging between 0.20 and 0.40% (Fig. 7C and D). Thus, a TA range corresponding to the recommended pH range (5.70 – 5.40) was not reached even after 8 h. It was therefore concluded that the *L. lactis* subsp. *cremoris* strains were low acid producers. Yawger & Sherman (1937) and Salama *et al.* (1995) showed that strains of this subspecies usually produce less acid in milk and in general have less acid fermentation activity than *L. lactis* subsp. *lactis* (Fig. 6). Farrow (1980) also showed that lactococci that ferment lactose only slowly contain both β-galactosidase and phospho-β-galactosidase, while those that ferment it rapidly only contain phospho-β-galactosidase (Cogan *et al.*, 1997).

Single strain starter culture – Maas production

After 8 h of activation, the addition of a 1% (v/v) inoculum was made of each of the 25 strains into one litre of pasteurised milk and incubated at 22°C for 16 h (Human, 1998) during which the pH and %TA was monitored at 2 h intervals (Fig. 8, 9 and 10).

Eight of the 12 *L. lactis* subsp. *lactis* biovar *diacetylactis* strains were found to be poor acidifiers, with a final Maas pH ranging between 6.25 and 6.42 (Fig. 8 A and B) and TA values between 0.23 and 0.28% (Fig. 8 C and D) after 16 h at 22°C. However, strains S1, S2, S3 and S5, lowered the milk pH to 4.96, 5.05, 4.59 and 4.51, respectively, after 16 h at 22°C. The amount of lactic acid produced by these strains during the period was 0.74, 0.78, 0.83 and 0.87%, respectively. These four strains also showed similar growth patterns during their activation periods (Fig. 5A and B). The decrease in pH and increase in TA after 16 h incubation at 22°C for the respective four strains indicated higher acidification activity by these four strains than after their individual activation periods.

Strain S1 had a final pH of 5.41 and TA value of 0.67% during activation and during the production of Maas a final pH and TA value of 4.96 and 0.74%. This showed that although a drop in pH of 0.45 units was obtained during the Maas production, it produced 0.07% more TA than during the activation. It was therefore

figure8

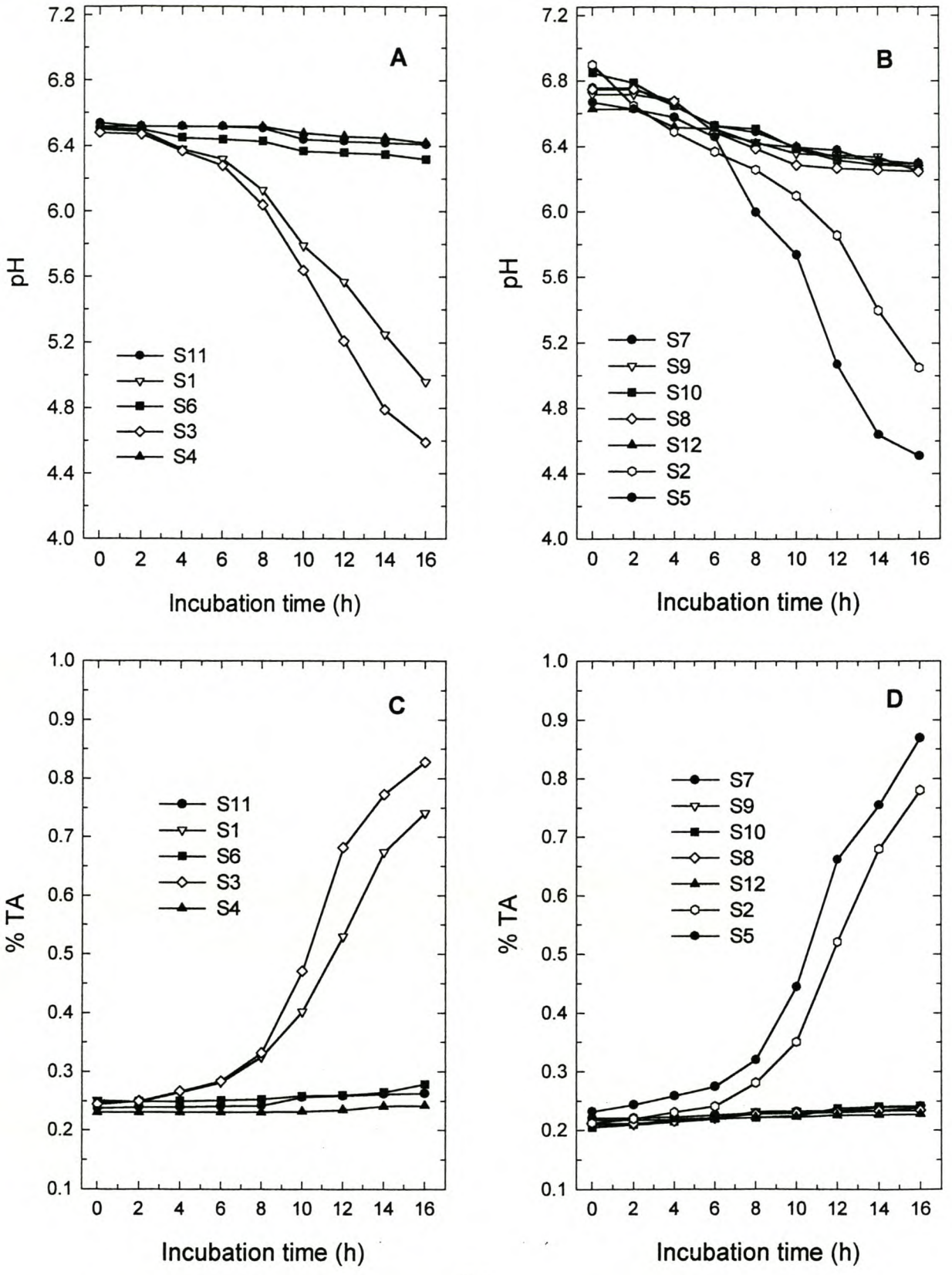

Figure 8. Changes in pH and %TA of Maas incubated at 22°C for 16 h and produced with 1% (v/v) activated *L. lactis* subsp. *lactis* biovar *diacetylactis* strains.

concluded that strain S1 was a weak acid producer even during the Maas production.

Strain S2 showed a stronger decrease in pH after 16 h at 22°C (pH = 5.05; TA = 0.78%) compared with its activation period (pH = 6.36; TA = 0.36%). The possibility, during the activation period, that strain S2 would reach a lower pH, was thus accomplished. In spite of the TA value, the final pH value was still higher than the other three strains (S1, S3 and S5) and also too high to give a product of sufficient viscosity (Mutukumira, 1996). Consequently strain S2 was not considered a good acidifier during Maas production.

Although strains S3 and S1 only reached the recommended pH range after an extended activation period, S3 reached a lower pH (pH = 4.59) and higher TA (0.83%) during the 16 h incubation at 22°C than S1. Strain S3 also showed, in addition to its flavour producing qualities, a further pH drop of 0.99 units and TA increase of 0.30% in comparison to that found during the activation period, and could thus be used as a successful acidifier during Maas production.

Strain S5 did not reach the recommended pH range during activation, but it showed a pH decrease of 1.44 units to a final pH of 4.51 after 16 h at 22°C during the Maas-making procedure. The final TA value also increased during this period to 0.87%. These results suggested that strain S5 could be suitable as an acid producer during Maas production.

According to Mutukumira (1996), coagulation during the manufacture of fermented milk products is basically due to quiescent acidification resulting from specific bacterial fermentations. As the pH decreases during acidification, the viscosity of the coagulum increases dramatically as the pH drops to below 5.20. At a pH lower than 5.20, casein reaggregates into the protein network with the other caseins. The viscosity then reaches a maximum at a pH of about 4.60 to 4.70. Thus, when the final pH values of the four strains of *L. lactis* subsp. *lactis* biovar *diacetylactis* (S1, S2, S3, S5) are considered, only strains S3 and S5 would lead to the production of a more viscose product. Strains S3 and S5 were found to be faster acid producers than S1 and S2 as they exhibited a faster pH decrease and TA increase over the 16 h incubation time (Fig. 8).

In the dairy industry *L. lactis* subsp. *lactis* strains are generally used for acidification of milk (Cogan, 1983; Daly, 1983). The data in Fig. 9 show that strains

figure9

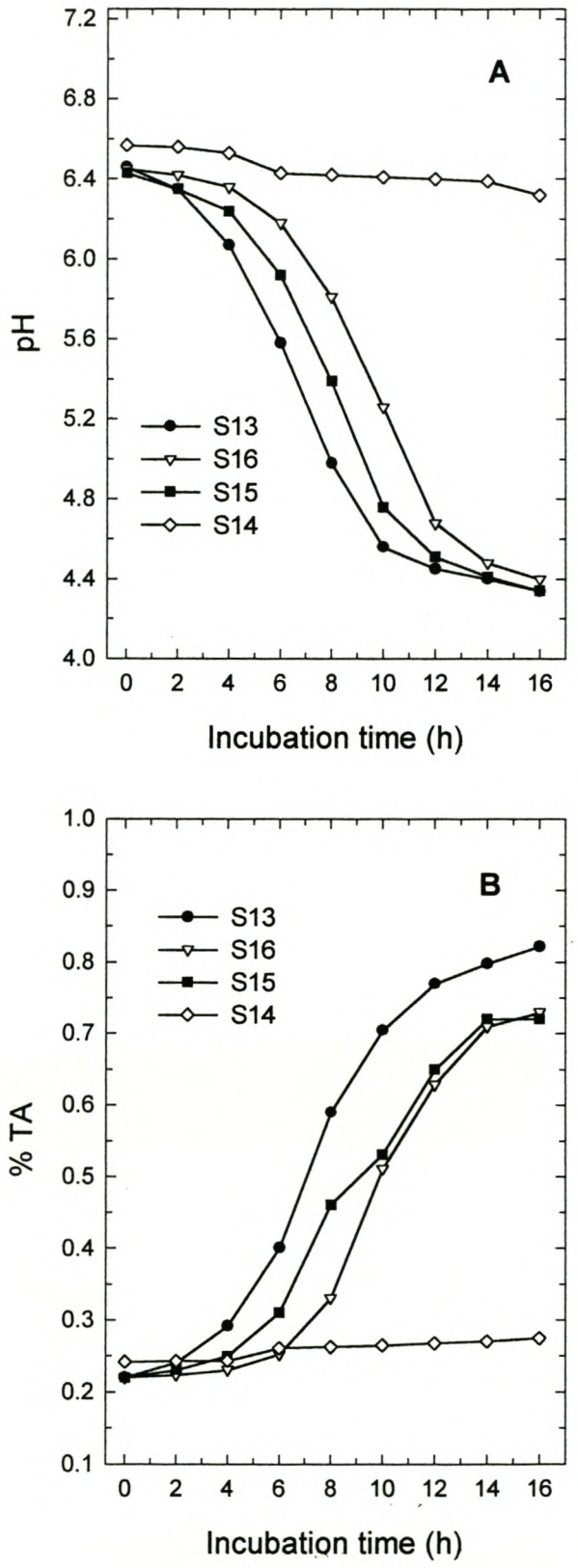

Figure 9. Changes in pH and %TA values during Maas production, incubated at 22°C for 16 h and produced with 1% (v/v) activated *L. lactis* subsp. *lactis* strains.

S13, S15 and S16 are the best acid producers of the four *L. lactis* subsp. *lactis* strains after 16 h at 22°C. These strains reduced the pH of the milk to 4.34, 4.40 and 4.34 (Fig. 9A) with TA values of 0.82, 0.73 and 0.72% (Fig. 9B), respectively. When the 8 h activation (Fig. 6) is compared to the 16 h Maas production incubation (Fig. 9) period of the *L. lactis* subsp. *lactis*, the latter shows the same pattern of pH and TA changes during the two phases. However, its evident from Fig. 9 that strain S13 resulted in the fastest pH decrease and TA increase over the 16 h incubation period. This variation in acid production may also be explained by the presence of different enzymes (Farrow, 1980). Strain S14 showed a minimum pH reduction even after 16 h at 22°C, reaching a final pH of 6.32 and TA of 0.28%. The amount of lactic acid (expressed as %TA) produced by strain S14 shows its weakness as an acid producer.

Strains S18, S19, S20, S21, S23, S24 and S25 of the *L. lactis* subsp. *cremoris* showed no significant pH decreases over the 16 h period at 22°C (Fig. 10A and B). However, strains S17 and S22 (Fig. 10B) showed a pH decrease over the 16 h at 22°C to a final pH of 4.61 and 4.33, respectively. The TA values that correspond to the pH decrease by these two strains after 16 h at 22°C are 0.73 and 0.81% (Fig. 10D), respectively. Although strain S22 showed little acidification during activation (pH = 6.48; TA = 0.28%), higher acidification activity was found after the further 16 h incubation at 22°C. From the strains that showed a possibility of further acidification during activation (S17, S23, S25), only S17 decreased the milk pH after 16 h at 22°C. Hunter (1939) and Salama *et al.* (1995) also observed this type of acid production variation by some strains of *L. lactis* subsp. *cremoris*.

Using acid production as a selection criterion, it can be concluded that nine cultures (S1, S2, S3, S5, S13, S15, S16, S17 and S22) are suitable for Maas production.

Single strain starter cultures – Metabolite production

The flavour of fermented milks is mainly determined by the presence of single or different combinations of volatile organic compounds (VOC) and organic acids (Badings & Neeter, 1980). The compounds considered most important for the flavour quality of fermented milk products are acetaldehyde, diacetyl, lactic and acetic acids. However, studies have shown that other compounds (diacetyl and 2-

figure10

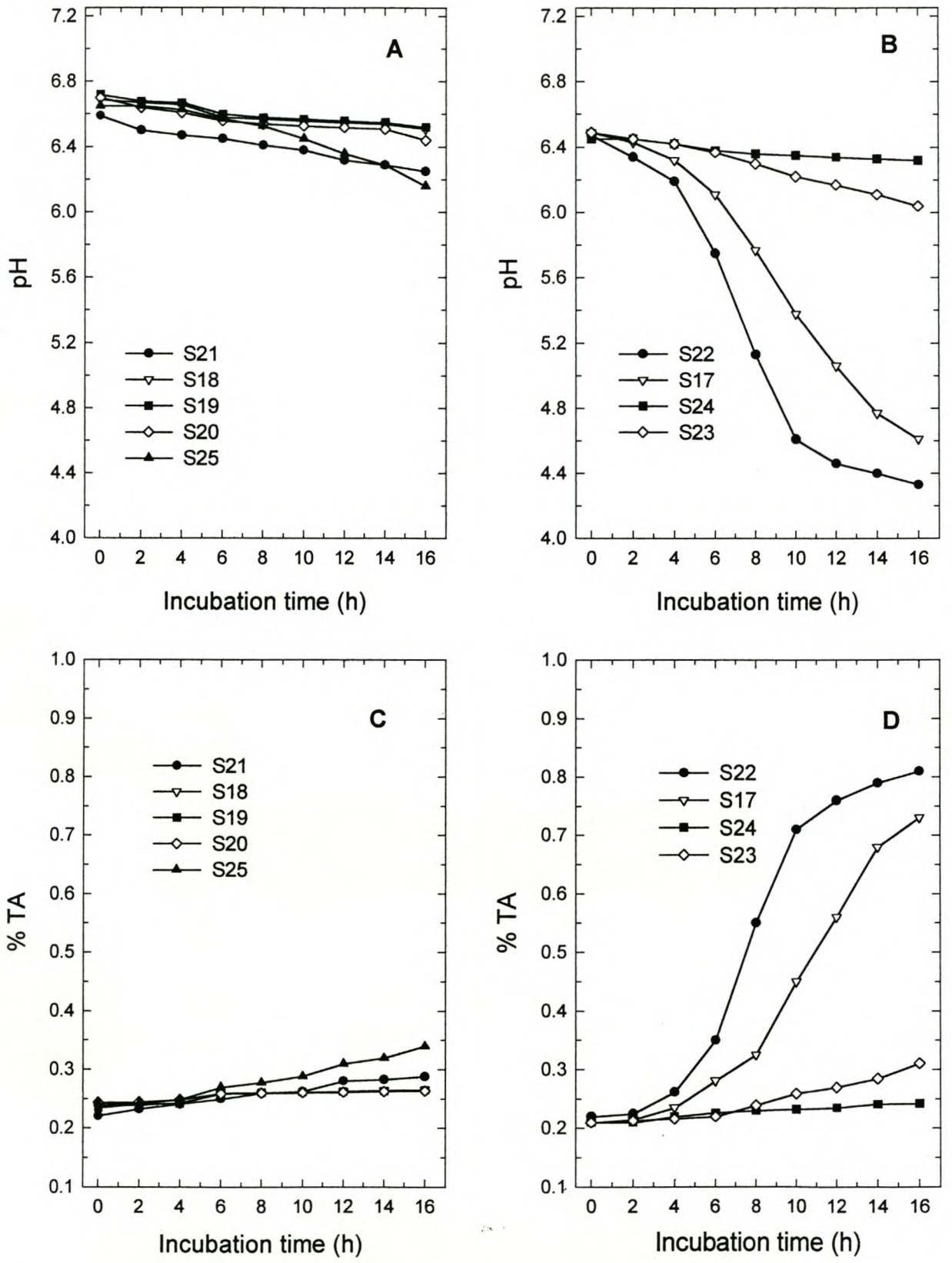

Figure 10. Changes in pH and %TA values during Maas production, incubated at 22°C for 16 h and produced with 1% (v/v) activated *L.lactis* subsp. *cremoris* strains.

butanone) when present in low concentrations, may also strongly contribute to flavour and quality of fermented milks (Kneifel *et al.*, 1992b; Imhof *et al.*, 1994a and 1995).

The concentrations of the specific VOC's (acetaldehyde, ethanol, acetone, diacetyl and 2-butanone) in each of the different Maas products made with the 25 single strains, were sampled after 16 h at 22°C and the data summarised in Table 4.

The commercial starter culture (Table 4) produced the following metabolites after 16 h at 22°C ($mg.l^{-1}$): acetaldehyde (2.4); ethanol (2.5); acetone (0.3); and diacetyl (0.3) but no 2-butanone.

Nine single strain starter cultures (S1, S2, S3, S5, S13, S15, S16, S17 and S22) were chosen for further evaluation because of their ability to strongly acidify the milk to a low final pH which resulted in a sour, well coagulated and/or creamy and/or viscose Maas. The results obtained from this study (Table 4) showed a large variation in acetaldehyde production between these nine strongly acidifying strains of lactococci. After 16 h at 22°C, the nine lactococci strains produced acetaldehyde concentrations ranging between 8.5 and 164.0 $mg.l^{-1}$ at final pH values of between 4.33 and 5.05. These concentrations were much higher than the acetaldehyde concentration reached by the control. The acid producing *L. lactis* subsp. *lactis* biovar *diacetylactis* strain S3 was found to produce the lowest acetaldehyde concentration (8.5 $mg.l^{-1}$) and *L. lactis* subsp. *lactis* strain S16 the highest concentration (164 $mg.l^{-1}$). The accumulation of acetaldehyde by the strains in this study could be the result of the decarboxylation of pyruvic acid, which is formed by transamination of alanine (Tawfik *et al.*, 1993). Another attribute, according to Rash (1990), to increase acetaldehyde levels in fermented milk products could also be high threonine and low glycine concentrations.

Acetaldehyde, which is also commonly produced by LAB species in fermented milk products, is normally a result of the metabolism of pyruvate and threonine (Lees & Jago, 1978). Kang *et al.* (1988) reported that acetaldehyde concentrations of 23 to 41 $mg.l^{-1}$ gave optimal flavour and aroma at pH values between 4.00 and 4.40. It was also reported in the literature that some lactic strains produce this compound only in small concentrations, e.g. *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* and others in larger quantities (Lindsay *et al.*, 1965; Tawfik *et al.*, 1993). Mutukumira *et al.* (1996b) reported an acetaldehyde concentration of about 8 $mg.l^{-1}$ produced by

Table 4. Final pH, metabolite concentration and sensory evaluation of the control and mesophylic strains during Maas production. A 1% (v/v) activation and starter inoculum was transferred to the milk and incubated at 22°C for 16 h.

Strain	Final pH	Metabolite concentration ($mg.l^{-1}$)					Sensory evaluation
		*Acetald	*EtOH	*Acet	*Diacet	*2-But	
Control (commercial starter)	4.57	2.4	2.5	0.3	0.3	-	Effervescent, creamy
L. lactis ss. *lactis* biovar *diacetylactis*							
S1	4.96	66.7	-	1.6	-	-	Slightly creamy, sour, sweet
S2	5.05	36.4	9.1	-	-	-	Sour, watery, effervescent
S3	4.59	8.5	1.6	0.6	-	-	Apple, sour, creamy
S4	6.42	-	1.8	5.5	-	-	Neutral-taste, sweet, watery
S5	4.51	11.5	111.6	1.4	-	-	Sour, creamy, buttery, gritty
S6	6.32	1.6	1.7	4.6	0.8	-	UHT-taste, pleasant smell
S7	6.30	2.9	-	2.1	-	-	Watery, slightly sour, sweet
S8	6.25	4.8	-	2.3	-	-	Watery, sour, sweet
S9	6.25	1.8	3.3	3.6	-	-	Watery, slightly sour, sweet
S10	6.28	2.9	6.6	1.8	-	-	Watery, gritty, sweet
S11	6.41	5.9	56.2	1.6	-	-	Medicine-taste, sweet
S12	6.30	3.3	19.1	5.1	-	-	Watery, slightly sour, sweet
L. lactis ss. l*actis*							
S13	4.34	12.7	9.6	1.9	1.3	0.6	Effervescent, creamy, apple
S14	6.32	9.7	51.9	27.1	-	-	Sweet, watery, cowy
S15	4.34	89.9	838.1	44.5	-	-	Creamy, sour
S16	4.40	164.0	236.7	20.1	-	-	Creamy, apple, ethanolic-taste

Table 4. cont./

Strain	Final pH	Metabolite concentration ($mg.l^{-1}$)					Sensory evaluation
		*Acetald	*EtOH	*Acet	*Diacet	*2-But	
L. lactis ss. *cremoris*							
S17	4.61	23.6	2.0	1.0	0.2	-	Creamy, effervescent, green apple
S18	6.51	2.9	-	24.3	-	-	Milk taste, sweet, watery
S19	6.52	-	-	24.9	-	-	Watery, milk taste, sweet
S20	6.44	13.9	-	17.6	-	-	Cowy, watery, slightly sour
S21	6.25	-	32.1	0.6	-	-	Sulphur smell, sweet
S22	4.33	118.9	348.1	37.0	-	-	Very sour, creamy
S23	6.04	-	3837.1	25.1	-	-	Ethanol-taste, H_2S-taste
S24	6.32	167.5	190.0	47.4	-	-	Cowy, watery, grassy
S25	6.16	-	1300.7	30.6	-	-	Sweet, watery, ethanolic-taste

*Acetald = Acetaldehyde; EtOH = Ethanol; Acet = Acetone; Diacet = Diacetyl; 2-But = 2-Butanone

L. lactis subsp. *lactis* biovar *diacetylactis* and about 1 $mg.l^{-1}$ produced by *L. lactis* subsp. *lactis*.

The ethanol concentration (Table 4) in the Maas after 16 h at 22°C was found to be higher for the single strain starter cultures than for the commercial starter culture (2.5 $mg.l^{-1}$). The maximum ethanol concentration in the Maas made with the nine acid forming strains after 16 h at 22°C was 838.1 $mg.l^{-1}$ (strain S15) with a lowest of 1.7 $mg.l^{-1}$ (strain S3). The presence of high levels of ethanol after 24 h incubation and the low concentration of acetaldehyde suggests that the acetaldehyde produced by these members of the genus *Lactococcus* was possibly, as suggested by Mutukumira *et al.* (1996c), metabolised to ethanol by alcohol dehydrogenase activity.

The acetone levels (Table 4) detected in the Maas after 16 h at 22°C for the nine acid forming single strains were higher than the levels reached by the commercial starter culture. The acetone concentrations ranged between 0.6 and 44.5 $mg.l^{-1}$.

Diacetyl in dairy products is normally formed by the fermentation of milk citrate by *Leuconostoc* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* strains (Jordan & Cogan, 1988). The citrate fermentation ability of these species only occurs after the pH is lowered below 6.00 by the acid producing species (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*). However, it was found in this study that all 25 strains examined were citrate negative (Table 2). Thus, it was expected that the nine single strains would not produce diacetyl. However, *L. lactis* subsp. *lactis* strain S13 (1.3 $mg.l^{-1}$) and *L. lactis* subsp. *cremoris* strain S17 (0.2 $mg.l^{-1}$) did produce diacetyl after 16 h at 22°C. According to Vasavada *et al.* (1985), 1 – 2 $mg.l^{1}$ are considered normal while 8 – 10 $mg.l^{-1}$ are considered excessive quantities of diacetyl in cultured buttermilk. Bottazzi & Dellaglio (1967) also reported that some strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* were found to produce small amounts of diacetyl. Strain S6 produced a diacetyl concentration of 0.8 $mg.l^{-1}$, which according to Vasavada *et al.* (1985), is regarded as a low concentration.

According to Marshall (1984), a lack of diacetyl in the fermented milk products may also occur when the pyruvate is metabolised via "active aldehyde" to acetoin. Other possibilities could possibly be the complex formations between thiamine pyrophosphate and acetaldehyde to form "active aldehyde" (Speckman & Collins,

1968) and furthermore, if NADH is available, diacetyl would be absent (Marshall, 1984).

Keen & Walker (1974) found the presence of only trace amounts of 2-butanone in fermented milk products and in this study only *L. lactis* subsp. *lactis* strain S13 produced a small amount of 2-butanone (0.6 $mg.l^{-1}$) after 16 h at 22°C.

Single strain starter culture – Sensory evaluation

The results of the sensory characteristics of the Maas produced by the 25 single strain starter cultures are also summarised in Table 4. Comments by the panel members show a preference for a product that is creamy, highly viscous with a mild to strong lactic acid taste. These product characteristics are similar to the characteristics recommended during the commercial production of South African Amasi or Inkomasi (Keller & Jordaan, 1990). In fermented milk products, the main flavour compounds are generally assumed to be diacetyl and acetaldehyde (Mutukumira *et al.*, 1996c). The flavour ratio of these two compounds are, however, a subject of controversy in view of their presence and the strong influence of other organic compounds (Kneifel *et al.*, 1992a,b). A diacetyl to acetaldehyde ratio of about 4 : 1 has been suggested to give a typical buttery flavour in cultured milks (Badings & Neeter, 1980; Vedamuthu, 1994). However, the ratios obtained in this study are in contrast to reports in the literature. The Maas produced with the nine strong acid single strains gave ratios higher than 4 : 1 because of the high acetaldehyde concentration. According to Imhof & Bosset (1994b), this is an unfavourable ratio as a "green flavour" could develop in the product. Bodyfelt *et al.* (1989) also reported that the "green flavour" is a characteristic off-flavour due to the formation of high concentrations of acetaldehyde by the starter cultures and will be associated with the flavour of "green apples" or "plain" yoghurt.

From the sensory evaluation it was also evident that, except for the aroma and taste characteristics, a cultured product should appear firm and uniform in appearance. The panel's observations were that the Maas products made from strain S2 had a thin body texture (watery). According to Bodyfelt *et al.* (1989), a frequent cause of thin body textures is inadequate culture activity. Only strains S2, S13 and S17 produced effervescent (gassy) Maas products due to the formation of CO_2. Again, according to Bodyfelt *et al.* (1989), this is commonly found with certain

lactic cultures, but the use of lower incubation temperatures or reduced incubation times may prevent or minimise gassy products. From the sensory evaluation it was concluded that with some process manipulations, *L. lactis* subsp. *lactis* biovar *diacetylactis* (S1, S2, S3, S5), *L. lactis* subsp. *lactis* (S13, S15, S16) and *L. lactis* subsp. *cremoris* (S17, S22) strains would be best suited for the production of a well-balanced, creamy, sour Maas product.

Mixed strain cultures – Two strain combinations

It is common practice to combine an acid producer and an aroma producer during the production of mesophylic fermented milk products (Cogan, 1995). In this section the aroma producing *L. lactis* subsp. *lactis* biovar *diacetylactis* strains (S1, S2, S3, S5) is combined with strains of *L. lactis* subsp *cremoris* (S17, S22) and *L. lactis* subsp. *lactis* (S13, S15, S16), respectively.

The data summarised in Table 5 show the final pH, metabolite concentration and sensory characteristics of Maas produced when using the four *L. lactis* subsp. *lactis* biovar *diacetylactis* strains (S1, S2, S3, S5) in combination with the *L. lactis* subsp. *lactis* strains (S13, S15, S16) and the *L. lactis* subsp. *cremoris* strains (S17, S22), respectively. The milk was inoculated with 1% (v/v) of the respective activated single strain culture required for the combinations and then incubated for 16 h at 22°C.

The pH ranged from 4.65 to 6.47 for the strain combinations of the *L. lactis* subsp. *lactis* biovar *diacetylactis* with *L. lactis* subsp. *cremoris*. According to Marshall *et al.* (1982), a pH range of 4.24 - 4.50 is acceptable for plain, unsweetened yoghurt. However, for bacterial fermentations to reach a maximum viscosity, a pH range of about 4.60 – 4.70 is required (Parnell-Clunies *et al.*, 1986; Dannenberg & Kessler, 1988; Van Vliet & Keetels, 1995). Combination S5S17 reached the lowest pH value (4.65) while the highest pH (6.47) was reached with combination S2S17.

The combinations S1S17, S1S22, S2S17 and S2S22 reached a final pH of 5.61, 5.49, 6.47 and 4.95 after 16 h at 22°C, respectively. Due to the final pH that is "too high" these combinations cannot be used for Maas production because of their low acidification activity and inability to coagulate the milk. It was also observed that the degree of acidification by the combined strains was less than that obtained as individual single strains. Combinations S5S17, S5S22, S3S17 and S3S22

Table 5. Final pH, metabolite concentration and sensory evaluation of two-strain culture combinations used for Maas production. A 1% (v/v) activation and starter inoculum was used and incubated at 22°C for 16 h.

Culture combination	Final pH	Metabolite concentration ($mg.l^{-1}$)					Sensory evaluation
		*Acetald	EtOH	*Acet	*Diacet	*2-But	
S1S17	5.61	5.6	2.6	0.8	0.2	0.7	No coagulation, sweet taste
S1S22	5.49	121.7	-	0.9	0.2	0.4	No coagulation, sweet, apple
S2S17	6.47	3.0	4.0	1.4	-	-	No coagulation, milk flavour and taste
S2S22	4.95	34.3	3.2	1.0	-	-	Effervescent, not thick, pleasant smell, after-taste
S3S17	4.77	25.3	1.0	1.4	0.4	0.4	Smooth, glossy, pleasant smell, creamy, slightly sweet
S3S22	4.85	19.0	0.8	0.7	0.3	0.8	Thick, chunky, strong lactic acid flavour, slightly sour
S5S17	4.65	15.9	11.6	1.3	1.6	0.5	No flavour, not glossy, not creamy, little after-taste
S5S22	4.80	137.0	0.6	0.9	0.4	0.2	Green apple, chunky, slightly creamy
S1S13	5.39	6.6	0.1	0.9	0.7	0.3	No coagulation
S1S15	5.53	2.7	2.5	1.0	-	-	No coagulation
S1S16	5.77	50.1	0.5	0.9	0.4	1.2	No coagulation
S2S13	4.36	12.7	14.6	0.8	-	-	Gritty, more sour, fatty
S2S15	4.38	8.7	45.0	0.9	-	-	Gritty, sour, fatty
S2S16	6.34	-	9.8	0.9	-	-	No coagulation, fatty
S3S13	4.45	21.7	6.8	0.8	0.3	-	Creamy, sour, sweet
S3S15	4.60	14.6	2.3	1.1	0.8	0.2	Glossy, smooth, sweet sour taste, apple smell
S3S16	4.64	70.1	7.1	1.1	0.3	0.1	Sweet, glossy, smooth, apple
S5S13	4.41	6.4	6.8	0.3	0.4	0.2	Fine granular texture
S5S15	4.52	28.3	0.1	1.3	0.1	0.1	Gritty, sweet, sour
S5S16	4.52	3.6	4.4	0.3	1.4	0.3	Gritty, sour, fruity taste

*Acetald = Acetaldehyde; EtOH = Ethanol; Acet = Acetone; Diacet = Diacetyl; 2-But = 2-Butanone

reached final pH values of 4.65, 4.80, 4.77 and 4.75 after 16 h at 22°C, respectively, which were sufficient for the coagulation of the milk.

The strain combination of *L. lactis* subsp. *lactis* biovar *diacetylactis* with *L. lactis* subsp. *lactis* produced a final pH ranging between 4.36 to 6.34 after 16 h at 22°C. The combination of S2S13 reached the lowest final pH, which resulted in the coagulation of the milk. The final pH of S2S13 corresponded with the low final pH of the single strain culture S13 (pH = 4.34) which suggests that the acid producing strain S13 could be the dominant acidifying strain in the combination. The single strain S15 also reached a final pH of 4.34 and suggests that the dominance of this acidifying strain in the combination of S2S15 contributed to a final pH of 4.38. However, the combination of S2S16 only reached a final pH of 6.34 and as a result the milk did not coagulate. Thus, strain S16 which reached a final pH of 4.40 as a single strain, showed less interaction with strain S2 compared with the single strains of S13 and S15 in their respective combinations with strain S2.

The interaction between the three *L. lactis* subsp. *lactis* (S13, S15, S16) and strain S1 showed acidification activity reaching final pH values of 5.53, 5.77 and 5.39, respectively. These acidity values were consequently insufficient to coagulate the milk (Marshall *et al.*, 1982).

The combinations of strains S3 and S5 with strains S13, S15 and S16, respectively, reached a final pH ranging between 4.41 – 4.64. Although these combinations reached higher pH values than the individual acid producing *L. lactis* subsp. *lactis* strains (S13, S15, S16), they did show interaction with the *L. lactis* subsp. *lactis* biovar *diacetylactis* strains (S5, S3) that lead to coagulation of the milk. In the combinations, the acid producing strains of *L. lactis* subsp. *lactis* were probably responsible for the pH decrease as shown by their high acidification activity as single strain cultures.

The production of volatile organic compounds (VOC) using the combinations of *L. lactis* subsp. *lactis* biovar *diacetylactis* with *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* (Table 5) were generally lower compared to the concentrations produced by the single strains (Table 4). In spite of the overall lower VOC concentrations, the acetaldehyde concentrations were, in most cases, still higher than the other volatile organic compounds, ranging between 2.7 – 137.0 $mg.l^{-1}$. Acetaldehyde concentrations of 23 – 41 $mg.l^{-1}$ were reported by Kang *et al.* (1988) to

give optimum flavour in fermented milk products. The high acetaldehyde concentrations of 121.7 and 137.0 $mg.l^{-1}$ produced by the combinations S1S22 and S5S22, respectively, suggest that little acetaldehyde was reduced to ethanol which might lead to the "green flavour" defect in these combinations (Bodyfelt *et al.*, 1989). The single *L. lactis* subsp. *cremoris* strain S22 (Table 4) produced high acetaldehyde concentrations (118.9 $mg.l^{-1}$) and was probably responsible for the high acetaldehyde levels in these combinations. The acetaldehyde concentrations produced by the *L. lactis* subsp. *lactis* biovar *diacetylactis* and *L. lactis* subsp. *lactis* combinations of S1S16 and S3S16 reached 50.2 and 70.1 $mg.l^{-1}$, respectively, suggesting low alcohol dehydrogenase activity (Mutukumira *et al.*, 1996c). The high acetaldehyde concentration of the combinations could possibly be ascribed to the presence of strain S16, which produced 164.0 $mg.l^{-1}$ acetaldehyde as a single strain (Table 4).

Ethanol concentrations (Table 5) of between 0.1 – 45.0 $mg.l^{-1}$ were found for the combinations of L. *lactis* subsp. *lactis* biovar *diacetylactis* with L. *lactis* subsp. *cremoris* and L. *lactis* subsp. *lactis*, respectively, after 16 h at 22°C. In most of the combinations the ethanol concentrations were lower than the acetaldehyde suggesting that low alcohol dehydrogenase activity was present in the combinations (Mutukumira *et al.*, 1996c). The 45.0 $mg.l^{-1}$ of ethanol produced by combination S2S15 could be due to the inclusion of strain S15, which produced high ethanol levels as a single strain (Table 4). However, high levels of ethanol in combinations could not always be accounted for by the high levels in the single strains. The single strains S5 and S22 also produced high ethanol levels (Table 4), but in the respective combinations they did not lead to similar high ethanol concentrations.

The acetone levels (Table 5) produced by the combinations were between 0.3 – 1.4 $mg.l^{-1}$ after 16 h at 22°C. The combinations produced less acetone than the single strains (Table 4) used in the combinations. Only *L. lactis* subsp. *cremoris* strain S22, as single strain culture, produced a high acetone concentration (37 $mg.l^{-1}$), but when it was combined with the *L. lactis* subsp. *lactis* biovar *diacetylactis* strains, lower amounts of acetone were detected.

A maximum diacetyl concentration (Table 5) of 1.6 $mg.l^{-1}$ was reached after 16 h at 22°C by combination S5S17. The combined strains of *L. lactis* subsp. *lactis* biovar *diacetylactis* and *L. lactis* subsp. *cremoris* generally produced more diacetyl

than the respective single strain cultures (Table 4). The quantities of diacetyl produced by the combinations of *L. lactis* subsp. *lactis* biovar *diacetylactis* and *L. lactis* subsp. *lactis* were also more than the diacetyl produced by the single strains (Table 4). The highest level of diacetyl (Table 5) was reached by combination S5S16, producing 1.4 $mg.l^{-1}$ after 16 h, although both strains did not produce diacetyl when cultured as single strains. When the *L. lactis* subsp. *lactis* biovar *diacetylactis* strain S2, which produced no diacetyl as a single strain culture (Table 4), was combined with the respective L. *lactis* subsp. *cremoris* and L. *lactis* subsp. *lactis* strains no diacetyl was detected (Table 5).

The 2-butanone (Table 5) produced by the combinations ranged between 0.1 – 1.2 $mg.l^{-1}$ after 16 h at 22°C. Of all the strains examined as single strains only *L. lactis* subsp. *lactis* strain S13 produced 2-butanone (Table 4), but when combined with the *L. lactis* subsp. *lactis* biovar *diacetylactis* strains no or lower levels were detected. More 2-butanone was however, produced by the combinations than the single strains. The production of 2-butanone therefore appears to depend on the combination used, and the interaction between the single strains involved.

A little after-taste was observed in all the combinations during the sensory evaluation of the Maas. The two-member panel suggested that the after-taste could be due to the MRS-medium due to the fact that the single strain cultures were propagated in the MRS-medium and inoculated directly into the milk for activation. In spite of the flavour defect some combinations were still observed by the panel members to be acceptable in terms of glossiness and creaminess. The combinations of *L. lactis* subsp. *lactis* biovar *diacetylactis* strains S3 and S5 with the *L. lactis* subsp. *cremoris* strains (S17, S22) and *L. lactis* subsp. *lactis* strains (S13, S15, S16) produced sufficient acid levels to coagulate the milk. However, combinations with strain S1 showed no interaction with *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* strains, producing insufficient acid levels to coagulate the milk. On the other hand, combinations of S2 with the respective *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* strains coagulated the milk, but resulted in an unacceptable fatty, gritty body texture, as reported by the panel.

Mixed strain cultures – Three strain combinations

Mixed cultures to produce Maas were formed by combining the two good acid

forming strains of *L. lactis* subsp. *lactis* biovar *diacetylactis* (S3, S5) with two *L. lactis* subsp. *cremoris* strains (S17, S22) and three *L. lactis* subsp *lactis* strains (S13, S15, S16). The milk was inoculated with 1% (v/v) of each of the activated single strain cultures and incubated for 16 h at 22°C. The quantitative results of these experiments are summarised in Table 6.

The combinations reached a final pH range of 4.37 – 4.91 after 16 h at 22°C (Table 6). The combinations of S3S22S16 and S3S17S16 attained the highest and lowest pH values, respectively. According to Mutukumira (1996), a maximum viscosity can be reached at a pH of about 4.60 - 4.70 while over-acidification (pH < 4.50) could result in gritty products whereas a pH higher than 4.70 could result in watery products, depending on the starter cultures and the combinations used.

The results in Table 6 show that acetaldehyde production range between 25.8 – 64.4 $mg.l^{-1}$. Similarly to the studies with the single strain cultures and two-strain combinations, the three-strain combinations also produced high acetaldehyde concentrations. A combination consisting of single strain S16 and the two-strain combination of S5S22 produced the highest level of acetaldehyde. The single strain S16 (Table 4) and two-strain combination S5S22 (Table 5) both produced high acetaldehyde concentrations in the previous studies.

Comparison of the respective two-strain combinations of S5S17 and S5S22 in combination with the *L. lactis* subsp. *lactis* strains (S13, S15, S16), shows that higher concentrations of acetaldehyde were produced with the S5S22 than with the S5S17 combinations. The higher acetaldehyde concentrations could be the result of variations among the different *L. lactis* subsp. *lactis* strains (S13, S15, S16), where single strain S16 produced more acetaldehyde than strains S13 and S15 (Table 4). This probably explains why the data shows that the three-strain combination of S5S17 and S5S22 with strain S16 produces more acetaldehyde than those containing strains S13 and S15. However, in contrast with the S5S17 and S5S22 observations, the combinations containing two-strain S3S17 produced higher acetaldehyde concentrations than combinations with S3S22 when combined with the *L. lactis* subsp. *lactis* strains (S13, S15, S16). The two-strain combinations of S3S17 and S3S22 with single strain S16 produced less acetaldehyde than those containing single strains S13 and S15. Thus, where the single strain S16 was combined with the two-strain combination of S3S22 (S3S22S16), which produced the lowest

Table 6. Final pH, metabolite concentration and sensory evaluation of three-strain culture combinations used for Maas production. A 1% (v/v) activation and starter inoculum was transferred to the milk and incubated at 22°C for 16 h.

Culture combination	Final pH	Metabolite concentration ($mg.l^{-1}$)					Sensory evaluation
		*Acetald	*EtOH	*Acet	*Diacet	*2-But	
S5S17S13	4.64	34.0	5.7	1.6	0.6	-	Chemical smell, sweet, slightly effervescent, bitter, watery
S5S17S15	4.70	29.5	28.8	2.7	2.0	1.3	Slightly effervescent, gritty, flat taste, chemical smell, thick texture
S5S17S16	4.66	45.6	33.7	3.9	4.3	1.2	Chemical taste, slightly gritty, milk taste
S5S22S13	4.62	58.0	47.8	4.3	2.1	-	Sharp bitter taste, slightly effervescent, sour taste, creamy
S5S22S15	4.64	61.6	25.0	4.0	2.8	0.3	Slightly effervescent, bitter, after-taste, creamy
S5S22S16	4.62	64.4	23.4	4.0	0.8	-	Very effervescent, sweet, chemical taste, creamy
S3S17S13	4.40	53.8	47.9	3.0	1.0	-	Thick texture, acid taste, creamy
S3S17S15	4.65	51.1	23.4	3.1	0.9	-	Glossy, watery, slight chemical taste
S3S17S16	4.91	32.4	14.5	2.1	2.3	0.8	Effervescent, watery, sweet, milk taste
S3S22S13	4.43	42.1	18.0	2.5	0.6	-	Cowy, nice consistency, bitter taste
S3S22S15	4.59	30.4	21.2	4.4	1.8	0.6	Effervescent, tasteless, glossy, slight after-taste
S3S22S16	4.37	25.8	23.7	2.1	-	-	Gritty, bad chemical smell, smooth

*Acetald = Acetaldehyde; EtOH = Ethanol; Acet = Acetone; Diacet = Diacetyl; 2-But = 2-Butanone

acetaldehyde concentration (25.8 $mg.l^{-1}$), the single strain S16 did not influence the net production of acetaldehyde.

In this study (Table 6), the three-strain combinations produced ethanol concentrations ranging between 5.7 – 47.9 $mg.l^{-1}$ after 16 h at 22°C. However, the levels of ethanol in relation to the high acetaldehyde concentrations suggest that low alcohol dehydrogenase activity existed in the combinations (Mutukumira *et al.*, 1996c).

Acetone concentrations (Table 6) for the three-strain combinations were found to range between 1.6 – 4.4 $mg.l^{-1}$ after 16 h at 22°C. The acetone detected was also higher than the acetone produced by the two-strain combinations (Table 5). The highest acetone concentrations were reached by combinations S3S22S15 and S5S22S13.

The diacetyl concentration (Table 6) formed by the combinations ranged between 0.6 – 4.3 $mg.l^{-1}$, with combination S5S17S16 producing the highest concentration. The three-strain combinations produced more diacetyl than the respective single strains (Table 4) and two-strain combinations (Table 5). This finding suggests a better synergetic effect when these specific combinations (Table 6) are used.

The 2-butanone concentrations (Table 6) produced by the three-strain combinations were fairly low and ranged between 0.3 – 1.3 $mg.l^{-1}$. The combination of S5S17S15 produced the highest 2-butanone concentration, while all the combinations containing single strain S13 produced no 2-butanone.

Maas made with the three-strain combinations and then sensory evaluated were also observed to have a "chemical" after-taste. Once again this was probably due to the culturing in the MRS-medium. The results of the sensory evaluation also showed that some of the Maas samples were slightly effervescent (gassiness). However, a moderate degree of carbonation is generally produced in some fermented milk products (Bodyfelt *et al.*, 1989) and gassiness is normally not considered a defect in a fermented milk product. The Maas from combinations S3S17S15, S3S22S15, S3S22S13 and S3S22S16 was observed to be either too watery, gritty, cowy or sweet.

The evaluation of the combinations containing single strain S5 showed poor physical characteristics with a gritty and watery body texture. According to Bodyfelt

et al. (1989), an inadequate culture activity is mostly responsible for thin body textures. In contrast, the appearance and consistency of the Maas made with the combinations containing single strain S3, especially S3S17S13, had a very thick body texture under the time-temperature conditions used in this study. The physical characteristics of the Maas made with this combination also showed that the rate of acid production at 22°C resulted in a good body texture, which was not affected by the specific combination of strains. Thus, combination S3S17S13 was found to produce an acceptable Maas that showed the typical characteristics of commercially produced Maas, but the highly acceptable flavour was not pronounced enough.

Influence of the MRS-medium on the final Maas product

In the previous sensory evaluations of the Maas samples (Tables 5 and 6) a slight, but bitter chemical taste and smell were detected. In the panel members' opinion, the MRS-medium on which the stock cultures were grown was probably the cause. To exclude this from the final Maas product, the starter cell suspensions that were cultured in MRS-medium were centrifuged and the cell precipitate washed twice with a sterile saline solution. After decanting the supernatant, the cells were suspended in pasteurised milk (the volume of milk used equalled the volume of MRS-medium decanted) and the mixture vortexed for use in the subsequent studies. Sensory evaluation using four combinations (Table 7) was then again implemented. These combinations included combination S3S17S13 to determine if any improvement in taste and smell could be facilitated. The data obtained from this second sensory evaluation (Table 7) showed that the bitter chemical taste and smell were absent. In the case of the S3S17S13 combination, where the bitter chemical taste was absent in the first evaluation, the removal of the MRS-medium by washing the cell suspension did not cause any change in the Maas characteristics. Therefore, it was concluded that the bitter chemical taste is caused by the MRS-medium present in the final product. It is recommended that after culturing the starter cultures in the MRS-medium, the MRS-medium must be replaced with milk.

Optimization of production parameters

Due to the absence of a highly acceptable flavour, good body texture and a too strong acid taste in the final Maas from the previous study, certain production

Table 7. Sensory evaluation of four starter culture combinations for Maas production. A 1% (v/v) activation and starter inoculum was transferred to the milk and incubated at 22°C for 16h after centrifuging the cell suspension. Centrifugation and subsequent suspension in full cream milk was used to exclude the MRS-medium taste and smell from the Maas production.

Starter culture combination	Sensory evaluation*
S3S17S15	Slightly gritty, glossy, lactic acid taste
S3S22S15	Effervescent, glossy, sweet
S3S22S13	Gritty, sweet, ethanol taste
S3S17S13	Thick, creamy, lactic acid taste

*No chemical taste or smell detected.

manipulations were necessary to elevate flavour production and increase body texture. This was accomplished by increasing the inoculation percentage from 1 to 2% (v/v) and then reducing the incubation time from 16 to 14 h, for both the culture activation and starter inoculum (Maas production) steps.

It is known that the inoculation level has an impact on the incubation and vice versa. According to Champagne *et al.* (1992), mesophylic lactic acid bacteria have a generation time of 140 min at 21°C. Thus, if the milk was inoculated with twice the normal amount, it can be expected that a slightly reduced incubation time would be required. A 2% (v/v) inoculum was also used to prevent the production of too thin body textures, which is frequently caused by inadequate culture activity (Bodyfelt *et al.*, 1989). When the inoculation rate for cultivating starters is changed, the incubation time must also be considered (Meriläinen, 1988). Therefore, the incubation time was reduced from 16 to 14 h to compensate for the increase in inoculation size. The results of these changes are given in Table 8 and discussed in the next section. It has also been reported that a reduction in the incubation time could minimise gas (CO_2) formation in the final product (Bodyfelt *et al.*, 1989). Furthermore, in this study the CO_2 formation was pronounced in the single strain cultures, S13 and S17 and could thus also be minimised by the reduction in incubation time.

Maas production using combination S3S17S13 and additional flavour producing cultures

Cultures used in the production of fermented milk products should impart a pleasing "bouquet flavour" which results from the overall blend of a delicate, diacetyl (buttery-like) aroma and a distinctly clean acid taste (Bodyfelt *et al.*, 1989). From the previous study, it was evident that combination S3S17S13 was the most acceptable for Maas production. The Maas production using this combination had a creamy, thick body texture and acid taste, but lacked the typical Maas flavour. This reflects the product characteristics of commercially available Maas, but not the flavour. The "lacks flavour" defect, according to Bodyfelt *et al.* (1989), is characterised by the absence of the desired aroma and is often associated with an acid taste. Also, it may simply be due to the selection of inappropriate flavour producing strains.

Lactococcus lactis subsp. *lactis* biovar *diacetylactis* and *Leuconostoc* species

Table 8. Final pH, metabolite concentration and sensory evaluation of four-, five- and six-strain culture combinations used for Maas production. A 2% (v/v) activation and starter inoculum was transferred to the milk and incubated at 22°C for 14 h.

Starter type	Culture combination	Final pH	Metabolite concentration ($mg.l^{-1}$)					Sensory evaluation
			*Acetald	*EtOH	*Acet	*Diacet	*2-But	
Control		4.57	2.4	2.5	0.3	0.3	-	Effervescent, creamy
A	S3S17S13 L1	4.52	69.2	16.4	1.5	1.4	0.3	Slightly watery, pleasant aroma, glossy, buttery
B	S3S17S13 L2	4.49	33.6	22.5	1.9	1.6	-	Gritty, no aroma, creamy
C	S3S17S13 L1L2	4.57	26.6	8.1	0.9	-	-	Smooth, creamy, glossy
D	S3S17S13 L1L2S22	4.50	89.3	28.9	3.6	0.4	-	Creamy, sour, slightly sweet

*Acetald = Acetaldehyde; EtOH = Ethanol; Acet = Acetone; Diacet = Diacetyl; 2-But = 2-Butanone
L1 = *Leuconostoc mesenteroides* subsp. *dextranicum* 235; L2 = *L. mesenteroides* subsp. *citrovorum* 147

are of the most common flavour producers found in fermented foods (Cogan & Jordan, 1994). However, according to Levata-Jovanovic & Sandine (1997), the associative culturing of lactococci and leuconostocs need compatible strains and also sufficient numbers of the bacteria that produces acid and flavour. The *Leuconostoc* species do not produce significant amounts of acid in milk, but they produce aromatic end-products during citrate utilisation in association with the acid producing lactococci strains (Cogan & Jordan, 1994; Vedamuthu, 1994).

To enhance flavour production in the final Maas product, *Leuconostoc mesenteroides* subsp. *dextranicum* 235 (starter A) and *L. mesenteroides* subsp. *citrovorum* 147 (starter B) (Table 8) were added (2% v/v inoculum) to combination S3S17S13, respectively. Furthermore, a combination of S3S17S13 with both *Leuconostoc* species (starter C) was also formed. The S3S17S13 combination with both *Leuconostoc* species (starter C) was also combined with *Lactococcus lactis* subsp. *cremoris* strain S22 (starter D). The inclusion of strain S22 (2% v/v inoculum) was based on its favourable acid and metabolite production and sensory characteristics with the three-strain combinations (Table 6). The full cream pasteurised milk that had been inoculated with each of the starter types (A, B, C and D) was then incubated for 14 h at 22°C.

Data for the final pH, metabolite concentration and sensory evaluation of the control (commercial starter) and the four starter types (A, B, C and D) are summarised in Table 8. The four starters (A, B, C, D) reached pH values ranging between 4.49 – 4.57 after 14 h at 22°C (Table 8) with the lowest value obtained with starter B. It is the primary aim of a dairy fermentation to generate lactic acid (Davidson & Hillier, 1994), which lowers the pH to a level that inhibits the growth of many spoilage organisms thereby adding to the flavour of the product. It was therefore concluded that, in terms of acid production, all four combinations reached acceptable pH levels.

From the data summarised in Table 8, it can be seen that the control culture produced lower concentrations of all the metabolites determined when compared to the four starters (A, B, C and D). The higher metabolite concentrations produced by the combinations could possibly be the result of favourable competition between the different microorganisms in the four starters to metabolise the various primary sources for the flavour compounds (Table 9).

Table 9. Flavour compounds produced by microorganisms in fermented milk products (Blanc, 1984).

Metabolite	Strain(s) responsible	Source	
		Primary	Secondary
Acetaldehyde	*Lactobacillus delbrueckii* subsp. *bulgaricus* *Streptococcus salivarius* subsp. *thermophilus*	Lactose transformation	Amino acid transformation
Diacetyl	*S. salivarius* subsp. *thermophilus*	Citric acid transforamtion	Lactose transformation
Acetone	*L. delbreuckii* subsp. *bulgaricus* *L. acidophilus* *S. salivaruis* subsp. *thermophilus*	Citric acid transformation Lactose and fat transformation	Lactose transformation Thermal degradation of fat
2-Butanone	Various lactic strains	Lactose and fat transformation	Thermal degradation of fat
Ethanol	Various lactic strains	Lactose transformation	–

Higher acetaldehyde concentrations (Table 8) were produced by starters A and D (69.2 and 89.3 $mg.l^{-1}$), respectively, when compared to that produced by the similar three-strain combination S3S17S13 (Table 6) without the respective *Leuconostoc* species and strain S22. It must be taken into consideration that the parameters in this study had been changed to 2% (v/v) inoculums (Table 8) instead of 1% (v/v) (Tables 4, 5, 6 and 7) and the incubation time reduced to 14 h (Table 8) instead of 16 h (Tables 4, 5, 6 and 7). According to Badings & Neeter (1980), high acetaldehyde concentrations could also be produced if the ratio of the *L. lactis* strains to the *Leuconostoc* population is higher. Lower acetaldehyde concentrations of 33.6 and 26.6 $mg.l^{-1}$ were detected with starters B and C, respectively. This suggests that a further metabolisation of acetaldehyde to other flavour metabolites in the presence of the *Leuconostoc* species, as shown in Fig. 11, had taken place. It has also been reported in the literature that dairy leuconostoc strains (Keenan & Lindsay, 1966; Lees & Jago, 1978) have high alcohol dehydrogenase activity and could efficiently reduce acetaldehyde to ethanol.

Starter D produced the highest amount of ethanol (28.9 $mg.l^{-1}$) (Table 8), while the concentration for the other combinations ranged from 8.1 – 22.5 $mg.l^{-1}$. The amounts produced by the four culture combinations were lower than the amounts produced by the three-strain combination of S3S17S13 (Table 6). The ethanol could be produced by the *Leuconostoc* strains during heterofermentation of the lactose present in the milk (Mutukumira *et al.*, 1996c) as these strains have high alcohol dehydrogenase activities (Keenan & Lindsay, 1966; Lees & Jago, 1978).

Acetone concentrations (Table 8) were found to vary between 0.9 – 3.6 $mg.l^{-1}$ after 14 h incubation at 22°C. The highest acetone concentration was produced by culture combination D, which was also higher than the concentration produced by the three-strain combination S3S17S13 (Table 6).

No diacetyl (buttery flavour) was produced (Table 8) by starter C, while starters A, B and D produced low diacetyl concentrations of 1.4, 1.6 and 0.4 $mg.l^{-1}$, respectively. In starters A and B, relatively more diacetyl was produced in comparison with that produced by the three strain combination S3S17S13 (1.0 $mg.l^{-1}$), but their acetaldehyde concentrations were still overwhelming. A diacetyl:acetaldehyde ratio of 4:1 (Badings & Neeter, 1980; Vedamuthu, 1994) has been suggested to give an acceptable buttery flavour, but in this study acetaldehyde

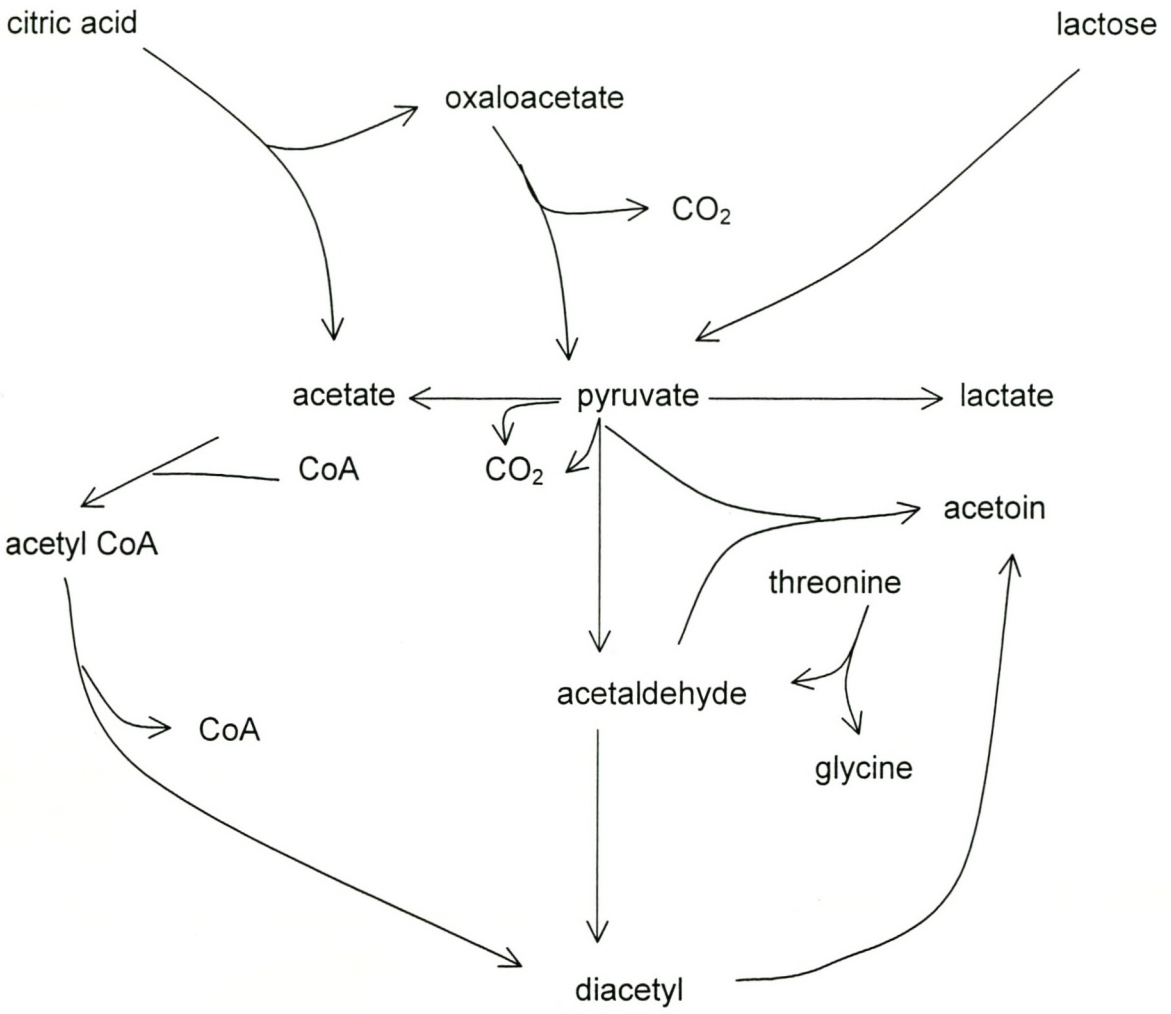

Figure 11. The main metabolic pathways for flavour production in fermented milk products (Marshall, 1982).

was produced in much higher concentrations than diacetyl. Combining the two *Leuconostoc* strains with S3S17S13 in starter C clearly influenced the ability of combination S3S17S13 to produce diacetyl from the citrate metabolic pathway. This can be seen from Table 6 where combination S3S17S13, in the absence of the two *Leuconostoc* subspecies, produced a diacetyl concentration of 1.0 $mg.l^{-1}$. Starter C with the two *Leuconostoc* strains (Table 8) did not produce any diacetyl. This could be due to the conversion of puryvate from citrate metabolism to lactate, acetate, ethanol and CO_2 (Marshall, 1982; Hugenholtz, 1993; Bellengier *et al.*, 1994; Cogan & Jordan, 1994). Although starter D produced lower diacetyl concentrations than the three strain combination S3S17S13 (Table 6), acetaldehyde concentrations were still higher than the diacetyl concentrations. Again, it must be pointed out that a 2% (v/v) inoculum and 14 h incubation time was used instead of the 1% (v/v) inoculum and 16 h incubation time for the three strain combinations. The production of diacetyl in fermented milk products by pure cultures of the leuconostocs occurs at pH values lower than 5.2 (Vedamuthu, 1994), but from this study it can be seen that in combinations with other cultures their ability to produce diacetyl was possibly suppressed.

In this study, the presence of only trace amounts of 2-butanone were detected in the Maas made with starter A (0.3 $mg.l^{-1}$). Scarpellino & Kosikowski (1962) described a pathway for the formation of 2-butanone from diacetyl via 2,3 butyleneglycol. Thus, in the absence of diacetyl as precursor, the production of 2-butanone would not be possible, as the data for starter C (Table 8) shows. However, results obtained by McGugan *et al.* (1968) suggested that the presence of 2-butanone could arise from the inclusion of an organism that is able to produce 2-butanone by other pathways.

The results of the sensory evaluation of the Maas made with the multiple strain cultures are also shown in Table 8. The Maas made with starters A, C and D were characterised as having a pleasant aroma with a creamy, glossy body texture. This was achieved by using pasteurised full cream milk, fermented at 22°C for 14 h and with a 2% (v/v) inoculum, which is double the recommended inoculum size (Human, 1998). However, in spite of having a creamy body, starter B had no strong aroma development and a gritty texture. It could thus be concluded that with starters A, C and D, it was possible to make an acceptable Maas product having a pleasant

sour taste and delicate flavour.

Conclusions

The data from this study showed that the best pH decrease was obtained with nine of the 25 mesophylic single strain starter cultures. These strains were able to reach pH levels between 4.33 - 5.05 after 16 h incubation at 22°C using a 1% (v/v) inoculum. The study also showed that specific *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* strains (flavour producers) showed promise as acid producers. It was thus concluded that these nine single strains, based only on acid production, would be suitable for acceptable acid formation during Maas production.

Data obtained on the two-strain combinations formed between *L. lactis* subsp. *lactis* biovar *diacetylactis* strains (S1, S2, S3, S5), *L. lactis* subsp. *lactis* strains (S13, S15, S16) and *L. lactis* subsp. *cremoris* (S17, S22), showed a good pH decrease with certain combinations. Thus, the combination of flavour and acid producers was in some instances suitable for acid production depending on the symbiotic relationship between the strains. The metabolites formed in the Maas made using the two-strain combinations showed that acetaldehyde was mostly produced in higher concentrations. Low (< 1 $mg.l^{-1}$) to normal quantities of diacetyl, which formed one of the major flavour metabolites in fermented milk products, were also detected in the Maas. The Maas made using the two-strain combinations, (S3S17, S3S22, S5S17 and S5S22) resulted in thick, creamy and smooth characteristics from the sensory evaluation with an average pH of 4.77.

Results of the three-strain combinations formed between the four two-strain combinations and the *L. lactis* subsp. *lactis* strains (S13, S15, S16) showed a further decrease in the pH to an average of 4.60. Metabolite concentrations were also higher for the three-strain combinations than the two-strain combinations with an increase in the ethanol, acetone, diacetyl and 2-butanone concentrations. However, acetaldehyde was still the major flavour metabolite produced by the three-strain combinations. Sensory evaluation of the three-strain combination showed that S3S17S13 produced the most acceptable Maas under the time-temperature conditions with thick, creamy and acid-taste characteristics. Thus, this combination produced sufficient acid for coagulation of the milk, as well as fairly high quantities of

the flavour metabolites but the Maas flavour was still too weak.

The change in production parameters (2% (v/v) inoculum and 14 h incubation time) resulted in a final pH similar to that reached with the 1% (v/v) inoculum and 16 h incubation time parameters. Combinations of S3S17S13 with *L. mesenteroides* subsp. *dextranicum* and two *Leuconostoc* strains resulted in lower and more acceptable acetaldehyde concentrations. It is therefore concluded that starters A, C and D produced a very acceptable Maas product. These combinations will be further investigated in a full-scale sensory evaluation of fruit flavoured Maas.

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CHAPTER 4

SENSORY EVALUATION OF MAAS PRODUCED WITH DIFFERENT FRUIT FLAVOURS

Abstract

The acceptance of dairy products by consumers is generally determined by the product's sensory attributes. These characteristics are a result of various changes in the product and the surrounding environment. The results of this study showed that the type of flavours (apple, naartjie, strawberry, banana, fig, watermelon, caramel, mango, peach, grape and blackcurrant) used significantly influenced the appearance, smoothness, flavour intensity and overall acceptability of the final Maas product. The sensory evaluation showed that banana flavoured Maas had preference and the mango and peach flavoured Maas were least preferred. The flavoured Maas made with the three starter culture combinations (A, C and D) were found to differ in sweetness and overall acceptability, with culture combination C being more acceptable and sweeter than culture combination D. The shelf-life of the Maas prepared with the three starter culture combinations (A, C and D) displayed a decrease in the pH-value to 4.45, 4.50 and 4.44, whilst the TA increased to 0.86, 0.88 and 0.83% after 22 d, respectively. During storage at 5°C, no coliforms were detected during the shelf-life studies. Sensory evaluation done during the shelf-life period showed that yeasty and effervescent values gradually increased over the 22 d period. The Maas prepared with the three starter culture combinations (A, C and D) was found to be the most favourable after 15 d during the 22 d shelf-life study.

Introduction

The consumers' acceptance of dairy products is generally determined by the products' sensory characteristics (Watson & McEwan, 1995). These characteristics are influenced by various intrinsic and extrinsic factors. It has even been reported

that the tactile properties of some food products can influence consumer acceptability to the same extent as taste and aroma (Schultz *et al.*, 1967). Many of these factors change during storage, thus altering the sensory characteristics (Watson & McEwan, 1995). These changes are important, especially in deciding the product's shelf-life.

The general sales successes of fermented milk products are normally not based on the nutritive and medical aspects, but rather on the organoleptic qualities (Mocquot & Hurel, 1970). In fact, fermented foods are pleasant to consume because of their freshness, their flavour and consistency. It is therefore, imperative that fermented milk products exhibit a delicate, desirable aroma in addition to the perceived clean, lactic acid taste (Bodyfelt *et al.*, 1989). Moreover, it is easy, with the addition of varying flavourings and fruits to produce a wide range of highly acceptable products which can satisfy the most varied taste (Mocquot & Hurel, 1970). This is probably the reason for the considerable development in many countries over the last few years of fermented milk products prepared on an industrial scale.

Traditional firm yoghurts are normally flavoured with fruity extracts, suitable flavours and colours, and then sweetened. These substances are usually added before pasteurisation and they slightly slow down the fermentation process. The addition of artificial flavours makes the production of flavour by the yoghurt cultures less important (Mocquot & Hurel, 1970). However, a new consumer trend is developing where the consumer wants a natural product without any additives.

Sensory analysis is an analytical tool, which can be used in all areas of food and drink production, including quality control and the measurement of shelf-life (Lyon *et al.*, 1992; Watson & McEwan, 1995). It has been shown that sensory analysis has a definite role to play in the dairy industry. Mutukumira (1995) reported that sensory evaluation panels, when evaluating natural fermented dairy products, showed a preference for a product that was creamy and highly viscous with a mild lactic acid acidity. Claassen & Lawless (1992) proposed a more descriptive sensory profile and developed a consumer oriented vocabulary that was found to be simpler and more sensitive than the traditional dairy judging.

In this study three unflavoured Maas samples prepared with three different starter culture combinations (A, C and D), were flavoured with 10 fruit and one outlandish flavour and then sensory evaluated with the three unflavoured Maas samples serving as controls. The shelf-life of the three natural unflavoured Maas products was then tested over a 22 d period.

Materials and methods

Milk preparation

Full cream pasteurised milk was obtained from local supermarkets and given an additional heat treatment in a thermostatically controlled waterbath at 80°C for 20 min and then cooled to 22°C before inoculation. The additional heat treatment led to the whey proteins having a greater binding activity and gave a better structure and texture to the final product (Training Board for the Dairy Industry, 1997). The heat treatment was also necessary to standardise the microbial quality (Human, 1998).

Starter cultures

From the previous study (Chapter 3 of this thesis), three starter culture combinations (A, C and D) containing six different bacterial strains, were used (Table 1). These cultures consisted of strains of the species *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp *cremoris*, *L. lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *dextranicum* and *L. mesenteroides* subsp. *citrovorum*.

The bacterial strains were incubated in MRS-medium at 30°C until a cell concentration of 1×10^8 cfu.ml^{-1} was reached. The cell suspension was then centrifuged and the cell precipitate washed in a sterile saline solution. After decanting, the cells were suspended in pasteurised milk and vortexed. A 2% (v/v) inoculum of each of the six single strains was then made into pasteurised milk and incubated for 8 h at 22°C. A 2% (v/v) inoculum of the activated starter culture combination was then inoculated into one litre of pasteurised milk and incubated for 14 h at 22°C. The Maas was then kept at 4°C for 24 h before each sensory evaluation session started.

Table 1. The three culture combinations used in this study.

Starter culture	Combination and strains
A	S3S17S13L1: *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (S3); *L. lactis* subsp. *lactis* (S13); *L. lactis* subsp. *cremoris* (S17); *Leuconostoc mesenteroides* subsp. *dextranicum* (L1)
C	S3S17S13L1L2: *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (S3); *L. lactis* subsp. *lactis* (S13); *L. lactis* subsp. *cremoris* (S17); *Leuconostoc mesenteroides* subsp. *dextranicum* (L1); *L. mesenteroides* subsp. *citrovorum* (L2)
D	S3S17S13L1L2S22: *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (S3); *L. lactis* subsp. *lactis* (S13); *L. lactis* subsp. *cremoris* (S17); *Leuconostoc mesenteroides* subsp. *dextranicum* (L1); *L. mesenteroides* subsp. *citrovorum* (L2); *L. lactis* subsp. *cremoris* (S22)

Treatment design

A 3 × 12 factorial design was used giving 36 treatment combinations, with the following experimental factors: the culture combinations (A, C or D); the fruit flavours; and the unflavoured Maas as control. The fruit and outlandish flavours are listed in Table 2.

Sensory evaluation

The sensory panel consisted of 13 trained members who evaluated the Maas products for flavour intensity, appearance, sourness, sweetness, smoothness and overall acceptability. At least 10 members of the panel were used in every evaluation session depending on the availability of each panellist, as lower numbers would make the data statistically negligible (Snedecor & Cochran, 1989).

The quantitative assessment of the Maas was carried out under white illumination in a well air-conditioned room to minimise potential bias (Bodyfelt *et al.*, 1989). The Maas was cooled to 4°C before being presented to the members at room temperature (20° ± 3°C) in 125 ml sample cups with lids, filled with 80 ml Maas. The samples were presented monadically and in random order, with three samples per session. Each of the three samples, which represented the three different starter culture combinations, was inoculated with the same flavour with every evaluation session.

The quantitative evaluation was undertaken by recording the perceived intensity of each attribute on a 100 mm (1 mm = 1 unit) line scale on which only the lowest and highest values for each attribute were indicated (Fig. 1). Panel members were required to indicate their preference by making a vertical tick on each line. The distance from the left of the tick was subsequently measured. The sensory profiling of the Maas took place in triplicate over a three week period.

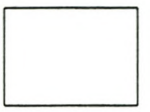
Experimental design

A split-plot design (Snedecor & Cochran, 1989) with the flavourants as main treatment and the three culture combinations as the sub-plot treatments was

Table 2. Type of flavours used to flavour the Maas prepared with the three starter culture combinations (A, C and D).

Type of flavour	Flavour	Brand name*	Dosage (%) (m/v)
1	Apple 316608	H&R	0.2
2	Naartjie 3696 B7	IFF	0.2
3	Strawberry 1805	H&R	0.2
4	Banana 390008	H&R	0.2
5	Fig 3568	H&R	0.2
6	Watermelon 5793	H&R	0.2
7	Caramel (outlandish) 20984	H&R	0.2
8	Unflavoured (control)		-
9	Mango 14484	H&R	0.2
10	Peach 15887	H&R	0.2
11	Grape FV 4028	DW	0.2
12	Blackcurrant 64570	H&R	0.2

*H&R = Haarman & Reimer; IFF = International Flavours and Fragrances; DW = Duckworth

Panellist: _____ Date: _____ Sample 

1. Sourness

None — Extreme

2. Sweetness

None — Extreme

3. Appearance

Dull — Glossy

4. Smoothness

Gritty — Smooth

5. Flavour Intensity

Very Weak — Very Strong

6. Overall acceptability

Not acceptable — Just acceptable — Very acceptable

7. Name of flavour

_____

8. General comments:

Figure 1. Sensory evaluation form used by the panel member to evaluate the flavoured Maas products.

repeated in three blocks. The advantage of this design was that each member had only three samples to evaluate during each tasting session.

Statistical analysis

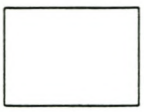
Data were tested for normality and heterogeneity of variance prior to analysis and it was found that the data had a normal distribution and no transformation was needed. An analysis of variance for the split-plot design was performed on the data using SAS software (SAS/STAT Software: Release 6.12, 1996). All significant effects ($p \leq 0.05$) were further investigated by means of the pairwise Student t-LSD (Least Significant Difference) test ($p \leq 0.05$) for specific treatment differences (Snedecor & Cochran, 1989).

Shelf-life determination

The shelf-life evaluation was done over a period of 22 d (Liebenberg, 1998) on the three culture combinations used to make the natural Maas. According to Venter (1999), a shelf-life of 22 d is commonly found for commercially available fermented milk products. The determination of the pH, percentage titratable acidity (James, 1995), total coliforms (Harley & Prescott, 1993) and sensory evaluation of the Maas was used as shelf-life parameters. The sensory evaluation was done using the evaluation form presented in Fig. 2.

Analytical procedures

The percentage titratable acidity (TA) was determined by titrating 10 ml of each Maas sample against standardised sodium hydroxide (0.1 M NaOH) with phenolphthalein as indicator (James, 1995). The pH of each Maas sample was measured using a Knick Portamess 751 Calimatic (West Germany) pH meter. The coliform test was done according to Harley & Prescott (1993) with Violet-Red Bile Agar (Merck).

Panellists: _____ Date: _____ Sample 

1. Sourness

None — Extreme

2. Sweetness

None — Extreme

3. Yeasty (cheesy)

None — Extreme

4. Effervescent (gassy)

None — Extreme

5. Smoothness

Very Weak — Very Strong

6. Appearance

Dull — Glossy

7. Overall acceptability

Not acceptable — Very acceptable

Figure 2. Sensory evaluation form used by the panel members to evaluate the natural Maas prepared with the three starter

Results and discussion

Choice of parameters

From previous data shown in Chapter 3, it was observed that fermentation periods of 16 h resulted in overacidification and gritty products because of the metabolic breakdown of the volatile organic compounds. As a result, the fermentation periods for this study were reduced to 14 h at 22°C. In this study, the three culture combinations (A, C and D) were furthermore used with an activation and starter inoculum of 2% (v/v). It was concluded from the data in Chapter 3, that an inoculum concentration of 1% (v/v) at 22°C resulted in Maas properties that lacked the typical Maas flavour. With these production parameters (2% (v/v), inoculum and 14 h incubation), the final Maas products were found to be highly acceptable with the three culture combinations (A, C and D) and each developed a highly acceptable characteristic Maas taste and aroma.

Panel members

Although differences occurred due to personal preferences, the panel members were able to consistently evaluate the samples over the three replications thereby making all the data obtained from the sensory evaluation usable.

Characteristics that are generally associated with commercially available products were identified as creamy, highly viscous and a mild lactic acid taste (Mutukumira, 1995). The panellists were therefore asked to identify the primary attributes that they considered important for an acceptable Maas product, before performing the sensory evaluation of the Maas. The panellists subsequently identified characteristics like sourness, sweetness, firm coagulum (appearance and smoothness) and flavour to be important when considering the final acceptability of the various Maas samples. The panellists were also trained each week to differentiate between the 10 different fruit flavours (Table 2), an outlandish flavour (caramel) and the control (natural unflavoured Maas). Only one flavour was used in every evaluation session of the three Maas combinations (A, C and D).

Analysis of variance – main effects

The main effects that were found to be significant ($p \leq 0.05$) included: the type of fruit flavour (Table 3), which directly influenced the appearance, the smoothness, the flavour intensity and the overall acceptability; and the culture combination (Table 3), which influenced the sweetness and the overall acceptability.

The 'appearance' values for this study were defined as values between glossy and dull, where a glossy product was thought to be appropriate for Maas production. A maximum value of 10 units was used to indicate a glossy product whereas a value of 0 labelled a dull product. The data in Fig. 3 shows the 'appearance' values for the Maas with each fruit flavour used in this study. No significant difference in 'appearance' values was observed between the three culture combinations (A, C and D) over the three week period. It is, therefore, an indication that the Maas prepared with the three culture combinations had similar appearances, irrespective of the fruit flavour used. The 'appearance' value for the Maas with flavour 1 (apple) was significantly different to that with flavours 3 (strawberry), 4 (banana), 5 (fig), 6 (watermelon) and 10 (peach) (LSD = 0.52) which all produced a more glossy product. However, none of the above mentioned fruit flavoured Maas products differed significantly in 'appearance' from the natural unflavoured Maas (flavour 8, control) and fruit flavours 2 (naartjie), 7 (caramel), 9 (mango), 11 (grape) and 12 (blackcurrant).

The data in Fig. 4 shows the 'smoothness' ratings for each fruit flavour used with the Maas. A value of 10 units was used to indicate that the product is smooth whereas a value of 0 indicated a gritty product. The panellists rated the smoothness of the Maas sample with flavour 12 (blackcurrant) to be significantly smoother than Maas produced with flavour 3 (strawberry) (LSD = 0.96). Flavour 12 (blackcurrant) was rated the highest, with a value of 8.4 units, by the panellists, while flavour 3 (strawberry) had a value of 7.3 units. The 'smoothness' value of the control Maas (flavour 8; 7.8 units) did not differ significantly from any of the other fruit flavoured Maas products. However, the Maas produced with flavour 12 (blackcurrant) and flavours 1 (apple), 2 (naartjie), 9 (mango) and 11 (grape) were all rated higher by the panellists in terms of 'smoothness' values when compared with the natural Maas.

Table 3. The main effects that influenced the variables in the study.

Variables	Main Effects
Sourness	-
Sweetness	Culture combination
Appearance	Type of fruit flavour
Smoothness	Type of fruit flavour
Flavour intensity	Type of fruit flavour
Overall acceptability	Culture combination
	Type of fruit flavour

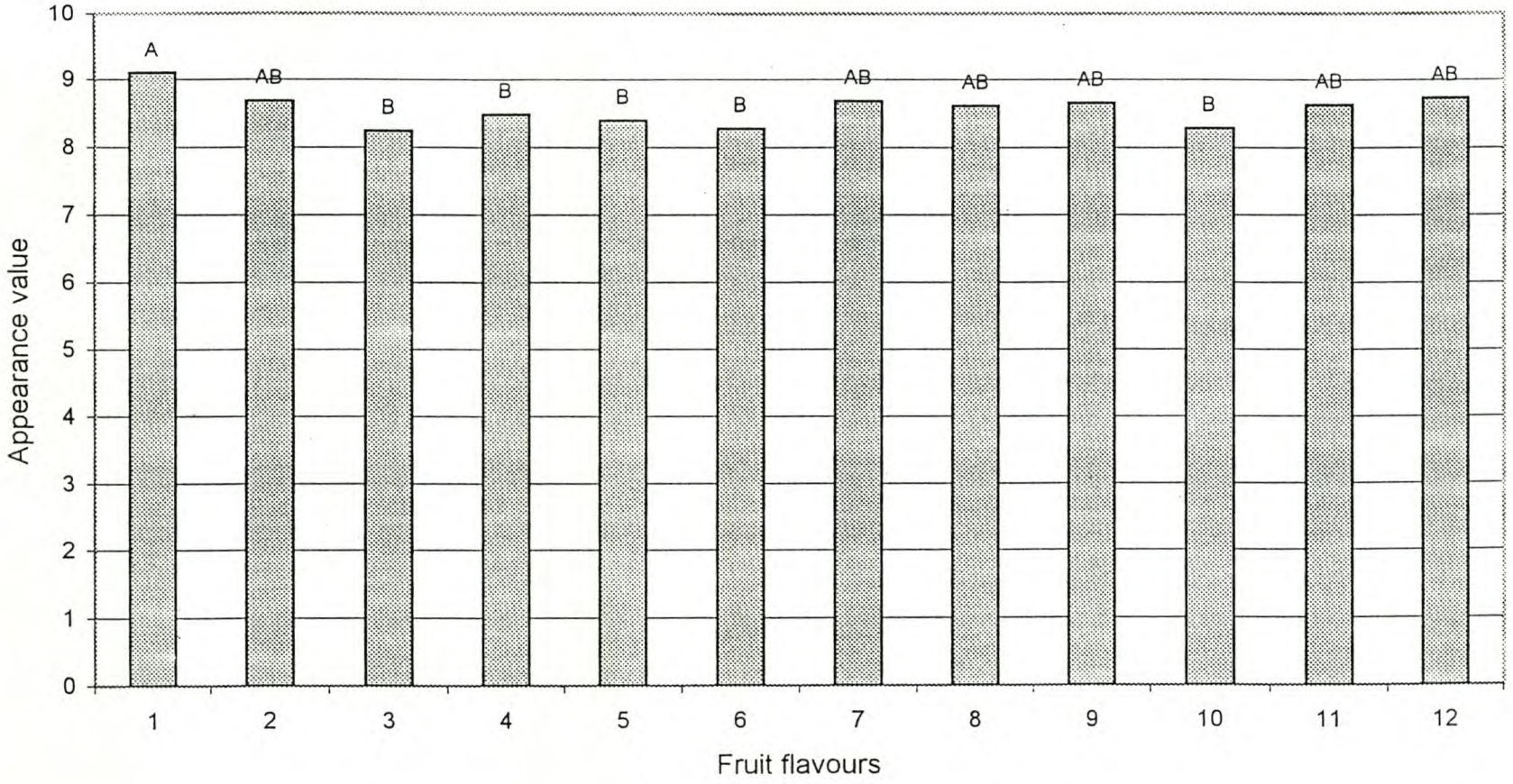


Figure 3. Effect of fruit flavour on appearance values. Bars with the same letters do not differ significantly ($p \leq 0.05$). (Flavour 1 = apple; flavour 2 = naartjie; flavour 3 = strawberry; flavour 4 = banana; flavour 5 = fig; flavour 6 = watermelon; flavour 7 = caramel (outlandish); flavour 8 = unflavoured; flavour 9 = mango; flavour 10 = peach; flavour 11 = grape; flavour 12 = blackcurrant).

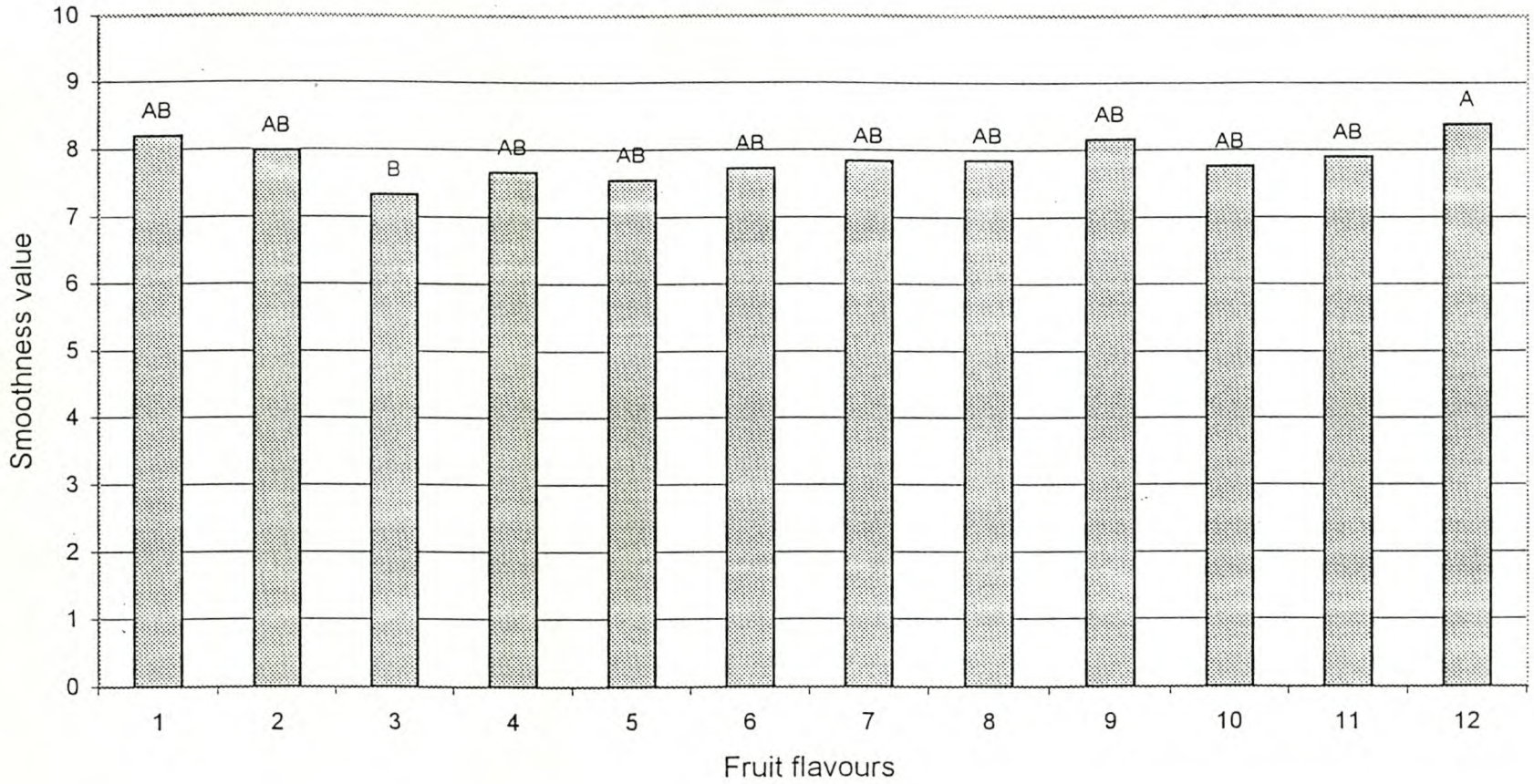


Figure 4. Effect of fruit flavour on smoothness values. Bars with the same letters do not differ significantly ($p \leq 0.05$). (Flavour 1 = apple; flavour 2 = naartjie; flavour 3 = strawberry; flavour 4 = banana; flavour 5 = fig; flavour 6 = watermelon; flavour 7 = caramel (outlandish); flavour 8 = unflavoured; flavour 9 = mango; flavour 10 = peach; flavour 11 = grape; flavour 12 = blackcurrant).

The 'flavour intensity' values for the 12 fruit flavours are shown in Fig. 5 and a value of 10 and 0 units indicate a very strong and very weak flavour, respectively. The data showed that the panellists rated the 'flavour intensity' of the Maas with fruit flavours 2 (naartjie), 3 (strawberry), 4 (banana), 6 (watermelon), 9 (mango) and 12 (blackcurrant) to be significantly different from that of the control (flavour 8) (LSD = 1.94). The highest 'flavour intensity' value of 4.8 units was assigned to flavour 12 (blackcurrant). Panellists observed a difference in 'flavour intensities' between the control and some of the fruit flavours and hence rated them higher than the control. However, the control did not differ significantly from the Maas with flavours 1(apple), 5 (fig), 7 (caramel), 10 (peach) and 11(grape), according to observations made by the panellists.

The data from this study indicated that, in contrast to the other variables, culture combination was regarded as the main effect (Table 3) that influenced the variable 'sweetness'. When evaluating 'sweetness' it must be taken into consideration that this attribute is masked by the sweet taste associated with the fruit flavours used in the Maas samples. The influence of the culture combination on the 'sweetness' values can be seen from the data in Fig. 6 where a value of 10 units indicate extreme sweetness. The 'sweetness' value for culture combination C was noted as significantly sweeter (LSD = 0.62) than for culture combination D, but both these combinations did not differ significantly from culture combination A. However, none of the culture combinations had an extreme sweetness level as the panellists only indicated a maximum 'sweetness' value of 2.4 units.

The statistical data of the sensory evaluation also showed that the 'overall acceptability' values were influenced by the fruit flavour used and the specific culture combination (Table 3). The 'overall acceptability' values for the Maas prepared with the different fruit flavours used in this study are shown in Fig. 7A. An 'overall acceptability' value of 10 or 0 units is used to indicate a very acceptable or an unacceptable product, respectively. It was evident that the most unacceptable Maas, produced with fruit flavour 12 (blackcurrant), was significantly different from that produced with the other fruit flavours, including flavour 8 (control) (LSD = 1.11). The panellists evaluated the Maas with fruit flavour 12 (blackcurrant) to be less acceptable with 3.9 units, whereas the Maas with other flavours had an average

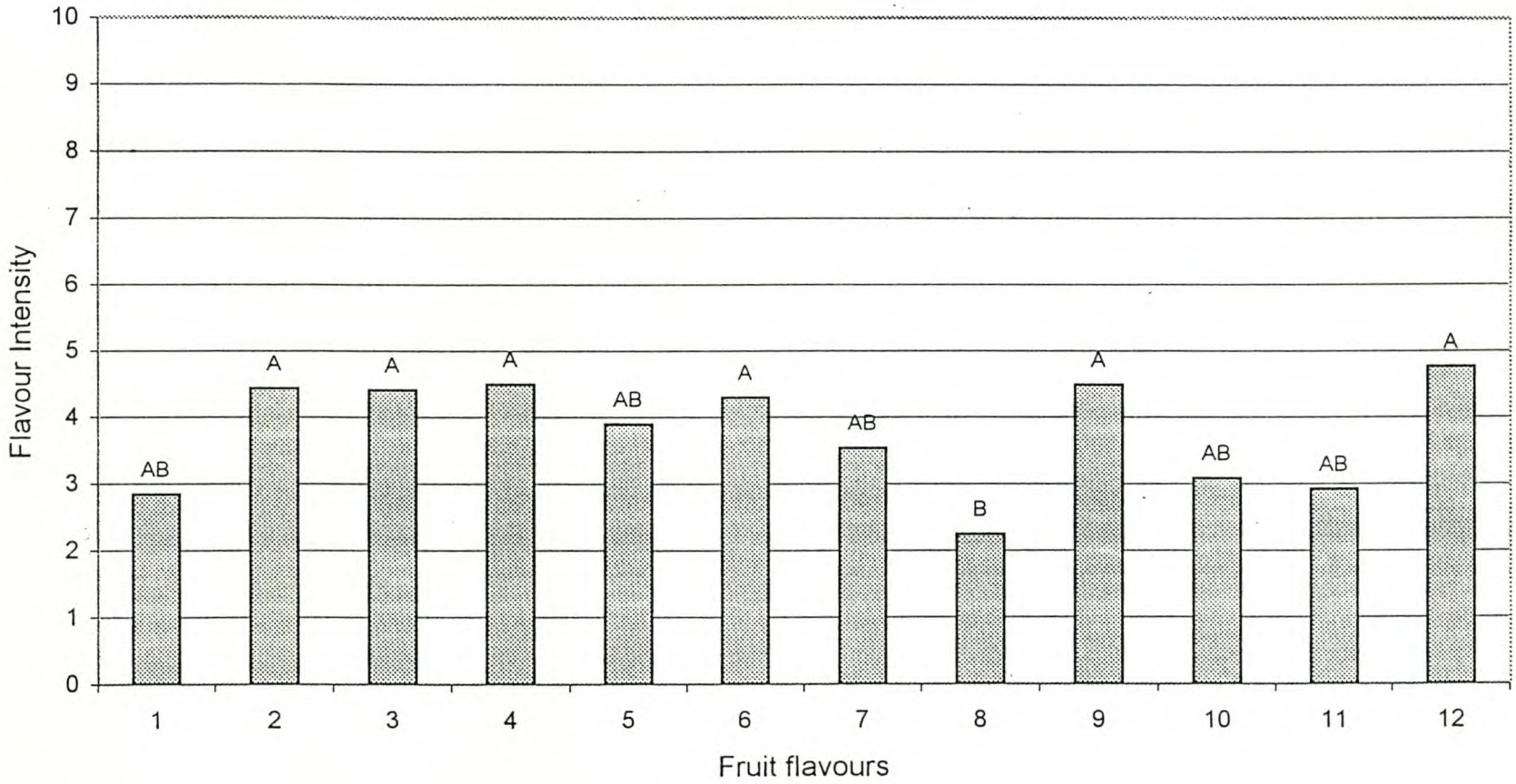


Figure 5. Effect of fruit flavour on flavour intensity values. Bars with the same letters do not differ significantly ($p \leq 0.05$). (Flavour 1 = apple; flavour 2 = naartjie; flavour 3 = strawberry; flavour 4 = banana; flavour 5 = fig; flavour 6 = watermelon; flavour 7 = caramel (outlandish); flavour 8 = unflavoured; flavour 9 = mango; flavour 10 = peach; flavour 11 = grape; flavour 12 = blackcurrant).

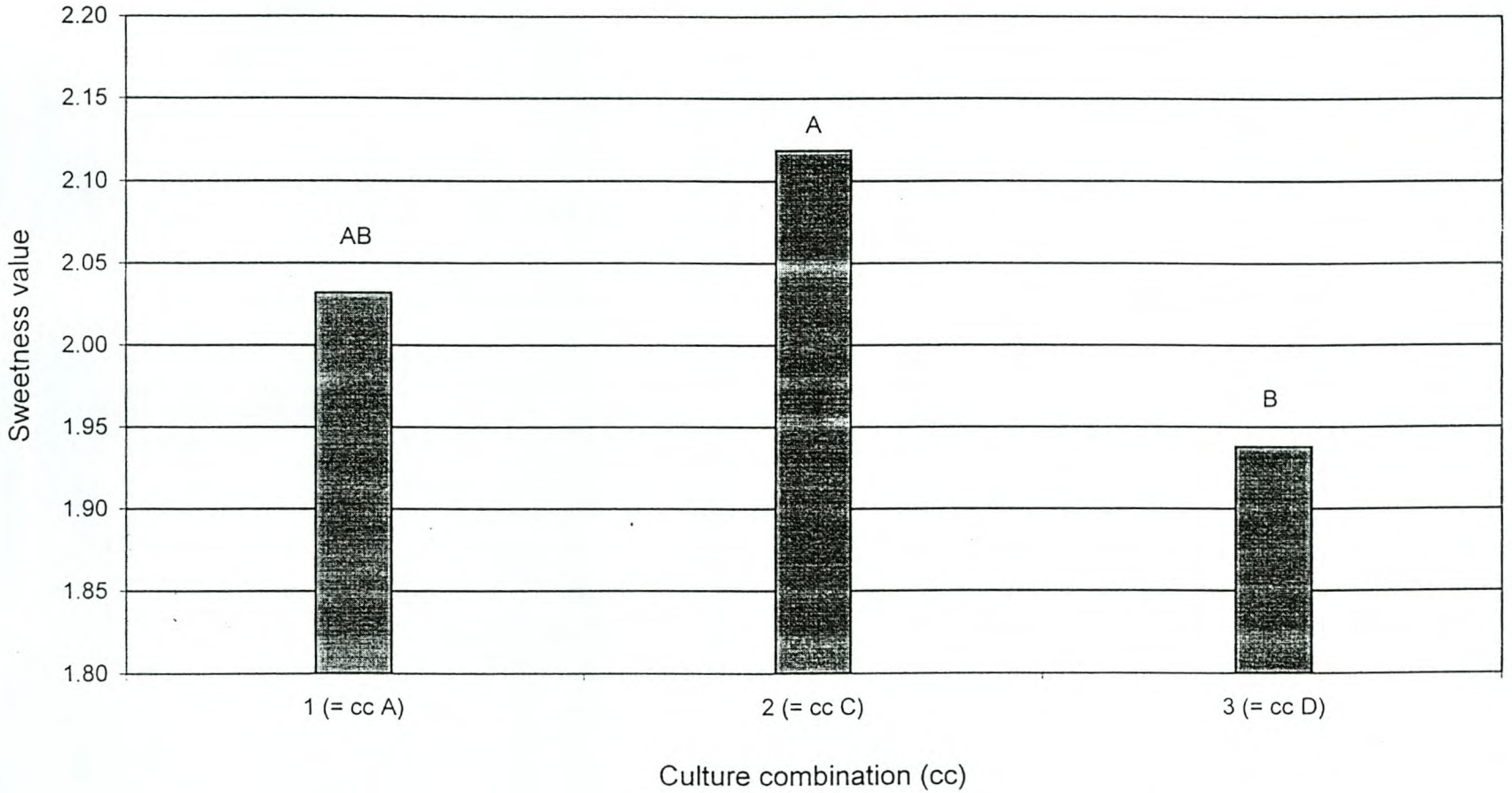


Figure 6. Effect of culture combination on sweetness value. Bars with the same letters do not differ significantly ($p \leq 0.05$). (Flavour 1 = apple; flavour 2 = naartjie; flavour 3 = strawberry; flavour 4 = banana; flavour 5 = fig; flavour 6 = watermelon; flavour 7 = caramel (outlandish); flavour 8 = unflavoured; flavour 9 = mango; flavour 10 = peach; flavour 11 = grape; flavour 12 = blackcurrant).

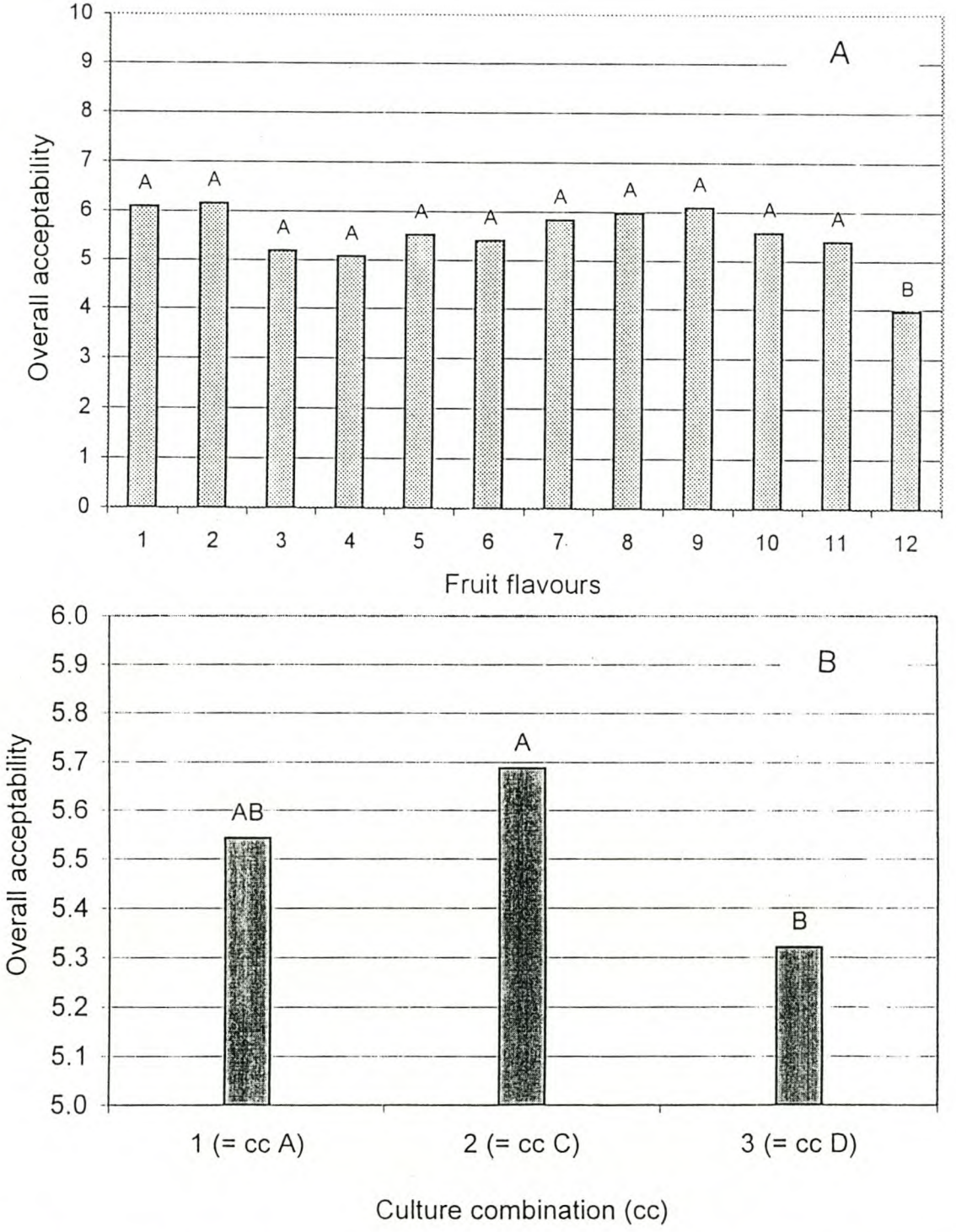


Figure 7. Effect of fruit flavour (A) and culture combination (B) on overall acceptability. Bars with the same letters do not differ significantly ($p \leq 0.05$). (Flavour 1 = apple; flavour 2 = naartjie; flavour 3 = strawberry; flavour 4 = banana; flavour 5 = fig; flavour 6 = watermelon; flavour 7 = caramel (outlandish); flavour 8 = unflavoured; flavour 9 = mango; flavour 10 = peach; flavour 11 = grape; flavour 12 = blackcurrant).

value of 5.7 units. The panellists also evaluated Maas with flavours 1 (apple), 2 (naartjie) and 9 (mango) with a better 'overall acceptability' than the control Maas with 'overall acceptability' values of 6.0, 6.1 and 6.0, respectively.

The 'overall acceptability' values for the culture combinations (A, C and D) used to prepare the Maas, were also scored as significantly different (LSD = 0.32) by the panellists (Fig. 7B). Culture combination C differed significantly in 'overall acceptability' from that of combination D, with combination C being preferred by the panellists and thus scoring 5.7 units. However, the data showed that culture combination A was not significantly different in 'overall acceptability' from culture combinations C or D.

Flavour recognition

The panellists were also requested to clearly identify the 10 fruit flavours and the outlandish flavour (caramel) used during the Maas production and to then distinguish them from the control (flavour 8), in addition to evaluating the Maas samples on sourness, sweetness, appearance, smoothness, flavour intensity and overall acceptability. A percentage value of 100% indicated that the flavour was correctly recognised.

From the data shown in Fig. 8, it is clear that the panellists were consistent in identifying the three control samples and separating them from the other fruit flavours. For the duration of the three weeks, the panellists continuously scored 100% for the controls.

The data clearly indicated that the panellists showed a preference for specific fruit flavours (banana, caramel and watermelon) despite the fact that the same flavour concentration (0.2% m/v) was used for all the fruit flavours. This is probably due to the taste perceptions of the individual panel members. Mean values for the fruit flavours showed that flavour 4 (banana) was the most preferred fruit flavour (93%) by the panellists. This was followed by the outlandish flavour (caramel, 89%). Positive recognition of fruit flavours 5 (fig) and 6 (watermelon) gradually increased over the course of the three week study with mean recognition values of 60 and 69%, respectively.

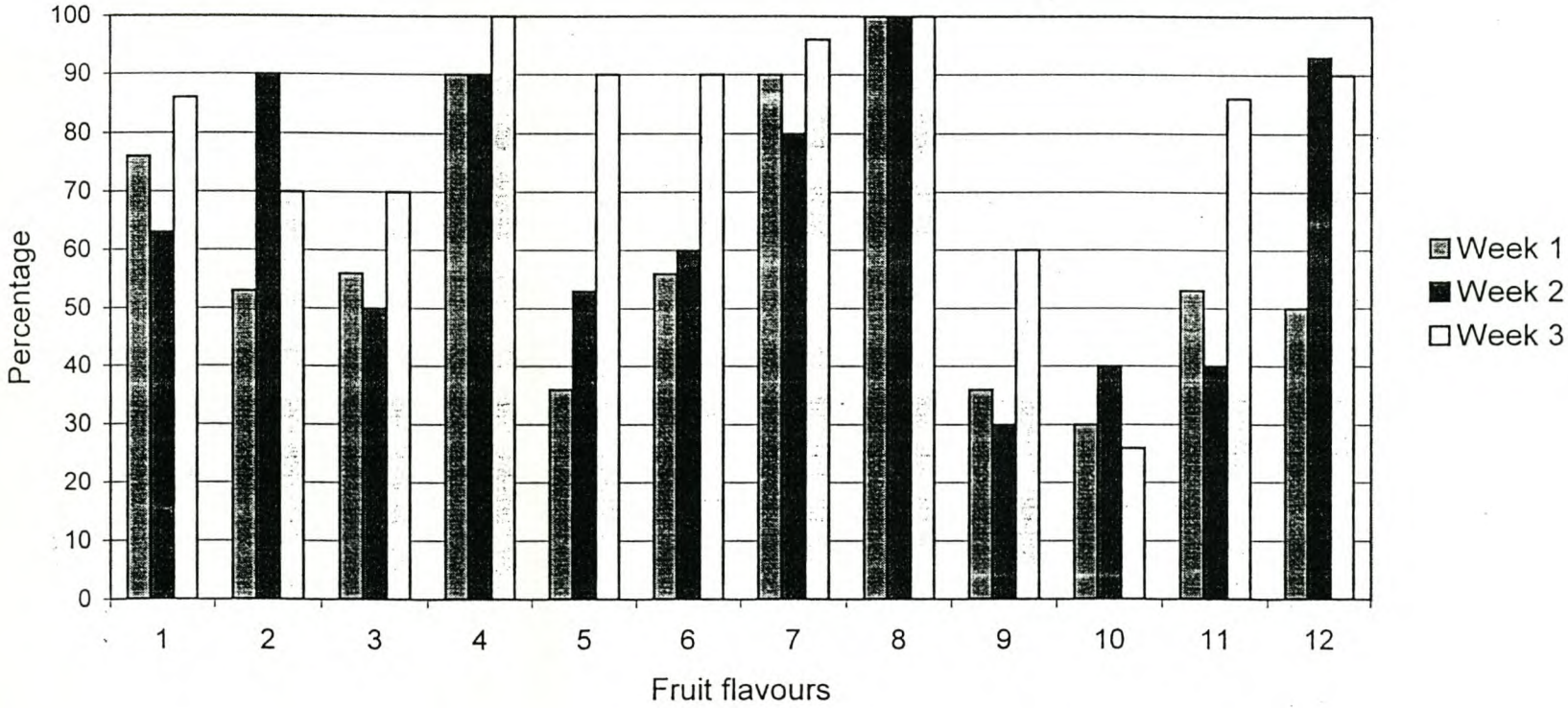


Figure 8. Aroma recognition values (%) of the sensory evaluation of the fruit flavoured Maas. (Flavour 1 = apple; flavour 2 = naartjie; flavour 3 = strawberry; flavour 4 = banana; flavour 5 = fig; flavour 6 = watermelon; flavour 7 = caramel (outlandish); flavour 8 = unflavoured; flavour 9 = mango; flavour 10 = peach; flavour 11 = grape; flavour 12 = blackcurrant).

Most of the fruit flavours scored mean values of above 60%, with the exception of flavours 9 (mango) and 10 (peach). These fruit flavours only scored mean values of 42 and 33%, respectively. However, the low recognition values for these two flavours could be the result of the panellists not being able to distinguish them from the other flavours or because the flavour concentration was under the threshold value. (This was not determined in this study.) The threshold value is the concentration where the flavour can easily be recognised. In this study, the flavour concentration for all the fruit flavours added to the Maas was 2% (m/v) and this is in agreement with good manufacturing practises recommended in the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972).

Shelf-life of Maas prepared with the three starter culture combinations

According to Venter (1999), a shelf-life of commercial Maas products is normally regarded as 22 d at refrigerated temperature (5°C), whereas the shelf-life of fresh milk at the same temperatures, is 14 d (Anonymous, 1999). Very little growth of the mesophylic cultures occur below 10°C, but according to Champagne *et al.* (1992), microbial growth, albeit slow, may still occur at 4° - 5°C.

The data in Fig. 9 shows the changes in pH and titratable acidity (TA) values during the shelf-life of the Maas produced with the three starter culture combinations. The data shows, as expected, a slight decrease in pH and small increase in titratable acidity for all three starter combinations over the 22 d period at the refrigerated temperature. The pH value of the three Maas products decreased only slightly by 0.09, 0.07 and 0.07 units for starter combinations A, C and D, respectively. The TA values for the three starter combinations (A, C and D) increased by 0.07, 0.07 and 0.05%, reaching final TA values of 0.86, 0.88 and 0.83% over the 22 d at 5°C, respectively.

No coliforms were detected in the Maas prepared with the three starter combinations during the 22 d storage period at 5°C, indicating the high hygienic standards obtained during production. High coliform counts in products indicate low hygienic standards during the handling of milk and the preparation of the fermented product (Mutukumira, 1995).

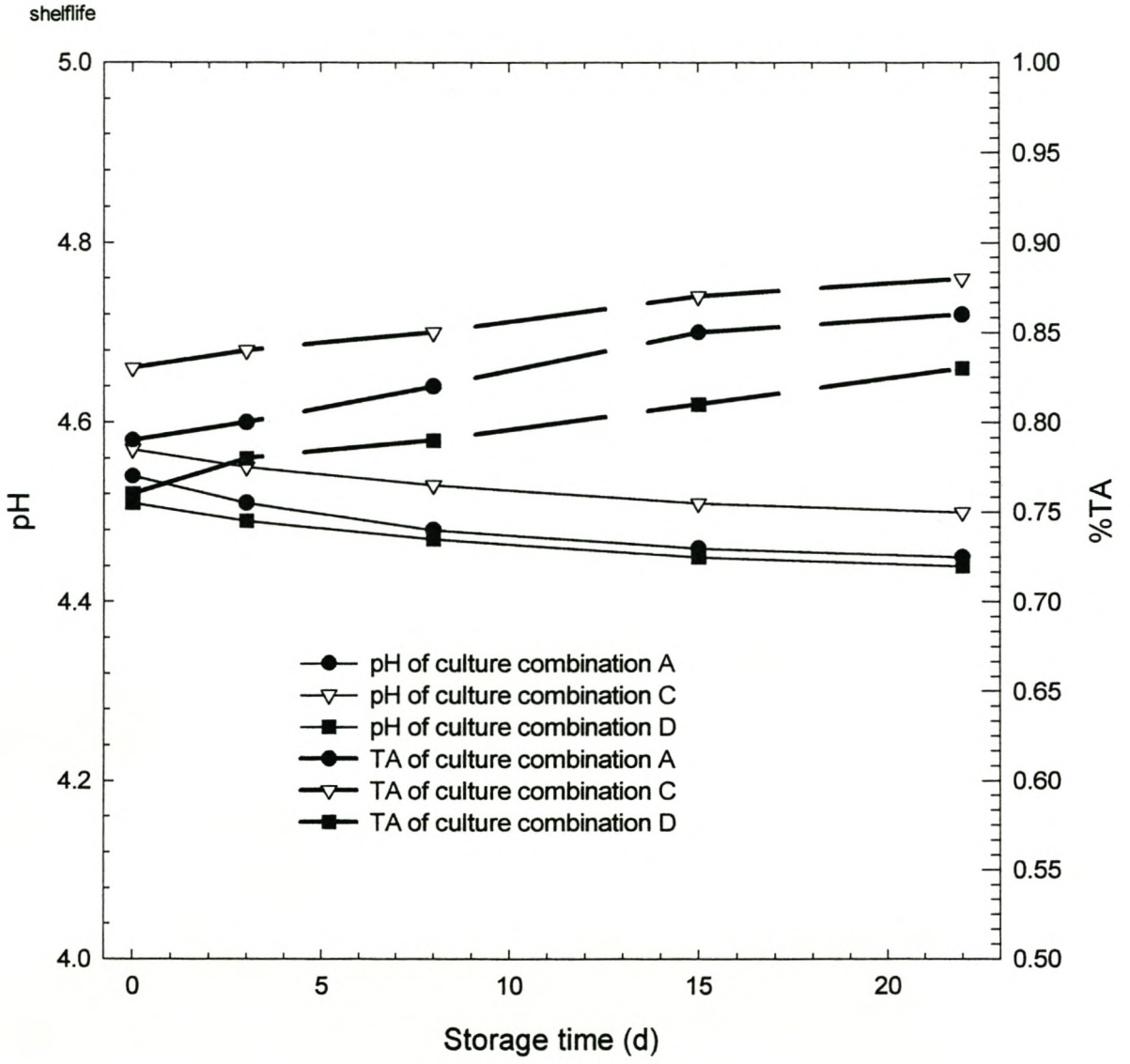


Figure 9. Changes in pH and TA during the shelf-life studies of Maas prepared with either starter culture combination A, C or D.

The results of the sensory evaluation of the Maas made with the three starter combinations (A, C and D) during the shelf-life studies are shown in Table 4. An increase in 'sourness' was observed for all three Maas products over the 22 d period with mean values ranging between 6.5 – 8.9 units. Panellists rated the three Maas products to be inferior in terms of 'sweetness', with mean 'sweetness' values of <1 over the 22 d period. The 'yeasty' values scored by the panellists gradually increased over time for the Maas produced with all three starter combinations (A, C and D). Although these were not extreme values, mean 'yeasty' values of 3.5, 2.5 and 3.3 units were scored for the three starter combinations. According to Bodyfelt *et al.* (1989), a 'cheesy off-flavour' (yeasty) starts to develop in products that have been stored for some time. The panellists also observed that a slight 'gassy' character developed in the Maas made with all three starter combinations over the 22 d period. Maas made with either starter combinations A, C or D scored final mean 'effervescent' values of 1.4, 2.9 and 0.7 units, respectively.

The mean 'smoothness' and 'appearance' values of all the Maas products ranged between 8 and 10 units (Table 4). 'Overall acceptability' values were observed to reach a maximum after 15 d of storage at 5°C, with mean values of 7, 8.4 and 8.9 units for starter combination A, C and D, respectively. After 22 d of storage, the 'overall acceptability' of the products decreased, which was ascribed to the development of a 'cheesy off-flavour' and 'gassiness' in all three Maas products. It was thus concluded that all three starter culture combinations had a shelf-life of at least 15 d. Longer storage of the Maas would lead to less acceptable products due to the development of off-flavours (cheesy) and a gassy taste.

Conclusion

The results of the sensory evaluation showed that the type of fruit flavour used had an influence on the appearance, smoothness and overall acceptability of the Maas. Each fruit flavour affected different attributes of the Maas, but none of the fruit flavours changed the sourness. Results of the sensory evaluation showed that there were no significant differences in sourness between the type of fruit flavour and the culture combination used. The three starter culture combinations (A, C and D) used to prepare the Maas, influenced the sweetness and overall acceptability of the final

Table 4. Mean sensory evaluation scores** for shelf-life studies done on the attributes of the natural unflavoured Maas prepared with three starter combinations (A, C and D) after 1, 8, 15 and 22 d. The mean score of three panel members were used.

Storage time (d)	Combination	Sourness	Sweetness	Yeasty (cheesy)	Effervescent (gassiness)	Smoothness	Appearance	Overall Acceptability
1	A	7	0	0.3	0	10	10	5.4
	C	6.8	0.3	0	0	9	10	6.3
	D	7.4	0.2	0.2	0.2	8.9	8.8	7.3
8	A	7.6	0.2	2.8	0.2	10	8.5	5.9
	C	6.5	0	0.8	0	10	10	7.2
	D	7.9	0.7	1.2	0.5	8.5	8.9	7.7
15	A	8.1	0.5	3	0.3	8.9	10	7
	C	7.9	0	1.5	1.5	9.4	9.4	8.4
	D	8.4	0.8	2.1	0.8	9.8	9.8	8.9
22	A	8.5	0.4	3.5	1.4	9.3	9	3.2
	C	7.5	0.8	2.5	2.9	8.5	8.2	5.4
	D	8.9	0.6	3.3	0.7	9.5	9.5	5.2

**A score of 0 units = not acceptable and a score of 10 units = very acceptable.

product. The sensory panellists indicated that culture combination C gave the best overall acceptability over the three week study period. The sensory evaluation also showed that six fruit flavours were preferred which could be easily distinguished from the control flavour. Fruit flavour 4 (banana) was the most preferred flavour. The control (flavour 8) was not significantly different from most of the flavoured Maas attributes evaluated, except for its flavour intensity that was significantly different from flavours 2 (naartjie), 3 (strawberry), 4 (banana), 6 (watermelon), 9 (mango) and 12 (blackcurrant). The overall acceptability value of the control was found to be significantly different from flavour 12 (blackcurrant). The blackcurrant flavour was given the highest flavour intensity value during the sensory evaluation and its flavour was regarded to be too sweet to be associated with the sour character of Maas.

The shelf-life of the Maas prepared using the three starter culture combinations (A, C and D) showed that Maas could be kept refrigerated for 15 d and still show an acceptable appearance, taste and good microbiological quality. After 15 d, defects like yeasty and gassiness influenced the quality of the final Maas product. The sensory evaluation data, that was statistically evaluated, showed that optimum characteristic Maas could be made using a 2% (v/v) inoculum of culture combination C and incubating the product for 14 h at 22°C. The Maas produced under these conditions gave the best acceptability, sourness and smoothness values.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

Background

A wide range of fermented dairy products can be found in most countries of the world. These vary from fresh products such as yoghurts, to products that have been matured for several months. The experimental knowledge on the production of fermented milk products is enormous. Yet, the scientific understanding of the detailed mechanisms and metabolic pathways followed and the end-products formed during formation of the characteristic aroma of each of the respective products, despite all the scientific research done over the past 50 years, is only just beginning (Imhof & Bosset, 1994).

Milk is highly nutritious, but vulnerable to spoilage which not only limits the shelf-life but also leads to significant quantities being rejected daily by the consumer resulting in economic losses to the industry. Fermented milk products, like our indigenous Maas, are the solution. Consumed over thousands of years, the popularity of fermented milks is ever increasing. This is mainly due to its convenience, versatility and image as a healthy food. The production of Maas also allows the small-holder producers to process surplus milk on the farm or in centralised small scale units.

Maas is an indigenous South African fermented milk product that has been made by the Black community for many generations. Traditionally, the herd boys milked the cows and when the wooden milking pails were full, they poured the milk into calabashes or into leather milk sacks to curdle. This production method has been carried over for generations and the traditional Maas is still made in clay pots and calabash (Coetzee, 1982). Commercially manufactured Maas has a retail price of about R 3-50 for 500 ml (August 1999 price) which is fairly expensive for the low socio-economic groups in South Africa. In order to target these groups, Maas has to become more affordable and available by encouraging the development of small businesses (Myburgh, 1995). Small businesses can also diversify the natural Maas

product range by producing fruit flavoured Maas, a more appealing finished product, thereby expanding the choice of South African consumers.

The objective of the study was therefore to screen potential mesophylic starter culture strains for use in the production of natural Maas and diversifying the natural Maas to include fruit flavoured Maas products.

Starter culture selection

The species and strain of microorganisms used during the fermentation dictate the resultant variety of end-products accumulated in fermented milk products. The lactic acid bacteria used to manufacture fermented milks are either mesophylic or thermophylic and the majority of manufacturers now use inocula of known identity. The type of bacteria used in the production of fermented milk and, to a large extend, the success of the product type on the market mainly depends on consumer appeal.

In this study, the potential of single strain, two-strain and three-strain combinations for Maas production was investigated to construct an acceptable starter culture. Twenty-five mesophylic single strain cultures were chosen from the University of Stellenbosch Food Science Culture Collection and evaluated for possible use in Maas production. Selection of the single strains was based on their ability to produce acid under controlled time-temperature conditions. Acid production forms an integral part of Maas production for it produces a tangible acid taste that is normally associated with Maas products. A final pH range of 4.60 – 4.70 would produce high viscosity final products (Mutukumira, 1996). However, a wide range of pH values (4.33 – 6.51) was found for the 25 single strain cultures screened in this study. It was found that only nine of the 25 single strain cultures produced acid in a pH range of 4.33 – 5.05, which could be used in Maas production. These included four *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* strains (S1, S2, S3 and S5), three *L. lactis* subsp *lactis* strains (S13, S15 and S16) and two *L. lactis* subsp *cremoris* strains (S17 and S22).

Twenty, two-strain culture combinations were then formed using the nine single strain cultures to stimulate further growth and activity. The two-strain culture combinations consisted of a *L. lactis* subsp. *lactis* biovar *diacetylactis* combined with

the *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, respectively. It was observed in this study and from other reports (Mutukumira, 1996) that in certain combinations, acid production was not stimulated. Furthermore, it was found that certain two-strain combinations had sensory defects, like fatty and gritty body textures. However, more flavour metabolites were produced with the two-strain combination than with the single strains. From the two-strain combination data obtained, it was found that combinations S3S17, S3S22, S5S17 and S5S22 produced sufficient acid levels to coagulate the milk. These four culture combinations were therefore able to reach a pH range between 4.65 – 4.85. However, a pH range of 4.60 – 4.70 is necessary to produce maximum viscosity products (Mutukumira, 1996). These combinations (S3S17, S3S22, S5S17 and S5S22) were then combined with the acid producing strains of *L. lactis* subsp. *lactis* (S13, S15 and S16) to form three-strain combinations.

The purpose of the *L. lactis* subsp. *lactis* strains was to further increase acid levels during Maas production. During the evaluation of these combinations, a pH range of 4.37 – 4.91 was reached after 16 h at 22°C and flavour metabolite production was increased. The increase in flavour metabolites could be ascribed to the further metabolic breakdown of lactose by *L. lactis* subsp *lactis* biovar *diacetylactis* strains. Sensory evaluation of the combinations showed a wide range of physical characteristics developed during the subsequent Maas production. However, only combination S3S17S13 produced sufficient acid (final pH = 4.40) that resulted in a good body texture with typical characteristics of commercially produced Maas, but flavour production was not pronounced enough.

The sensory evaluation of the product also yielded a slight, bitter chemical taste and smell. The chemical taste and smell was removed by centrifuging the cell suspensions that were cultured in MRS-medium and re-suspended in milk and then used in the production of Maas. Sensory evaluation of these products showed a vast improvement in taste and smell. It was concluded that the bitter chemical taste and smell could be attributed to the MRS-medium in which the cultures were cultured.

Due to the absence of a pronounced flavour in the three-strain combination S3S17S13, production parameters were manipulated to elevate flavour production.

In optimising the parameters, the inoculation percentage was increased to 2% (v/v) and the incubation time reduced from 16 h to 14 h during both the activation and Maas production steps. Two flavour producing strains, *Leuconostoc mesenteroides* subsp. *dextranicum* and *L. mesenteroides* subsp. *citrovorum*, were further added to combination S3S1713, respectively. From the data obtained in the sensory evaluation, it was clear that the inclusion of the two leuconostocs lead to a superior aroma with an excellent creamy, glossy body texture.

It is therefore possible to produce excellent Maas products with the use of three different starter culture combinations (A, C and D). These products are all highly viscous and have a pleasant sour taste and delicate flavour, superior to traditional South African Amasi or Inkomasi.

Sensory evaluation of fruit flavoured Maas

The use of fruit flavourings in fermented milk products has gained popularity over the last two decades (Tuorila *et al.*, 1993). Flavour is the most important ingredient of the product because, as it helps to sell the product. In this study, 10 fruit flavours (apple, naartjie, strawberry, banana, fig, watermelon, mango, peach, grape and blackcurrant) and one outlandish flavour (caramel) were used to flavour the Maas.

Sensory evaluation was done on the fruit flavoured Maas, with the inclusion of the natural unflavoured Maas, after 14 h of incubation at 22°C. The test objectives of the sensory evaluation were to assess the sourness, sweetness, flavour intensity, appearance, smoothness and overall acceptability of the respective Maas samples using the three best culture combinations (A, C and D) from the previous study.

Significant differences in appearance, smoothness and overall acceptability values were observed between Maas with the different fruit flavours. Appearance values for the apple flavoured Maas was significantly different from that of the strawberry, banana, fig, watermelon and peach flavoured Maas. The apple flavoured Maas scored the highest appearance value. The smoothness value of the blackcurrant Maas significantly differed from the strawberry Maas. Although the blackcurrant Maas was smoother, the sensory evaluation showed that its overall acceptability was lower. The fruit flavours also significantly influenced the flavour

intensity of the various flavours in the Maas. The natural Maas (unflavoured) was evaluated as being significantly different from the flavoured Maas samples, scoring the lowest flavour intensity value. It was also shown that because the blackcurrant flavour is perceived as a sweet flavour, the sourness of the Maas was not associated with the sweet flavour. This would mean that in the production of blackcurrant Maas, the product has to be further sweetened to compliment the sweet smell of the blackcurrant, but then again its all about personal taste.

The three starter culture combinations (A, C and D) significantly influenced the final sweetness and overall acceptability of the flavoured Maas samples. However, in both the sweetness and overall acceptability, culture combination C was evaluated as being significantly sweeter and more acceptable than culture combination D. It was thus concluded from the statistical evaluated sensory data that the best flavoured Maas could be produced using culture combination C.

The ability of the panellists to distinguish between the different flavours and the natural Maas was also tested during the sensory evaluation. The results showed that in all the experiments the natural Maas was correctly identified. The panellists did, however, find it difficult to distinguish between specific flavours, especially with the mango and peach flavours. It was found that the identification of the other flavours improved over the course of the study.

Shelf-life of Maas prepared with the three starter culture combinations

The shelf-life of fermented milk products and the consistency of organoleptic, physical and microbiological characteristics depend largely on their storage at refrigerated temperatures. The shelf-life of the Maas prepared with the three starter culture combinations at refrigerated temperatures, was evaluated over 22 d. The pH and TA decreased and increased, respectively for all three culture combinations (A, C and D).

No coliforms were observed, which is an indication of the hygienic standards that were maintained during the study.

Results of the sensory evaluation over the 22 d storage period showed that the Maas prepared with the three culture combinations (A, C and D) became more

sour, yeasty, effervescent and smoother. This indicated that the microorganisms were not just producing acid, but also other flavour defects, that could shorten the shelf-life of the final Maas product. However, from the data of the sensory evaluation, all three combinations used to prepare the Maas were found to be acceptable up to 15 d at refrigerated temperatures.

Recommendations

The results of this study indicate that a starter culture combination consisting of selected strains of lactococci and leuconostocs could successfully be used for the production of excellent Maas. With these selected strains, it was found that an acceptable natural Maas with no additives could be made using the following method:

i. pasteurise one litre of homogenised full cream milk at 85° ± 3°C for 20 min;
ii. cool to 22°C within 10 min. in a waterbath;
iii. activate 2% (v/v) of the culture combination C for 8 h at 22°C;
iv. add 2% (v/v) of the activated starter culture combination C to the milk; and
v. incubate at 22°C for 14 h to a final pH of 4.5 – 4.6.

This procedure leads to an excellent Maas product that has a smooth, glossy, creamy body texture and buttery flavour, which is comparable and even superior to commercial Maas products. Furthermore, the Maas product has a shelf-life of at least 15 d at refrigerated temperatures without the addition of the additives found in most of the commercially available products.

It is also recommended, although personal taste has an influence, that when fruit flavoured Maas products are produced, the flavours have to compliment the sourness of the natural Maas (like naartjie), if however sweet flavours, like blackcurrant, are considered, the Maas should be sweetened additionally.

Future Research

Modern society demands foods that are safe, nutritious, aesthetically appealing, readily available, convenient to use and reasonably priced. Considerable progress in food science and technology and in many related areas over the past decades have made it possible to meet these challenges.

Future research work on the influence of secondary metabolites produced by the starter microorganisms and their influence on the formation and quality of the flavour of Maas must be consolidated. Knowledge about the interactions between species as well as single and mixed strain starter cultures, would be of enormous benefit. Qualitative and quantitative analyses of the volatile flavour compounds and their threshold value in the Maas can only be the first step towards achieving this goal. The threshold of each of the flavour compounds in the fermented milk product should also be determined in order to assess its aromatic influence in the resulting flavour and production of better quality Maas products.

It must also be remembered that when an unique starter culture combination has been found, as in this research where three usable culture combinations were constructed and evaluated, future research has to done in preserving the combination to make it more user friendly. A possible preservation method could be to freeze-dry the combination in order to prolong its shelf-life and usability. A marketer's cliché is: "if it does not look good, it won't sell". Product packaging is therefore another aspect that will require future development.

It is also worth mentioning that no product is fully explored until it is made as a "diet", "light" or "low-calorie" product. Thus, one aspect worth considering and on which future research could be conducted, is the dietetic properties of Maas and how it can be used to improve the knowledge of the health benefits of traditionally produced Maas.

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