POSSIBILITIES AND LIMITATIONS IN FLAVOURING THE CULTURED MILK PRODUCT MALA BY MEANS OF PREPARATIONS FROM FRUITS AVAILABLE IN KENYA

CHIE THESIS HAS BEEN ACCEPTED FOR FC THE DEGREE OF MASC 1987. AND ACCEPTED IN THE CNIVERSITY LIBRARY.

A thesis submitted in part fulfilment for an M.Sc. in the Department of Food Technology & Nutrition

July 1987

UNIVERSITY OF MAIROBI

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

John Wangoh

This thesis has been submitted with our approval as University supervisors.

Dr. W. Schulthess Department of Food Technology & Nutrition University of Nairobi

Date

Dr. P. Struebi Department of Food Technology & Nutrition University of Nairobi

17/12/87

Date

ACKNOWLEDGEMENT

I greatly appreciate the personal involvement instruction and constant guidance by my supervisors, Drs. W. Schulthess and P. Struebi, which were invaluable for successful completion of this thesis.

I also express my thanks to the Swiss Government for financing the M.Sc. program in general and student sponsorship in particular.

Lastly much thanks to Mrs. Ruth Gichanga for typing the work and to all those staff of Department of Food Technology & Nutrition, many to thank individually, who carried out the numerous sensory evaluations.

LIST OF CONTENTS

		Page
	ACKNOWLEDGEMENT	i
	LIST OF CONTENTS	ii
	LIST OF TABLES	viii
	LIST OF FIGURES	x
	ABSTRACT	xi
1.	INTRODUCTION	1
2.	LITERATURE REVIEW	6
2.1.	Flavouring of Cultured Milk	6
2.1.1.	Acceptability of Different Fruit	
	Based Flavours Used in Cultured	
	Milks	8
2.2.	Viscosity of Cultured Milk	8
2.2.1.	Definition and Measuring	8
2.2.2.	Optimisation of Viscosity of	
	Cultured Milk	10
2.2.2.1.	Increasing Solids-not-Fat	10
2.2.2.2.	Heat Treatment	11
2.2.2.3.	Incubation Time and Temperature	12
2.2.2.4.	Use of Slime Producing Cultures	13
2.2.2.5.	Addition of Stabilisers to	
	Cultured Milks	13
2.2.3.	Manufacture of Pourable Fruit-	
	Sugar Mixes for Addition to	
	Cultured Milks	14

ø

11

(iii)

2.2.4.	Viscosity of Fruit Flavoured	
	Cultured Milk	15
2.3.	Preservation of Fruit Mixes for	
	Addition to Cultured Milk	16
2.4.	Preservation of Fruit Flavoured	
	Cultured Milks	17
2.4.1.	Cooling	17
2.4.2.	Freezing	21
2.4.3.	Aseptic Processing	21
2.4.4.	Chemical Preservation	21
2.4.5.	Pasteurisation of Cultured Milks	22
2.4.6.	High Frequency/Ultra High	
	Frequency/Multiple Frequency	
	Method	24
3. MATERI	IALS AND METHODS	25
3.1.	Raw Materials	25
3.1.1.	Milk	25
3.1.2.	Sugar	25
3.1.3.	Mango Pulp	25
3.1.4.	Passion Fruit Juice Concentrate	25
3.1.5.	Bananas	25
3.1.6	Pineapples	26
3.1.7.	Strawberries	26
3.1.8.	Culture	26
3.2.	Chemicals	26

Page

		rage
3.2.1.	Sucrose	26
3.2.2.	Citric Acid, Caffeine and Sodium	
	Carbonate	26
3.2.3.	Sodium Hydroxide	27
3.2.4.	Potato Dextrose Agar	27
3.2.5.	Gelatine	27
3.3.	Methods of Preparation	27
3.3.1.	Starter Culture	27
3.3.2.	Mala	27
3.3.3.	Banana Pulp	29
3.3.4.	Pineapple	29
3.3.5.	Passion Fruit Juice	29
3.3.6.	Sodium Caseinate	29
3.3.7.	Fruit-Sugar Mixes for Preserva-	
	tion Trials	31
3.3.8.	Fruit Flavoured Mala	31
3.3.8.1.	Fruit Flavoured Mala for Fruit/	
	Sugar Level Optimisation	31
3.3.8.2.	Fruit Flavoured Mala for other	
	Experiments	32
3.4.	Methods of Storage	32
3.4.1.	Fruit Sugar Mixes	32
3.4.2.	Fruit Flavoured Mala	33
3.5.	Methods of Analysis	33
3.5.1.	рН	33
3.5.2.	Viscosity	33

.

		Page
3.5.3.	Brix	34
3.5.4.	Titrable Acidity	34
3.5.5.	Solids - not - Fat	34
3.5.6.	Yeast and Mould Counts	34
3.5.7.	Characterisation of Pectin Used	34
3.6.	Methods of Sensory Evaluation	34
3.6.1.	Panel Selection	34
3.6.2.	Sensory Evaluation for Fruit/	
	Sugar Level Optimisation in	
	Fruit Flavoured Mala	35
3.6.3.	Sensory Evaluation to Determine	
	Most Acceptable Fruit Flavoured	
	Mala	36
3.6.4.	Sensory Evaluation to Assess	
	Keeping Quality of Fruit/Sugar	
	Mixes	36
3.6.5.	Sensory Evaluation to Assess the	
	Keeping Quality of Fruit Flavoured	
	Mala	37
	IS AND DISCUSSION	38
4.1.	Optimisation of Fruit Sugar Levels	
	in Mala Flavoured With Preparation:	S
	From Different Fruits and Their	
	Acceptability	38

+

đ

4.1.1	Optimisation of Fruit Sugar	
	Levels	39
4.1.2.	Selection of the Best Fruit	
	Flavoured Mala	43
4.1.3.	Discussion	45
4.2	Viscosity Optimisation of Mala	
	Flavoured with Passion Fruit Juice-	
	Sugar Mix	46
4.2.1.	Addition of Pectin and Gelatine	
	to Passion Fruit Juice-Sugar Mix	46
4.2.2.	Addition of Sodium Caseinate to	
	Milk Prior to Calturing	49
4.2.3.	Discussion	50
4.3	Preservation of Some Fruit-Sugar	
	Mixes	51
4.3.1.	Discussion	53
4.4.	Storage of Mala Flavoured with some	
	Fruit-Sugar Mixes	54
4.4.1.	Mala Flavoured With Pineapple	
	Sugar Mix	55
4.4.2.	Mala Flavoured With Banana Pulp-	
	Sugar Mix	59
4.4.3.	Mala Flavoured With Passion Fruit	
	Juice-Sugar Mix Containing Gelatine	62
4.4.4.	Discussion	67

*

		Page
5.	CONCLUSIONS AND RECOMMENDATIONS	70
6.	REFERENCES	72
	APPENDIX	83
	APPENDIX I: Ballots and Materials	
	for Screening and Selection for	
	Taste and Odour	83
	APPENDIX II: Ballot for Flavoured	
	Mala Hedonic Scoring	94
	APPENDIX IIIA: Ballot for Flavoured	
	Mala Difference Evaluation	100
	APPENDIX IIIB: Ballots for Flavoured Mala	
	Difference and Hedonic Evaluation	101

(vii)

(viii)

Page_

LIST OF TABLES

TABLE 1:

1.	Yeast and mould counts in Flavoured	
	Yoghut at the point of distribution	
	recommended by Davis et al. (14)	20
2.	Brix value, citric acid and pH of fruit	
	preparations used	38
3.	Sensory evaluation scores of mala flavoured	
	with different fruit preparations at different	
	fruit and sugar levels	40
4.	Characteristics of fruit sugar 👻 mixes	
	and sensory evaluation of mala flavoured with	
	the fruit-sugar mixes at optimum levels	44
5.	Viscosity of unflavoured mala in relation	
	to mala flavoured with passion fruit juice-	
	sugar mix containing gelatine or pectin	48
6.	Viscosity of unflavoured mala and of mala	
	flavoured with passion fruit juice-sugar	
	mix made from milk with differenct levels	
	of sodium caseinate (17.9% dry matter)	49
7.	Characteristics and sensory evaluation	

of fruit mixes before and after 30 days storage at 25°C ----- 52

.

TABLE

- 8. Mean sensory evaluation scores of mala flavoured with pineapple fruit mix stored at 25^oC against that stored at 2-3^oC as reference ----- 57
- 9. Mean sensory evaluation scores of malas flavoured with banana preparation stored as 25°C against that stored at 2-3°C as reference. ----- 60
- 10. Duo-Trio difference evaluation of mala flavoured with passion fruit-sugar mix with or without sorbic acid, stored at 15°C against the control without sorbic acid stored at 2-3°C 63
- Hedonic scoring of mala flavoured with passion fruit juice-sugar mix without sorbic acid added, after
 6 days storage at 15°C against that stored at 2-3°C ----- 64
 12. Viscosity of mala flavoured with
 - passion fruit juice-sugar mix containing gelatine and stored at 2-3°C and 15°C. ----- 65

(ix)

Page

LIST OF FIGURES

FIGURES	age
1. Mala preparation	28
2. a&b. Pulp-sugar interaction in	
perception of odour in mala	
flavoured with strawberry pulp	41
3. Viscosity changes in passion	
fruit juice-sugar mix with	
varying levels of gelatine	
or pectin	47
4. a,b & c. Titrable acidity, pH and	
viscosity changes respectively of	
unflavoured mala and mala flavoured	
with pineapple mix and stored at	
2-3°C or 25°C	56
5. Yeast and mould development in	
unflavoured mala and in mala flavoured	
with some fruit preparations and	
stored at various temperatures	58
6. a,b & c. Titrable acidity, pH and	
viscosity changes respectively	
of unflavoured mala and mala	
flavoured with banana pulp.and	t
stored at 2-3°C or 25°C	61
7. a & b. Titrable acidity and pH respec- tively of mala flavoured with passion fruit juice-sugar mix and containing 0 or 200 ppm.sorbic acid stored at 2-3 C or 15°C*	66

6

Þ

ABSTRACT

1

This research was conducted to asses the possibilities and limitations in flavouring of the cultured milk product "mala" by preparations from fruits available in Kenya. Experiments were therefore carried out to:

- determine the sucrose and fruit pulp or juice levels in mala most acceptable to Kenyans and the comparative acceptability of different fruit based flavours;
- optimise the viscosity of mala flavoured with fruit juices;
- devise simple means for preparation and preservation of fruit-sugar mixes for aromatising and sweetening mala;
- assess the storage quality of mala flavoured with selected fruit-sugar mixes.

Samples of the cultured milk product mala flavoured with different fruits at varying fruit and sugar levels were tested for acceptability by adult Kenyans. Pulps of mango, banana and strawberry, passion fruit juice and pineapple juice together with its pieces were used to flavour mala. For the various fruit-sugar preparations the optimum flavouring effect was obtained with the following combinations:-

(xi)

Mango: 18% pulp and 12% sucrose Passion fruit: 12% juice and 10% sucrose Banana: 18% pulp and 10% sucrose Strawberry: 20% pulp and 12% sucrose Pineapple: 16% pieces and juice and 10% sucrose.

When various quality characteristics of mala flavoured with different fruit-sugar combinations at their optimum were compared, mala with mango pulp and with passion fruit juice respectively, had the best appearance; those flavoured with mango and strawberry pulp respectively were the most preferred for taste; mala flavoured with mango pulp had the best consistency. Seeds in strawberry mala were found objectionable. The same applied to specks and high viscosity of mala flavoured with banana pulp. The viscosity of mala flavoured with passion fruit juice was considered low. Odour hedonics for all the malas did not differ.

ř.

When 0.164% pectin and 0.9% gelatine respectively, were added the viscosity of the mala flavoured with optimum passion fruit juice-sugar mix was improved to that of the control/original mala. Sodium caseinate (17.9% dry matter) added at the rate of 0.6% on dry matter basis to the milk prior to acidification had the same balancing effect.

Raising the temperature of fruit-sugar mixes

(xii)

to 75°C, hot filling immediately after removal of bottles from boiling water and cooling to ambient proved adequate to preserve the mixes without refrigeration for one month. Banana pulp without heat treatment however had to be preserved by freezing. The possible levels of 330-500 ppm sorbic acid on fruit part, considering that the flavoured mala should not exceed 50 ppm, can protect the product from infections if not used immediately after opening.

Unflavoured mala and mala flavoured with different preparations became unacceptable when yeast and mould counts were of the order of 10⁷/ml. At the same storage temperature unflavoured mala or mala flavoured with different fruit preparations attained these counts after varying storage periods although the initial yeast and mould counts were approximately the same. Unflavoured mala, mala flavoured with pineapple preparation and mala flavoured with banana pulp attained the counts after more than 6 days, 6 days and 4 days storage at 25°C, respectively. The type of fruit preparation added determined the yeast and mould growth and thereby the keeping quality of the product at a given temperature.

Sorbic acid at 200 ppm of the final product prolonged the storage period of mala flavoured with passion fruit juice sugar mix from 6 to 8 days at 15°C by slowing down the initial proliferation of yeasts and moulds. Mala flavoured with fruit preparations other than passion fruit juice showed a decrease of pH and viscosity and an increase of titrable acidity over the storage period. On the other hand mala flavoured with passion fruit juice showed an increase in pH, a decrease in titrable acidity and a constant viscosity over the storage period. The latter may be due to the hydrocolloids added to improve the viscosity of the product.

1. INTRODUCTION

In most developing countries unfermented milk has played a minor role throughout history. Spontaneous fermentation invariably led to a sour milk product with the advantage of a longer shelf-life (1, 40). In addition various studies have shown advantages of sour milk over fresh milk with respect to consumer safely and nutrition (40,54,62,64).

The majority of low and middle income groups in urban areas in East Africa consume fresh milk only in tea and coffee but will usually consume sour milk or 'maziwa lala', a spontaneously fermented milk product (43). In 1975 Kenya Cooperative Creameries (KCC) adopted the trade name 'mala' for a product made by culturing milk with selected mesophilic lactic acid bacteria (43).

In this thesis the term 'mala' will be used for products made from heat treated milk, which is then inoculated with mesophilic lactic acid bacteria, i.e. <u>Streptococcus lactis or Streptococcus cremor</u> is with or without <u>Leuconostoc citrovorum</u>, <u>Streptococcus diace</u>tilactisetc. These bacteria offer some advantages over the thermophilic culture used in yoghurt in that they can be incubated at ambient temperature (18-25°C) and fermentation stops when a pH of 4.2-4.3 or 1-1.2% lactic acid is attained. This eliminates the need of cooling to stop further souring as is the case with Yoghurt(43).

- 1 -

After a trial period of two years, from 1975 to 1977, K.C.C. reintroduced mala on the market in 1985. The flavour, viscosity and keeping quality of such a product was decisively improved by Kurwijila (43). The success of this project is reflected in a very fast growth of sales figures.

Experiences elsewhere indicate that flavouring of cultured milk products with fruit preparations opens very important additional sales prospects. Fruit—based yoghurt accounted for 91% of the total market for yoghurt in Switzerland in 1981 (18), 86-88% of yoghurt sales in U.K. in 1971 (13) and were responsible for an increase of 22% in yoghurt sales between 1970 and 1971 when they were introduced in France (5). Fruit based cultured milks were also most preferred in a school milk programme in the Federal Republic of Germany and tests in U.K. gave similar results among boys and girls (48a). Together, 50% of total yoghurt consumed in the Netherlands, France, U.K., Switzerland, Belgium, Austria, Denmark and Italy is flavoured with fruit (48b).

Fruit flavoured cultured milks combine the improved nutritive value and therapeutic properties of fermented milk products as well as alleviation of lactose intolerance with the carbohydrates, vitamins and minerals of fruits (22).

The amount of fruit flavour and sugar added to cultured milk varies with the nature of flavour, type of fruit, type of cultured milk, source of milk

- 2 -

and the preference of the consumer (3, 17, 28, 29, 33, 49, 54, 71, 72).

The body and mouthfeel of products is markedly influenced by their viscosity. Products of very high viscosity are not always desirable (34,40) while too thin foods may be associated with low nutritive value (40,54).

The seasonal availability of many fruits and the need to minimise fruit spoilage requires that some preservation be done. In tropical developing countries where refrigeration or canning is an expensive venture, simple and effective methods of preparation and preservation should be adopted. The sugar content of fruit based flavours suitable for addition to cultured milks varies widely but three broad categories can be identified: unsweetened conserves which necessitates the addition of sugar to the cultured milk, conserves with the requisite sugar added to aromatize and fully sweeten stirred cultured milk and fruit conserves which may not contain any added sucrose for dietetic reasons. The last two offer an advantage to the dairy plant as no extra sugar is added to the cultured milk (49).

The manufacture of such fruit flavours is based on quality considerations, such as taste, aroma, consistency, viscosity, pH and simplicity of use.

Cultured milks *are not usually heat treated

- 3 -

after fermentation and will therefore contain lactic acid bacteria in high numbers together with varying amounts of contaminating yeasts, moulds and bacteria (54). Fruit—based flavours to be added to such cultured milks can also be a source of contaminating yeasts, moulds and bacteria if proper methods of their manufacture are not adopted (69,79).

The fruit-flavoured cultured milks are high in sugar and/or low in pH and may therefore selectively promote the growth of yeasts and moulds. At higher storage temperatures such growth will be fast, leading to rapid deterioration of the product (39,42,52). High temperatures will also promote protein and fat hydrolysis resulting in a bitter product and development of off-flavours (54).

The objectives of this work therefore were:

- (1) To optimise the sugar and fruit pulp or juice levels with respect to acceptability in the cultured milk product mala flavoured with preparations from some fruits available in Kenya.
- (2) To determine the acceptability of different fruit based flavours.
- (3) To optimise the viscosity of mala flavoured with fruit juices.

÷

- 4 -

- (4) To devise simple means for preparation and storage of fruit-sugar mixes.
- (5) To assess the storage quality of mala flavoured with selected fruit-sugar mixes.

*

2. LITERATURE REVIEW

2.1. Flavouring of Cultured Milk

The consumption of flavoured fermented milk is not new. The biography of the Roman Emperor Elagabalum (A.D. 218-222) mentions two recipes of flavoured soured milk, 'opus lactarum' flavoured with honey, flour and fruits and 'oxygala' flavoured with vegetables and spices (54). Over the last few decades cultured milks have been flavoured with coffee, chocolate, artificial or natural fruit essences and syrups, pulps, juices, concentrates, purees, preserves, whole fruit or pieces from diverse fruits (15, 17, 28, 29, 33, 51, 71, 72, 76).

Juices and pulps from many fruits can be used at normal strength (54,76), but sugar is added for a more satisfactory flavour (49,54). To some fruits, natural flavour concentrates or other fruits are added to balance flavour (54,76). For example citric acid or lemon juice is added to banana pulp (54).

The amount of flavour and sugar added varies with the nature of the flavour, type of fruit, type of cultured milk, the source of milk and consumer preferences. Literature

reports various flavour and sugar levels that have been found acceptable in cultured milks: 6-8% fruit syrup in yoghurt (72), 6% amazonian fruit pulp, 20% sugar and 10% water in yoghurt made from buffalo milk (29), 6% orange juice concentrate containing 50-60% sugar in cultured milk (33), 20% combination of coconut and pineapple or peach and passion fruit juice (3). In Central Europe, flavoured yoghurt is made by addition of 10-25% pulp or juice and 8-12% sucrose depending on the fruit. Pineapple and cherry flavoured yoghurts are optimal with 11-12% sugar, while yoghurtsflavoured with raspberries or strawberries need higher amounts of sugar (49). Natural aroma concentrates and single strength fruit flavours are equally acceptable but concentrates are used at much lower levels (17,51).

Many Legisla tions limit the quantities of fruit and sugar to 25% in the flavoured cultured milk (54). In some countries no legal requirements exist for fruit yoghurt. In Kenya, however, the Kenya Bureau of Standards limits to 30% the addition of natural flavourings to yoghurt (36).

- 7 -

Acceptability of Different Fruit Based 2.1.1. Plavours Used in Cultured Milks

- 8 -

In a study comparing the acceptability of sundae type yoghurt flavoured with honey, orange, strawberry, pineapple, passion fruit or grapefruit preserves, orange strawberry and honey flavoured yoghurts were most acceptable in that order and they had better appearance and texture (9). Frozen yoghurts flavoured with peach, cherry or strawberry were most acceptable in that order. Those flavoured with jams were more acceptable, as jams gave colour as opposed to aroma concentrates but they were more expensive, Those frozen yoghurts flavoured with coffee, apple, cloudberry, grapefruit, pineapple, pear or a banana-vanilla combination were found unsuitable (28).

- 2.2. Viscosity of Cultured Milk
- 2.2.1.

Definition and Measurement

The viscosity of a fluid refers to its resistance to flow, Fluids are classified as Newtonian or non-Newtonian. Newtonian fluids are those whose rate of flow is proportional to shear stress and their viscosity is independent of the shear rate. The viscosity of

non-Newtonian fluids depends on shear rate and may also be time dependent.

- 9 -

Various types of viscometers have been used to measure viscosity of non-Newtonian fluids. Falling ball viscometers are suited for measuring firmness of cultured dairy products with unbroken curd. Capillary and torsion spring (coaxial) viscometers can be used to measure viscosity of dairy products with a stirred curd (34,55,65,66).

Cream, sour cream, ice-cream mix, evaporated milk and yoghurt exhibit varying degrees of non-Newtonian behaviour. The latter also exhibits thixotropic behaviour, where viscosity decreases with shear rate and with duration in shear stress. It also exhibits a degree of pseudoplasticity (34). As a result, only values obtained under particular standard conditions can be compared (66). Measurements are also affected by the treatment of the curd and its temperature (66). Kaahwa (34) observed that the viscosity of the unstirred sour butter milk was always lower than that of the product stirred by an electric stirrer at 500 rpm for 1 minute.

2.2.2. Optimisation of Viscosity of Cultured Milk

Possibilities for achieving desired viscosity in cultured milks are: increasing solids not fat, manipulating processing procedures particularly temperatures and times of heat treatment, incubation and cooling, using specific cultures and addition of stabilisers.

2.2.2.1. Increasing Solids-not-Fat

Levels of 9-10% and 12-16% solids-notfat are considered optimal for achieving a good viscositv of cultured buttermilk (34,41,42) and yoghurt (48b) respectively. Yoghurt in Switzerland contains 3.4-4% butterfat, 15% solids-not-fat and 10-15% added sucrose. Additions such as sugar and fruit, however may not exceed 25% of the plain yoghurt (43). At one plant in Holland yoghurt is prepared from a base containing no fat, no added sugar and some added solidsnot - fat (43). In U.S.A. cultured buttermilk is made from milk with solids-not-fat of not less than 9% (43). A significant amount of buttermilk is made from milk with 2% dry skim milk added to skim milk, the resulting product contain not less than 10.5% solids-not-fat and is not excessively

viscous (43). In K.C.C., mala is made from milk standardised at 2.3% butterfat without addition of solids-not-fat, as declared on the label.

2.2.2.2. Heat Treatment

Raw material used for cultured milk products is often heated to 85°C with 30 minutes holding time or 88-91°C for 3-5 minutes (8,27,34,70). These heat treatments achieve 80% denaturation of whey proteins (26). Particularly good results are obtained in our pilot plant by heating to 90°C for 15 minutes. The resulting interaction between casein particles and whey proteins increase water binding capacity of casein thereby minimising wheying off during subsequent handling of the product (75). Heat treatment below 82°C and above 88°C for 30 minutes causes weak body and whey separation (23,41). Inadequate heat treatment of milk may often be the cause of weak bodied, curdy textured milk products which whey off easily (40,54,74).

Furthermore adequate heat treatment also causes destruction of bacteria and natural inhibitors, expulsion of oxygen and partial protein hydrolysis. These factors tend to

- 11 -

stimulate growth of starter cultures (75). Higher temperatures may inhibit starter growth due to formation of toxic volatile sulfides (50).

2.2.2.3. Incubation Time and Temperature

Within certain limits, sub-optimal incubation temperatures and consequent longer incubation periods appear to favour higher viscosity in yoghurt. Rapid acid production in the late stage of yoghurt fermentation at 44-46[°]C leads to a very firm compact coagulum with heavy subsequent separation of whey and weak viscosity upon stirring and addition of fruit (43). Slow acid development may result in grainy texture (43).

Milk products cultured by mesophilic lactic acid bacteria, such as buttermilk, are incubated at 21-22^OC for 14-16 hours. As the acidity increases to 0.75%-0.80% lactic acid, casein hydration and hence whey retaining capacity increases and the stability of the product is improved. Longer incubation time causes acidity and viscosity to increase, but with adverse effect on taste and whey retaining property of the curd (40,41).

2.2.2.4. Use of Slime Producing Cultures

Galesloot and Hassing (25) used a <u>lactobacillus bulgaricus</u> (RR) culture with high slime producing characteristic to increase the viscosity of stirred yoghurt to meet the demands of high speed production and packaging. Slime production may be augmented in cultures of mesophilic lactic acid bacteria by inoculating at low temperatures when certain strains of <u>Streptococcus cremoris</u> may form long chains (41). Cultures with none or excessive production of slime have been considered unsuitable for manufacture of yoghurt with good viscosity (54).

2.2.2.5. Addition of Stabilisers to Cultured Milks

In Switzerland addition of upto 0.2% stabiliser in yoghurt is allowed. In U.S.A. 0.5% gelatine is a popular choice in yoghurt (43). The use of 0.4-0.6% pregelatinised starch or 0.6% gelatine alone gave yoghurt of excellent viscosity (43). Gelatine and starch are used as jellying agents and stabilisers, the latter also as a thickener. Sodium caseinate at greater than 2% in milk gives undesirable thickening

of yoghurt (54). However, at 0.7% of the milk it improved organoleptic qualities, increased viscosity threefold and reduced syneresis by 20% in kefir and prostokvasha (53). Agar, carageeenan guar gum, gum arabic, carob's kernel flour or carboxymethylcellulose may damage flavour and lower acid production when added to milk especially at higher concentrations (54). Sodium citrate, added to milk in order to increase citrate content, interacts with milk phosphates and increases the viscosity of cultured milk (24,34). This effect also prevents wheying off. Davis (14) claims that the body and firmness of yoghurt can be improved by the use of skim milk powder with parallel improvement in nutritional value and that the use of non-nutritional stabilisers is not necessary when a natural product of high nutritional value can perform as well.

2.2.3. <u>Manufacture of Pourable Fruit Sugar Mixes for</u> Addition to Cultured Milks

The manufacture of fruit-sugar mixes for addition to cultured milks is a specialised field (7,15,37,49,68,76,78). Wigget <u>et al</u>. (78) recommend a mix with 30-50% fruit, 40-50% total solids made by adding sugar, 0.25-0.5% low methoxyl pectin, 0.05% gel thickener and 280-1000 ppm sorbic acid, while Szemplenski (68) recommends addition of sugar, water and stabilisers to the fruit and a heat treatment greater or equal to 70°C for three minutes, then filling in sterile containers. Hydrocolloids can also be added when fruits do not contain sufficient pectin. This prevents undesirable thinness, separation of juice or separation of fruit pieces (49).

2.2.4. Viscosity of Fruit Flavoured Cultured Milk

The addition of jams, heavy pulps and thick syrups to cultured milks does not adversely affect their viscosity. However, when fruit particles are present and their pH is less than 3, there is a risk of whey separation around the fruit particles (54). This will lower the viscosity of the product. The addition of fruit juices will dilute the yoghurt resulting in low viscosity and so they are rarely used (54). The fruit mix or juice should just be pourable so as not to affect product viscosity adversely and still be manageable (78).

- 15 -

2.3. <u>Preservation of Fruit Sugar Mixes for</u> Addition to Cultured Milk

Fruit preparations for addition to yoghurt stored well at 21°C when sugar, water and stabiliser were added and the mixture heated to 70°C for 3 minutes then filled hot in sterile containers (68). Kivi (37) asserts that such an aseptic process results in a more uniform product with better retention of colour, flavour and nutrients and requires no refrigeration. Other advantages are that the product is heated at lower temperatures and no colour-

ing, artificial flavours or preservatives are required (32). It is possible to avoid chemical preservatives altogether if flavouring ingredients are packed hot or reheated in the container and used soon after opening (49).

Chemical preservation of fruit to be added to cultured milks has also been tried. Mango, guava and pawpaw pulps preserved with sodium metabisulphite at 2000 ppm were found satisfactory after 18 months storage at 30°C. However, when required for use, desulphitation was necessary. This was done by either a 10 minute boiling for guava and mango pulps or

- 16 -

addition of 0.25% H₂O₂ to all pulps. This latter method may not be allowed in all countries. Both treatments resulted in no flavour changes but free sulphur ranged from 202 to 612 ppm and total sulphur from 259 to 767 ppm in both cases (17).

The 10 minute boiling is more than adequate to preserve the pulps (37,78) and the 2500 ppm H_2O_2 may leave high residual H_2O_2 levels which may in turn oxidise other compounds.

2.4. Preservation of Fruit Flavoured Cultured Milks

Methods available for increasing the shelflife of cultured milk products include cooling, freezing, aseptic processing, use of chemical preservatives, pasteurisation and treatment with high frequency and ultra high frequency alternate electromagnetic fields.

2.4.1. Cooling

Apart from secondary infections by yeasts, fungi and bacteria, the shelf-life of cultured milk products depends very much on the temperature (42,54). Deteriorative enzymatic and chemical reactions will also be faster at higher temperatures.

The continued growth of <u>Lactobacillus</u> bulgaricus results in increased acidity in yoghurt, not only at higher storage temperatures but also at temperatures as low as $6-5^{\circ}C_{, A}^{\text{though}}$ at a much slower rate (54). However, in the mesophilic culture used in mala fermentation stops when 1-1.2% lactic acid is attained and the risk of oversouring is greatly reduced (43). Overacidification can lead to curd syneresis and taste defects (41,43,54).

Yoghurt stored at over 15°C retained good flavour for 4-5 days and the most frequent defects thereafter were sourness due to overacidification and bitterness due to protein hyrolysis (58). The shelf-life of voghurt stored at 8-12°C or 20-25°C was 96 and 24 hours respectively, and yeast and mould counts were 1.2 X 10⁶ and 7 X 10⁶/ml respectively at the end of these storage periods (42). Yoghurt remained palatable for upto 3 months when stored at 4°C but only for one month at 8-10[°]C (39). When infected intentionally with non-sporulating yeasts Torula cremoris yoghurt became gassy, developed fruity flavour and was inedible after 10 days storage at 25°C (64). When cooled to 7°C 'Mladost' cultured cows milk kept in good condition for upto 24 hours at 16-18°C (16). Prostokvasha kept well

for 30 days at $2-4^{\circ}C$ (63).

Stoyanov et al. (67) stored yoghurt with 10% fruit syrup at 5°C, 10°C or 15°C and found the shelf-life to be 4 weeks , 1 week and 2-3 days respectively. Yoghurt flavoured by maple syrup kept well for 29 days at 4.4°C but only for 15 days at 10°C. Yeasts and moulds were the most serious contaminants (6). The storage period of a fruit yoghurt with 1.5% butter fat could be extended to 7 days at 8^OC (80). Farah et al. (22) reported that a beverage made up of 60% buttermilk and 40% orange juice and pasteurised at 75°C for 30 seconds kept well for 21 days at 4[°]C but only for 4 days at 25[°]C due to increased yeast and mould counts. When yoghurt with 12.5% fruit was inoculated with yeast at 2000/ml it had a shelf-life of 14 days at 5°C, 6 days at 8-10 °C and 1 day at ambient temperature (52). In a study on some marketed fermented milks, changes in organoleptic quality were detected after 3 days at room temperature or 7 days at 10 °C without microbial contamination during storage (11). In another study, yeasty aroma or taste became evident when yeast and mould count was $10^5/gm$ in quarg (19). Davis et al. (14) recommended microbial standards for fruit flavoured yoghurt at the point of

distribution (Table 1).

Table 1. Yeast and mould counts in flavoured yoghurt at the point of distribution recommended by Davis et al. (14)

Viable count	Satisfactory	Doubtful Unsatis- factory
mould/ml	< 1	1 - 10 >10
yeast/ml	< 100	100 - 1000 >1000

In yoghurt and other cultured milks, a storage life of 4 weeks at around 10 °C should be strived for (14). Temperature will also affect other properties of cultured milks. During cooling and storage of yoghurt an improvement in viscosity occurs for 48 hours after manufacture. This is due to hydration of proteins, solidification of the gel structure and the eventual thixotrophy of yoghurt (54). Cooling also reduces the activity of diacetyl reductase in buttermilk considerably, affecting flavour (56), while reduction of diacetyl to 2, 3 - butylene glycol at above 4°C together with volatilisation of diacetyl at higher temperatures contributes significantly to flavour loss (61,77).

2.4.2. Freezing

Freezing is only applicable to stirred cultured milks. Stirred yoghurt may be successfully frozen at 18°C provided that the total solids content is sufficiently high (20-25% in fruit yoghurt and 13-14% in unflavoured yoghurt). Addition of stabilisers has a similar effect to high total solids. (54).

2.4.3. Aseptic Processing

Aseptic processing involves complete prevention of contaminating micro-organisms. This requires special equipment and is therefore expensive. Hygienic manufacture, which involves application of common hygienic measures in each production step to exclude yeasts and moulds as much as possible results in a reasonably good storage life without special equipment (54).

2.4.4. Chemical Preservation

Addition of chemicals to cultured milk products to prevent microbial spoilage is feasible. The concentration of sorbic acid used in making cultured dairy products of prolonged shelf-life usually amounts to 0.025-0.15% (44). Beverages of prolonged shelf-life were produced by addition of

50-100% water, together with sorbic acid, to yoghurt after incubation followed by homogenisation, cooling, incorporation of CO, and bottling (10). Yoghurt with 12.5% fruit had a storage life of 14 days at ambient temperature when 300 ppm sorbic acid was added and 8 days at 10[°]C with the addition of 100 ppm. These levels of sorbic acid were not detectable by taste or smell. 150 ppm sulphur dioxide or sodium metabisulphite or sodium benzoate did not prevent the proliferation of yeasts (52). Fermented milks, however, are regarded as natural products which should not contain foreign chemicals (54), but in tropical developing countries there could be a justification for their use.

2.4.5. Pasteurisation of Cultured Milks

When cultured milks are pasteurised, all yeasts, moulds and most lactic acid bacteria are destroyed. Since microorganisms are more susceptible to heat in an acid medium, relatively low temperatures are germicidal (54). Lactic acid bacteria can be eliminated in yoghurt of pH 3.8-4.0 and 4.0-4.5 by heat treatments for 22 seconds at 65°C and 70°C respectively. Temperatures as low as 55°C are also effective but require about half an hour holding time depending on the pH (47,54). Normal pasteurisation temperatures range from 60-75^OC with holding times of 30-40 seconds depending on acidity, type of bacteria and the dry matter content of the product (47,60).

The main problems encountered in the heat treatment of sour curd are the shrinking of the acid casein leading to sandiness, whey separation and loss of aroma; the last is less pronounced in flavoured products (54). Shrinking of the casein can be overcome by adding stabilisers or adjusting the pH (46). At a pH value below 4 the majority of fermented milks can be pasteurised without any additives (54). The following measures will also increase the stability of cultured milk products towards heat: decreasing protein content, use of proteolytically active bacteria, preheating the original milk to very high temperatures and/or homogenisation (47). Cooling and holding milk at 18°C for 18 hours reduces the tendency to syneresis. When such additional cooling is uneconomical the fat content or the hydrocolloid level can be raised (47).

Since pasteurised fermented milks contain very few if any viable lactic acid bacteria, serious objections have been made concerning the use of names. For example, should the name yoghurt be used for a pasteurised product (38a)? FAO/WHO (21) make a distinction between flavoured yoghurt and flavoured yoghurt heat treated after fermentation. The method of pasteurisation however, has found widespread application in the manufacture of flavoured or fruit yoghurt to extend shelf-life (11, 38b, 60). Farah <u>et al</u>. (22) and Schulz and Voss (60) recommend pasteurisation of cultured milk products in warm developing countries. The shelf-life of such pasteurised products is long, in some cases one year or more (59).

2.4.6. High Frequency/Ultra High Frequency/Multiple Frequency Method

The method overcomes the drawback of none or few viable lactic acid bacteria in pasteurised products. It refers to fast bielectric treatment of the product, filled into plastic cups, by use of high frequency alternate electromagnetic fields. Yeasts and moulds are killed and lactic acid bacteria remain viable but 'shocked' and shelf-life increases to 6 weeks with no refrigeration (54).

- 24 -

3. MATERIALS AND METHODS

3.1. Raw Materials

3.1.1. Milk

Raw milk with a butter fat content >3.2% was obtained from farmers at the point of delivery to the KCC's Dandora Factory, Nairobi.

- 25 -

3.1.2. Sugar

Sugar produced locally was bought from the shops.

3.1.3. Mango Pulp

Canned unsweetened mango pulp was obtained from Trufoods Limited, Nairobi.

3.1.4. Passion Fruit Juice Concentrate

Passion fruit juice concentrate was obtained from Kenya Fruit Processors Limited, Thika.

3.1.5. Bananas

William Hybrid fully ripe bananas were bought from local markets.

3.1.6. Pineapples

Canned pineapples as bits in unsweetened juice were obtained from Kenya Canners Limited, Thika.

3.1.7. Strawberries

Strawberries were bought locally in bulk, pulped using the Homorex mixer, sealed in polythene bags and kept in a domestic deep freezer until required.

3.1.8. Culture

One mesophilic lactic acid culture containing <u>Streptococcus cremoris</u>, <u>Streptococcus</u> <u>diacetilactis</u> and <u>Leuconostoc citrovorum</u> (Betacoccus cremoris), B-CH:40, was obtained from Christian Hansen's Laboratorium, Denmark. It was selected after screening of several cultures in taste panels composed of adult Kenyans (43).

3.2. Chemicals

3.2.1. Sucrose

Alpha brand, standard laboratory reagent sucrose from Alpha Chemicals Limited, Nairobi was used.

3.2.2. <u>Citric 'Acid, Caffeine and Sodium Carbonate</u> Kobian brand laboratory chemicals from

Kobian (Kenya) Limited were used.

3.2.3. Sodium Hydroxide

HG brand laboratory chemical sodium hydroxide from Howse and McGeorge (Kenya) Limited was used.

3.2.4. Potato Dextrose Agar

Potato Dextrose Agar from E. Merck Darmstadt (Germany) was used.

3.2.5. Gelatine

Dominion brand, pure granulated gelatine packed by Erskine and Price (MFG) Ltd., Nairobi was used.

3.3. Methods of Preparation

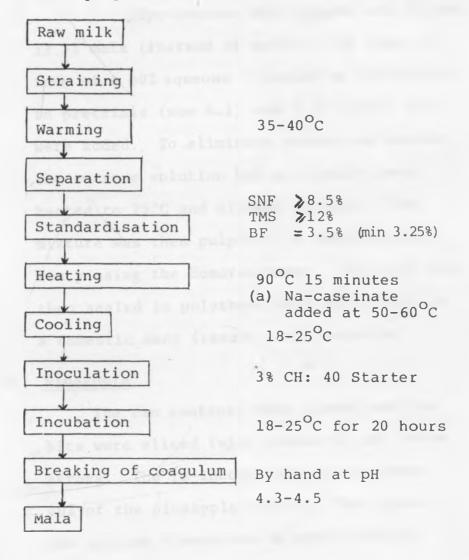
3.3.1. Starter culture

B-CH: 40 starter frozen during lag phase (43) was thawed and four transfers done to obtain the bulk starter. For each transfer, milk was heated to 90°C, held for 15 minutes, cooled to ambient temperature and inoculated with 3% culture, then incubated at ambient temperature for 20 hours and stirred.

3.3.2. Mala

Mala was prepared as indicated in Figure 1.

Fig. 1. Mala preparation



NOTE: BF = Butter fat

- SNF = Solids-not-fat
- TMS = Total milk solids

(a)	=	added for viscosity optimisation
		trials when passion fruit juice -
		sugar mix with no added hydrocolloids
		was used.

3.3.3. Banana Pulp

Ripe bananas were peeled and sliced, 33.3% mala (instead of water), 30% sugar in form of a 60% aqueous solution as determined in pretrials (see 4.1) and 0.3% citric acid were added. To eliminate yeasts and moulds the sucrose solution had previously been heated to 75°C and allowed to cool. The mixture was then pulped to a smooth consistency using the Homorex mixer. The pulp was then sealed in polythene bags and stored in a domestic deep freezer until required.

3.3.4. Pineapple

The can contents were sieved and the bits were sliced twice downwards and twice across. The re-incoporated juice formed 40% of the pineapple pieces. The pieces had average dimensions of approximately 1 X 1 X 0.5 cm.

3.3.5. Passion Fruit Juice

Passion fruit juice concentrate was diluted with water to 14° Brix to obtain a standard straight juice.

3.3.6. Sodium Caseinate (57)

Raw milk was heated to about 40 C, skimmed, pasteurised at 63°C for 30 minutes, cooled, inoculated with 2% starter and

incubated at 20-25°C for 18-24 hours. The curd was then stirred slowly under indirect heating to 55-58°C within half an hour. The whey was withdrawn and the acid casein washed twice with water at 50-60°C and at ambient temperature respectively; each wash was stirred for approximately 10 minutes. The casein was then put in moulds lined with cheese cloth and pressed for 16 hours with press weight of approximately 15kg/kg casein. The acidecasein was broken into small pieces and dried in layers of about 1 cm to 90-92% total solids using dry air current with a temperature not exceeding 60°C. To prepare sodium caseinate, one part dry casein and five parts water were mixed and stirred until a good homogenity was obtained. NaHCO3, at 4.5% of the casein by weight, was dissolved in a little water and slowly added to the mixture with constant stirring. The mixture was then stirred vigorously for 10 minutes, heated slowly to 60-65°C and held at this temperature for $l_{\frac{1}{2}}^{\frac{1}{2}} = l_{\frac{1}{2}}^{\frac{1}{2}}$ hours under constant stirrings, cooled and stored at 2-5°C. The sodium caseinate so made had 17.9% total solids.

÷

3.3.7. Fruit-Sugar Mixes For Preservation Trials

The required quantities of granular sugar, as determined in the pretrials (see 4.1) were added to fruit mixes except bananas. Ascorbic acid was also added to some of these mixes, ensuring that the resultant flavoured mala would not contain more than 50 ppm and in some specific experiments not more than 200 ppm. Each mix was then heated to 75°C, filled in hot glass jars, capped, allowed to cool and stored at ambient temperature. Different quantities of gelatine or pectin were added to some portions prior to the heat treatment.

1

3.3.8. Fruit Flavoured Mala

3.3.8.1. Fruit Flavoured Mala for Fruit/Sugar Level Optimisation

Different quantities of pulps from mango (see 3.1.3.), strawberry (see 3.1.7) and banana (see 3.3.3.) as well as pineapple fruit mix (see 3.3.4) and passion fruit juice

- 31 -

- 32 -

(See 3.3.5) together with different quantities of granular sugar were added to the mala and stirred by hand until homogenous.

The sugar and the mala added when preparing banana pulp were taken into account when determining the pulp and the sugar quantities to be added to the mala.

3.3.8.2. Fruit Flavoured Mala for Other Experiments

The required quantity of the appropriate fruit-sugar mix (see 3,3,7,) was added to the mala and stirred by hand until homogenous.

3.4. Methods of Storage

3.4.1. Fruit-Sugar Mixes

Enough fruit-sugar mixes (see 3.3.7) were stored in glass bottles with airtight caps at 25°C or 2-3°C. Three bottles were analysed before and after one month storage at 25°C for Brix pH, viscosity and acidity. The contents of these bottles were then used to flavour mala. Sensory difference evaluation was then done, whereby mala flavoured with fruit mixes stored at 2-3°C was used as a reference.

3.4.2. Fruit Flavoured Mala

Enough samples were packed in 500 ml polythene bags and stored at 25°C, 15°C or 2-3°C. 5 packets were selected randomly on the day of analysis. Three of them were each analysed for pH, acidity and viscosity, as well as yeast and mould counts. The remainder was then mixed with the other two for sensory evaluation. This represented a 15% and 25% sampling for chemical and sensory evaluation respectively. Samples stored at 2-3°C were used as controls.

- 33 -

3.5. Methods of Analysis

pH

3.5.1.

pH was measured by Metrohm Herisau (Switzerland), E 516, titriskop model pH meter.

3.5.2. Viscosity

The viscosity was measured at 25^oC by a torsion viscometer (Rheometer STV, Contraves AG, Switzerland). 120 ml were put into system A with speed settings I or II. Readings were taken 30 seconds after the spindle began to turn.

3.5.3. Brix

Brix was measured by Abbe's refractometer (Kikuchi, Tokyo) at 20⁰C.

3.5.4. Titrable Acidity

Samples were titrated with 0.1N NaOH to pH 8.4 and acidity expressed as ^OTh. for products with milk and as grams citric acid/100 -ml for products without milk.

3.5.5. Solids-not-Fat

Solids-not-fat in milk were found by determination of buttterfat according to Gerber, density and subsequent calculation using Fleischmann's formula (57).

3.5.6. Yeast and Mould Counts

The method adopted by the American Public Health Association (4) was used for yeast and mould counts.

3.5.7. Characterisation of the Pectin Used

The pectin was characterised by the method adopted from Escher and Denzler (20) and was found to have 43% esterification.

3.6. Methods of Sensory Evaluation

3.6.1. Panel Selection

17 panelists were screened on the basis

of recognition of the four basic tastes and of the aroma of the fruits to be used later. Selection was then done on the ability to rank pure solutions of sucrose and citric acid pure consistently (Appendix I). Further selection criteria were: motivation, willingness and ability to follow instructions (2). 3 of the 17 penelists were rejected, one for inability to identify odours of fruits to be used later and two for low ability to follow instructions and loss of interest respectively.

3.6.2. Sensory Evaluation for Fruit/Sugar Level Optimisation in Fruit Flavoured Mala

A factorial design with 3 sugar and 3 fruit levels was used to evaluate the optimum sugar and fruit levels for each of the fruit used to flavour mala. Samples were presented at random in glasses or plastic cups to any ten of the fourteen panelists; such tests require 8-25 semitrained panelists (31). Panelists were asked to fill the questionnaires (Appendix 1I) adopted from Amerine <u>et al.</u> (2) and Rasic and Kurmann (54). Data were subjected to analysis of variance with partitionea sum of squares for treatment and interaction (45).

UNIVERSITY OF MAIROR

3.6.3. Sensory Evaluation to Determine Most Acceptable Fruit Flavoured Mala

Mala was flavoured with the optimal quantities of fruit and sugar as determined for each fruit (see 4.1). The samples were then presented together in a completely randomised design to ten panelists. They were asked to fill a questionnaire similar to the one used in Sec. 3.6.2. Data were subjected to analysis of variance with partitioning of the relevant sums of squares (45).

3.6.4.

Sensory Evaluation to Assess Keeping Quality of Fruit/Sugar Mixes

Fruit sugar mixes stored at 25°C for one month were added to mala, stirred and then presented for difference evaluation by the Duo-trio test (2). Mala flavoured with fruit-sugar mixes stored at 2-3°C was used as reference. Any five panelists taken from the fourteen were presented with three sets of samples at random and asked to identify the one identical to the reference (Appendic IIIa). In this way 15 comparisons between sample and reference were achieved. 3-10 panelists have been recommended (31) and Collins et al. (12) presented three evaluations for each pair per judge when assessing the keeping quality of cottage cheese. Evaluation of results was done by reference to statistical tables (2).

3.6.5. <u>Sensory Evaluation to Assess the Keeping</u> Quality of Fruit Flavoured Mala

When no quantification of difference was envisaged the five panelist, did only a difference evaluation (Appendix IIIa) which was assessed by reference to statistical tables (2). When a quantitative difference was envisaged, a difference evaluation and a hedonic scoring were done (Appendix IIIb) with assessment of results by reference to statistical tables (2) and by t-test (45) respectively.

•

4. RESULTS AND DISCUSSIONS

4.1.

Optimisation of Fruit and Sugar Levels in Mala Flavoured with Preparations from Different Fruits and Their Acceptability

The brix value, titratable acidity (as citric acid) and pH of the fruit preparations used are shown in Table 2. On the whole the levels are within thelimits of those reported in literature except for the banana pulp.

Table 2. <u>Brix value, titratable acidity (as</u> <u>citric acid) and pH of fruit prepara-</u> <u>tions used</u>

Fruit preparation	^O Brix Titratab acidity (citric acid) (g/100 m	
Passion fruit juice	14.0 4.47	2.98
Banana pulp	37.0 * 0.70	4.15
Strawberry pulp	7.7 1.73	3.52
Pineapple mix	14.5 00.71	3.85
Mango pulp	14,9 1,27	3.46

Note* Banana pulp with sugar, citric acid and diluent added as described in 3.3.3.

4.1.1. Optimisation of Fruit Sugar Levels

Sensory evaluation scores of mala flavoured with different fruit and sugar levels are shown in Table 3.

Appearance appreciation due to fruitsugar combinations was not significantly different in all malas flavoured with different fruits.

Consistency appreciation due to fruitsugar combination was not significantly different in all except mala flavoured with passion fruit juice. The consistency of the latter was the best when 12% juice was added to the mala.

Odour appreciation between different fruit and sugar levels was not significantly different in mala flavoured with passion fruit juice nor with mango pulp or with banana pulp or with pineapple mix. Interestingly however, mala flavoured with strawberry pulp showed a significant interaction between pulp-sugar levels and odour appreciation. When 16% strawberry pulp was added, odour appreciation decreased when the amount of sugar increased from 10 to 18%. At 18% pulp it increased with increasing sugar concentrations and at 20% pulp, first increased and then levelled off (Fig. Increase in pulp levels from 16% to 18% 2a). decreased odour appreciation when sugar levels

Table 3.	Sensory evaluation scores of mala flavoured
	with different fruit preparations at diffe-
	rent fruit and sugar levels
	(Maximum score 210)

Sensory	Fruit						
criteria	preparation	10% P(8%	12% 10%	14%	16% P(12%	18% 14%	20% 16%)
Appearance	Passion	182	186	175	173	179	181
	Mango	175	178	176	176	174	179
	Pineapple	178	183	181	186	184	172
	Banana	171	170	170	171	171	169
	Strawberry	165	161	162	162	162	162
Odour	Passion	166	163	170	163	165	171
	Mango	169	166	172	176	165	169
	Pineapple	178	182	180	176	180	184
	Banana	168	167	167	172	176	178
	Strawberry	165	169	173	169	167	163b
Taste	Passion Mango Pineapple Banana Strawberry	$ \begin{array}{r} & 149 \\ \hline 170 \\ 180 \\ 182 \\ 152 \\ \end{array} $	181 180 172 168 179	161ac, 164 154 162 164 d	160 162 172 162 156	130 173 164 174 169	143 179 170 171 171
Consistency	Passion	166	166	169	181	165	156
	Mango	198	191	198	196	196	195
	Pineapple	181	178	183	185	177	179
	Banana	178	178	172	175	176	177
	Strawberry	160	163	169	166	161	165

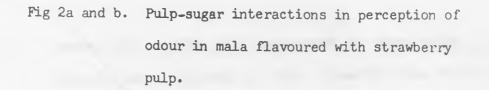
Notes:

- P are sugar and juice levels for mala flavoured with passion fruit juice.
- Figures in the row joined by the same line or underlined are significantly different from the rest in the row P(0.01).
- In rows lettered a significance is at P(0.05).

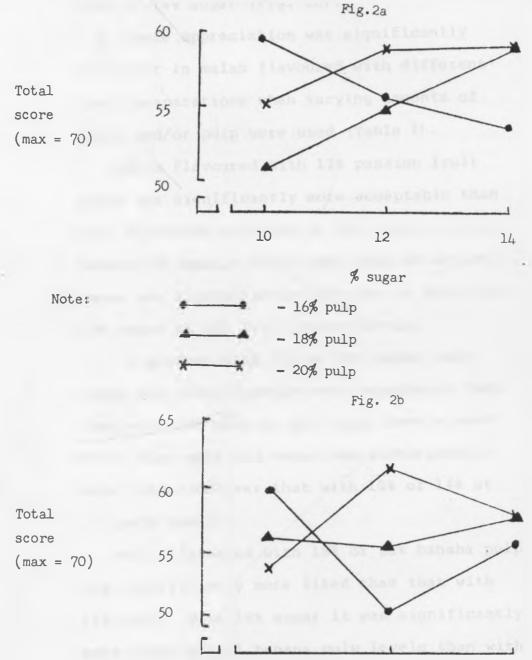
÷

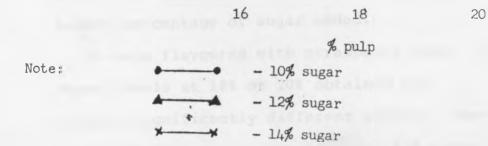
- b interactions significant P(0.01)
- c 12% sugar in mala flavoured with mango pulp scores significantly higher P(0.1) than 10% sugar.

d - 12% sugar in mala flavoured with strawberry pulp scores significantly higher P(0.05) than 14% sugar.



*





*Assessment on a 7 point hedonic scale by 10 panelists

were 10% or 12% and increased it when pulp levels were raised to 20%. The reverse held true at 14% sugar (Fig. 2b).

7

Taste appreciation was significantly different in malas flavoured with different fruit preparations when varying amounts of sugar and/or pulp were used (Table 3).

Mala flavoured with 12% passion fruit juice was significantly more acceptable than that flavoured with 14% or 16% juice at all levels of sugar, while that with 8% or 12% sugar was significantly inferior to that with 10% sugar at all fruit juice levels.

A product with 18% or 20% mango pulp added was significantly more acceptable than that with 16% pulp at all sugar levels used while that with 12% sugar was significantly more preferred over that with 10% or 14% at all pulp levels.

Mala flavoured with 18% or 20% banana pulp was significantly more liked than that with 16% pulp. With 10% sugar it was significantly more liked at all banana pulp levels than with higher percentage of sugar added.

In mala flavoured with strawberry pulp, sugar levels at 18% or 20% obtained the highest significantly different scores. However, the most acceptable odour at 12% sugar

- 42 -

was obtained when 20% strawberry pulp was used (Fig. 2b). Thus sugar at 12% and pulp at 20% were considered most acceptable in mala flavoured with strawberry pulp.

There were no significant interactions between taste appreciation and the fruit-sugar preparation levels in all fruit flavoured malas (Table 5). This was inspite of the significant differences due to sugar levels and due to fruit preparation levels in all except mala flavoured with pineapple fruit mix.

4.1.2. Selection of the Best Fruit Flavoured Mala

The results of sensory evaluation of mala flavoured with fruit-sugar combinations at optimum levels together with their pH, titrable acidity and viscosity are presented in Table 4.

The appearance of malas flavoured with mango pulp and passion fruit juice was significantly better than the others. 7 out of 10 of the panelists downgraded appearance of mala flavoured with strawberry pulp due to seed particles; 6 out of 10 judged the appearance of mala flavoured with banana pulp unattractive or having black specks. 4 out of 10 criticised mala flavoured with pineapple fruit mix due to presence of pieces.

- 43 -

Table 4. Characteristics of fruit -sugar mixes and sensory evaluation of mala flavoured with the fruit-sugar mixes at optimum levels

	Fruit							
Parameter		Passion fruit juice	Mango pulp	Strawberry pulp	Pineapple mix	Banana pulp		
% fruit preparation added		12	18	20	16	18		
% sugar added		10	12	12	10	10		
рH		3.81	4.09	4.01	4.15	4.25		
Titrable acidity (OTh)		166	111	109	108	113		
Viscosity (CP)a		62	98.8	94.8	85.7	128.8		
Sensory criteria		Sensory	evaluati	on scores	(max. 70)			
Appearance		62	66	46	51	51		
Odour		60	59	56	48	55		
Taste		52	59	58	51	51		
Consistency		45	66	54	51	54		
Notes: a -	set	scosity m tting II o eed setti	except ba	th Rheomat nana which	: system A was measu	and speed ared with		
	Fig	gures und	erlined i	n a row ar	e signific	antly		

different from the rest (P = 0.01).

The odour hedonics of malas flavoured with the different fruits were not significantly different.

Mala flavoured with mango and strawberry pulp tasted significantly better than the rest.

Mala flavoured with passion fruit juice was considered thin by 8 out of 10 panelists while mala flavoured with banana pulp was considered too viscous by 5 out of 10 panelists. The consistency of mala flavoured with mango pulp was most acceptable.

4.1.3. Discussion

The most acceptable fruit-sugar combinations in mala together with some properties of these products are summarised in Table 4. The optimal fruit-sugar levels in mala were different for each fruit. Various authors have reported similar results with preparations from different fruits to be added to different cultured milk products (3,17,29, 33,49,51,72). The quantities of fruit preparations and the respective sugar concentrations to be added to cultured milk products differ among regions (49).

÷

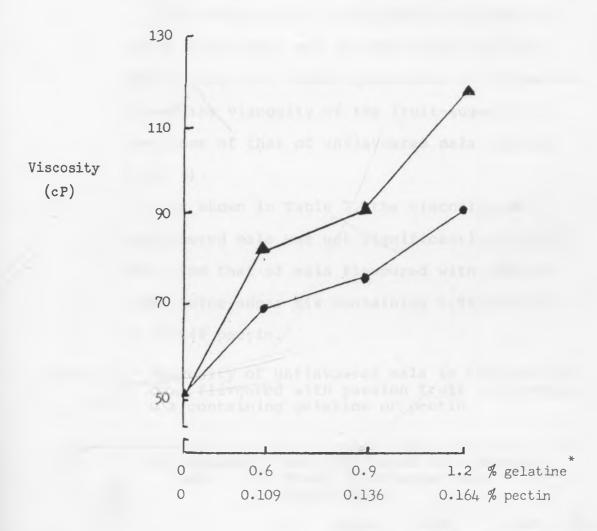
Malas flavoured with different fruit preparations were not equally acceptable. Hague <u>et al</u>. (28) and Chen <u>et al</u>. (9) when experimenting with frozen and sundae type yoghurts respectively, found similar results when flavouring their products with preparations from different fruits. It is necessary therefore to determine the optimum quantities of each fruit preparation and its sugar concentration to be added to mala. Furthermore, due consideration has to be given to the target consumer.

4.2. <u>Viscosity Optimisation of Mala Flavoured With</u> Passion Fruit Juice-Sugar Mix

The consistency of mala flavoured with passion fruit juice was scored low, as it had by far the lowest viscosity among the malas flavoured with different fruit preparations (Table 4). This made it necessary to try and improve the viscosity of mala flavoured with passion fruit juice-sugar mix by various means.

4.2.1. Addition of Pectin and Gelatin to Passion Fruit Juice-Sugar Mix

There was a significant increase in viscosity of passion fruit juice-sugar mix when gelatine or pectin was added. In order to



Note:

- gelatine and pectin as % age of passion
 fruit juice-sugar mix.
- each point represents an average of three values.
 - gelatine

- pectin

arrive at similar increases in viscosity of passion fruit juice-sugar mix, different quantities of gelatine or pectin were required (Fig. 3).

The addition of 0.9% gelatine to passion fruit juice-sugar mix is equivalent to 0.16% pectin (Fig. 3). These quantities of thickeners raised the viscosity of the fruit-sugar mix to the range of that of unflavoured mala (Tables 5 and 6).

As shown in Table 7, the viscosity of unflavoured mala was not significantly different from that of mala flavoured with passion fruit juice-sugar mix containing 0.9% gelatine or 0.164% pectin.

Table 5. Viscosity of unflavoured mala in relation to mala flavoured with passion fruit juice-sugar mix containing gelatine or pectin

	Unflavoured mala	Mala flavoured with passion fruit juice-sugar mix containing:					
		0	0.16% pectin	0.9% gelatine	1.2% gelatine		
Viscosity (cP)	99.9	64.0	95.7	98.6	103.8		
S.D.	11.2	10.8	11.5	11.1	6.7		

Note: Figures underlined in a row are not significantly different P(0.01)

S.D. = Standard deviation of three values from three batches of mala.

4.2.2. Addition of Sodium Caseinate to Milk Prior to Culturing

The viscosity of unflavoured mala and of mala flavoured with passion fruit juicesugar mix made from milk with different levels of sodium caseinate are shown in Table 6.

0.6% sodium caseinate added to milk for manufacture of mala would counter-balance the reduction in viscosity when mala is flavoured with passion fruit juice-sugar mix, which does not contain any added hydrocolloids.

Table 6. Viscosity of unflavoured mala and of mala flavoured with passion fruit juice-sugar mix made from milk with different levels of sodium caseinate (17.9% dry matter)

Mala	00	Sodium caseinate added to milk					
		0	matter bas	0.6			
Unflavo	ured mala						
viscosi		83.8	95.8	109.9			
S.D.		9.3	6.8	11.3			
Flavour							
viscosi	ty (cP)	64.5	75.2	91.1			
S.D.		8.2	13.0	9.4			
Notes:-	Viscosity different	-	in a row a	re significa	intly		
-			in a column different H).		
-	Underlined P(0.01)	d figure	s are signif	ficantly dif	ferent		
-			eviation of m three bate				

ŧ

4.2.3. Discussion

Gelatine at 0.9% or pectin at 0.164% added to passion fruit juice-sugar mix improved viscosity of the mix to within that of mala. This implies that on addition of the mix to the mala, the viscosity of the flavoured mala will not be adversely affected. The levels of pectin are comparable to those recommended by Wigget <u>et al</u>. (78) in the manufacture of fruit-sugar mixes to be added to yoghurt. Kurman and Rasic (54) report that pectin is usually added to fruits low in pectin which are used in such products.

The use of gelatine in fruit-sugar mixes to be added to cultured milks appears to be very limited but it has been used in milk to improve the viscosity of cultured milk products (43).

Sodium caseinate (17.9% dry matter) was added to the milk prior to incubation at the rate of 0.6% on dry matter basis. This improved the viscosity of mala so that when passion fruit juice-sugar mix, with no hydrocolloids added was used, the viscosity of the flavoured product was similar to that of unflavoured mala with no sodium caseinate added. The addition of 0.3% sodium caseinate proved to be inadequate (Table 6). Ramanauskas et al.

- 50 -

(53) added 0.7% caseinate to milk when preparing kefir and prostokvasha, the viscosity of both products was improved 20-fold. In our experiments viscosity values of mala increased by 30% when 0.6% sodium caseinate was added. This improvement in viscosity is lower in relative terms primarily because the initial viscosity of mala is much higher than that of kefir and prostokvasha.

4.3. Preservation of Some Fruit-Sugar Mixes

Preservation of most fruit pulps can be done by a combination of heat, chemicals and lowering of pH (69,79). The precondition for applying such methods is that appearance, flavour and texture of the preserved fruit mix are judged favourably. Banana pulp, however, when heated produces a modified flavour which has not found widespread acceptance among consumers (35). It is therefore usual to preserve banana pulp by freezing (79), unless fresh bananas can be obtained throughout the year.

Table 7 shows physico-chemical characteristics of fruit-sugar mixes and the results of sensory evaluation of mala flavoured with the appropriate quantities of the same, before and after 30 days storage. The Brix value,

- 51 -

Fruit and sugar percentage added to mala	Percentage sugar added to fruit	Storage period days	Sorbic acid ppm on fruit part (b)	Brix	Titrable acidity ml N/10 NaOH per 100 gms	pĦ	Viscosity cp (centi- poise) or p (poise)	Correct response out of 15 with respect to control
Passion		0	0	54.0	549	3.00	87.2cP	_
	0.0		500	54.1	551	3.00	85.9cP	-
2.20	83.3	30	0	54.0	550	3.00	89.2 CP	7 9
228			500	54.0	548	3.00	87.9 CP	9
Mango			0	48.4	303	3.60	8.3P	_
mango		0	360	48.4	303	3.61	8.3P	_
	67		0	48.8	304	3.61	8.3P	8
30%		30	360	48.6	306	3.61	8.3P	6
Pineapple			0	53.0	330	3.65	23.6cP	-
- THEOPPIC		0	400	53.0	330	3.65	24.5cP	-
	62.5	2.0	0	52.9	329	3.64	23.8cP	9
26%		30	400	53.0	331	3.65	23.1cP	9
Strawberry			0	40.9	385	3.55	5.4P	-
	60	0	330	41.0	387	3.57	5.2P	-
2.0.4		20	0	40.7	389	3.59	5.5P	8
32%		30	330	40.8	387	3.56	5.4P	10

Table 7. Characteristics and sensory evaluation of fruit mixes before and after 30 days storage at 25°C

Notes: 12, 13 and 14 correct matches are required for significance at P(0.05), P(0.01) and P(0.001) respectively. (b) Calculated so that when the respective percentage of the mix is added to mala the flavoured product does not contain more than 50 ppm sorbic acid.

pH, titrable acidity and viscosity did not change. There were also no significant identifications of malas flavoured with control fruit sugar mixes stored for one month at 2-3^oC as compared to those flavoured with fruit-sugar mixes stored for the same period at 25^oC. The addition of sorbic acid to the mix, at 330 to 500 ppm of the fruit part, did also not influence the sensory response to the flavoured mala.

4.3.1. Discussion

When filled at 75°C in hot bottles, capped and allowed to cool, the fruit mixes retained their quality over 30 days at 25°C when compared to controls stored at 2-3°C (Table 7). Since no specification was made to the panelists as to the criteria of differentiation, it can be concluded that all quality attributes were well retained over the storage period. This process is as suggested by Mueller (49) and similar to the aseptic process used by Wigget et al. (78) and Kivi (37). In all reports similar results were obtained.

At a pH below 4.2 the main aim is to eliminate yeasts and moulds. To achieve this, heat treatment of the product in containers for 3 minutes at 75[°]C or aseptic filling the

- 53 -

product into sterile containers after the same bulk heat treatment is adequate (68). Alternatively raising the temperature of bottles and caps to boiling in a water bath, filling the product at 75[°]C immediately, capping and allowing to cool sufficed. If fresh fruit is used, higher temperatures may be indicated with due regard to necessary inactivation of enzymes.

The level of sorbic acid in the fruit mixes is limited by IDF recommendation that fruit flavoured cultured milk products should not contain more than 50 ppm of chemical preservative(s) used in the fruit singly or in combination (30). However, the corresponding levels possible of 330-500 ppm on fruit part (Table 7) can protect the fruit - sugar mixes from secondary infections after opening. These levels are within the lower part of the 280-1000 ppm range that have been used by various authors (49,54,68,78).

4.4. <u>Storage of Mala Flavoured With Some Fruit-</u> Sugar Mixes

Due to low pH, presence of fruit pieces and non-heat treatment respectively, malas flavoured with passion fruit juice, pineapple or banana-sugar mixes were selected for storage trials.

4.4.1. Mala Flavoured with Pineapple-Sugar Mix

After two days storage at 25° C, mala flavoured with pineapple-sugar mix had a taste significantly inferior to that of the same product stored at $2-3^{\circ}$ C, but it was still acceptable (Table 8). After four days storage at 25° C all sensory parameters were around the middle of a hedonic scale which is regarded as a baseline of acceptability. The product was inedible after 6 days storage at 25° C (Table 8), when yeast and mould counts were 2 x 10^{7} /ml (Fig. 5).

There was a rapid increase in titrable acidity and decrease in pH in mala flavoured with pineapple-sugar mix stored at $25^{\circ}C$ as compared to unflavoured mala stored at the same temperature or flavoured mala stored at $2-3^{\circ}C$ (Fig. 4a and b).

The viscosity of mala flavoured with pineapple-sugar mix drops rapidly after 4 days storage at 25° C when compared to un-flavoured mala at the same temperature or flavoured mala, at $2-3^{\circ}$ C. (Fig. 4c).

Fig. 4. a, b and c. Titrable acidity, pH and viscosity changes respectively of unflavoured mala and mala flavoured with pineapple mix and stored at 2-3°C or 25°C.

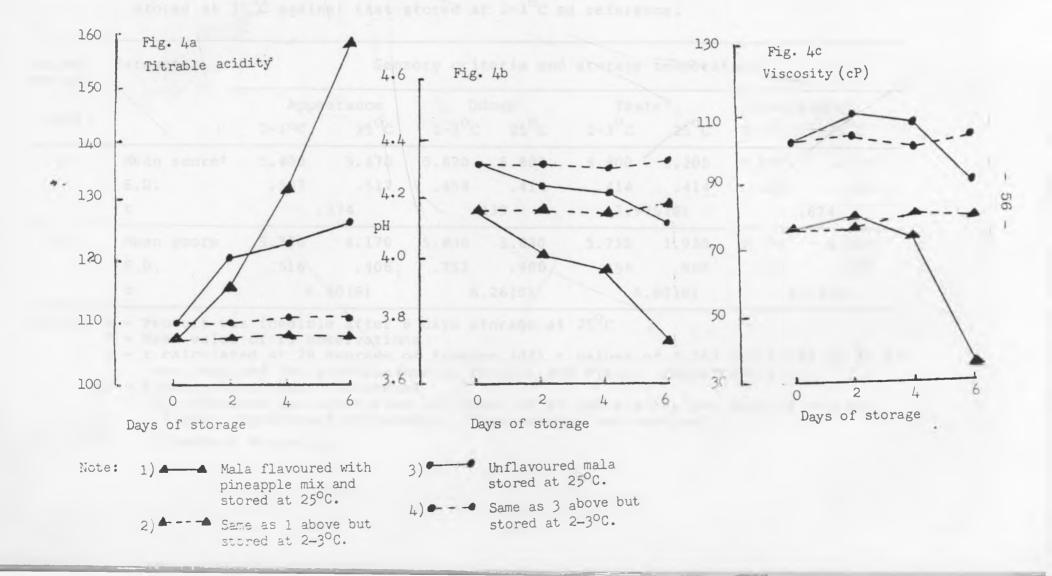


Table ⁸. Mean sensory evaluation scores^{**}of mala flavoured with pineapple fruit mix stored at 25[°]C against that stored at 2-3[°]C as reference.

Stored	Parameter	Sensory criteria and storage temperature											
(days)		Appearance 2-3°C 25 [°] C		Odour 2-3 ⁰ C 25 ⁰ C		Tast 2-3 ⁰ C		Consistency 2-3 ⁰ C 25 ⁰ C					
2	Mean score*	5.400	5.470	5.870	5.800	5.800	5.200	5.670	5.530				
+.	S.D.	.507	.517	.458	.414	.414	.414	.488	.640				
	t		374	. 4	39	3.9	75(S)		674				
4b	Mean score	5.330	4.170	5.830	3.830	5.730	3.930	5.730	4.260				
	S.D.	.516	. 408	.753	.980	. 458	900	.704	.884				
	t	6.80(S)		6.26(S)		6	.90(S)	5.02(S)					

Notes: b - Product was inedible after 6 days storage at 25°C

* - Mean value of 15 observations

t - t calculated at 28 degrees of freedom (df). t values of 2.763 and 2.048 at 28 df are required for significance at P(0.01) and P(0.05) respectively.

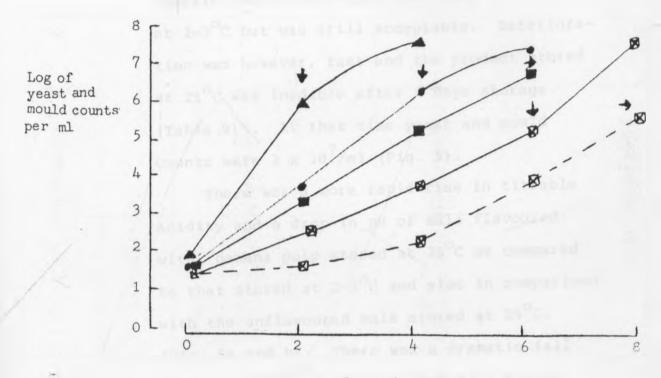
(S) - Highly significant difference

** - The reference was identified in 14 out of 15 pairs after two days of storage, a highly significant difference. Thus scoring was necessary.

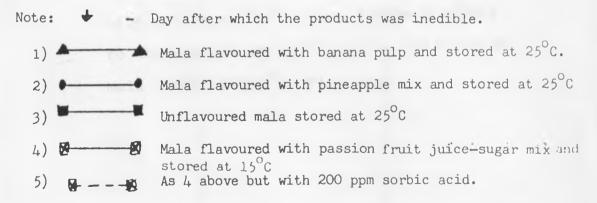
S.D. - Standard Deviation

- 57

Fig. 5. Yeast and mould development in unflavoured mala and in mala flavoured with some fruit preparations and stored at various temperatures.



Days of storage



4.4.2. Mala Flavoured With Banana Pulp-Sugar Mix

After two days storage at 25° C, mala flavoured with banana pulp-sugar mix had inferior taste and odour than that stored at 2-3°C but was still acceptable. Deterioration was however, fast and the product stored at 25° C was inedible after 4 days storage. (Table 9) . At that time yeast and mould counts were 3 x 10^{7} /ml (Fig. 5).

There was a more rapid rise in titrable acidity and a drop in pH of mala flavoured with banana pulp stored at 25°C as compared to that stored at 2-3°C and also in comparison with the unflavoured mala stored at 25°C. (Fig. 6a and b). There was a dramatic fall in viscosity of mala flavoured with banana pulp stored at 25°C after 4 days compared to the control at 2-3°C or the unflavoured mala stored at 25°C (Fig. 6c).

Mean sensory evaluation scores to mala flavoured with banana pulp stored at Table 9. $25^{\circ}C$ against that stored at $2-3^{\circ}C$ as reference

Storage period (days)	Parameter		Sens	sory cri	teria and	l storage	tempera	ture	•
		Appea	arance	Odo	our	Tast	te	Consistency	
		2-3 ⁰ C	25 ⁰ C	2-3 ⁰ C	25 ⁰ C	2-3 ⁰ C	25 ⁰ C	2-3°C	25 ⁰ C
4.	Mean score*	4.500	4.500	6.250	5.250	6.583	5.000	4.750	4.750
2b	S.D.	1.168	1.168	0.450	0.754	0.515	1.206	1.357	1.357
	t	C)	2	.790(S)	4.1	82(S)	0	

b - Product was inedible after 4 days storage at 25°C. Notes:

- * Mean value of 15 observations
- t t calculated at 28 degrees of freedom (df)
- t value of 2.763 and 2.048 at 28 df are required for significance at P(0.01) and P(0.05), respectively.
 (S) - Highly significant difference

- S.D. Standard deviation.
- ** The reference was identified in 13 out of 15 pairs after two days storage, a significant difference. Thus scoring was necessary.

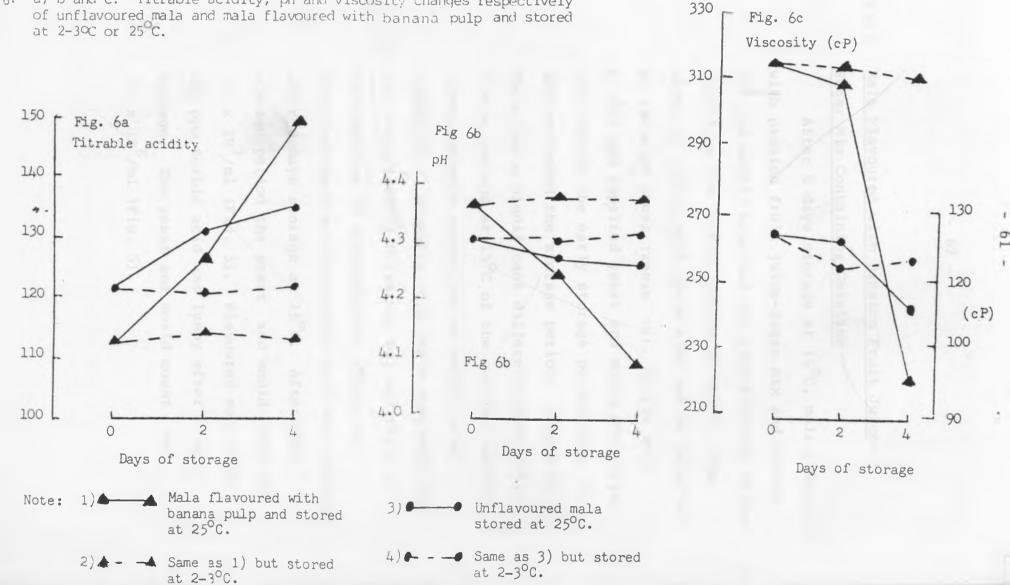


Fig. 6. a, b and c. Titrable acidity, pH and viscosity changes respectively of unflavoured mala and mala flavoured with banana pulp and stored

4.4.3. <u>Mala Flavoured with Passion Fruit Juice</u>-Sugar Mix Containing Gelatine

After 6 days storage at 15°C, mala flavoured with passion fruit juice-sugar mix and having 200 ppm sorbic acid was not significantly different from the control stored at 2-3°C. The level of sorbic acid could also not be detected by taste or smell (Table 10). Sorbic acid at 200 ppm impaired yeast and mould proliferation during the early storage period (Fig. 5) and prolonged the storage period. (Table 10). There was a significant differentiation after 6 days storage at 15°C of the control against flavoured mala containing no sorbic acid (Table 10). However, only taste acceptability was significantly different but was still at the baseline of acceptability (Table 11). Flavoured mala with no sorbic acid was inedible after 8 days storage at 15°C. After this storage period the yeast and mould count was $3.0 \times 10^{\prime}$ /ml (Fig. 5). Flavoured mala with 200 ppm sorbic acid was lumpy after 8 days storage. The yeast and mould count was 2.5×10^{5} /ml (Fig. 5).

Table 10. Duo-Trio difference evaluation of mala flavoured with passion fruit-sugar mix with or without sorbic acid, stored at 15^oC against the control without sorbic acid stored at 2-3^oC.

Storage period (days)	Correct identificat in 15 pairs of mala passion fruit juice at 15 ⁰ C	No. of identifications required for significance				
	No sorbic acid	200 ppm sorbic acid	P(0.1)	P(0.05)	P(0.01)	
2	8	10	12	13	14	
4	9	8	12	13	14	
6	14	9	12	13	14	
8	-		12	13	14	

Note : - indicates that the samples were not presented because of lumps or inedibility.

Table 11. Hedonic scoring of mala flavoured with passion fruit juice-sugar mix without sorbic acid added, after 6 days storage at 15°C against that stored at 2-3°C

Parameter	Sensory criteria and storage temperature											
	Appe	arance	Odo	our	Tas	ste	Consistency					
	2-3 [°] C	15 [°] C	2-3 ⁰ C	15 ⁰ C	2-3 ⁰ C	15 ⁰ C	2-3 [°] C	15 [°] C				
Mean score*	5.930	6.000	5.730	5.730	6.400	3.960	5.600	5.600				
S.D.	0.704	0.654	0.468	0.458	0.632	0.510	0.507	0.507				
t	0.2	82	()	11.9	505(S)	()				

Notes: * - Mean value of 15 observations

- t t calculated at 28 degrees of freedom (df).
- t values of 2.763 and 2.048 at 28 df are required for significance at P(0.01) and P(0.05) respectively
- (S) Highly significant difference
- S.D. Standard deviation
- ** The reference was identified in 14 out of 15 pairs, a highly significant difference. Thus scoring was necessary.

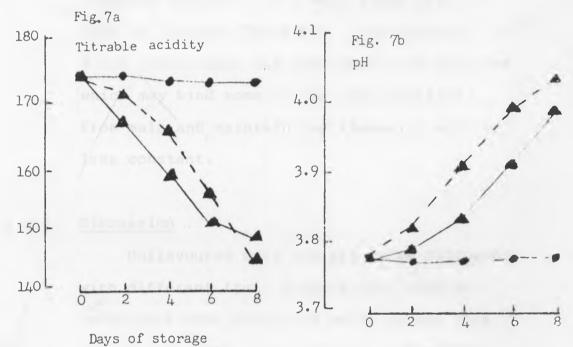
There was a drop in titrable acidity in mala flavoured with passion fruit juicesugar mix, whether it contained sorbic acid or not. (Fig. 7a). There was an increase in pH over the storage period which was more rapid in mala flavoured with passion fruit juice-sugar mix, without sorbic acid (Fig. 7b).

Mala flavoured with fruit preparations other than passion fruit showed a decrease of pH and an increase of titrable acidity over the storage period (Fig. 4a and b and Fig. 6a and b).

Table 12. Viscosity of mala flavoured with passion fruit juice-sugar mix containing gelatine and stored at 2-3[°]C and 15[°]C

Temper of sto			Vi 0	scosity 2		afte 4		of storage 8
2.	5		110.	2 112.4	4 1	11.0	115.6	114.2
15			110.	0 113.0) 10	07.2	111.5	- a
15	S		108.	8 115.6	5 1	14.0	109.1	-a
Note:	15 ^S	-	The p	roduct h	nad 20	00 pp	m sorbi	c acid
	а	-		roduct w ot taker		umpy	and vis	cosity

Fig. 7a and b. Titrable acidity and pH changes respectively of mala flavoured with passion fruit juice-sugar mix and containing 0 or 200 ppm sorbic acid and stored at 2-3°C or 15°C.



Days of storage

Note: 1) _____ mala flavoured with passion fruit juice mix and containing 200 ppm sorbic acid and stored at 15[°]C.

2) - - - Same as 1) above but with no sorbic acid.

3) Same as 2) above but stored at 2-3°C.

The viscosity of mala flavoured with passion fruit juice-sugar mix containing gelatine did not change after 6 days storage whetheror not it contained sorbic acid. The products stored at 15^oC were lumpy after 8 days of storage.(Table 12). The passion fruit juice-sugar mix contained 0.9% gelatine which may bind some of the whey expelled from mala and maintain the viscosity more or less constant.

4.4.4. Discussion

Unflavoured mala and all malas flavoured with different fruit preparations were unacceptable when yeast and mould counts were of the order of 10⁷/ml (Fig. 5). Kruschev and Mladenov (42) found yeast and mould count to be 1.2×10^6 /ml and 7 x 10^6 /ml at the end of shelf-life of yoghurt stored at 8-12°C and 20-25°C, respectively. At the same storage temperature unflavoured mala or mala flavoured with different fruit preparations attained yeast and mould count of the order of 107/ml after different storage periods although the initial yeast and mould counts were about the same (Fig. 5). Unflavoured mala, mala flavoured with banana pulp and that flavoured with pineapple preparation attained yeast and

mould count of the order of $10^7/ml$ after more than six days, after 4 days and after 6 days storage at 25°C, respectively (Fig. 5). Thus the type of fruit preparation added determines the yeast and mould growth and thereby the keeping quality of the product at a given temperature. Experiments by various authors indicate similar trends. A product with 40% orange juice and 60% buttermilk even when pasteurised at 75°C for 30 seconds kept for only 4 days at 25° C and spoilage was due to increased yeast and mould counts (22), while even when plain yoghurt was inoculated with yeasts it was edible for upto 10 days when stored at $25^{\circ}C$ (64). A fruit yoghurt had a shelf-life of 7 days at 8°C (80) In another trial the shelf life of yoghurt with 10% fluid was 2-3 days at 15°C (66). Plain yoghurt retained good flavour for 4-5 days at 15°C (58). Mala flavoured with either banana or pineapple preparations had a very significantly inferior taste after 2 days storage at 25°C (Table 8 and 9). A yoghurt containing 12.5% fruit and inoculated with yeast cells at 2000/ml had a shelf-life of 14 days 6 days and 1 day when stored at $5^{\circ}C$, $8-10^{\circ}C$ and ambient temperature, respectively (52). This is comparable to the results obtained by us.

Sorbic acid at 200 ppm prolonged the storage period of mala flavoured with passion fruit juice-

sugar mix from 6 to over 8 days at 15°C and arrested the initial proliferation of yeasts and moulds (Fig. 5). Osborne and Pritchard (52) were able to keep yoghurt containing 12.5% fruit and 300 ppm or 100 ppm sorbic acid, inoculated with yeasts at 2000/ml for 14 days at ambient temperature and 8 days at 10°C, respectively. The effect of sorbic acid appears to be more important than that of the temperature. When compared to a shelf-life of 4 days at 25°C for pasteurised, fruit flavoured butter milk, as reported by Farah and Bachmann (22), sorbic acid has better preservation potential, unless pasteurisation in the retail container of aseptic packing of the pasteurised product is done.

5. CONCLUSIONS AND RECOMMENDATIONS

The optimum quantity of fruit preparation ranged from 12-20% of the unflavoured mala depending on the type of fruit. The optimum amount of sugar was 10% or 12% of the unflavoured mala depending on the fruit.

Mala flavoured with mango pulp and strawberry pulp respectively proved to be the most liked. Malas flavoured with passion fruit juice and mango pulp respectively had the best appearance. Mala flavoured with mango pulp had the best consistency while that flavoured with passion fruit juice with no hydrocolloids added was considered too thin. Fruit particles in strawberry pulp and in pineapple preparation were considered undesirable. The black specks and the high viscosity of mala flavoured with banana pulp were objectionable.

The viscosity of fruit juices can be improved by addition of gelatine or pectin so that when they are added to mala the viscosity of the product is not adversely affected. Requisite quantities of gelatine or pectin may need to be determined for each juice. Sodium caseinate can be added to the milk to improve the viscosity of mala so that on addition of juices the viscosity of the product is not adversely affected. Fruit-sugar mixes when heat treated to 75°C, filled in hot bottles, capped and allowed to cool have an extended shelf-life at ambient temperature. Banana pulp however, can only be preserved by freezing, as heat treatment causes undesirable changes in taste. The possible levels of sorbic acid of 330-500 ppm on fruit part can protect the fruit-sugar mixes from secondary infections after opening of containers. If the fruit-sugar mixes are used immediately after opening, sorbic acid is not needed.

The shelf-life of mala flavoured with fruit sugar mixes is influenced by temperature and at a given temperature there is a definite fruit effect depending on how favourable the addition of a particular fruit-sugar mix makes the product for growth of yeasts and moulds. Yeast and mould growth is the single most important factor determining shelf-life of cultured milk products. Sorbic acid at 200 ppm delays initial proliferation of yeasts and moulds and extends shelf-life.

- 71 -

6. REFERENCES

- Alyward, F. 1974. Milk and milk products in in less developed countries. Proc. Symp.Milk Products of the Future. Publ. Soc. Dairy Technol.
- Amerine, A. M., Pangborn, R.M. and Roessler, E.B. 1965. Principles of Sensory Evaluation of Food. Academic Press, New York.
- ANUGA. 1981. A brief report. Dairy Sci. Abstr.
 44: 2702.
- 4. APHA. 1972. Standard Methods for the Examination of Dairy Products. ed. W.J. Hausler, Washington D.C. APHA.
- Auclair, J. and Mocquot, G. 1974. Cultured milk. Proc. Symp. Milk Products of the Future. Publ. Soc. Dairy Technol.
- 6. Bray, S.L. 1979. Shelf-life of all natural maple flavoured yoghurt. Dairy Sci. Abstr. 41: 7491.
- 7. Brown, L.D. 1979. Quality concerns of fruits and flavours for yoghurt. Dairy Sci. Abstr. 41: 7419.
 - 8. Burke, A.D. 1964. Milk dealer. <u>In</u>: "Dairy Bacteriology". John Wiley and Sons Inc.

- 9. Chen, M.T., Tseng, Y.Y. and Chen, H.H. 1981. Experiments on the preparation of semisolid yoghurt II. Effects of addition of honey and various fruit preserves on palatability and nutritive value of sundaetype yoghurt. Dairy Sci. Abstr. 44: 3510.
- 10. Chenier, R.P. 1961. Method for production of milk beverage. French Patent 1280713, Dairy Sci. Abstr. 25: 2522.
- 11. Chotickai, P. 1978. A study on the keeping quality of some marketed fermented milks. Food 11(3): 199.
- 12. Collins, E.B. and Moustafa, H.H. 1969. Sensory and shelf-life evaluations of cottage cheese treated with K-sorbate. J. Dairy Sci. 52(4): 439.
- 13. Davis, J.G. 1970. Fruit yoghurt. Dairy Industry, 35(10): 676.
- 14. Davis, J.G. 1971. Standards for yoghurt. Dairy Industry. 36(8): 56.
- 15. Diaz, D. and Villalobos Cruz, M. 1975. Preservation of tropical fruits. Dairy Sci. Abstr. 37: 3289.
- 16. Dinev, V. 1974. Technology, microflora and storage life of "Mladost" cultured cows milk. Dairy Sci. Abstr. 30: 5416.

- 17. Dodine, A.B. and Martin, J.H. 1979. Consumer acceptance of flavoured sweetened butter milk. Cult. Dairy Prod. J. 14(4): 20.
- 18. Egli, F. 1981. Fruit contraining milk products in Switzerland. Dairy Sci. Abstr. 43: 4124.
- 19. Engel, G., Teuber, M., Hoffmenter, G. and Neumann, K.I. 1980. Development of yeasts and moulds in quarg. Dairy Sci. Abstr. 42: 5073.
- 20. Escher, F. and Denzler, A. 1974. Lebensmitteltechnologisches Praktikum fuer Lebensmittel-Ingenieure. Institut fuer Lebensmittelwissenschaft ETH Zurich, Switzerland.
- 21. FAO/WHO. 1977. Code of principles concerning milk and milk products. Standard number Alla, Step 7, 1977.
- 22. Farah, Z. and Bachman, M. 1981.

Buttermilk flavoured with natural fruit juice - an example of product for developing countries. Lebensm.wiss. & Technol. 14: 276.

23. Forster, E.M. et al. 1964.

Dairy Microbiology. Publ. Prentice Hall Englewood Cliff. N.J.

24. Furia, T.E. 1977. Handbook of Food Additives CRC Press. 1977.

- 25. Gallesloot, T.E. and Hassing, F. 1968. Manufacture of stirred yoghurt of high viscosity. Dairy Sci. Abstr. 30: 2260.
- 26. Harland, H.A., Coulter, S.J. and Townley, V.H. 1975. A quantitative evaluation of changes occurring during heat treatment of skimmilk at temperatures ranging from 170-300^oF. J. Dairy Sci. 38: 1199.
 - 27. Harper, W.J. and Hall, C.W. 1976. Dairy Technology and Engineering. AVI Publ. Co., Westport, CT.
- 28. Hauge, B., Steinshelt, K. and Abrahansen, R.K. 1981. Flavour in frozen yoghurt. Dairy Sci. Abstr. 44: 1480.
- 29. Hunns, Lourenco Junior J. de B., Carvalho, L.O.D. de M., Nascimento, C.N.B. do and Viera, L.C. 1981. Buffalo milk yoghurt with amazonian fruit flavour. Dairy Sci. Abstr. 44: 7296.
- 30. IDF 1967. Compositional Standards for Fermented Milks. IDF General Secretariat, Brussels, Belgium.
- 31. IFT Committee on Sensory Evaluation. 1964. Panel evaluation of foods and beverages. Food Technol. 18: 1135.

- 32. Jebwabnik, M. 1981. Aseptic fruits advantages over regular fruits as seen by a dairy processor. Dairy Sci. Abstr. 44: 1483.
- 33. Jooste, Ke., Bertelsen, E. and Bresby, G. 1982. Flavour additives to fruit flavoured cultured milk. Dairy Sci. Abstr. 26: 1877.
- 34. Kaahwa, R.M. 1978. Variation in viscosity of cultured buttermilk. Dissertation Saskatchewan Univ., Canada.
- 35. Kay, D.E. 1967 . Banana Products. Tropical Products Institute, London.
- 36. KBS 1977. Specification for yoghurt. Kenya Standard 1977 KS-05-35 parts 1-2.
- 37. Kivi, G.C. 1981. Why process fruits aseptically? Dairy Sci. Abstr. 44: 1485.

38a. Klupsch, H.J. 1972 .

New knowledge on cultured milk products. Dairy Sci. Abstr. 34: 3572.

- 38b. Klupsch, H.J. 1972. Manufacture of cultured milks of long storage. Dairy Sci. Abstr. 35: 2803.
- 39. Kondratenko, M., Goranova, L., Simeonova, R. and Gosheva, B. 1979. Changes in yoghurt during storage at 4^oC. Dairy Sci. Abstr, 43: 3073.

40. Kosikowski, F.V. 1977.

Cheese and Fermented Milk Foods. Publ. F.V. Kosikowski. Ithaca. N.Y.

- 41. Kosikowski, F.V. and Brueckner, J.H. 1941. The Manufacture of Cultured Buttermilk. Cornell Univ. Agric. Ext. Bull. 723.
- 42. Krushev, B. and Mladenov, M. 1970. Effect of storage conditions on some characteristics of yoghurt. Dairy Sci. Abstr. 33: 1525.
- 43. Kurwijila, R.L.N. 1980. Low cost optimisation of the flavour, consistency and keeping quality of fermented milk with particular reference to consumer acceptability in Kenya. M.Sc. thesis, Nairobi Univ. Kenya

44. Lawrence, R.L. and Perry, G.A. 1962.

Process for improving the flavour of cultured milk products and the resulting product. US pat. 3022176 cit. Dairy Sci. Abstr. 24: 2216.

- 45. Little, T.M. and Hills, F.J. 1978. Agricultural Experimentation: Design and Analysis. Wiley, New York.
- 46. Luck, H. 1970. Which dairy products have a good chance in future. Dairy Sci. Abstr. 39: 1157.

- 47. Lück, H. and Moestert, J.F. 1971. Pasteurisation of fermented milk products. S. African Dairy Technol. 3(2):75
- 48a. Mann, E.J. 1981. School milk. Dairy Ind. Intern. 46 (1): 11.
- 48b. Mann, E.J. 1981 . Yoghurt. Dairy Ind. Intern. 46(3): 28.
- 49. Mueller, H.P. 1973 . Aufbereitung von Fruchtund Gewuerzzutaten fuer Sauermilchprodukte. Milchtechn. Inst. ETH, Zurich.
- 50. Neish, A.C. 1952. Rep. Nat. Res. Council, Canada. No. 46-83. Analytical Methods for Bacterial Fermentations, 2nd ed.
- 51. Obert, G., Tomka, G. and Szakaly, S. 1974. Experiments on flavouring milk beverages and cultured milk by different fruit syrups. XIX Intern. Dairy Congr. 1974.
- 52. Osborne, R.J.W. and Pritchard, E.W. 1974. Preservation of fruit yoghurt by preservatives and by storage at low temperatures. Dairy Sci. Abstr. 37: 2394.
- 53. Ramanauskas, R. and Urbene, S. 1982. Possibilities of using sodium caseinate in production of cultured milk products. Dairy Sci. Abstr. 44: 187.

54. Rasic, J.L. and Kurmann, J.A. 1978. Yoghurt. Publ. Rasic, J.L. and Kurmann, J.A., Freiburg, Switzerland.
55. Reid, W.E.H. and Welch, F.F. 1930. Factors influencing the properties of cultured

reconstituted milk. J. Dairy Sci. 13: 124.

- 56. Ritcher, R.L. 1977 . Manufacture of superior quality buttermilks. Cult. Dairy Prod. J. 12: 22.
 - 57. Schulthess, W. 1986. Practicals Manual in Dairy Chemistry. Dept. of Food Technol. and Nutrition. University of Nairobi.
 - 58. Schulz, M.E. and Voss, E. 1955. Kieler Milchw. Forschungsber., 8, I. Dairy Sci. Abstr. 18: 606d.
 - 59. Schulz, M.E., Moller, W., Raeth, J. and Schmanke, E. 1969. Recipes for improving keeping quality of cultured milk products. Dairy Sci. Abstr. 31: 4089.
- 60. Schulz, M.E. and Voss, E. 1970. International aspects of preservation of fermented milk products by pasteurization. XVIII Int. Dairy Congr. IE: 417.

÷

61. Seitz, E.W., Sandine, W.E., Elliker, P.R. and Day, E.A. 1963. Distribution of diacetyl reductase among bacteria. J. Dairy Sci. 46(3): 186.

62. Shahani, K.M. and Chandan, R.C. 1978.

Nutritional and healthful aspects of cultured and culture containing dairy foods. J. Dairy Sci. 62: 10.

- 63. Shidlovskaya, V.P. and Nasanova, L.M. 1981. Effect of storage conditions on changes in physico-chemical and organoleptic characteristics of prostokvasha. Dairy Sci. Abstr. 44: 5739.
- 64. Soulides, D.A. 1956. Lactose fermenting yeasts in yoghurt and their effects upon the product and bacterial flora. Dairy Sci. Abstr. 19(2): 136.
 - 65. Steinholt, K. 1977. Calculation of fundamental viscosity constants on the basis of measurements with Brookfield viscometer. Fd. Sci. and Technol. Abstr. 9: 1920.
 - 66. Stogards, T. 1964. Heat treatment of milk prior to the addition of cultures. IDF bull. 1964.
 - 67. Stoyanov, S., Obretenova, S., Panova, L.,
 Andreinski, H., Radulov, R. and Dimitrova,
 M. 1978. Production of long keeping
 yoghurt. Dairy Sci. Abstr. 41: 7489.

- 68. Szemplenski, T.E. 1981. Aseptic processing of fruit for yoghurt. Dairy Sci. Abstr. 44: 1484.
- 69. Tressler, D.K. and Joslyn, M.A. 1971.
 Fruit and vegetable juice processing technology. AVI Publ. Co. Westport, CT.
 70. Trestasavan, W.J. 1947. Manufacturing cul-

tured creams. Milk plant monthly. 36(7): 24.

- 71. Vancura, V. 1968. Yoghurt flavoured with fruit syrup. Dairy Sci. Abstr. 30: 1203.
- 72. Vancura, V. 1970. Yoghurt flavoured with fruit syrup. Dairy Sci. Abstr. 33: 1287.
- 73. Vaisey, G., Moskowitz, H., Solms J. and Roth, H.J. 1977. Sensory response to food - a sensory workshop. Forster Verlag AG/Forster Publishing Limited, Switzerland.
- 74. Webb, B.H. and Whittier, E.O. 1970. Byproducts from Milk. AVI Publ. Co. Westport, CT
- 75. Webb, B.H., Johnson, A.H. and Alford, A.J. 1974. Fundamentals of Dairy Science. AVI Publ. Co. Westport, CT.
- 76. Werry, P. 1981. Flavouring creation and application. Dairy Ind. Intern. 47:
- 77. White, C.H. 1978. Manufacturing better buttermilk. Dairy Prod. J. 13(I): 16.

78. Wigget, A.R., Webblewaite, P.E. and Peter, D.J. 1982. Fruit composition and method of manufacture thereof. Dairy Sci. Abstr. 44: 8085.

79. Woodroof, J.G. and Luh, B. 1975.

Commercial Fruit Processing. AVI Publ. Co, Westport CT.

80. Sobkora, Z.S. and Plush, C.L. 1979. Effect of the storage period of 1.5% fat fruit yoghurt on its characteristics. Dairy Sci. Abstr. 41: 7488.

APPENDIX I

1. Screening tests for taste and odour

1.1. Ballot for screening tests for taste

Name or code number

Instructions: In front of you are 6 cups containing weak water solutions of chemicals representing the basic taste sensations. One or more of these may be a blank or a repeat. Your task is to identify the dominant taste in each cup.

Please rinse your mouth with water before you taste each sample. Please taste the samples in the order indicated on a special sheet. For each sample please record on the ballot below if the sample is tasteless or has a sweet, salty, sour, or bitter taste. When you have finished consult an instructor about the accuracy of your results, score them (correct = 1, incorrect = 0, and proceed to the odour test

Sample code number Taste description Score

Total score

1.2. Ballot for screening test for odour

Name or code number

Instructions: In front of you are 5 bottles each containing a common fruit. Your task is to identify each of these. Please sniff each sample in turn, working from left to right. Wait approximately 15 seconds between samples. Please record your description of each sample on the ballot below. When you have finished, consult an instructor about the accuracy of your results and score them. Directions for scoring

5 = correct

4 = nearly correct - item named has significant overlap of components with the stimulus.
3 = somewhat correct - item named is in the same narrow class as the stimulus.

2 = nearly incorrect - item in the same mediumsized class as the stimulus.

1 = incorrect

0 = no response

Sample Code	Number	Odour	Descriptio	on	Score
0					
Р					
Q					
R					
S					

Total score

correct	Taste %	Sample totals	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	Panelist	
																												Sweet	
																												Salty	Tas
																												Sour	ste
																												Bitter	SC
																												Blank	Cor
	49																											Blank	P
×I	Odor																											Total Correct	
																												Pineapple	
																												Strawberry	0
										L																		Banana	Odour
																												Mango	r s
																												Passionfruit	COL
																													re
																												Total	

1.3. Tabulation of results

- 85 -

Now, please consider:

- (a) Are any of the candidates ageusic (taste blind)? If yes eliminate them at this stage.
- (b) Are any of the candidates anosmic (odour blind)? If yes, eliminate them at this stage.

You have been given a summary of the results of the two screening tests. On the form below, please rank the candidates for performance in each modality, i.e. list first the Judge number on the candidate with the greatest accuracy.

1.4. Candidate's Performance Order in Screening Tests

	correct	st total	Odor (highest lowest total score Maximum = 50					
Panelist	Score	Rank	Panelist	Score	Rank			
		*						

- 87 -

2. Selecting

Ballot for magnitude estimation

Name or code number

Instructions: In front of you are 3 test sets, numbered 1, 2 and 4, each consisting of 5 coded cups containing weak water solutions of the same chemical. Your task is to judge the intensity of the taste in each sample in relation to the reference sample (R). For each judgement, taste the reference and score it 10; expectorate, rinse your mouth, wait 10 seconds, then taste the sample and decide how much stronger or weaker it is than the reference and assign a score. First taste the samples within Set 1 in order specified on separate sheet provided to show the concentrations. Convert your score to rank (highest score = 1, lowest = 5). Assess your performance on the following page. Then return to this page and complete Set 2 and Set 4.

Name or code number

First assess your performance in the exercise with Set 1 as described in the following. Then proceed to exercise and assessment of Sets 2 and 4. In each magnitude estimation set, calculate the number of inversions you made in rank ordering. If your ranking differs from the expected order, the number of inversions is calculated as follows: Start from the left of your series and, proceeding to the right, take each rank in turn and count to the right of it the number of ranks that are smaller. There is an example in the table. When you have counted your inversions, calculate your reward (or penalty) as follows:

Reward.

Penalty

0-2 inversions - win 30 cts.
12-15 inversions - pay 30 cts
3-5 inversions - win 20 cts.
9-11 inversions - pay 20 cts.
6-8 inversions - win 10 cts.

Then taste Set 2 and again calculate your reward or penalty and do the exercise with Set 4.

Set	Code	Score	Rank order*	Decode conc.	Expected rank**
1					
2					
4					

* smallest rank for highest score

÷

** smallest rank for highest concentration

Summary of rank order from intensity judgements and calculations of inversions

	Str	ŧ	Weak	est	Inversions	Reward or Penalty	
Concentrations:							-
Expected Rank	1	2	3	4	5		
Example:	3	1	2	5	6	2+0+0+1+1 = 4	+ 20 cts
Set 1:							
Set 2:							
Set 4:							

Panelist	Numbe	r of ir	nversio	ons	Candidate rank
	Set 1	Set 2	Set 4	Total	l = best
1					
2				-	
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
Total					
Mean x					

Form for evaluation of panelists (Sensitivity to acid and sucrose)

2.4. Materials and Methods.

2.4.1. Appendix I, 1.1 Screening for taste. Label H I K L M Ν Sucrose Blank Caffeine Blank NaCl Citric acid 1.0% 0.015% 0.18 0.01% Order of presentation I L H Ν M K 2.4.2. Appendix I, 1.2 Screening for odour Closed dark cups with small openings containing the following items:

Label.	Item
0	Banana
Р	Strawberry
Q	Passion fruit
R	Mango
S	Pineapple

2.4.3. Appendix I, 2. Selection. Citric acid concentrations of 0.02, 0.04, 0.08, 0.16, and 0.24% and sucrose concentrations of 1, 2, 4, 8 and 16% were used.

Citric acid and sucrose concentrations were presented separately with the following labelling in order of increasing concentration in each case.

Set 1: E, A, D, C, B.
Set 2: D, C, E, A, B.
Set 4: A, E, B, C, D.
The order of presentation for each was
Set 1: A D B E C; Set 2: B E D A C ; Set 4: A C D B E
(Adopted from Vaisey et al. (73)

- 93 -

APPENDIX II: FLAVOURED MALA HEDONIC SCORING

Date -----

Name or code no. -----

Please evaluate each sample for the following quality attributes (one attribute at a time for all the samples):

- a) appearance
- b) odour
- c) taste/flavour
- d) consistency, according to the following scale: 7 - like very much 6 - like moderately 5 - like slightly 4 - neither like nor dislike 3 - dislike slightly 2 - dislike moderately 1 - dislike very much

You are asked to say about each sample how much you like or dislike each of the listed quality attributes. Write the code number of the sample and the number that corresponds to the phrase that best describes your assessment of the attribute. Give reasons (remarks) with aid of guide phrases below score card for each attribute whenever you dislike an attribute.

Keep in mind you are the judge. You are the only one who can tell what you like or dislike and why you dislike. Nobody knows whether these products should be considered good, bad or indifferent. An honest expression of your assessment will help us decide.

- 95 -

i) APPEARANCE SCORE CARD

Sample code	Number given	Remarks
	<u></u>	
		+

Descriptive phrases

(appearance)

	Desirea	ble	attributes		Undisireable attri-
					butes
a)	Surfac	e -	Homogenous	-	unhomogenous
		-	no whey separation	-	whey separation
		-	sufficiently stirred	-	insufficiently stirred
b)	Fresh	cond	lition		
			resh ppearance	-	unattractive non- fresh appearance
C)	Colour		orresponding to the fruit	-	atypical
	U	used	-	too pale	
				-	over coloured

ii) ODOUR SCORE CARD

Sample code	Number given	Remarks

Descriptive phrases (Odour)

	Desirable		Undesiable
-	typical of fruit	-	atypical
	used		
-	full	-	faint
-	sourish (mala like)	-	flat or empty
-	pleasant	-	repulsive

iii) TASTE SCORE CARD

Sample code	Number given	Remarks

Descriptive terms (Taste)

Des	sirable attributes	Und	desirable attributes
-	typical of fruit used	-	atypical
-	mild to light sourish	-	over-sour
-	full and pleasant	-	empty and weak
-	balanced sweetness	-	too sweet
		-	little sweetened

*

11	1	
Sample code	Number given	Remarks
		<u> </u>

iv) CONSISTENCY SCORE CARD

Descriptive phrases (consistency)

- creamy
- viscous
- nonpasty

Desirable attributes Undesirable attributes

- thin, milky
- too viscous
- sticky, gummy
- lumpy
- whey separation

APPENDIX IIIA . FLAVOURED MALA DIFFERENCE EVALUATION

DIRECTIONS

- You are presented with three sets of fruit flavoured mala and a reference R.
- <u>Taste</u> the two samples in each set and indicate which is the same as R.

Set	Sample	Same as R
Set I		
Set II		
Set III		

APPENDIX III B. FLAVOURED MALA DIFFERENCE AND HEDONIC EVALUATION

DIRECTIONS

- You are presented with three sets of fruit flavoured mala and a reference R.
- <u>Taste</u> the two samples in each set and indicate which is the same as R.

Set	Sample code	Same as R
Set I		
Set II		
Set III		

- 3. Evaluate the two samples in each set of the following attributes:
 - a) appearance c) taste
 - b) odour d) consistency
 - as in Appendix II