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Detection of bacterial spoilage in some fruit flavoured ultra-high temperature milks in Nigeria

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Summary

Statistical analyses of pH measurements and microbiological methods have been employed to monitor microbial activity (spoilage) in a brand of non-refrigerated mango and vanilla flavoured ultra-high temperature (UHT) milk drinks in Nigeria. The mango flavoured milks were more sensitive to changes in storage conditions than was the vanilla flavoured milks. The aerobic bacterial flora in the spoiled flavoured milks was dominated by Gram-positive cocci, both catalase-positive and catalase-negative types. Coliforms and pseudomonads were also detected. The importance of this study to the methods for detecting spoilage in quality control and to the storage conditions of such products in the tropics is discussed.

Résumé

Des analyses statisques des mésures pH et les méthodes microbiologiques ont été employées pour contrôller l'activité microbiologique dans une espèce de boisson laitière traitée a haute température, assaisonnée avec de la mangue ou de la vanille et conservé au chaud. Le lait assaisonné avec de la mangue était plus sensible au changement dans les conditions de conservation que le lait qui a été assaisonné avec de la vanille. L'aerobie de la flore microbienne dans le lait gâté était dominée par les cocci Gram-positifs, tous les deux types a savoir le catalase positif et le catalase-négatif étaient presents. Les coliformes et les pseudomonads furent également detectés. L'importance de cette étude pour detecter les déchets dans le contrôle de la conservation de tels produits dans les tropiques etait discuté.

Introduction

The process of heating fresh milk at 133°C for a few seconds (3–5 sec) followed by aseptic packaging is a convenient method of producing commercially sterile milk (Westhoff, 1981). Ultra-high temperature (UHT) milk should have a shelf-life of some months and needs little or no refrigeration. The UHT process has been shown to destroy *Escherichia coli* (Read, Schwartz & Litsky, 1961); sporing organisms including *Bacillus* species (Segner, Frazier & Calbert, 1963); the mycobacteria (Harrington & Karlson, 1965) and *Staphylococcus aureus* (Zottola & Jezeski, 1966). Organisms involved in UHT milk spoilage are post-pasteurization Gram-negative psychrotrophic contaminants belonging to the genera *Pseudomonas*, *Flavobacterium*, *Chromobacterium* and also coliforms (Thomas & Sedkar, 1946; Thomas & Druce, 1969). Some species of *Bacillus* have also been found to survive pasteurization (Weckback & Langlois, 1977).

UHT milk has achieved considerable success, and its popularity in Nigeria, for example, may be attributed to (a) lack of pasteurized milk supply, (b) storage without refrigeration and (c) consumer acceptance for the product in handy packages. A recent introduction into the market has been UHT milk flavoured with mango and vanilla, both from fruit sources.

Although occurrence and the distribution of bacteria in UHT milk during storage had been reported (Hankin, Dillman & Stephens, 1977; Credit *et al.*, 1972; Mourgues & Auclair, 1973) such studies have been confined to low temperatures near to refrigeration. Studies on microbiological analyses of fruit flavoured drinks are limited, furthermore procedures involving pre-incubation at elevated conditions have been recommended for UHT milk pro-

ducts (Westhoff, 1981). This study therefore reports the detection of spoilage in mango and vanilla flavoured UHT milk drinks using the conventional recommended conditions and at elevated storage conditions which may be encountered in the tropics during storage of products. The work involves statistical analyses in pH levels of samples and an attempt to study the distribution of organisms in the products after the pre-incubation periods.

Materials and methods

Sample

The sample consisted of a batch of a particular brand of non-refrigerated mango flavoured (MF) and vanilla flavoured (VF) UHT milk drinks. The products in 200-ml paper packages were obtained from retailers of similar storage conditions and were tested at least 4–5 weeks prior to the expiry dates as shown on the packages. Products were randomly divided into three groups each of twenty samples; pre-incubation at (a) 30–32°C for 7 days (Marth, 1979); (b) 37°C for 1 day and (c) 37°C for 7 days, before testing.

Measurement of pH

Packages were first opened after the appropriate incubation period and pH was measured immediately using a pH meter 702 (Electronic Instruments Ltd, Kent, U.K.), standardized with buffer solutions of pH 4 and pH 9 (BDH Chemicals Ltd, Pools, U.K.).

Bacteriological examination

Packages were opened aseptically and aliquots of sample (0.1 ml) were taken directly from container and cultured by streaking onto nutrient agar plates and incubated at 30–32°C for 2 days. The streaked plates were examined for growth and the number of colonies developed along the line of streaks were counted.

Individual colonies from each plate were removed and examined for Gram-reaction and morphology. Gram-negative rods were characterized as presumptive coliforms using the criteria of growth in or on MacConkey medium (Oxoid, Ltd, Basingstoke, U.K.), pseudomonas on agar with 1% cetrimide, growth at 4°C and other tests as described by Cowan (1974) and by Collins and Lyne (1976). Cocci when encountered were characterized simply into catalase-positive and catalase-negative. Gram-positive rods and spore-bearing bacteria were not encountered. Complete identification of isolated colonies was not attempted.

Results

The effect of temperature and the length of storage (pre-incubation conditions) on the pH of the milk products are shown in Table 1. The results which were statistically analysed by multiple regression at 5% probability level are

Table 1. pH values of milk samples after pre-incubation conditions (sample size $n = 20$)

Product	Pre-incubation period (storage)	Mean pH $\bar{X}$	Standard deviation (S)
MF	30–32°C for 7 days	6.82	0.050
	37°C for 1 day	6.78	0.060
	37°C for 7 days	6.69	0.153
VF	30–32°C for 7 days	6.81	0.043
	37°C for 1 day	6.79	0.030
	37°C for 7 days	6.74	0.139

pH was measured when the milks were first opened after various pre-incubation periods.

shown in Table 2. Increase in temperature and length of storage lowered the pH of both milks. The higher absolute *t*-values for the mango flavoured milks indicated that temperature and length of pre-incubation period had relatively more pronounced effects on the pH of the MF than the VF milks. Temperature however had a more detrimental effect than the duration of storage on the MF milk. The R^2 value for the MF results implied that about 22% of the variations in the pH was explained by the combined variations in the two parameters of storage. The highly significant *R*-value at 5% probability level indicated the significance of the combined effect of temperature and length of pre-incubation period on product pH.

With the VF products only temperature had any significant effect on the pH, duration of storage had no effect. Furthermore, about 10% of the variations in the pH of the VF products was explained by the combined effects of temperature and length of pre-incubation period. The *p*-test was not significant at the 5% level of probability. This implied that there were other factors that would affect variations in the pH of the vanilla flavoured milk held at the different storage conditions.

Table 2. Analyses of multiple regression on pH values after various pre-incubation conditions. Effect of temperature and length of pre-incubation (sample size $n = 20$)

Variables	Regression coefficient	*t*-Value
Product: Mango flavoured milks		
Constant term 7.455		
Temperature	−0.01786	−3.8738
No. of days	−0.01416	−2.6312
$R^2 = 0.21547$; *F*-value = 7.8278		
Product: Vanilla flavoured milks		
Constant term 7.410		
Temperature	−0.0093	−2.351
No. of days	−0.0035	−1.808
$R^2 = 0.0962$; *F*-value = 3.032		

Tests of significance were conducted at 5% probability level.

MF: Temperature and length of pre-incubation period had significant effect on product pH.

VF: Only temperature had any significant effect of product pH.

Tables 3 and 4 show the results of the bacteriological examination of the flavoured milk products. No colonies were detected from samples of both the mango and vanilla flavoured milk which were pre-incubated at 30–32°C for 7 days. Bacteria were detected in 4/20 and 6/20 mango flavoured milks stored at 37°C for 1 day and 37°C for 7 days, respectively, while bacterial colonies were also detected in 3/20 and 4/20 vanilla flavoured milks under the two similar storage conditions. Generally, more colonies were obtained from cultures of milk samples which were pre-incubated at 37°C for 7 days before testing. Isolated colonies characterized by Gram reaction, morphology and other tests showed that spoilage was associated mainly with aerobic Gram-positive cocci. Low numbers of presumptive coliforms and pseudomonads were also detected.

Discussion

This study shows that during storage at elevated temperatures bacteria can grow in non-refrigerated mango and vanilla flavoured UHT milks and the growth was associated with a fall in product pH. A fall in pH value of a food

Table 3. Bacteriological examination to detect microbial spoilage

Sample no.	Pre-incubation period	pH	Growth
MF3	37°C for 1 day (a)	6.7	++
MF8	a	6.7	+
MF13	a	6.7	++
MF16	a	6.8	+
MF1	37°C for 7 days (b)	6.5	++
MF7	b	6.4	+++
MF10	b	6.5	++
MF16	b	6.5	++
MF18	b	6.5	+++
MF20	b	6.4	++
VF6	a	6.7	+
VF11	a	6.8	+
VF20	a	6.8	+
VF2	b	6.5	++
VF7	b	6.4	++
VF10	b	6.6	++
VF17	b	6.4	+++

\+ Up to 10 colonies.
++ 10 –20 colonies;
+++ 20–30 colonies

No colonies were detected from MF and VF samples pre-incubated at 30–32°C for 7 days.

Larger numbers of colonies were associated with products with lower pH values

Table 4. Characterization and distribution of colonies isolated in bacteriological examinations of milk products

Organism	*Distribution (%) MF	VF
Pseudomonads	20	20
Presumptive coliforms	10	10
Gram-positive catalase-positive cocci	40	50
Gram-positive catalase-negative cocci	10	10
Gram-negative rods	20	10
Spore bearing bacteria	none	none

*Based on the identification of all isolates from positive plates. No significant difference in distribution of organisms was observed from products pre-incubated at 37°C for 1 day and 37°C for 7 days.

product has been found to be an indication of spoilage (Halls, Aitken & Henry, 1980; Ackland *et al.*, 1981; Magnusson & Traudstadottir, 1982) which may be due to the formation of short chain organic acids by the organisms in the environment (Ackland *et al.*, 1981). Packages from which colonies were isolated after growth on agar had their pH significantly lower than those products which gave no colonies. In the present study, the milk samples which were pre-incubated at 30–32°C for 7 days were considered to act as 'controls'. Colonies were not detected when cultured but products in which pH fell below the mean of such 'controls' were associated with microbial spoilage, which was more pronounced at the longer pre-incubation period of 7 days at 37°C.

The distribution of organisms in the spoiled products showed that the products were dominated by organisms common to both the mango flavoured and the vanill flavoured milks. A paired *t*-test also showed that while a higher temperature had detrimental effect on both types of milks, the period of pre-incubation had more effect on the mango flavoured milks than the vanilla flavoured milks. No extensive work on bacteria associated with the spoilage of fruit flavoured UHT milk on storage has been reported, but published data on UHT milk had revealed that spoilage of such products on storage even at near refrigeration temperatures (Thomas & Sedkar, 1946; Credit *et al.*, 1972) was associated with some organisms similar to those obtained from this study.

These experiments show that temperature and length of storage for non-refrigerated fruit flavoured UHT milks play an important part in the shell-life of such products in the tropics, as in the country where temperatures and humidities can be particularly high during most part of the year. In the production of products such as UHT milks, an industry where post-processing contamination, including packaging, has been identified as a major problem (Speck & Busta, 1968; Westhoff, 1981), the traditional storage conditions of UHT milk products (non-refrigeration) in the temperates is certainly not ideal in such warm climates, because higher storage temperatures will easily encourage spoilage. Rapid techniques which should incorporate statistical sampling of a large number of packages at elevated temperature conditions will also be an essential part of monitoring

activity in such non-refrigerated products after processing. Measurement of pH of large numbers of products after accelerated storage conditions can be a simple and effective technique of assessing the effect of storage conditions on the quality of such products after processing.

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