

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: [editorpjn@gmail.com](mailto:editorpjn@gmail.com)

## Proximate Analysis and Microbiological Quality of Cheese Produced from Raw Cow Milk Obtained from Fulani Settlement in Ogun State Nigeria, Using Lactic Acid Bacteria and Extract from Sodom Apple Leaf (*Calotropis procera*)

P.O. Uaboi-Egbenni<sup>1</sup>, P.N. Okolie<sup>2</sup>, T.I. Akintunde<sup>3</sup>, O. Bisi-Johnson<sup>4</sup>, L. Enwe<sup>2</sup> and P.O. Bessong<sup>5</sup>

<sup>1</sup>Department of Microbiology, School of Mathematics and Natural Science,  
P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa

<sup>2</sup>Department of Food Technology, Yaba College of Technology, P.M.B. 2011, Yaba Lagos

<sup>3</sup>Department of Public Health, University of Venda,  
P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa

<sup>4</sup>Department of Medical Microbiology, Walter Sisulu University, P.B. X1,  
Mthatha 5117, Eastern Cape Province, South Africa

<sup>5</sup>Department of Microbiology, School of Mathematics and Natural Science,  
University of Venda, P.M.B. 5050, Thohoyandou, Limpopo Province, South Africa

**Abstract:** Raw cow milk obtained from Fulani settlement in Ogun State, Nigeria was inoculated with pure cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* with extract from Sodom apple leaf (*Calotropis procera*) as coagulant in the absence of rennin. Fermentation was done for four (4) days for the development of necessary aroma and coagulation. Physicochemical analysis of the fermenting sample showed a gradual drop in pH from 5.8-3.20 and an increase in total titratable acidity from 0.049-0.137%. Proximate analysis of the cheese sample showed a moisture, ash, fat, protein and carbohydrate (by difference) of 64, 0.60, 13.4, 12.86 and 9.14% respectively. Microbiological analysis of the cheese product revealed that the sample was completely free of coliforms, mould and yeasts and hence safe for consumption. This Sodom Apple produced cheese is hereby recommended for both growing children and adult due to the retention of a high percentage of protein after fermentation and its expected ability to correct protein deficiencies. The microbial production and nutritional analysis of the cheese sample is discussed.

**Key words:** *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Calotropis procera*, coagulation, cheese,

### INTRODUCTION

Cheese is the curd or hard substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms from which part of the moisture has been removed by cutting, warming and/or pressing, which has been shaped in a mould and then ripened by holding for sometime at suitable temperatures and humidity. The conventional method for the production of cheese has been discussed extensively by Frazier and Westhoff (1988). Standard cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been employed as starter cultures for cheese production (Frazier and Westhoff, 1988). For the production of high quality cheese rennet enzyme is added for effective curdling and in its absence it is possible to use extract from leaves of Sodom Apple (*Calotropis procera*) as alternative. The need arise to develop an appropriate formulation for the production of cheese due to inconsistency in the uniformity of cheese being produced locally.

From the writings or submission of Helen and Elisabeth (1990), there is no real origin of cheese or cheese making but in the earliest records of human activities refers to cows and milk. These may be found in Sanskrit writings of the Sumerians 4000BC, in Babylonian records, 2000BC. It is also found in Vedic hymns.

The preparation of cheese probably dates back many centuries to the time when nomadic tribes of Eastern Mediterranean countries carried milk of domesticated mammals in sacks made from animal skins or gourds or in vessels such as stomachs or bladders (Helen and Elisabeth, 1990). When the milk is kept warm, it rapidly became sour and separated into curds and whey. In the absence of liquid milk, the curd is supplied as supplement as much of the milk value is retained.

According to Helen and Elisabeth (1990) cheese is classified into three categories-soft, blue veined and hard-pressed cheese. This vary in moisture content and therefore in keeping quality and method of ripening. Soft cheese retains a high proportion of moisture (whey) 55-80% and these varieties are eaten fresh (Cambridge, Coulommier Bondon, etc.), whilst others are ripened,

usually by growth of surface moulds (Brie, Camembert, Pont (Eveque, etc.). Semi-soft cheeses (Limburger, Tilst, Brie) are made from slightly firm curds 45-55% moisture and are ripened by surface growth of micro organisms particularly by *Brevibacterium linens*. These are the smear-ripened cheese (Helen and Elisabeth, 1990). Blue-veined cheeses such as Stilton, Roquefort and Gorgonzola are made from semi-soft/semi-hard curd 42-52% moisture and are ripened by species of *Penicillium*, which grow with the cheese. Semi-hard cheeses such as Edam and Gouda are made from firmer curd with moisture content within ranges of 45-50%. These are ripened by bacteria and consumed within 2-3 months.

The hard-pressed cheeses are made from relatively dry curd 35-45% moisture, ripened by bacteria and mature slowly within 3-12 months. The very hard, grating cheeses such as Parmesan, Romano and Asiago are made from curd below moisture 26-34%, made partly from skimmed milk and are ripened by bacteria slowly over period of one to two years (Specialist Cheese Makers Association, 2002).

Prentice and Neaves (1986) have argued that the variety of cheese to be produced in any class is strictly determined by the type of milk used, preparation of the young curds and inclusion in the milk or curds of certain microorganisms responsible for the development of acidity during manufacture and development of characteristic features and flavours during ripening.

Cheese is generally made from cow milk, but in some countries and for making certain varieties of cheese, milk of other mammals is used (Helen and Elisabeth, 1990). For example, Ewe's milk is used for making Roquefort cheese and varieties such as Feta, Ricotta, Pecorino, etc.; goats' milk for making varieties of cheese in Italy and Greece and Buffalo's milk in India and Egypt. Ewe and goat's milk are increasingly used for cheese making in UK (Prentice and Brown, 1983).

Milk intended for use in cheese production must be stored at 40°C and transported to factory where it is stored in insulated silos until it is used (FAO, 2008). Prentice and Neaves (1986) observed that raw milk on arrival at the creamery will have total counts of  $10^3$ - $10^7$ /ml depending on the levels of hygiene at the farms. They also observed that organisms present consist of psychrotrophs mostly *Pseudomonas*, *Aeromonas*, *Alcaligenes*, small number of lactic acid bacteria, spore-forming gram-positive rods, coryneform bacteria, *Micrococcus* and coli forms. Of these, only the psychrotrophs will multiply during transport and storage, particularly if temperature in insulated tanks and milk silos is allowed to rise.

The temperature employed could also determine the type of starter to be employed. For example, a temperature of 38-40°C will attract the use of a thermophilic starter, whereas, a temperature of 32-45°C

may attract the use of a mesophilic starter (<http://www.ys.f403.2004>). Starter culture used for cheese fermentation have been back slopping (Prentice and Brown, 1983).

Sharp (1979) stressed that species of lactic acid bacteria use as starters in cheese making belong to the genera *Streptococcus*, *Leuconostoc* and *Lactobacillus*. It is possible to use single strain starter as in the case of *Streptococcus lactis* or a combination of both (Billie *et al.*, 1985). It is also possible to employ multi-strain starters (Timson *et al.*, 1982) or mixed strain starters involving mixture of strains of *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus diacetylactis* and *Leuconostoc* (Timson *et al.*, 1982).

Starter cultures in cheese making is a medium of harmless, active microorganisms, which by growing in cheese milk and curd assist the development of mature cheese with desirable characteristics of flavour, aroma, pH, texture and body (Scott *et al.*, 1998). Billie *et al.* (1992) observed that the rate of acid production is crucial/critical in the manufacture of certain product like cheddar cheese. Mullan (1986) observed that in addition, antibiotic substances now referred to as bacteriocins, produced by starters e.g., nisin may also have a role in preservation.

## MATERIALS AND METHODS

**Sample collection:** Fresh milk was obtained from the shorthorn of the Fulani at Abeokuta, Ogun State, Nigeria in a sterile 4 litres keg container. The sample after collection was kept in an ice-frozen container and immediately transported to the laboratory and kept in the refrigerator at 4°C until it was ready for use. Sodom apple leaves (*Calotropis procera*) were obtained from the premises of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.

**Starter cultures:** *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from culture collection unit in the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi. The isolates were maintained on MRS Agar slant for *Lactobacillus bulgaricus* and Nutrient Agar slant for *Streptococcus thermophilus*. The slants were kept in the refrigerator at 4°C until they were used.

**Preparation of the inoculum (Seeding culture):** Starter cultures of the selected organisms were grown in separate flasks. About 50 ml of MRS broth was used for the growth of *Lactobacillus bulgaricus* and 50 ml of nutrient broth for the cultivation of *Streptococcus thermophilus*. Both flasks were agitated in a shaker incubator at 100 rpm for 18 h after which they were centrifuged at 5000 rpm for 30 min. The supernatant liquid was decanted and used.

**Fermentation of milk sample:** Freshly collected cow milk previously refrigerated was pasteurized at 68°C for 30 min, cooled to 45°C and thereafter inoculated with the microorganisms. The culture slurry was aseptically inoculated into the pasteurized milk sample at 45°C. Fermentation was allowed to proceed for 24 h at 43°C. After fermentation, the fermented milk sample was heated to boiling point with extract from Sodom apple leaves for the formation of curd at 85°C for 10 min. The fermented curd was then allowed to remain in the whey for the development of necessary yoghurt cheese-like aroma.

**Analysis of fermenting sample:** The fermenting samples were analyzed after 24 h for the following parameters:

**pH determination:** About 10 ml of the fermenting milk sample was dispensed into conical flask and its pH determined using the pH (Unicam 9450 Model). The pH meter was standardized using standard buffer of pH 4.0 and 7.0.

**Determination of the total titrable acidity:** This was done by dispensing 10 ml of the fermenting milk into conical flasks and adding 3 drops of phenolphthalein indicator. Thereafter, 0.1 N NaOH was used for titration to a noticeable pink colour for endpoint determination. The acidity was calculated as lactic acid using the relationship:

$$\text{Lactic acid (\%)} = \frac{\text{Titre value} \times \text{Normality of Alkali} \times 9}{\text{Volume of sample}}$$

Normality of alkali = 0.1, Volume of sample = 25 ml

#### **Proximate analysis of cheese sample**

**Determination of moisture content of cheese:** The procedure employed was that of AOAC (1990). About 5 g of the cheese sample was weighed into pre-weighed aluminum dry dishes and the sample was leveled carefully in the dish. The dish and its content were then transferred into the oven at a temperature of 105°C and were dried for 3 h. This was then allowed to cool in a desiccator and weighed. The dish was returned into the oven for another half hour and again cooled and reweighed. The process was repeated until a constant weight was reached.

**Determination of ash content:** About 5 g of the cheese sample was weighed into porcelain crucible previously ignited and weighed. The material was ignited in the fume cupboard until no fume was seen charred of organic matter. This was then transferred into muffle furnace at 550°C using a pair of tongs and was ignited for 3 h, cooled in a desiccator and weighed immediately.

#### **Determination of fat content by soxhlet extraction**

**method:** About 5 g of cheese sample was weighed and put in thimbles using a dry paper and plugged with cotton wool. The thimbles were dried and inserted into a soxtec system HT2. The extraction cups were dried and weighed and then 50 ml solvent (petroleum ether) was added in each cup. The cups were inserted into the soxtec. The samples were extracted for 15 min in boiling position. The extraction was carried out continuously for 3 h. This was cooled and reweighed.

#### **Determination of protein content of fermented cheese:**

Kjedahl nitrogen method was employed for the determination of protein content of the fermented cheese. About 1.0 g of the cheese sample was weighed into the digestion flask. Kjedahl catalyst (5 selenium tablets) was added to the sample. About 20 ml of concentrated tetraoxosulphate VI acid was added to sample and then fixed for 8 h in the digestion unit (450°C) of the Kjedahl apparatus in fume cupboard. The digest, pure yellow after cooling changed into a colourless liquid that was transferred into 100 ml volumetric flask and made up to mark with distilled water. About 20 ml of 4% boric acid solution was pipetted into conical flask. A drop of methyl red was added to the flask as indicator. The sample was thereafter diluted with 75 ml of distilled water. About 10 ml of the digest was made alkaline with 20 ml of NaOH (20%) and distilled. The steam exit of the distillatory was closed and the change of colour of boric acid solution to green was timed. The mixture was distilled for 15 min (AOAC, 1990). The filtrate was then titrated against 0.1 N HCl. The protein content was calculated from the relationship:

$$\text{Total protein (\%)} = \frac{\text{Titre} \times \text{Normality of acid} \times 0.014}{\text{Sample weight}} \times 100$$

Protein conversion factor = 6.38 for Milk

Protein (%) = % Nitrogen x 6.38

Normality of acid (HCl) = 0.1 N

Sample weight = 1.0 g

#### **Determination of the carbohydrate content of fermented cheese:**

This was determined by subtracting from 100 the sum of the percentage moisture, ash, protein and fat. The remainder value gives the carbohydrate content of the sample.

$$\text{Carbohydrate (\%)} = 100 - (\text{sum of moisture, protein, ash and fat})$$

#### **Microbiological analysis of fermented cheese sample:**

The standard method of Harrigan and McCance (1976) was employed. About 1 g of the cheese sample was aseptically weighed using a weighing balance and carefully introduced into 9 ml of sterile distilled water. This was shaken manually in order to have a

homogeneous suspension. About 1 ml of this was taken and introduced into the second tube, followed with series of dilutions up to  $10^{-10}$  dilution. One ml was taken from  $10^{-4}$  dilution and introduced into sterile plates and molten agar (50°C) added by pour plate method using the following agar and incubation periods:

**Nutrient agar:** This was used for the determination of total viable bacteria in the sample. The plates were incubated at 37°C for 24-48 h.

**MacConkey agar:** This was used for the enumeration of total coliform organisms in the sample. The plates were incubated at 35°C for 24-48 h.

**Sabouraud dextrose agar:** This was used for the enumeration of mould and yeast in the sample. The plates were incubated at 30°C for 24 h for yeasts and 3-5 days for mould.

## RESULTS

The results of the various analysis done on fermented cheese sample are as displayed in the tables below. All results are average values of four (4) determinations. Table 1 shows the gradual decrease in the pH of fermenting milk during cheese production over a 72-h period from 5.80-3.20. This final value is the mean value over four (4) determinations. This value is far lower than those obtained in previous studies. The total titratable acidity of fermenting sample during cheese production increased from an initial value of 0.049-0.137 over 72 h (Table 2). The mean moisture content of the cheese product was 64.0% (Table 3). The mean ash content of the fermented cheese was 0.6% (Table 4). The mean % fat content of cheese was 13.4%. The mean protein content of the fermented cheese was 12.8% (Table 6). The mean carbohydrate content was 9.14% (Table 7). The microbiological level as determined from this study (Table 8) shows the total plate count was  $1.1 \times 10^1$ . There were no coliforms isolated from the cheese, neither were moulds or yeasts isolated from the final cheese product. This finding shows the high quality of the laboratory-made cheese.

## DISCUSSION

The production of fermented products with special reference to cheese and yoghurt has been discussed by several workers (Frazier and Westhoff, 1988; Helen and Elisabeth, 1990; Prentice and Neaves, 1986; Specialist Cheese Makers Association, 2002; Prentice and Brown, 1983; Billie *et al.*, 1992). The use of starter cultures have also been mentioned by several workers; *Streptococcus thermophilus* (Mullan, 1986; Helen and Elisabeth, 1990); *Lactobacillus delbrueckii subspecies bulgaricus* (Timson *et al.*, 1982); *Lactobacillus acidophilus* (Rogosa *et al.*, 1951); *Lactococcus* (Mullan, 1986); *Streptococcus cremoris* / *Leuconostoc* (Billie *et al.*, 1985; Timson *et al.*, 1982).

Table 1: pH of fermenting milk sample during cheese production

Time (h)	pH values
0	5.80
24	4.2
48	3.70
72	3.20

Table 2: Total titratable acidity of fermenting sample during cheese production (samples per h)

Time	0	24	48	72
1st titre (ml)	1.30	3.30	3.50	3.80
2nd titre (ml)	1.40	3.40	3.70	3.80
Average titre (ml)	1.35	3.35	3.60	3.80
Lactic acid (%)	0.049	0.121	0.129	0.137

Table 1 is the pH of the fermenting milk sample, which shows a gradual drop in pH value from 5.80-3.20. The low acid pH value obtained in this study is similar to the observation made by Seo *et al.* (2009) in their studies of yoghurt samples in which they obtained a pH value of 3.82. This difference in the acid level may have stemmed from the fact that yoghurt is more of liquid than cheese. The acid level of this Sodom Apple leaves extract coagulated cheese is far lower than Rennet coagulated cheese (pH 5.8-6.5). The decrease in acid level of leaf extract of *Calotropis procera* fermented cheese may be attributed to presence of acid in the extract that enhance a fall in the acidic content of the ferment or that the cultures had higher potential at producing high level of lactic acid. This needs to be seriously investigated. Egan *et al.* (1988) in their analysis explained that pH is the measure of the acidity and alkalinity of the fermenting medium.

The total titratable acidity of the fermenting milk sample (Table 2) reveals that there is a gradual increase in the titratable acid from 0.049-0.137%. The enhanced titratable acidity is due to the presence of lactic acid produced by lactic acid culture during fermentation. The titratable acidity obtained in this study is in line with those reported by other workers in which they obtained a value of 0.127% (Davies and Wilkinson, 1973; Davis *et al.*, 1993; Aworh and Akinniyi, 1989). This attribute enhances acidity of fermenting sample to the milk as well as to the buffering capacity of the product.

Proximate analysis revealed that the moisture content of the cheese sample (Table 3) was 64.0%. The result obtained is similar to those reported by Aworh and Akinniyi (1989) and Fasakin and Unokiweri (1992) who obtained 61.3% and 60.8% respectively. Egan *et al.* (1988) and Frazier and Westhoff (1988) had stressed that the moisture content is a measure of the water content and accounts for the texture of cheese. Analysis showed that the ash content of the cheese sample (Table 4) is 0.6%. This result is also similar to those obtained by Aworh and Akinniyi (1989) in which they obtained a value of 0.8%. The fat content of the cheese (Table 5) was 13.4%. Similar observation was made by Wong *et al.* (1988) where they obtained a value

Table 3: Moisture content of fermented cheese sample

Sample	Wt of sample (g)	Wt of sample+dish before drying (M1g)	Wt of dish + sample after drying M2 (g)	Moisture (%)
Cheese sample	5.0	47.22	44.22	64.0

Table 4: Ash content of fermented cheese sample

Sample	Wt of sample (g)	Wt of empty crucible	Wt of crucible + Ash	Ash (%)
Cheese	5.00	65.00	65.03	0.6

Table 5: Fat content of cheese sample

Sample	Wt of sample (g)	Wt of empty cup	Wt of cup + extracted oil (g)	Fat (%)
Cheese	5.00	30.00	30.67	13.4

Table 6: Protein content of fermented cheese sample

Sample	Wt of sample (g)	1st titre	2nd titre	Average titre	N <sub>2</sub> (%)	Protein (%) = %N <sub>2</sub> x 6.38
Cheese	1.00	14.3	14.5	14.4	2.016	12.86

Table 7: Carbohydrate content of cheese sample

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (by difference) (%)
Cheese	64.0	0.6	13.4	12.86	9.14

Table 8: Microbiological analysis of the cheese sample

Type of analyses	Colony forming Unit/g
Total plate count	1.1 x 10 <sup>1</sup>
Coliform count	Nil
Mould and yeast count	Nil

Above readings were average values of four (4) determinations

of 12.7% for heat-acid coagulated cheese. However, the result of Fasakin and Unokiwedi (1992) (47.50%) were at variance with those obtained in this study and previous studies. This may be due to the fact that their product was not heat-treated. Significantly, fat is important as a source of energy to the body (Hannon *et al.*, 2006). The study of the protein content of cheese gave a mean value of 12.86%. This value is higher than those reported by earlier workers on cheese (Frazier and Westhoff, 1988, 5.33%) and lower than the value reported by Fasakin and Unokiwedi (1992) (44.5%). The high protein content of this product shows that its consumption will help eliminate protein deficiencies that have become the bane of poor nations, Nigeria inclusive. The carbohydrate content of the fermented product (Table 7)

shows it contain 9.14%. Similar observation was reported by Fasakin and Unokiwedi (1992) during the chemical analysis of cheese from milk and melon milk. Microbiological data of the cheese sample (Table 8) revealed that the total plate count was 1.1 x 10<sup>1</sup> cfu/g with the absence of coliforms, moulds and yeasts. The total aerobic count of 1.1 x 10<sup>1</sup> cfu/g is within the acceptable limit and hence the product is safe for human consumption. The high quality of this product was possible because good laboratory practice was maintained throughout the study process culminating in the production of an acceptable product of low microbial quality.

This report has shown that in the absence of rennet, extract from *Calotropis procera* (Sodom Apple) leaves can be used to coagulate milk product without necessarily destroying the nutritive value of milk but

rather improve its quality. It also shows that such extracts are adequate replacement for rennet as the plant are available everywhere. It is hoped that the chemical characteristics of the Sodom Apple leaves will be analyzed in subsequent studies to ascertain its constituent.

## REFERENCES

- AOAC, 1990. Association of Official Analytical Chemists, Washington DC., USA.
- Aworh, S. and A. Akinniyi, 1989. Proximate analysis and nutritional component of locally produced cheese. *Nig. J. Food. Sci. and Tech.*, 9: 220-227.
- Billie, P.G., W.E. Espie and W.M.A. Mullan, 1992. Evaluation of media for the isolation of *Leuconostocs* from fermented products. *Milchwissenschaft*, 47: 640.
- Billie, P.G., W.M.A. Mullan and W.E. Espie, 1985. Observation on the effectiveness of agar media for the enumeration of *Leuconostocs* from cheese and starter cultures. *Irish J. Food Sci. Technol.*, 9: 78.
- Davis, P.A., J.F. Platon, M.F. Gershwin, G.M. Halpern, C.L. Keen, D. Dipaolo, J. Alexander and V.A. Ziboh, 1993. A Linoleate-enriched cheese reduce low-density lipoprotein in moderately hypercholesterolemic adults. *Ann. Intern. Med.*, 119: 555-559.
- Davies, F.L. and Wilkinson, 1973. *Bacillus cereus* in milk and dairy products. In; B.C. Hobbs and J.H.B. Christian. The microbial safety of food. Proceedings of the Eighth International Symposium on food microbiology (pp: 57-67). London, Academic Press.
- Egan, H., R.S. Kirk and R. Sawyer, 1988. *Pearson's Chemical Analysis of Foods*. Bath. Aron., pp: 15-20.
- FAO, 2008. *Milk for Health and Wealth*. FAO, Rome, Italy.
- Fasakin, A. and C. Unokiwedi, 1992. Chemical analysis of fermented cheese obtained from Cow milk and melon. *Nig. J. Microbiol.*, 5: 559-566.

- Frazier, W.C. and D.C. Westhoff, 1988. Food Microbiology. Tata-McGraw-Hill Publishers Co., New Delhi, pp: 410.
- Harrigan, W.F. and M.E. McCance, 1976. Laboratory Methods in Food and Dairy Microbiology, Academic Press, London, pp: 400.
- Helen, R. and M. Elisabeth, 1990. Microbiology of Cheese. Institute of Food Research Shirfield Berkshire, UK., pp: 409.
- Mullan, W.M.A., 1986. Bacteriophage induced starter problems. Dairy Industries Int., 51: 40-43.
- Prentice, G.A. and J.V. Brown, 1983. The microbiology of cheddar cheese manufacture. Dairy Industries Int., 48: 23-26.
- Prentice, G.A. and J. Neaves, 1986. J. Appl. Bacteriol., Symp., 8: 435-575.
- Rogosa, M., J.A. Mitchell and R.F. Wiseman, 1951. Media for the isolation of Lactic Acid Bacteria. J. Bact., 62: 132-133.
- Scott, R., R.K. Robinson and R.A. Wilbey, 1998. Cheese Making Practice. 3rd Edn., Applied Science Publication, Ltd., London.
- Seo, M.H., S.Y. Lee, Y.H. Chang and H.S. Kwak, 2009. Physicochemical, microbial, and Sensory properties of yogurt supplemented with nanopowdered chitosan during storage. J. Dairy Sci., 92: 5907-5916.
- Sharp, M.E., 1979. Identification of the Lactic Acid Bacteria: In: Identification methods for Microbiologists. 2nd edn. (Eds. F.A. Skinner and D.W. Lovelock) pp: 233-259, London, Academic Press.
- Specialist Cheese Makers Association, 2002. The specialist cheesemakers code of best practice. Available from <http://www.specialistcheesemakers.co.uk/about.html>
- Timson, W., M.C. Broome, A.J. Hillier and G.R. Jago, 1982. Metabolism of *Streptococcus thermophilus*. Aust. J. Dairy Technol., 37: 14-16.
- Wong, H.C, L.Y. Chen and C.L. Chen, 1988. Growth germination and toxigenic activity of *Bacillus cereus* in milk productions. J. Food Protection, 51: 707-710.