NUTRITIONAL EVALUATION OF ACCELERATED RIPENED CHEDDAR CHEESE

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN

Human Nutrition & Dietetics

TO THE KURUKSHETRA UNIVERSITY
KURUKSHETRA

1982

By

VEENA RANI

DIVISION OF HUMAN NUTRITIO N AND DIETETICS
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL (Haryana) INDIA

Registration No. 79-dk-130

NUTRITIONAL EVALUATION OF ACCELERATED RIPENED CHEDDAR CHEESE

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN

Human Nutrition & Dietetics

TO THE KURUKSHETRA UNIVERSITY
KURUKSHETRA

1982

By

VEENA RANI

DIVISION OF HUMAN NUTRITIO N AND DIETETICS
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL (Haryana) INDIA

Registration No. 79-dk-130

WITH LOVE AND GRATITUDE

TO

MY EVER LOVING PARENTS, BROTHERS AND SISTER

Dr.A.D.Deodhar, M.Sc., Ph.D.
Scientist S-2

DIVISION OF HUMAN NUTRITION & DIETETICS
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL (HARYANA)

Dated the 27th May, 1982

I, hereby, certify that the thesis entitled "NUTRITIONAL EVALUATION OF ACCELERATED RIPENED CHEDDAR CHEESE" submitted in partial fulfilment of the requirement for the Degree of M.Sc.(Dairying) in Human Nutrition and Dietetics to the Kurukshetra University, Kurukshetra, embodies the result of a bonafide research carried out by MISS VEENA RANI under my guidance and supervision.

I further certify that such help or source of information as has been availed of in this connection, is duly acknowledged by her.

A.D.Deodhar

(A.D.DEODHAR)

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to Dr.A.D.Deodhar, Scientist (S-2), Division of Human Nutrition and Dietetics for his unstinted and competent guidance that was meted out to me during the entire period of this investigation.

The facilities provided by Dr.I.S.Verma, Director and Dr.D.S.Sundaresan, Ex-Director, National Dairy Research Institute, Karnal, are gratefully acknowledged.

I am immensely thankful to Dr.S.Singh, Scientist (S-3) and Mr.S.K.Kanawjia, Scientist (S-1), Division of Dairy Technology for providing with the samples required for the present study and their unstinted help extended to me during the entire course of the present study.

The help and cooperation received from Mr.Surinder Singh, Scientist (S-1), Division of Economics, Statistics and Management and Mr.Madhu Sudan are duly acknowledged.

Thanks are also due to Saroj didi, Mr.B.K.Datta and my friends for their constant encouragement extended to me during the entire period of this study.

Veena Rani

(VEENA RANI)

C O N T E N T S

****

CHAPTER - I

INTRODUCTION

INTRODUCTION

Among different dairy products, cheese is one of those ancient food items known to human civilization. Records of its use go beyond 4000 years back when it was made and eaten in biblical period (Kon, 1972). Myriad studies carried out since then, have established this product to be semisolid, delactosed, dewatered form of milk with a casein and para-casein matrix embedded with other milk constituents especially, fat, calcium and whey.

Cheese consumption has increased immensely during the past decade. The cheese production showed a remarkable increase from 7,731,808 metric tons in 1969-71 to 10,483,713 metric tons in 1978 (FAO, 1978). In United States alone, cheese production showed a remarkable increase from 0.68 billion kg in the year 1960 to 1.2 billion kg in 1973 (Nelson, 1975). Likewise, in Hungery during the same period, cheese production almost doubled to 28,000 tons. Viability of cheese industry in most western countries is further evident from the increase in per capita consumption of cheese. In the United States, total consumption steadily rose from 5.1 kg per year to 9.9 kg during a span of over two decades (Olson, 1981).

Cheese is recognised as the food of high nutritive value by virtue of its richness in good quality proteins as well as several other nutrients. It is also acclaimed to be an ideal source of vitamin A, riboflavin, calcium and phosphorus. Thus, consumption of an ounce of cheese fulfills 3.8 percent of calorie, 12.7 percent of protein, 7.8 percent of vitaminA, 7.8 percent of vitamin B_2 and 25 percent of calcium requirements, of an adult human subject (Robinson, 1977). Such attributes, however, cannot be solely ascribed to mere concentration of nutrients during curd-setting. Lactic starter cultures further significantly modify proteins, lipids and lactose in such cultured dairy products, thereby, producing desirable qualities. However, several other biochemical changes such as synthesis of antibiotics, vitamins and certain enzymes by lactic microflora which, hitherto, remained overlooked, have come into prominance in recent time. Besides this, removal of most of the lactose in whey during curd separation and elaboration of an enzyme lactase by certain lactic cultures during ripening, further endorse unique position of cheese in the diet of lactose intolerant subjects (Sellars, 1981).

Cheese, like other fermented milk products, possesses one distinct advantage, in that, it helps in achieving an excellent public health record. The presence of natural inhibitory substances arising from lactic acid fermentation, suppress the proliferation of pathogenic bacteria. The safety factor is greater in the ripened cheese because of low moisture, lack of air, intermediate products of ripening and high salt concentration (Kosikowsky, 1966).

From the scientific point of view, cheddar cheese making can be categorised into following steps viz., (i) coagulation of milk by rennet, (ii) syneresis of the coagulum and (iii) curing under controlled temperature and humidity conditions.

Conventionally ripening of cheddar cheese usually takes about 3 to 12 months. While such longevity may be a valuable attribute of cheese when it is used in its natural form, the cost of storing until it acquires an acceptable flavour and consistency, adds substantially to the capital cost of cheese making plants as well as to its running cost. According to an estimate, the storage cost contributes near about 8.44 percent of the total cost of cheese production (Singh and Kalra, 1979). This underscores the pressing need to cut down the production cost of cheese so that it could remove constraints and eventually become more popular in countries like India. This prompted several investigations to modify the conventional method of cheese making to accelerate the ripening of process. These included addition of certain proteolytic and lipolytic enzymes, increased proportions of starter, ripening at elevated temperature, use of lactose hydrolysed milk etc. (Kosikowski and Iwasaki, 1975; Sutherland, 1975; Thompson and Brower, 1976; Sood and Kosikowski, 1979 and Law and Wigmore, 1982). However, these techniques equally pose problems in marketing product as there was every chance of product getting overripened. Since the means to arrest ripening changes were not readily available. To certain extent this could be achieved by cooling cheese. But in that case, the cost of refrigeration would as well outweight the saving gains of acceleration.

A process has been developed by which a liquid cheese product of characteristic cheddar flavour can be obtained from fresh curd in five to seven days. Basically the process involves blending 2 part of one day old salted unpressed cheddar curd with 1 part of 5.2 percent sterile sodium chloride solution and storage of homogenous slurry at 30°C. The slurry contains 40-41 percent total solids, and its pH ranges from 5.15 - 5.30 (Kristoffersen et al., 1967). This process was found to be economical because it by passes subsequent refrigeration, otherwise required, to control over-ripening.

Several biochemical reactions including partial degradation of proteins, fats and carbohydrates as well as synthesis of certain vitamins of B-group, take place during ripening of cheese, that profoundly alter the nutritional characteristics of milk. As mentioned earlier, controlled acceleration of ripening of cheddar cheese adopted in this investigation depends on several factors such as moisture level, temperature and salt concentration which markedly differ from those required for conventional ripening. All these factors would undoubtdly influence the metabolic course of starter cultures and in turn, further influence the nutritional value of the product. In view of the ultimate difference in periods for ripening by two different procedures, it becomes highly essential to understand the nutritional value of cheese made by using this process.

A study was, therefore, undertaken to study the proximate composition of cheese samples prepared by using either conventional or accelerated process. Attempts have also been made to find the changes in the level of certain vitamins of B group during accelerated ripening. Conventionally, cheese is consumed in its processed form. Studies were, therefore, further extended to compare the proximate composition as well as protein qualities of cheese proteins obtained using these processes. Results obtained are presented in this report.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

During ancient periods, cheese making was primarily considered as a means to preserve milk in a safe and palatable manner. Despite this, in the subsequent period, cheese has gained enormous popularity in most of European countries as well as regions where milk production has been in abundance. With such continuously growing popularity, cheese manufacture had become the capital intensive industry benefiting from a high turnover rate. Running cost alongwith substantial interest charges involved in cheese storage has been shown to represent a significant proportion to the total cost of production of cheese. In order to cut down overall cost of production, accelerated ripening has been the field of intensive research during the past couple of decades. The reduction in the ripening period can be attained in several ways. Large number of studies conducted on these lines and also investigations undertaken to probe biochemical changes occurring during cheese ripening, have been reviewed in this section and are presented under following three subtopics viz.

(i) Methods for acceleration of ripening of cheese

(ii) Biochemical changes during ripening of cheese

(iii) Nutritive value of cheese

1. Methods for acceleration of ripening of cheese

Ripening of curd is an important step in cheese making which imparts desirable flavour and texture to the end product. However, conventional ripening requires fairly long period for its proper accomplishment. It is known to depend on several factors as

(A) Starter cultures

In early days, the function of lactic acid bacteria was thought primarily to produce lactic acid to establish the pH of the cheese (Evans et al.,1914; Evans, 1918; Kelly, 1932 and Sherwood and Whitehead, 1934). However, subsequently it became apparent that the production of lactic acid is not the only function of a starter culture. These organisms, used as starter cultures, are extremely important in hydrolysing proteins and fat, thereby, contributing to the development of characteristic flavour (Kelly, 1932; Allen and Knowles, 1933-34; Sherwood and Whitehead, 1934; Dahlberg and Kosikowsky, 1947; Zielinska and Hiscox, 1954; Emmons et al., 1960; Perry, 1961 and Rieter et al., 1967).

(a) Increased rate of cultures

While during conventional preparation of cheese, starter cultures are added at the rate of 0.5 percent (Davis, 1976). Menshikov (1966) observed that the rate of ripening can be significantly accelerated when lactic streptococci starter culture was usedat 6 percent concentration. Law *et al.* (1976), on the other hand, failed to find out any influence of increased starter proteinase and peptidase activities on the flavour production. On the contrary, Lowrie *et al.* (1974) reported bitterness in cheese as the rate of starter culture was increased.

(b) Mutant strains

Dilanyan *et al.* (1975) studied the possibility of increasing the biochemical activity of lactic acid bacteria during cheese production by using highly active X-ray induced mutant of the lactic acid bacteria. The use of X-ray mutant (having proteolytic activity upto 60 percent higher than that of parent strains) significantly shortened the ripening stage by about 25 percent and yielding cheese of similar or perhaps of better quality.

Armyansk cheese containing the mutants was reported to be ripened more quickly than control cheese and mature flavour developed in 45 days instead of 60 days (Dilanyan and Sarkisyan, 1970).

Dulley *et al.* (1978) used mutants of *Streptococcus lactis* C_2 which did not produce acid from lactose, to increase (by approximately 2X) the number of starter bacteria in cheddar curd.

(c) Treatment for starter cells

Various treatments are known to be given to starter cells for accelerating the ripening of cheese.

(i) Heat shocked starter cells

Sublethal heat treatment at 59°C and 69°C substantially has been reported to delay lactic acid production of mixed strain mesophilic starter cultures or thermophilic streptococci and lactobacilli while only reducing their proteolytic activity by 10-30 percent and the rate of proteolysis increased in Swedish Houshold cheese containing heat shocked cells equivalent to 4-5 times the normal starter population (Petterson and Sjostrom, 1975).

(ii) Solvent treated cells

Exterkate (1979) reported that treatment of starter cells suspension with organic solvents resulted in the activation of some membrane bound proteolytic enzymes. After further development of this work, it is now possible to produce cells which do not produce acid but have peptidase activities upto 10 times greater than the normal cells. It was considered possible that the increased proteolysis and flavour intensity can be induced in Gouda cheese by using washed solvent treated starters to the acid producing starter inoculum.

(iii) Hydrolysed bacterial cells

Alexeev (1966) reported the rate of ripening to be approximately 1.5 times higher in experimental cheese using hydrolysed bacterial cells due to active development of microbiological and biochemical processes. The duration of ripening for cheese manufacture with hydrolysed bacterial starter may therefore, be shortened by 15-20 days without affecting the quality of the product.

(d) Use of modified starter

Allen and Knowles (1933-34) showed that the effect of a vigorous starter in inducing subsequent proteolysis is very marked as compared to that of a weak starter. It was concluded that the effect of a mixture of starter organisms induced greater proteolysis than the action of the species. In another study, slurries of excellent and intense Feta flavour were obtained from curd which was manufactured with a culture of S.lactis C_{10} or a 1:1 mixture of this culture and Lactobacillus casei, when the slurries were stored at 30°C for 8 - 9 days (Zerfiridis and Kristoffersen, 1970).

Shaligina et al. (1974) observed the shortening of ripening period of hard cheese from usual 1.5 to 6 months to 30 - 40 days by using special "Bukovinska" cheese starter (consisting of 50 percent *Streptococcus cremoris* and 50 percent *Streptococcus diacetilactis*) and selecting optimum temperature conditions for scalding the curd (32° - 35°C).

Use of a disintegrated frozen bacterial cell concentrate (DFCC), its crude cell free extract (CFE) or the cell debris (CD) as a supplement to the normal starter in order to accelerate cheddar cheese ripening was investigated by Davide and Foley (1979). A higher retention of the supplement was achieved by incorporating in the curd rather than in the milk. Supplementation with CFE, CD or DFCC increased protein breakdown in the cheese, liberating small peptides and amino acids which increased during ripening.

(B) Use of enzymes to accelerate cheese ripening

Studies have been reported on the acceleration of cheese ripening by the addition of various enzymes. Arsenova and Chebotarev (1972) used purified pancreatin in the manufacture of Rossiiskii cheese and obtained pronounced flavour in 45 to 50 days. The degree of ripening was found to be directly proportional to the amount of pancreatin added to the milk.

Nakanishi and Itoh (1974) added a mold protease prepared from *Aspergillus oryzae* to Gouda cheese before ripening at 13° - 15°C. It was observed that after 30 days, the index number of the cheese was 30.5. The characteristic flavour of the cheese was retained although amino acid pattern was different from that of commercial Gouda cheese.

Nofal and Abou-Dawood (1975) reported that the addition of pepsin at a rate of 1.8 g/20kg milk, accelerated the ripening of Domaiti cheese.

Sullivian and Infantino (1975) observed that ripening time of cheddar cheese was accelerated significantly by spraying a solution containing adenosine 3'-5' cyclic monophosphate at concentration of 0.2 - 200 mg/lb curd, over salted curd particles before filling the curd into cheese hoops.

Free volatile acids, soluble proteins and flavour production were found to be accelerated in young ripened cheddar cheese by adding various combinations of proteolytic and lipolytic commercial enzyme preparations with salt to curd before pressing (Kosikowski and Iwasaki, 1975).

Abd-El-Salam <u>et al</u>. (1978) studied the effect of added gastric lipases on the ripening and flavour development in Ras cheese and found the enzyme to accelerate the development of flavour, particularly when capalase K was used. In the subsequent year, the same workers found that cheese with large quantities of added enzymes ($<$ 4g/10kg curd) developed sharp flavour intensity after 45 days of storage, however, flavour defects developed on advanced storage. The addition of small amount of the enzymes improved the quality of cheese with little or no flavour defects.

Marschke *et al*. (1980) described that the addition of 'Maxilact' (a commercially available B-galactosidase extract of the yeast *Kluyveromyces lactis*) to cheese milk resulted in shortening of the ripening time of cheddar cheese.

Addition of various fungal and mold proteases, peptidases and lipases have been reported to accelerate the ripening ofcheddar cheese (Sood and Kosikowski, 1979; Kalinowski *et al*., 1979; Malkki and Mattson, 1981; Law and Wigmore, 1982).

(C) Pre-treatment of cheese milk

Many workers have reported acceleration in cheese ripening by pre-treatment of milk. Hofi *et al*.(1973) reported that the addition of 0.5 percent of casein acid hydrolyzate to cheese milk prior to its making shortens the ripening period to 60 days.

Thompson and Brower (1976) reported that by using milk which has 65 - 80 percent of its lactose hydrolysed by lactase, cheddar cheese can be manufactured which after 3 - 4 months showed the flavour, body and texture of characteristics of 6 - 8 months old cheese.

Weaver (1977) compared the hydrolysed lactose cheddar cheese made by a new process involving a prehydrolysis of lactose with lactase, with control cheddar cheese made by traditional methods. The increase in proteolysis resulted in a more rapid texture improvement in the hydrolysed lactose cheese so that at 3 months, it was equivalent to a conventional cheddar cheese ripened for 6-9 months.

Benraadt _et al_. (1973) also reported a decrease in ripening time of Grama cheese made from a milk hydrolysed with lactase. Anon (1977) have described accelerated cheese manufacturing process based on lactose hydrolysis (at least 60%) with lactase from _Sacchromyces lactis_.

Labuschagne and Nieuwoudt (1978) suggested that the addition of Beta-galactosidase at the rate of 0.3 g/lt milk at 30°C and left for 30 min before the addition of starter, accelerated the ripening of cheddar cheese.

Marschke and Dulley (1978) studied the effects of varying the hydrolysis conditions on the quality of cheddar cheese and found that 30°C for 1 hr was more effective than 4°C for 18 hr.

Hefi *et al.* (1979) reported that the addition of filtrates from a 2 days old culture of *Bacillus circulans* to cheese milk before renneting, increased lipolysis and proteolysis during ripening of Ras cheese. Further, the filtrates from 5-days culture of *B.pumilus* and *B.cereus* did not accelerate the ripening process.

Maqdoub *et al.* (1980) reported that the addition of cell-free filtrates from 2-days old milk culture of *Bacillus cirulans* (NRRL B-4284) to cheese milk prior to renneting caused a highly significant increase in counts of total bacteria, lactic acid bacteria and lactobacilli during ripening, thereby, accelerating the ripening.

(D) Use of minerals

Besides modifications of macromolecules, addition of certain minerals to enhance ripening of cheese has been attempted by several workers.

Hunter (1950) recommended the use of potassium and manganese ions to enhance the growth of lactobacilli and thus, to improve the flavour quality of cheddar cheese. Furthermore, potassium counteracts inhibitory action of higher concentration of sodium ions on the growth of lactobacilli. Similar observations were made by Peters (1960) and Demon and Galesloot (1962) who showed that the addition of manganese to milk had a stimulatory effect on betacocci (Leuc. cremoris) and enhanced citric acid fermentation.

Hofi et al. (1973) studied the effect of addition of trace elements on the ripening of Ras cheese using two mix of several trace elements such as Mix-I (B, Mn, Cu, Zn, I, Be, Ti, Su, Co, Si and Ni) and Mix-2 (Fe, Mn, Cu, Zn, Co and I). Such additions accelerated lipolysis and proteolysis and further hastened the ripening. Subsequently, they studied the effect of various dilutions of Mix-1 and Mix-2 added to milk at three different levels 1:500, 1:5000 and 1:10000. It was seen that 1:500 dilution decreased the development of acidity, lipolysis and protein degradation and resultant cheese had inferior properties. The 1:5000 and 1:10000 ratio accelerated cheese ripening and the development of typical flavour in both of the two mixture was superior to the control. Mix-2 was found to be more effective than Mix-1 in this respect.

It would, thus, appear that trace elements act as activaters or inhibitors of enzymes responsible for cheese ripening.

(E) Use of artificial systems

The level of moisture in the curd has been shown to influence the ripening process while

Sammis (1923) observed enhanced ripening of cheddar cheese when the moisture content was high. Van Slyke and Hart (1903) observed more proteolysis at higher moisture level in cheese than at lower levels. On the basis of such observations, Kristoffersen (1967) developed a method for accelerating development of flavour in cheddar cheese by raising the moisture level to 60 percent and further incubating at elevated temperature of 30°C. Such treatment produced strong flavour in about 5 to 7 days instead of 3-months period required during conventional ripening.

Singh and Kristoffersen (1969) applied the accelerated curd ripening process to Swiss cheese made from pasteurized milk. Slurries which contained 40 percent curd solids, 1.61 percent NaCl and 1.3 percent culture of propionic acid bacteria, developed full flavour after 4 to 6 days of storage. The addition of glutathione (100 ppm) developed flavour much faster, approximately 1 day earlier than slurries without it.

In the subsequent year, Singh and Kristoffersen (1970), reported a new method in which conventional starter and salt concentration of 3 percent as well as high ripening temperature of 30 to 35°C were used. The cheese ripened in 5 to 7 days.

Shanley and Sutherland (1975) reported slurries with 40 percent total solids with 3.2 percent salts. A mild cheddar cheese flavour was obtained when slurry was stored for 12 - 17 days at 25°C.

Dulley (1976) used slurried curd to accelerate cheese ripening. The normally manufactured cheddar curd was blended with 5 percent sodium chloride and 3 percent sorbate. This was stored at 30°C and incorporated into cheese by adding it to the curd before cheddaring.

(F) Microencapsulation of cheese ripening system

Besides the use of ripening enzymes as additives, attempts have been made to encapsulate them. When the solutions of enzymes were added to cheese milk, the distribution of enzymes was homogenous, though it resulted in a considerable loss of enzymes in whey. Law et al. (1975) reported loss of 60 percent enzymes in whey which becomes economically unfeasible.

Kosikowski and Iwasaki (1975) added enzymes in powder form using salt as a carrier and reported that there was approximately 9-times less enzyme requirement. However, a heterogenous enzyme distribution may become a difficulty in practice.

Enzyme microencapsulation provides another possible means of accelerating cheese ripening. Magee _et al_. (1979) reported the encapsulation in milk fat of a cell free extract of _Streptococcus diacetilactis_ together with substrates and cofactors required for diacetyl production. This method was found to have a great potential in accelerating the cheese ripening process.

Other devices/approaches

Klimovskii (1954) described a method by which the biochemical processes in cheese are speeded up so that product can be used for the manufacture of processed cheese after 15-20 days of ripening. Pasteurized milk is coagulated at 36°C within 15-18 min. by the addition of large amounts of starter culture (2-4%), the usual quantity of rennet, the coagulum cut and the curd scalded at 48°C for 50-60 min. After draining off whey, the curd is held for 30-60 min. till the pH falls to 5.1 - 5.3. It is, then, ripened at 20°C for 15 - 20 days.

Von Bochlemann and Loidin (1974) made some experiments in which emulsified ripened cheese of good quality was used as a "sourdough" additive in cheese making.

Govaryutkina _et al_. (1975) described a method for accelerating the ripening of Sovetsky cheese.

In this process, cheese was ripened at 10-12°C for 35-40 days and then, at 20-25°C during the subsequent 20 days which stimulated the growth of propionic acid bacteria.

Kalmikova and Prodanski (1976) suggested the use of buttermilk for accelerating the ripening in Dutch type hard cheese. Cheese, made with 30 percent butter milk added to skim milk, had higher percentage of soluble nitrogen than control cheese and was ready for processed cheese in 30 days. Addition of 1:2 mixture of Na_2HPO_4 + NaH_2PO_4 and sodium citrate in cheese grains, especially cheddar curd, accelerated ripening and eliminated the use of emulsifying salts in subsequent processing.

Biochemical changes

The ripening of cheese is a complex phenomenon. The physical state and concentration of the original milk constituents as they exist in cheese, the enzyme systems involved (both intra and extracellular) alongwith additives such as sodium chloride and the physical environment in which cheese is placed, all influence the ripening process. The problem of elucidating changes occuring during ripening is further complicated because milk constituents are changed by enzymatic action and by other chemical reactions.

Water

Water is necessary in cheese for the activities of biological agents of ripening. Whereas part of it gradually evaporates during ripening, considerable portion becomes more intimately associated with the proteins as ripening progresses (Van Slyke and Price, 1952). Such association is either physical or chemical in nature.

Proteins

Proteins undergo proteolysis resulting in peptones and peptides which on further decomposition may yield theindivisual amino acids.

As the proteolysis progresses in cheese, the insoluble constituents are to some extent changed to soluble forms. In early researches, the rate and extent of proteolysis was investigated extensively as a measure of ripening. Van Slyke and Hart (1903) found that during one year ripening period, the water soluble nitrogen comprised 44.7 percent, amino nitrogen 28.4 percent and ammonia nitrogen 5.4 percent of the total nitrogen.

Kosikowsky (1951) studied the role of production of free amino acids, amides and amines in American cheddar cheese from the date of manufacture over a ripening period of 180 days at 60°F. The appearance of many free amino acids was very rapid in both raw and pasteurized milk cheese. At the end of two days incubation at 60°F, 40 pervent of the number of free amino acids that were found in the fully ripened cheese had appeared.

Jacquet and Lenoir (1954) determined nitrogen distribution of Cammembert cheese at different intervals during ripening and reported the proportion of soluble nitrogen as peptide increased while that as proteose-peptone decreased. Ammonia was first detected at the 18th day and rapidly increased to 25 percent of soluble nitrogen after a month.

Cremer (1974) found that in all the cheese varieties examined, $_{5}$-casein was degraded more quickly than beta-casein by the milk coagulation enzyme used in the manufacture. Degradation of beta-casein was significant in the first month of ripening only in Gouda cheese but almost all beta-casein in 3 year old cheddar cheese was degraded.

Lee (1974) studied cheddar cheese at various stages of ripening and found that water soluble proteins increased by 6.07, 4.73, 8.24, 16.80 and 25.34 percent respectively at Ist, 2nd, 7th, 30th and 90th day of ripening.

A number of studies have been conducted on the relationship between products of proteolytic characteristic and the flavour of cheese. Harper and Swanson (1949) concluded that amino acids definitely contributed to the typical cheddar flavour. However, it is now generally accepted that amino acids do not contribute directly to typical cheese flavour. Kosikowsky (1951) found 16 amino acids in cheddar cheese. The number varied to a certain extent with the age of the cheese, yet for aged cheese the concentration of the major amino acids differed between various lots of cheese.

Various amines such as histamine, tryptamine and tyramine are produced as a result of the action of bacterial decarboxylase on amino acids such as histidine, tryptophan and tyrosine. It was observed that their presence in cheese varieties was responsible to cause some toxic effects in individuals who were unable to metabolise these amines. Doeglas _et_ _al_. (1967) reported the presence of 85 mg histamine in Gouda cheese variety. Sen (1967) reported the presence of 1-2 mg/g of tyramine, in cheddar, Mycella, Boursault and Blue Stilton cheese. Eitenmiller and Koehler (1974) reported the presence of 71.0 mg/100g of histamine in 3 samples of cheese out of 156 samples examined.

Voigt _et_ _al_. (1974) reported that the highest amount of tyramine and histamine in cheddar cheese samples were 0.7 mg/g and 1.3 mg/g respectively.

Tryptamine was uniformly low or completely absent in cheddar cheese.

Fat

Some degree of lipolysis and formation of free fatty acids is important in almost all varieties of ripened cheese. In some varieties, lipolysis plays an overriding role in typical cheese flavour and in others such as cheddar, lipolysis is less predominant. Obviously, cheddar cheese made from skim milk does not develop characteristic cheese flavour (Ohren and Tuchley, 1965).

Peterson and Johnson (1949) reported that in both raw and pasteurized milk cheese during the first 30 days of ripening, caperic, caprylic and capronic acid were absent while n-butyric acid was present at slightly lower levels than those of some cheese at 420 days. Acetic acid levels for both types of cheese are approximately one half than that of the same cheese at 420 days.

Sheuring and Tuckey (1947) made a study of the changes occurring in the fat constant values of cheese fat during the ripening of cheddar cheese. The RM values were found to be higher at the conclusion of study than at the beginning, indicating an increase in water soluble volatile fatty acids during aging.

The saponification number also tended to increase. The Polanskē values, on the other hand, decreased during the same period showing that water soluble volatile acids were being reduced. The percentage of soluble acids increased and the percentage of insoluble acid tended to decrease, giving further evidence of hydrolysis of the cheese fat.

The total free fatty acids resulting from the lipolysis have been shown to increase, although erratically, in the control cheese. These fatty acids may combine with ethanol, known to be present in cheese to form esters or they may be enzymatically oxidized to ketones. Such reactions may explain the declining trends in respect of free fatty acids (Jensen _et_ _al_., 1975).

Lactose

During ripening of cheese, residual lactose retained in the curd is metabolised by starter cultures resulting in the production of lactic acid.

Kristoffersen and Falls (1965) reported lactic acid concentration ranging from 1.1 to 2.0 percent and an average concentration of 1.7 percent in commercial samples of ripened cheddar cheese with no apparent relationship between the acid content, the age and the quality of the cheese.

Singh (1968) reported that initial lactic acid concentration in cheddar curd slurries (0.56 to 0.79%) were dependent upon the acidity at milling.

Nutritional value of cheese

Per capita consumption of cheese has been reported to increase from 5.1 kg in 1955 to 9.9 kg in 1978 (Olson, 1981).

The nutritional importance of cheese in comparison with other animal foods is presented in the following table (M.A.F.F., 1959):

Food item	Quantity	Protein (g)	Calcium (mg)	Vit. A (I.U.)	Riboflavi (mg)
Cheese	2 oz	14.2	460	738	0.28
Milk	7 oz	6.3	238	224	0.35
Egg	1(No.)	7.0	34	568	0.22
Fish(White)	6 oz	27.0	42	0	0.24
Meat	3 oz	14.4	9	42	0.21

It is evident that cheese is highly effective in providing certain nutrients, as compared to other products. It was evident that 2 oz of cheese fulfills requirement of protein, calcium, vitamin A and riboflavin more effectively as compared with 7 oz milk, 1 egg and 3 oz of meat. A reasonable serving of cheese (approx. 2 oz) would provide a moderately active man, over half his daily requirement for calcium, nearly a third of vitamin A and about a sixth of those for protein and riboflavin. The importance of cheese in the diet depends on its contribution towards dietary proteins, calcium and vitamin A as these valuable constituents of milk are retained in the curd (Nilson et al., 1965).

Protein content in different varieties of cheese has been reported to exhibit wide range from 17.5 percent in Camembert and 36 percent in Parmesan. The protein content in cheddar cheese was found to be 25 percent (Anonymus, 1973). Kosikowski (1966) reported 8 percent protein in cream type and 25 percent in cheddar cheese. Moderate variations were observed within a single type of cheese as evident from 16.5 percent for Camembert cheese (Anonymus, 1973) to 20.9 percent as reported by Eckhof-Stork (1976). In general, the protein content in cheese appears to depend on the factors, mainly the quality of raw material used in respect of SNF content and the moisture content in cheese.

Despite an extensive consumption of cheese, there are only limited number of observations on the protein quality of cheese.

In 1933, Beadles reported that digestibility of proteins in cheddar, Gruyere, Umbueger and Roquefort cheese was identical as that of milk proteins. The biological value appeared to be unchanged in Gruyere cheese and slightly decreased in the case of Umbueger. Reports of the 'University of Reading' (1941) further confirmed that digestibility and biological value of proteins remained same as those present in milk from which cheese had been manufactured. On the contrary, Mitchell (1948) showed that the maximum biological value of Gruyere cheese to be 73 percent as compared to 90 percent for milk proteins.

Dearden _et al_. (1945) reported biological value of 72 percent, digestibility coefficient 98 percent and protein efficiency ratio 3.3 for cheddar cheese.

Henry and Kon (1946) reported biological value of 76 and digestibility of the order of 98 percent when cheddar cheese was fed at 8 percent level to rats.

Steen and Eggum (1968) reported that biological value and net protein utilization for different types of cheese ranged from 64.8 to 77.2 and 64.9 to 75.7 percent respectively. The difference in biological value could not be ascribed to amino acid composition. Further, the degree of protein or fat decomposition did not influence the protein value.

Poznanski and Siudak (1971) observed net protein utilization to vary between 53 to 64 percent for hard, semihard and mold ripened varieties of cheese and between 60 - 76 percent for Edam, Rorepal and mold ripened cheese. It may further be observed that net protein utilization values were lower than those for skim milk powder. As regards digestibility coefficient, the range between 90 - 95 percent was observed in different varieties. It was seen that the digestibility of cheese was neither influenced by technology nor by the duration of ripening or direction of technology. Sehgal (1982) reported the biological value 83.72 percent, digestibility coefficient 87.91 percent, PER_D 3.42 and N.P.U. 60.33 percent. The proximity of biological values observed in various studies clearly suggested that near uniformity in casein to fat ratio in starting material, difference in technology adopted during various kinds of cheese preparation did not influence the biological value of milk proteins. Relatively higher values reported by Sehgal (1982), however, pose a question about the influence of other experimental factors such as the age of the animal as well as the extent of depletion of protein reserves.

Apparently, there is slight decrease in respect of both these parameters compared with the values reported for milk. It may be pointed at this stage that though cheese proteins are essentially milk proteins in a broad sense, these are distinctly devoid of whey protein, known to exhibit excellent amino acid make up. The change in the protein quality cannot, thus, be solely attributed to the effect of fermentation treatment during ripening (Pozananski and Siudak, 1971).

Fat

Fat content in the cheese is known to depend on thekind of milk used in its preparation (Arsenova and Chebotarev, 1972). It was observed that fat content in cheese exhibited wide variations among different varieties of cheese as is evident from 22 percent in Camembert, 26 percent in Japanese, 28 percent in Swiss, 29 percent in Gauda and 30 - 32 percent in cheddar cheese. Wong et al. (1977) reported 31.5 percent fat in cheddar cheese. It could be seen from the data given on fat content that the kind of cheese whether soft, semi hard or hard showed same degree of relationship with fat content. Thus, cheese varieties either semi hard or hard were found to have relatively higher fat content than other varieties. Close similarities in the fat content for any given variety of cheese is well understood in the light of near uniformity in casein to fat ratio universally adopted. As regards another nutritionlly important factor namely, free and total cholesterol, their content respectively, were reported to be 2.07 and 2.7 mg/100g of hard cheese (Ismail and Ahmad, 1978).

Carbohydrate

Most of the lactose present in milk is lost in the whey during milk coagulation. Lactose content in cheese was reported to vary between 0.01 to 0.48 percent by Frater et al. (1977). Kosikowski (1966), however, reported very high values of lactose (2%). Although, the small amount of carbohydrate in cheese may not have nutritional significance but their products are available and more directly concerned in the nutritional well being of human subjects (Kosikowski, 1966). The low lactose content in cheese further renders it suitable in the nutrition of lactose intolerants.

Minerals

Cheese is an excellent source of certain minerals, particularly calcium and phosphorus. In the cheddar cheese, 80 percent of milk calcium and 70 percent of milk phosphorus are retained and concentrated. Water soluble salts of potassium, acid, 144–2946 µg; biotin, 0.84–5.7 µg; folic acid 6.4 – 12.0 µg (Shahani et al., 1962); vitamin B_2, 0.01 – 0.955 mg (Anagama and Knaya, 1968); Berger-Grove, 1966; vitamin B_{12}, 1.48 – 4.25 µg (Hartman and Dryden, 1965; Rasic and Panic, 1961) have been reported per 100g of various varieties of cheese. Besides the inter-varietal differences, the variation within the same variety could also be seen in respect of several vitamins such as vitamin B_1, 0.012 – 0.55 mg; folic acid, 5 – 17 µg (Hartman and Dryden, 1965) B_6, 0.043 – 0.129 mg (Polanske and Tarpfer, 1965) and B_2, 0.3 – 0.9 mg (Kon, 1972) per 100g of cheddar cheese. Such a difference could be explained on the basis of duration of ripening.

The contents of certain vitamins are known to change during cheese ripening. Nilson et al. (1965) reported a decrease in vitamin B_{12} content during the initial period of ripening then, an increase, while folic acid has been observed to increase rapidly during the first week of ripening and decrease rapidly for next 6 weeks followed by a further decrease though at a slower rate, for the remaining period of ripening. Niacin and vitamin B_6 were further reported to increase during the initial period of ripening which may be related to lactose metabolism in cheddar cheese.

As regards vitamin B_2, Deardon et al. (1945) reported no changes during the curing of cheddar cheese. Others noted with cheddar cheese an initial decrease in B_2 that changed to a gradual increase so that the values at the end of six month were same as in the begining (Irvine et al., 1945). In the ripening of Gruyere cheese, riboflavin was reported to destroy... rapidly during the first month, following which the values stabilized (Randoin and Causeret, 1953). In Swiss cheese, the content of the vitamin increased slightly from third to sixth month (Hietaranta and Antila, 1953). Some disappearance of vitamin was also noted in the curing of Stilton cheese (Deardon et al., 1945).

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was conducted to assess the nutritional quality of accelerated ripened processed cheese and see changes during ripening of cheddar cheese by this process.

Collection of samples

Five batches of processed cheese made from conventionally ripened cheddar cheese and accelerated ripened cheddar cheese were obtained from the Division of Dairy Technology of the Institute.

Manufacture of conventionally ripened cheddar cheese and accelerated ripened cheddar cheese

The cheddar curd was manufactured by the standard procedure (Davis, 1976). In the case of accelerated ripened cheddar cheese, after milling (at 0.5 percent lactic acid) and salting, the curd was left in the cheese vat overnight for the completion of lactic acid fermentation.

Following day the curd was shredded in cheese shredder used for grinding natural cheese during processing. Two parts of shredded curd were mixed with one part of 5.2 percent sterile sodium chloride solution at 45°C and ground in a micropulverizer. The slurries were incubated at 30°C in 20 litres aluminium cans. Slurry were agitated alternate day to ensure proper aeration and to permit representative sampling for analysis.

Manufacture of processed cheese

For the manufacture of processed cheese, procedure outlined by Kosikowski (1966) was followed. After about 8th day of storage at 30°C, cheddar curd slurry was processed. Processing was done by blending of slurry with normally 9 month old ripened cheddar cheese. Sodium citrate (2.5%) was added as an emulsifier. In case of accelerated ripened processed cheese, 75 percent of ripened slurry was mixed with 25 percent of 9 months old cheddar cheese on total solid basis. In the case of conventionally ripened processed cheese 60 percent of young cheese was mixed with 40 percent of old cheese (9 month onwards).

Determination of proximate principles

The following constituents namely, protein, lactose, fat, moisture and total ash were determined from cheese samples as described below:

Determination of protein

The protein content in the sample was determined by using conventional microkjeldahl digestion and distillation procedure as described in AOAC (1980).

Cheese sample (1 g) was digested with 10 ml of conc. H_2SO_4 with the aid of a pinch of digestion mixture (K_2SO_4, $CuSO_4.5H_2O$ and SeO_2 in the ratio of 40 : 8 : 1). The digested sample was suitably diluted. Five ml aliquot of the diluted digest was taken for distillation and ammonia was liberated by adding 5 ml NaOH (80%) and collected in 25 ml of boric acid (3%). This was further determined by titrating against 0.02N HCl with 2 drops of mixed indicator (0.3 g Methyl red and 0.1g methylene blue dissolved in 100 ml ethyl alcohol).

Determination of total ash

Total ash content of the sample was determined according to procedure described in ISI bulletin (1967).

About 3 g of sample was accurately weighed in a silica crucible and heated initially on a low flame, followed by subsequent heating in a muffle furnace at 550°C for three hours.

Determination of moisture

Moisture content was determined according to the method described in AOAC (1980).

Weighed quantity of cheese samples (3 g) was taken in duplicate in a flat aluminium dish and uniformly spread. It was heated in an oven maintained at 100°C ± 1°C, till the constant weight was obtained.

Determination of fat

Fat was estimated following Gerber method described in British Standards (1969).

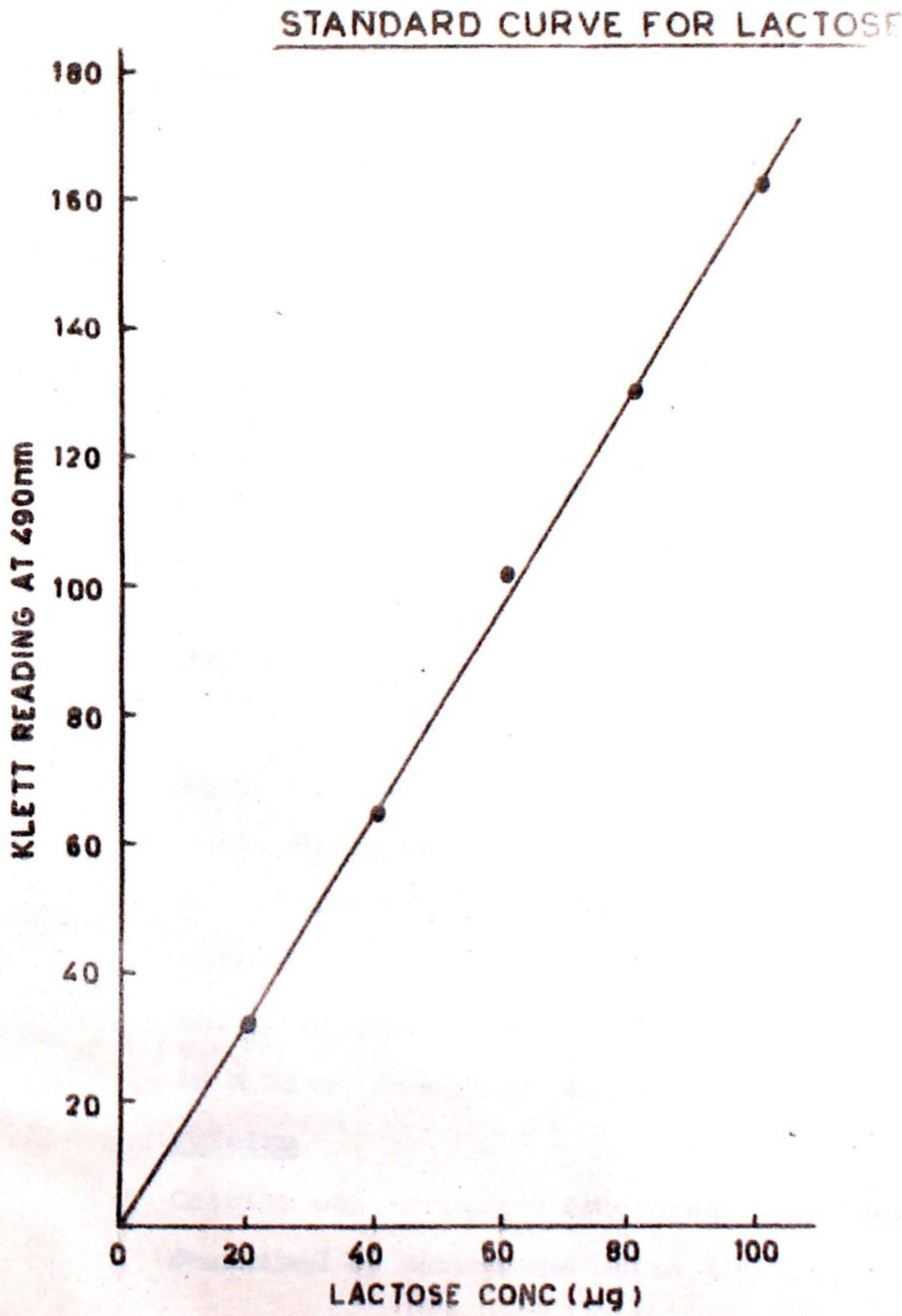
Three g of cheese sample was taken in duplicate in cheese cups and inserted in cheese butyrometer. To this was added 10 ml Gerber acid (Sp.gr.1.80), 1 ml amyl alcohol and water to adjust the volume. Having mixed thoroughly, butyrometer was centrifuged for 5 minutes and the fat level was recorded on the butyrometer scale. The fat percentage was calculated using the following formula:

$$\text{Fat \%} = \frac{\text{Butyrometer reading} \times 3}{\text{Wt. of the sample}}$$

<u>Determination of lactose</u>

Lactose was estimated according to the method of Acton (1977).

Two g of cheese sample was suspended in 10 ml of 0.5 M NaOH. On mixing thoroughly, the volume was adjusted to 100 ml with water. Further, 10 ml of $ZnSO_4$ solution (10%) was added and the suspension was made to 200 ml. The protein precipitate was removed by filtration through Whatman filter paper No.1. In duplicate, 1 ml of filtrate was mixed with 1 ml of 5 percent phenol solution in 150 x 25 mm test tubes. To this 5.0 ml of concentrated H_2SO_4 was rapidly added. Tubes were heated at 100°C in a water bath for 10 minutes. On cooling to room temperature, the coloured complex formed, was read



STANDARD CURVE FOR LACTOSE
KLETT READING AT 490nm
180
160
140
120
100
80
60
40
20
0
20
40
60
80
100
LACTOSE CONC (µg)

at 490 nm in a Klett Summerson photoelectric colorimeter. Lactose content was calculated by extrapolating from standard curve, prepared in the range of 0 - 100 ug lactose monohydrate/ml.

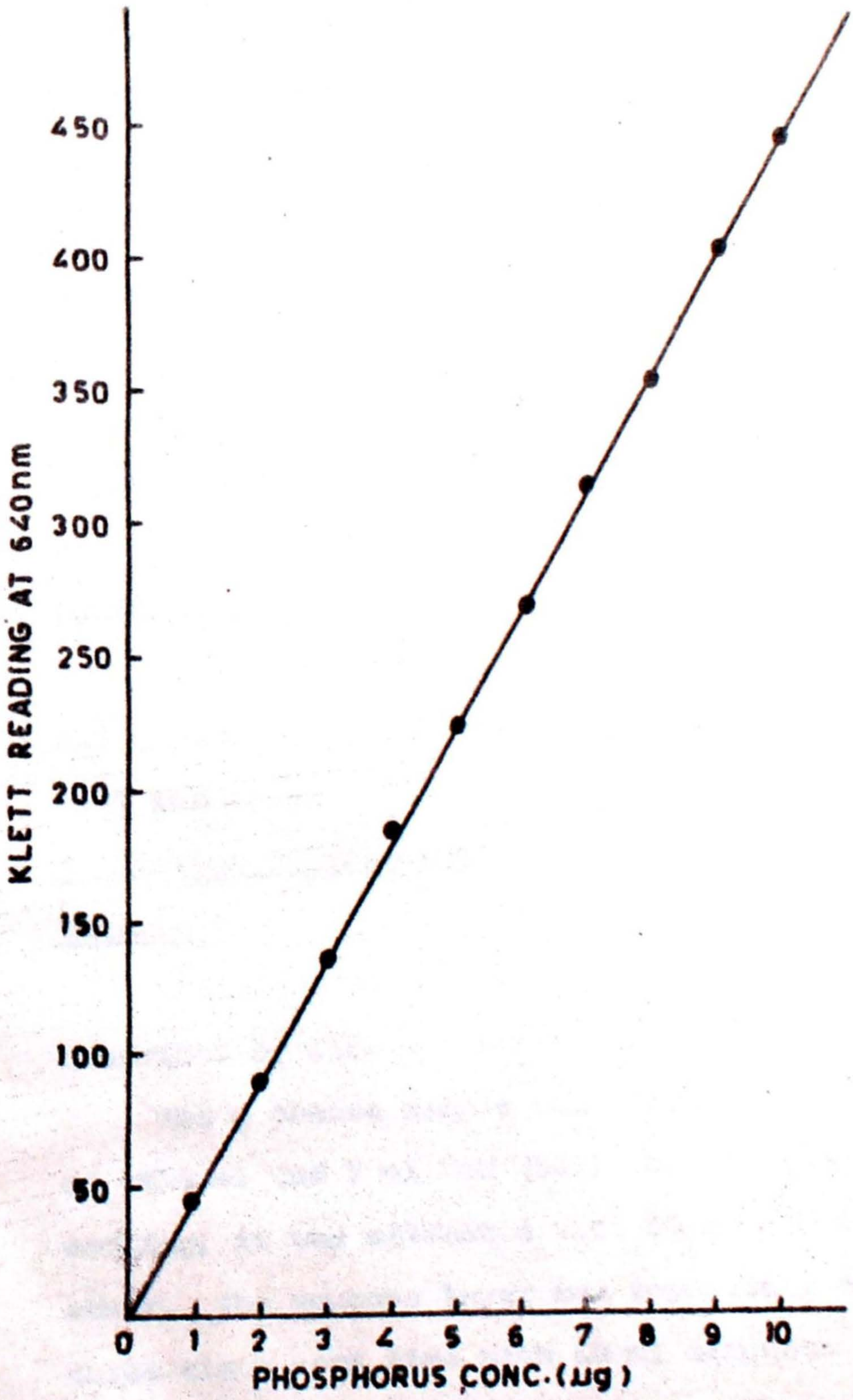
Determination of minerals

The procedure for the estimation of phosphorus was used as described by Ahovocova and Odavic (1969). The cheese sample (1 g) was digested with 15 ml of triacid mixture (HNO_3, conc. H_2SO_4 and $HClO_4$ taken in the ratio of 9 : 3 : 1). The digest was suitably diluted with water to give the final concentration of 10 ug/ml of phosphorus. Aliquots of diluted samples containing 1 ug - 10 ug phosphorus were taken in different tubes and the volume was made to 4.0 ml with glass distilled water. One ml of colour reagent (made by dissolving 3.5 g sodium molybdate, 200 mg hydrazine sulphate and 50 ml of conc. H_2SO_4 in a total volume of 500 ml) was added to all the tubes. On heating tubes in the boiling water bath for 30 minutes, the intensity of blue colour of phosphomolybdic acid produced was measured in a Klett Summerson photoelectric colorimeter at 640 nm.

Calcium

Calcium was estimated according to the method described by Davies and White (1962).

STANDARD CURVE FOR PHOSPHORUS



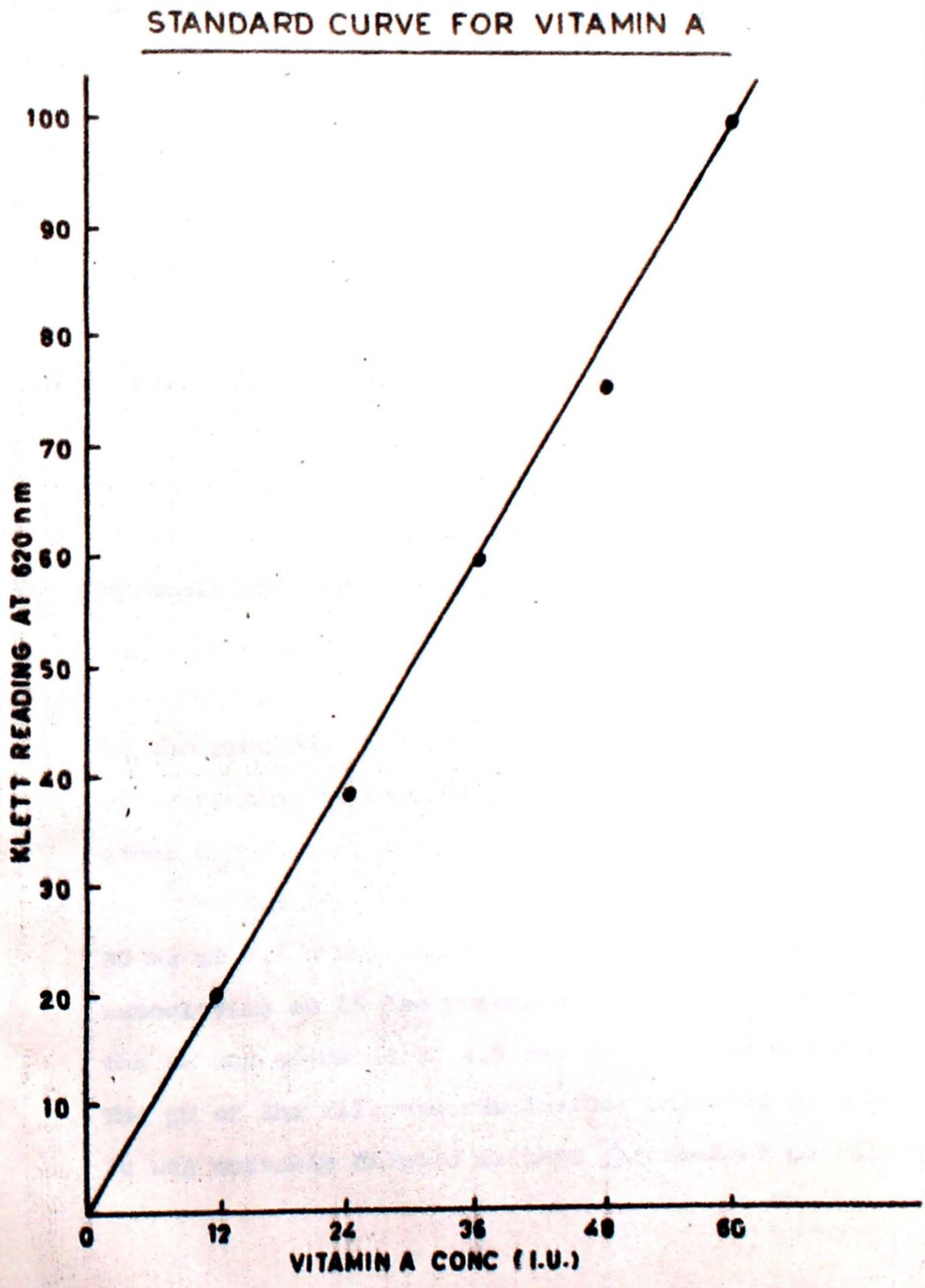
One g sample was digested with 15 ml of triacid mixture and the digest was suitably diluted with water. To 2.5 ml of digest, 0.5 ml of ammonium oxlate (4%) was added with a drop of methyl red. The solution was made alkaline by adding NaOH (20%), till a pale yellow colour was attained. HCl (1N) was added till the colour further changed to pink. The precipitate formed was allowed to settle at room temperature for 4 hours and centrifuged at 1400 rpm for 10 min, and saved. It was dissolved in 0.5 ml of 1 N HCl and quantitatively taken for titration after mixing with one ml of 0.05 M ethylene diamine tetra acetic acid and 1 ml of NH_4OH-NH_4Cl (pH 10) against 0.015 M magnesium acetate using two drops of Erichrome black T indicator (0.5 g erichrome T and 4.5 g hydroxylamine hydrochloride in 100 ml alcohol) till the colour changed from blue to violet.

Estimation of vitamins

Vitamin A

Vitamin A was estimated according to the method described by Carr and Price (1926).

Ten g cheese sample was saponified with 50 ml of ethanol and 7 ml KoH (50%) for 40 minutes. On cooling, it was extracted with 50 ml of diethyl ether. The aqueous layer was repeatedly extracted three times each time with 50 ml aliquots of diethyl ether. The ethereal extracts were collected, pooled and washed with water until free from alkali. On



STANDARD CURVE FOR VITAMIN A
KLETT READING AT 620 nm
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
VITAMIN A CONC (I.U.)

passing through anhydrous sodium sulphate, the ethereal layer was evaporated on a water-bath. The residue was dissolved in 5 ml chloroform.

Aliquots of the extract containing 20 - 100 ug of vitamin A were taken and volume was made to 1 ml with chloroform. On adding 4 ml of saturated $SbCl_3$ solution in chloroform, the intensity of colour produced was measured in Klett Summerson photoelectric colorimeter at 620 nm.

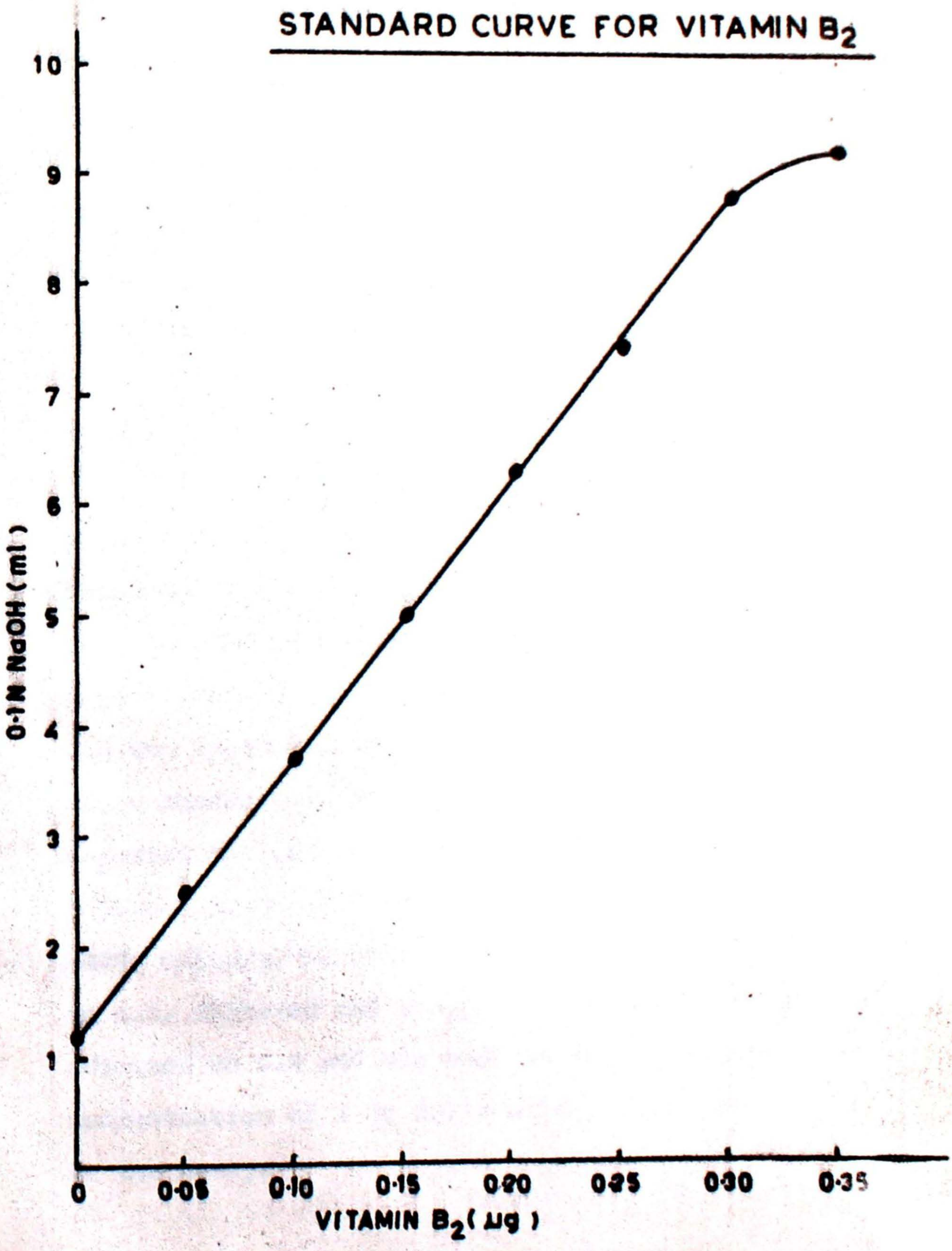
Riboflavin

Riboflavin content in cheese samples was determined microbiologically using *Lactobacillus casei* (ATCC 7469) as the test organism according to the method descrobed by Snell and Strong (1939).

Preparation of samples

Five g of cheese sample was defatted according to the procedure described by Rose Gottlieb (1959), by extracting it with 30 ml mixture of petroleum ether and solvent ether(taken in a ratio of 1:3).

The defatted sample was further hydrolysed with 30 ml of 0.1 N HCl and 5 ml of 2.5 M sodium acetate by autoclaving at 15 lbs pressure for 15 min. On cooling, the pH was adjusted to 4.5 and hydrolysate was filtered. The pH of the filtrate was further adjusted to 6.8. It was suitably diluted so that the content of riboflavin


STANDARD CURVE FOR VITAMIN B2
0·1N NaOH (ml)
10
9
8
7
6
5
4
3
2
1
0
0·05
0·10
0·15
0·20
0·25
0·30
0·35
VITAMIN B2 (µg)

was 0.1 ug per ml and used for assay. After 72 hrs of incubation, the growth response was measured titrimetrically with 0.1 N NaOH using bromothymol blue (0.04%) as indicator.

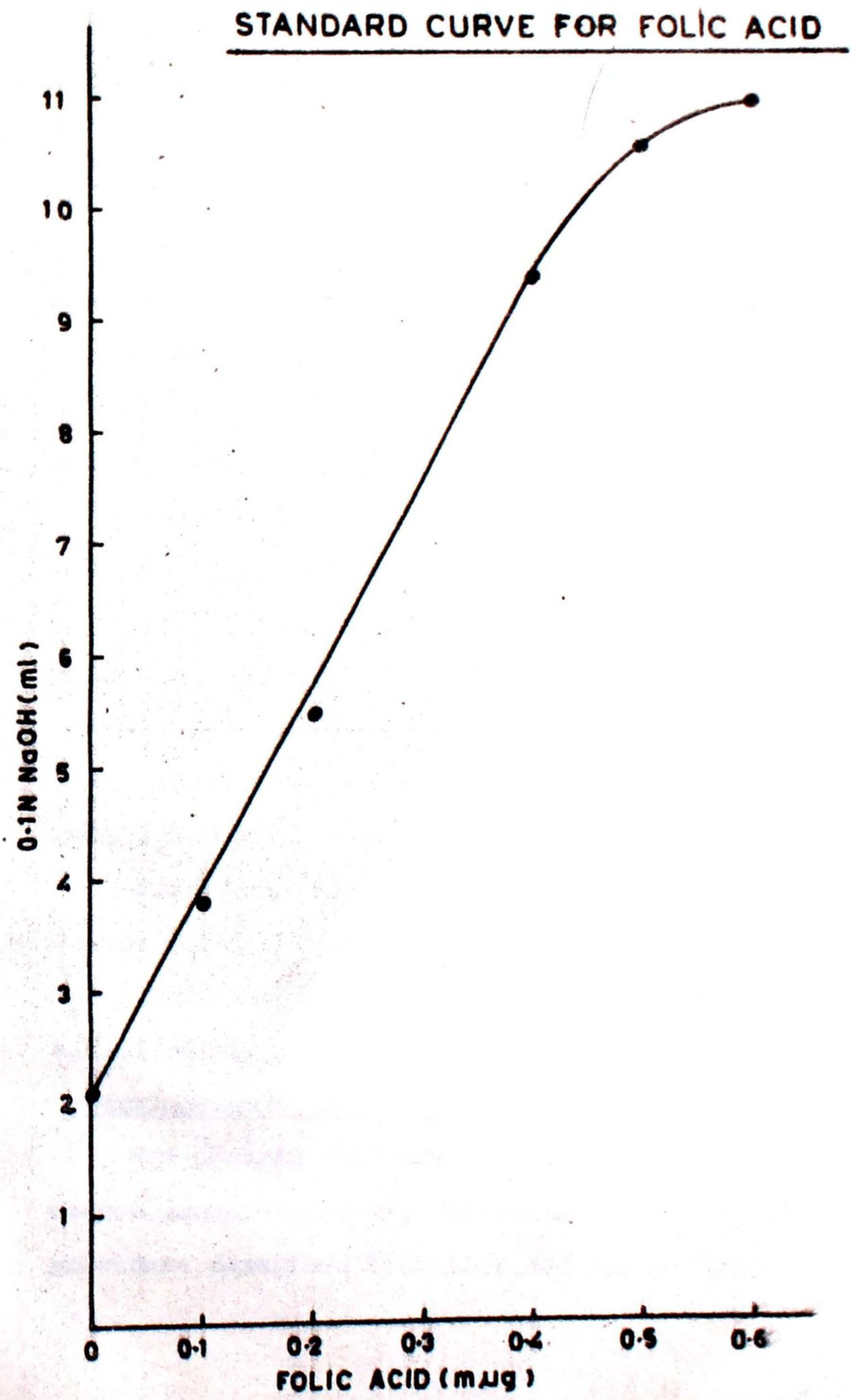
Folic acid

Folic acid was determined microbiologically using *Streptococcus faecalis* (ATCC 8043) as the test organism as described by Freed (1966).

Preparation of sample:

Two g cheese sample was defatted according to Rose Gottlieb method (1959) as described earlier.

The vitamin was liberated enzymatically by the action of pepsin followed by the action of trypsin. Defatted sample was suspended in 10 ml, of 0.1 N HCl and the pH was adjusted to 2. To this, 30 mg pepsin (1:3000, SD's Lab Chemical Industry) was added and the system was incubated at 37°C for 2 hrs. After incubation, 50 mg $CaCl_2$ was added and pH was adjusted to 7.2 and 30 ml water was added. This was further incubated at 37°C for 2 hours with 32 mg trypsin (2000 units/g, Merck). The pH of the sample was adjusted to 4.6, filtered and the pH of filtrate was finally adjusted to 6.8 and was made to volume to get the concentration of 2 ug folic acid per ml, and used in the assay.


STANDARD CURVE FOR FOLIC ACID
0·1N NaOH (ml)
11
10
9
8
7
6
5
4
3
2
1
0
0·1
0·2
0·3
0·4
0·5
0·6
FOLIC ACID (mμg)

The growth response was measured titrimetrically with 0.1 N NaOH using bromothymol blue as indicator.

Determination of free fatty acids

Free fatty acid content was determined according to method of Rao *et al.* (1972).

The fat from cheese sample was extracted by Mojonnier's method. Five g of cheese was made into a slurry with distilled water. It was digested with 5 ml of conc. HCl for 5 minutes at 60°C. The contents were transferred to Mojonnier tube, and extracted three times with a mixture of ethyl alcohol (95%), solvent ether and diethyl ether (1:2:2). The extract was washed with distilled water to make it acid free and dried in an oven at 100°C. The residue was dissolved in 5 ml of ethanol and titrated against sodium hydroxide (0.02 N) using phenolphthalein as indicator.

Free fatty acid was expressed as oleic acid and calculated by formula V x N x 28.8 where V is the titre (ml) and N is the normality of alkali solution.

Determination of net protein utilization

Net protein utilization value for different cheese preparations was determined according to the procedure described by Miller and Bender (1955).

Twenty four young female albino rats weighing between 50-55 gms obtained from the Small Animal House of the Institute were used in this study. Animals were divided randomly into three groups with eight rats in each. The average weight of animals in each group was 52.5 g. They were housed individually in anodized aluminium cages. Respective groups of animals, identical in body weight, were given either protein-free diet or test protein diet providing 10 percent protein for 10 days. The composition of the diet is given in Table I. Animals were fed <u>ad libitum</u> and had free access to water. At the end of experimental period, the animals were sacrificed. Incisions were made in the skull, thoracic region and the carcass was dried in the oven at 105°C, till a constant weight was attained. The dried carcass was powdered to uniform fineness.

One g of powdered carcass was taken for the estimation of nitrogen as described earlier.

$$NPU = \frac{\text{Body nitrogen of test group} - \text{Body nitrogen of protein free group} + \text{N consumed by protein free group}}{\text{Nitrogen intake}} \times 100$$

<u>Determination of biological value</u>

Biological value was determined according to the procedure described by Mitchell (1924).

TABLE - I

Composition of test protein and protein free diet per kg.

Ingredients	Accelerated ripened processed cheese	Conventional ripened processed cheese	Casein diet	Protein free diet [x]
Product	199.2*	191.9*	133.33	-
Sucrose	70.0	70.0	70.0	70.0
Fat (oil)	-	-	80.0	80.0
Cellulose	10.0	10.0	10.0	10.0
Vitamin mix**	10.0	10.0	10.0	10.0
Salt mix**	40.0	40.0	40.0	40.0
Starch	670.8	678.7	656.7	730.0

* Freeze-dried samples were used for the preparation of diet.

x Composition as given by Eggum and Jacobsen (1976)

** Composition as in AOAC (1980).

Sixteen female albino rats weighing between 40-45 g were obtained from the Small Animal House of the Institute. Animals were divided randomly in two groups of eight rats each and housed individually in metabolic cages made of anodized aluminium. The average weight of animals in each group was 45 g. Animals were maintained on a protein free diet for a period of 10 days and then on a test protein diet providing 10 percent protein for following period of 10 days. Animals were fed <u>ad libitum</u> and had free access to water. During both dietary regime of 10 days, first three days were for adaptation. Urine and faeces were collected separately and individually during the next seven days. Food intakes were recorded daily. The urine was collected under few millitres of toluene and 2 N HCl. Faeces were collected during the observation period of 7 days. These were pooled individually and the nitrogen was determined using micro-Kjeldahl digestion and distillation procedure described earlier. Biological value was calculated using the following formula:

$$\text{B.V.} = \frac{\text{Nitrogen digested} - \text{Nitrogen loss in metabolism}}{\text{Nitrogen digested}} \times 100$$

$$\text{Nitrogen digested} = I_n - (F_n - F_e)$$

Where :

I_n = Nitrogen intake

F_n = Nitrogen in faeces of test group

F_e = Nitrogen in faeces of protein free group

Nitrogen lost in metabolism = $U_n - U_e$

Where:

U_n = Nitrogen in urine of test group

U_e = Nitrogen in urine of protein free group

$$\text{Thus, B.V.} = \frac{I_n - (F_n - F_e) - (U_n - U_e)}{I_n - (F_n - F_e)} \times 100$$

On the basis of observations made on the nitrogen intake and faecal nitrogen during biological value experiment, digestibility coefficient was determined according to Mitchell (1924) as follows:

$$\text{D.C.} = \frac{\text{Nitrogen intake} - (\text{Faecal nitrogen} - \text{Endogenous N})}{\text{Nitrogen intake}} \times 100$$

From the data on protein intakes and gains in body weight obtained during the determination biological value, modified protein efficiency ratio (PER_D) was calculated as given by Venkata Rao (1964).

$$PER_D = \frac{\text{Gain in body weight} - \text{Loss during protein repletion based on 10 days}}{\text{Protein intake (g)}}$$

<u>Protein Efficiency Ratio</u>

Protein efficiency ratio was determined according to the procedure described in AOAC (1980).

Weanling albino rats of either sex weighing 30 - 40 g obtained from the animal house of the institute were divided in the groups with eight rats in each. The average body weight of rats in a group was 35.0g.

The animals were housed individually in anodised aluminium cages and fed ad libitum test protein diet providing 10 percent protein for a period of 28 days.

A group of eight animals identical in body weights with the test group was maintained on a protein free diet (Eggum and Jacobsen, 1976), to calculate net protein ratio (Bender and Doell, 1957).

$$\text{NPR} = \frac{\text{Gain in body wt. of test group} + \text{Loss in body wt. of protein free group}}{\text{Protein intake (g)}}$$

<u>Statistical analysis</u>

The data obtained were subjected to statistical analysis to verify the trends that were observed from results and to ascertain if the difference between two types of cheese is significant. This was done according to the method suggested by Snedecor and Cochron (1967).

One way classification of analysis of variance was used to study the significance of the difference between the effect of two types of rennets used for the purpose of study.

The model used was:

$$Y_{ij} = u + \alpha_i + E_{ij}$$

Where:

Y_{ij} = The observation corresponding to i^{th} treatment (rennet) for j^{th} replicates (animals)

u = The overall mean

α_i = The effect of i^{th} treatment

E_{ij} = The random error

The analysis of variance tables were tabulated and the influence of effect was tested for statistical significance by F test.

Further, the means for each treatment and for each character, under study, were calculated with respective standard errors and are presented.

CHAPTER - IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

It was seen in the earlier section that sizable information exists on the nutritional characteristics of conventionally ripened cheddar cheese. However, relatively little is known about an accelerated ripened product. Whereas several biochemical reactions are known to ensue during conventional ripening, the extent of changes that could be seen in the end product depends considerably on the period for which starter cultures act. As mentioned earlier, the process of ripening is accelerated so as to attain the desired organoleptic characteristics within a shorter period in contrast to prolong period of three to six months required for conventional ripening. This factor, comprised of variations in respect of moisture, temperature as well as other additives, could distinctly distinctly influence the nutritional character of the resultant product.

Data obtained on these lines are presented in this section.

Proximate composition

Data on the proximate composition of two types of cheese samples are given in Table 1 and its statistical analysis in Table 1A and 1B. Samples from

TABLE 1. Proximate composition* of conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese.

Product	Batch No	Moisture	Drymatter	Protein	Fat	Lactose	Total ash	Protein	Fat
								(on dry matter basis)	
	I	37.49	62.51	25.00	33.0	0.14	3.38	39.88	52.79
	II	39.00	61.00	25.36	32.0	0.19	3.36	41.57	52.46
Type A	III	39.06	60.94	24.29	33.5	0.11	2.36	39.86	54.97
	IV	37.56	62.44	25.90	33.0	0.12	2.54	41.16	52.85
	V	35.00	65.00	26.26	33.0	0.20	3.86	40.50	50.77
Mean ± S.E.		37.62 ± 0.73	62.38 ± 0.73	25.36 ± 0.34	32.9 ± 0.24	0.15 ±0.02	3.15 ±0.29	40.62 ± 0.33	52.77 ± 0.67
	I	60.00	40.00	14.29	20.0	0.21	4.00	35.72	50.00
	II	60.46	39.54	14.64	19.0	0.24	3.88	37.02	48.05
Type B	III	59.67	40.33	15.72	20.0	0.20	3.20	38.98	49.59
	IV	63.67	36.33	14.11	18.0	0.25	2.00	38.83	49.54
	V	61.66	38.34	15.18	19.0	0.26	3.40	39.59	49.55
Mean ± S.E.		61.09 ± 0.72	38.91 ± 0.72	14.78 ± 0.29	19.20 ± 0.37	0.29 ±0.10	2.77 ±0.13	38.03 ± 0.72	49.35 ± 0.33
C.D.		2.98	3.00	1.50	1.42	0.04	N.S.	1.99	1.92

* Expressed as g/100g, except stated, otherwise.

C.D. - Critical difference

N.S. - Non significant

TABLE 1A. Analysis of variance for proximate principles

Source of variation	d.f.	M.S.S.							
		Moisture	Drymatter	Protein	Fat	Lactose	Ash	Protein (on drymatter basis)	Fat (on drymatter basis)
Between four types of cheese	3	580.8	581.4	141.3	197.5	0.046	0.243	60.7	50.4
Between samples within one type of cheese	4	2.0	2.02	0.22	1.36	0.001	0.364	1.29	2.28
Error	12	2.39	2.42	0.60	0.54	0.0005	0.279	1.07	0.99
Total	19								

TABLE 1B. Analysis of variance for proximate principles

Source of variation	F value: Moisture	Dry matter	Protein	Fat	Lactose	Ash	Protein (on dry matter basis)	Fat (on dry matter basis)	F tab.
Between four types of cheese	242.3**	239.3**	235.4**	361.9**	79.1**	0.87^{NS}	56.7**	50.6**	$F_{0.01}=5.95$ $F_{0.05}=3.49$
Between samples within one type of cheese	0.83	0.83	0.36	2.49	2.55	1.30	1.21	2.29	$F_{0.01}=5.41$ $F_{0.05}=3.96$

** Significant at 1% level

NS Non significant

five different batches of cheese preparation were taken for this study in order to eliminate possible batch to batch variations. It was found that batch to batch variations in respect of all parameters studied were non-significant.

Although the protein and fat contents were markedly low in case of type B cheese, moisture and lactose content were significantly higher ($P < 0.01$). On the other hand, total ash content did not vary significantly. However, when data were expressed on dry matter basis, these differences in respect of fat and protein contents were obliterated. Higher values observed for lactose content in Type B cheese despite of shorter ripening period, could possibly be the reflection of its less utilization by starter cultures.

It was further observed that values for different constituents obtained in the present study and expressed on wet weight basis, compared well with those reported earlier by different workers (Kosikowski, 1966; Anonymus, 1973; Eckhof,Stork, 1976; Wong <u>et al</u>., 1977 and Sehgal, 1982).

Such similarities, particularly in respect of protein and fat content, are understandable in view of the near uniformity in the casein to fat ratio in milk preparations used in different studies. Also values for lactose content fall within the wide range of 0.01 to 0.48 percent reported earlier by Frater et al. (1977).

Higher moisture content in the case of Type B cheese is the result of special treatment given to the product to facilitate ripening.

Calcium and phosphorus

Keeping in mind the potential value of cheese in supplying calcium in the diet, contents of calcium and phosphorus were determined in both types of cheese and data are presented in Table 2 and its statistical analyses in Table 2A. The statistical analysis showed that there was no significant batch to batch variation. However, values for Type A cheese were higher when compared with those for Type B. This could be primarily due to higher moisture content in Type B cheese as mentioned earlier.

Values for these two minerals were comparable to those reported earlier by Kosikowski (1966); Anonymus (1973) and Sehgal (1982).

Changes in vitamin contents during accelerated ripening

Ripening is an extremely important step during cheese preparation. Besides several chemical changes responsible to impart organoleptic characteristics to the product, there is a significant increase in the nutritional value of cheese, particularly due to

TABLE 2. Calcium and Phosphorus contents of conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese.

Product	Batch No.	Calcium	Phosphorus
Type A	I	886	569
	II	886	587
	III	1002	631
	IV	1117	594
	V	1115	618
Mean ± S.E.		1001 ± 51.68	600 ± 11.26
Type B	I	770	344
	II	770	356
	III	770	350
	IV	654	375
	V	539	350
Mean ± S.E.		701 ± 46.52	355 ± 5.46
C.D.		214.20	29.31

Expressed as mg/100g

TABLE 2A. Analysis of variance for calcium and phosphorus

Source of variation	d.f.	M.S.S.		'F'	
		Calcium	Phosphorus	Calcium	Phosphorus
Between four types of cheese	3	116499	60067	9.47**	144.9**
Between samples within one type of cheese	4	15459	444.3	1.25^{NS}	1.07^{NS}
Error	12	12291	414.3		
Total	19				

* Significant at 1% level

NS Non significant

synthesis of certain vitamins of B group. As mentioned earlier, since the two procedures adopted for ripening in this study markedly differed in respect of several factors, experiments were conducted to see the extent to which the ripening was accomplished by measuring the level of free fatty acids produced. Further changes in the levels of vitamin B_2, folic acid as well as fat soluble vitamin A were determined and data are presented in Tables 4 to 6(i) alongwith their statistical analyses in Tables 4A to 6A(i), respectively.

Total free fatty acids

The degree of ripening is often judged by the extent to which levels of soluble nitrogen, free tyrosine and free fatty acids are altered. Of these measurements, free fatty acids have aroused interest in recent years in view of the association of these fatty acids or their derivatives with organoleptic properties of cheese (Nieuwoudt, 1977). Levels of free fatty acids were, therefore, determined in Type A and Type B cheese at different stages of ripening and data are given in Tables 3 and 3A.

It was observed that as the ripening progresses in case of Type B cheese, there was a progressive increase in the level of free fatty acids changing

TABLE 3. Total free fatty acid contents in conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese at different stages of ripening.

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	115.2	23.04	36.92	46.08
II	112.89	120.73	35.71	47.23
III	116.35	21.88	38.21	46.08
IV	110.59	19.58	34.56	48.38
V	115.20	23.08	35.71	46.08
Mean ± S.E.	114.04 ± 1.03	21.66 ± 0.0678	36.22 ± 0.624	46.77 ± 0.461

Expressed as mg oleic acid/100g

TABLE 3A. Total free fatty acid contents in conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese when expressed on dry matter basis.

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	184.28	60.09	94.68	115.20
II	185.06	53.15	90.40	119.44
III	190.92	56.10	97.48	114.25
IV	177.11	53.64	93.40	133.16
V	177.23	61.05	93.85	120.18
Mean ± S.E.	182.922 ± 2.62	56.806 ± 1.627	93.962 ± 1.141	120.440 ± 3.39

Expressed as mg oleic acid/100g

TABLE 3(i). Analysis of variance for total free fatty acids.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	25087	8362	3626 **
Between samples within one type of cheese	4	14.66	3.66	1.58 NS
Determination of samples	12	26.66	2.30	
Total	19	25129.32		

** Significant at 1% level

NS Non Significant

TABLE 3A (i). Analysis of variance for total free fatty acid expressed on dry matter basis.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	42208	14069	625.88**
Between samples within one type of cheese	4	18.33	4.58	0.203 NS
Determination of samples	12	269.75	22.47	
Total	19	42496		

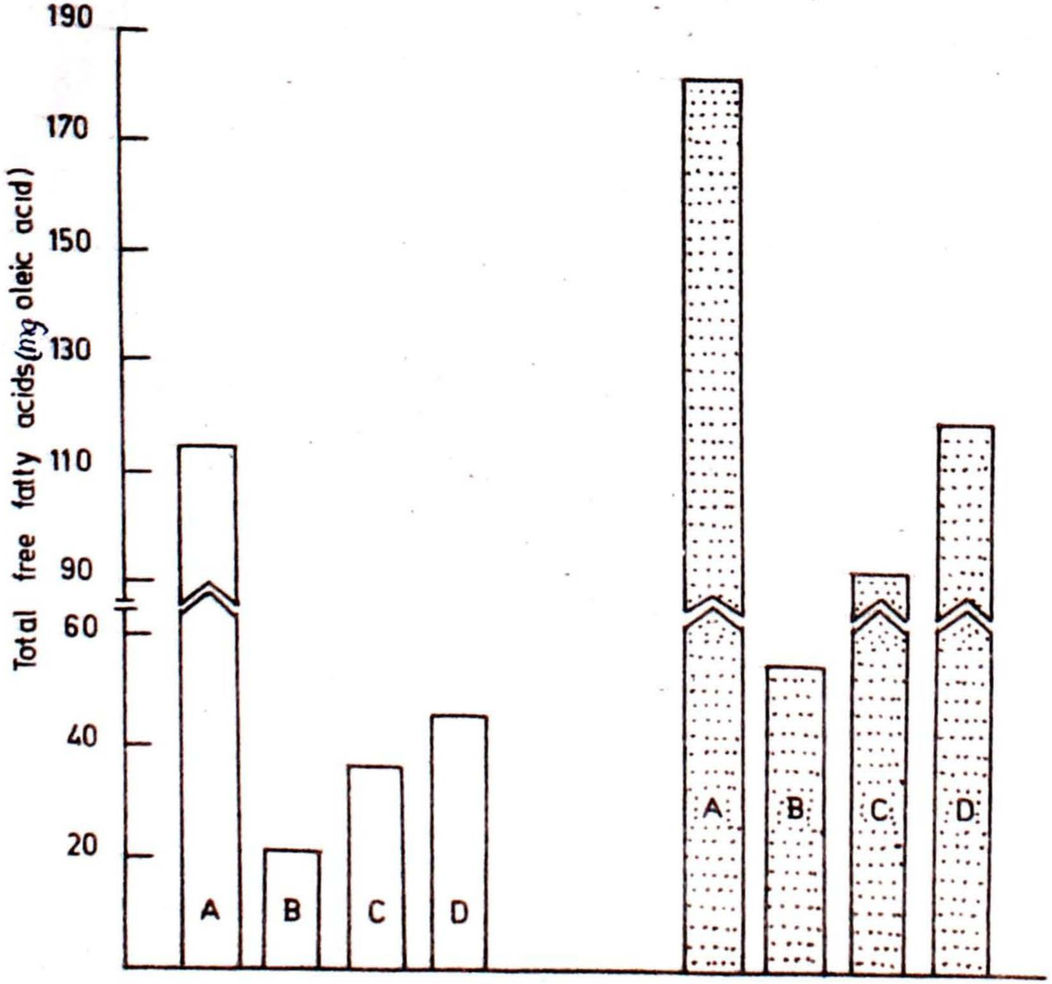
** Significant at 1% level

NS Non Significant

Fig.1. Effect of ripening period on total free fatty acids in accelerated ripened cheddar cheese.

A–Conventionally ripened cheddar cheese
B–Accelerated ripened cheddar cheese (0 Day)
C–Accelerated ripened cheddar cheese (4th Day)
D–Accelerated ripened cheddar cheese (8th Day)

(▭ On wet weight basis., ▦ On dry matter basis)



from 21.6 mg on 0 day to 46.7 mg on 8th day. However, even at this stage, the contents were markedly lower than those present in Type A cheese at 6 to 8 months of ripening. Such difference is possibly due to the result of steady accumulation of free fatty acids during the course of 6 to 8 months in case of Type A cheese.

Although the values of total free fatty acid were low for Type B cheese, yet it was having more lipolytic flavour as compared to Type A cheese. This may be due to further enzymatic oxidation of fatty acids to ketones or combibation of these acids with ethanol, known to be present in cheese, to form esters (Jensen _et al_., 1975).

As mentioned above ripening has been demonstrated to influence the levels of different vitamins of B-group in the curd. Beside starter cultures, other external factors like temperature, moisture as well as the lactose content initially present in the curd, markedly influence the levels of certain B-group vitamins. While the dependence of nicotinic acid and vitamin B_6 were shown to depend on the lactose content in the curd (Nilson, et al 1965), little is known about its effect on other important vitamins like riboflavin and folic acid. Though, most of lactose originally present in milk is lost in whey during curd separation, the residual amounts that remain could still exhibit effect on the levels of these vitamins. A study was therefore, extended to see, how the levels of these vitamins change during the course of accelerated ripening. Analysis on samples collected on 0 day, 4th day and 8th day were made and data were compared with values observed in the case of conventionally ripened cheese Tables 4 to 6A(1).

It could be seen from the data on folic acid that the contents markedly increased as ripening progressed. The values were 11.06 ug, 33.99 ug and 44.13 ug/100g at 0, 4th and 8th day of ripening as against 18.04 ug/100g in the case of conventionally ripened cheese. Data when expressed on dry matter basis, too, exhibited identical trend in the vitamin content. It was further observed that levels of folic acid in cheese ripened conventionally for 6 to 8 months, were very close to those present initially (0 day) in the accelerated ripened cheddar cheese. Data on conventionally ripened cheese obtained in this study, do not reflect the intermediate changes in the concentration of vitamins undergone during this process which were reported to be prominent by Nilson et al (1975). It would appear

TABLE 4. Folic acid content in conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese at different stages of ripening

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	19.00	12.33	36.11	44.33
II	20.44	11.33	32.44	42.66
III	17.00	8.77	30.55	47.33
IV	19.66	11.11	34.88	43.67
V	14.11	11.77	36.00	42.66
Mean ± S.E.	18.04 ± 1.13	11.06 ± 0.61	33.99 ± 1.08	44.13 ± 0.86

C.D. = 4.32

Expressed as ug/100g

TABLE 4A. Folic acid content in cheese samples expressed on dry matter basis.

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	31.14	32.15	92.58	110.82
II	33.51	29.05	82.12	107.89
III	27.00	22.50	77.93	117.35
IV	31.48	30.43	94.28	120.20
V	21.70	31.11	94.61	111.26
Mean ± S.E.	28.96 ± 2.10	29.04 ± 1.71	88.30 ± 3.47	113.50 ± 2.27

C.D. = 10.26

Expressed as ug/100g

TABLE 4(i). Analysis of variance for folic acid

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	3383	1127	224.2**
Between samples within one cheese	4	11.29	2.82	0.56 NS
Determination of samples	12	60.4	5.02	
Total	19	3454		

** Significant at 1% level

NS Non Significant

TABLE 4A(i). Analysis of variance for folic acid expressed on dry matter basis.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	27434	9145	323.2**
Between samples within one type of cheese	4	150.5	37.61	1.32 NS
Determination of samples	12	339.6	28.29	
Total	19	27923		

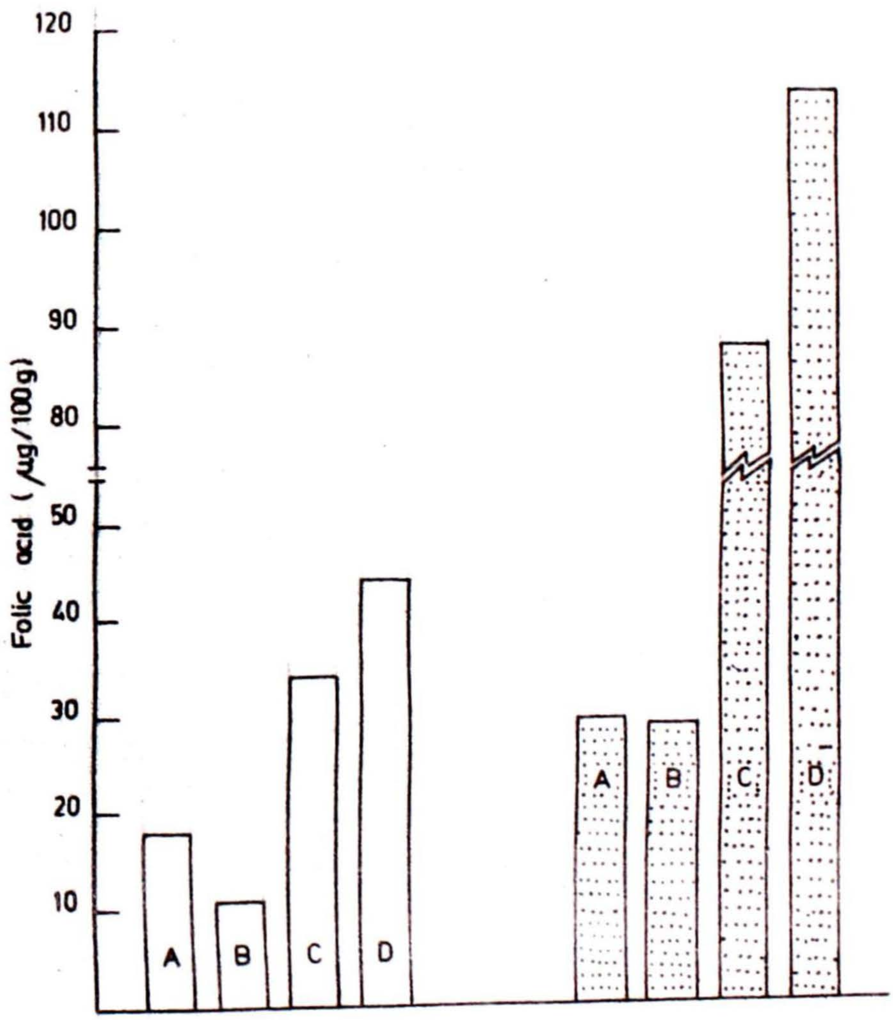
** Significant at 1% level.

NS Non Significant

Fig. 2. Effect of ripening period on the folic acid content in accelerated ripened cheddar cheese.

A—Conventionally ripened cheddar cheese
B—Acceleraled ripened cheddar cheese (0 Day)
C—Acceleraled ripened cheddar cheese (4th Day)
D—Acceleraled ripened cheddar cheese (8th Day)

(□ On wet weight basis., ▦ On dry matter basis)



on this account, that accelerated ripened cheese holds an edge over conventionally ripened cheese.

Relatively little data are available on the variations in the vitamin content occurring during accelerated ripening.

Values obtained for this vitamin compared well with those reported earlier by Shahani _et al_. (1962); Gregory (1975) and Shegal (1982).

As regards vitamin B_2, it was observed that when data were presented on wet weight basis, Type A cheese showed significantly higher values ($P < 0.01$) than Type B cheese at any stage of ripening. However, when expressed on dry matter basis, these differences were found to be non-significant. It was further observed that vitamin B_2 content remained virtually unchanged during ripening. Possibly, starter cultures either do not exhibit vitamin synthesizing capacity or the time chosen for ripening was not adequate to make changes, if any, visible.

Values obtained for this vitamin compared well with those reported earlier by Hartman and Dryden (1965) who recorded 0.5 mg vitamin B_2 per 100g of cheddar vheese. Whereas in another study, the content was 0.46 mg/100g (Anonymus, 1973).

The trend in the change of vitamin B_2 level observed in accelerated cheese was almost similar

TABLE 5. Vitamin B_2 content in conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese at different stages of ripening.

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	0.42	0.19	0.19	0.19
II	0.43	0.18	0.24	0.23
III	0.47	0.24	0.26	0.25
IV	0.37	0.24	0.25	0.24
V	0.48	0.25	0.23	0.23
Mean ± S.E.	0.43 ±0.01	0.22 ±0.01	0.23 ±0.01	0.23 ±0.01

C.D. = 0.045

Expressed as mg/100g

TABLE 5A. Vitamin B_2 content on dry matter basis in cheese samples

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	0.64	0.50	0.49	0.48
II	0.70	0.47	0.53	0.58
III	0.77	0.63	0.65	0.62
IV	0.60	0.67	0.69	0.68
V	0.75	0.68	0.66	0.68
Mean ± S.E.	0.69 ±0.03	0.58 ±0.04	0.60 ±0.03	0.60 ±0.03

C.D. = Non significant

Expressed as mg/100g

TABLE 5(i). Analysis of variance for vitamin B_2 content

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	0.1596	0.0532	84.56**
Between samples within one type of cheese	4	0.0106	0.0020	4.22*
Determination of samples	12	0.0075	0.0006	
Total	19	0.1778		

* Significant at 5% level

** Significant at 1% level

TABLE 5A(i). Analysis of variance for vitamin B_2 content on dry matter basis.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	0.0335	0.0111	3.30^{NS}
Between samples within one type of cheese	4	0.0778	0.0194	5.74**
Determination of samples	12	0.0400	0.0033	
Total	19	0.1520		

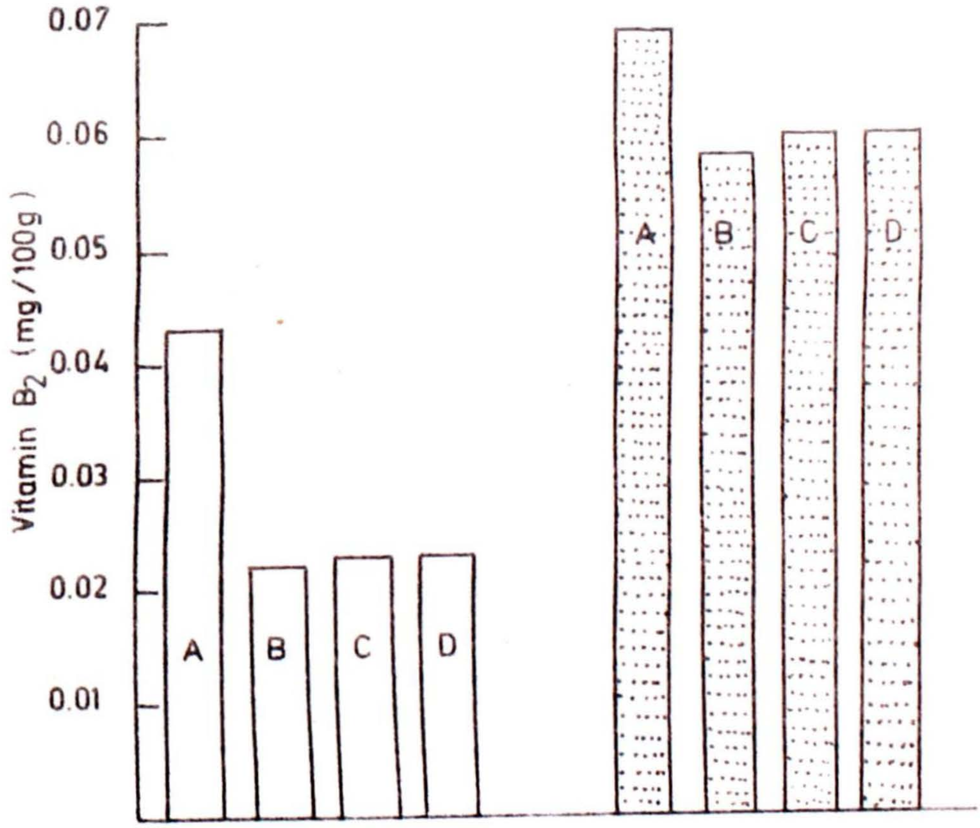
** Significant at 1% level.

NS Non Significant

Fig. 3. Effect of ripening period on the Vitamin B_2 content in accelerated ripened cheddar cheese.

A – Conventionally ripened cheddar cheese
B – Accelerated ripened cheddar cheese (0 Day)
C – Accelerated ripened cheddar cheese (4th Day)
D – Accelerated ripened cheddar cheese (8th Day)

(□ On wet weight basis ; ▨ On dry matter basis)



to that reported for conventionally ripened cheese (Deardon et al., 1945).

Observations were also made in respect of fat soluble vitamin A. It was seen that vitamin A content was significantly higher (P 0.01) in Type A cheese as compared to Type B when expressed on wet weight basis. The values when expressed on dry matter were also higher for Type A cheese. Such difference could be partly explained on the basis of higher fat content in Type A cheese, in the light of observations made by Davies and Moore (1939) and Deardon et al. (1945) who showed vitamins content to be dependent on fat content of cheese. Further, the difference in vitamin A contents in statting material, which was different for Type A and Type B cheese preparations, would also reflect in such differences.

There was no significant change in vitamin A content during the different stages of ripening of Type B cheese. Similar observations were made in other studies by Guerrant (1938); Higuchi and Peterson (1946) and Todhunter (1942) in the case of cheddar cheese.

Values obtained for vitamin A content of Type A cheese compared well with those reported earlier by

TABLE 6. Vitamin A content in conventionally ripened (Type A) and accelerated ripened (Type B) cheddar cheese at different stages of ripening

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	1112	612	637	625
II	962	625	612	625
III	1087	562	625	625
IV	1112	550	562	600
V	1150	550	587	600
Mean ± S.E.	1084.6 ± 32.34	579.8 ± 16.12	604 ± 13.53	615 ± 6.14

C.D. = 89.78

Expressed as I.U./100g

TABLE 6A. Vitamin A content in cheese samples expressed on dry matter basis.

Batch No.	Type A	Type B		
		0 day	4th day	8th day
I	1779	1597	1634	1562
II	1577	1602	1550	1580
III	1783	1442	1570	1549
IV	1780	1506	1520	1651
V	1769	1506	1544	1564
Mean ± S.E.	1737.6 ± 40.32	1530.6 ± 30.55	1563.6 ± 19.37	1581.2 ± 18.18

C.D. = 127.53

Expressed as I.U./100g

TABLE 6(i). Analysis of variance for vitamin A.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between five types of cheese	3	884636	294878	136.4**
Between samples within one typeof cheese	4	4459	1114	0.51 NS
Determination of samples	12	25923	2160	
Total	19	915020		

** Significant at 1% level.

NS Non significant

TABLE 6A(i). Analysis of variance for vitamin A expressed on dry matter basis.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between four types of cheese	3	126931	42310	9.39**
Between samples within one type of cheese	4	10923	2730	0.606 NS
Determination of samples	12	54036	4503	
Total	19	191891		

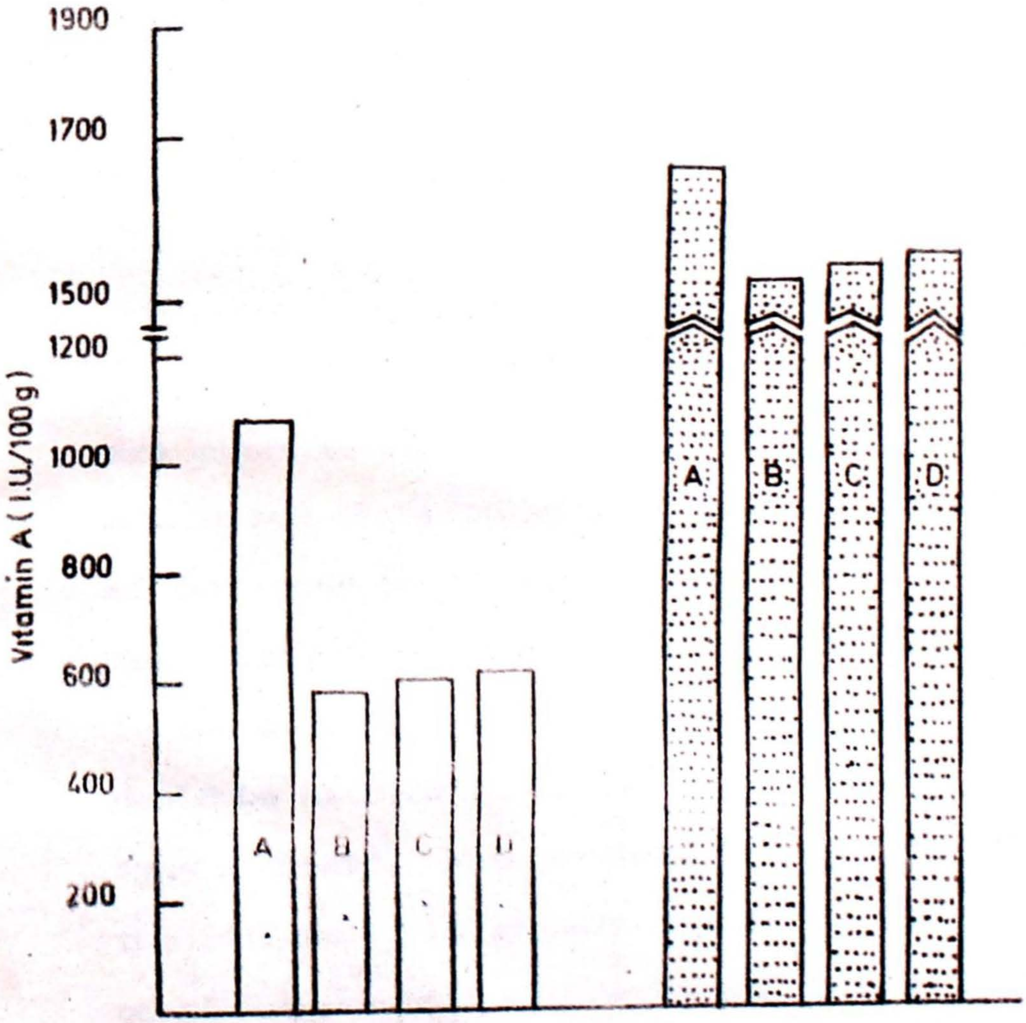
** Significant at 1% level

NS Non Significant

Fig.4. Effect of ripening period on the Vitamin A content in accelerated ripened cheddar cheese.

A—Conventionally ripened cheddar cheese
B—Accelerated ripened cheddar cheese (0 Day)
C—Accelerated ripened cheddar cheese (4th Day)
D—Accelerated ripened cheddar cheese (8th Day)

(□ On wet weight basis, ▩ On dry matter basis)



Hartman and Dryden (1965) who recorded 1129 I.U. vitamin A/100g and in another study, the contents were 1310 I.U. vitamin A/100g (Anonymus, 1973).

Proximate composition of processed cheese

Cheddar cheese is rarely consumed in its original form and is often processed by mixing ripened cheese with young cheese and further giving different processing treatments to obtain processed cheese. The study was further extended to see the nutritional value of processed cheese with special reference to its composition and protein quality, since such information was considered to be quite important.

Data obtained on the proximate composition of process cheese made from conventionally ripened (Type C) and accelerated ripened (Type D) cheese, are given in Table 7. It was seen that there was slight but significant difference in the composition of two types of cheese (Tables 1A, 1B and 7). Values for proximate principles were observed to be higher in the case of Type C cheese than Type D cheese. This could be attributed to the high moisture content of Type D cheese. When expressed on dry matter basis, the difference in protein content in the two types of cheese was markedly narrowed, making it non-significant. However, slightly, but significant

TABLE 7. Proximate composition* of conventionally ripened (Type C) and accelerated ripened (Type D) processed cheese.

Product	Batch No.	Moisture	Dry matter	Protein	Fat	Lactose	Total ash	Protein (on dry matter basis)	Fat (on dry matter basis)
Type C	I	40.22	59.77	26.80	29.0	0.30	2.56	44.82	48.51
	II	39.95	60.05	27.51	28.0	0.29	3.12	45.81	46.63
	III	41.66	58.34	25.90	28.0	0.32	3.00	44.39	47.99
	IV	42.30	57.30	26.61	27.0	0.26	2.80	46.11	46.79
	V	41.33	58.67	26.08	29.0	0.30	2.40	44.46	49.43
Mean ± S.E.		41.09 ± 0.44	58.90 ± 0.55	26.58 ± 0.29	28.2 ± 0.37	0.29 ±0.01	2.77 ±0.13	45.12 ± 0.35	47.87 ± 0.53
Type D	I	50.22	49.78	22.33	23.0	0.37	3.26	44.86	46.21
	II	53.78	46.02	21.43	20.0	0.40	3.00	46.56	43.46
	III	54.00	46.00	20.54	21.0	0.35	2.90	44.65	45.65
	IV	51.55	48.45	21.61	22.0	0.38	3.40	44.60	45.40
	V	53.77	46.23	20.54	21.0	0.40	3.10	44.43	45.43
Mean ± S.E.		52.66 ± 0.76	47.29 ± 0.77	21.29 ± 0.34	21.4 ± 0.51	0.38 ±0.01	3.13 ±0.09	45.03 ± 0.39	45.15 ± 0.43
C.D.		2.98	3.00	1.496	1.42	0.043	N.S.	1.99	1.92

* Expressed as g/100g, except stated, otherwise.

C.D. - Critical difference

N.S. - Non significant

difference persisted in case of fat content.

As regards calcium and phosphorus contents, it was evident from the data (Tables 2A and 8) that the calcium content in two types of cheese did not differ significantly while there was found to be a significant difference ($P < 0.05$) in the phosphorus content of Type C and Type D cheese.

Biological evaluation of protein quality

Data presented in the previous section showed that when expressed on dry matter basis, protein contents were identical in both types of cheese preparations. The quality of proteins, however, is not reflected by the quantity present in the food. The difference in the processes adopted during ripening could profoundly influence the quality of protein, particularly in respect of its digestibility or degradation and utilization of one or more essetial amino acids by starter cultures. In order to see if protein quality, in any manner, differed in these two preparations, biological evaluation of protein quality was made using young albino rats as the experimental model. This has been done by measuring (i) their growth promoting abilities, (ii) studying the nitrogen balance and (iii) determining nitrogen deposited in body carcass, when experimental animals were maintained

TABLE 8. Calcium and Phosphorus contents of conventionally ripened (Type C) and accelerated ripened (Type D) processed cheese.

Product	Batch No.	Calcium mg/100g	Phosphorus mg/100g
	I	770	344
	II	770	356
Type C	III	770	350
	IV	655	375
	V	539	350
Mean ± S.E.		701 ± 46.3	355 ± 5.36
	I	542	375
	II	799	394
Type D	III	770	350
	IV	683	381
	V	770	419
Mean ± S.E.		713 ± 47.05	384 ± 11.36

C.D.	=	214.20	29.31 at 1% level
C.D.	=	152.88	28.05 at 5% level

on diets containing these test protein sources. Data are presented in this section.

Protein efficiency ratio (PER)

Data on protein intake as well as gains in body weight in animals when maintained on test diets are present in Table 9. It was seen that protein consumption in groups maintained on test cheese diets was very close and did not differ significantly (Table 9A). However, under these conditions, gains in body weight were apparently higher for animals maintained on diet containing of conventionally ripened processed cheese (Table 9B). This was further reflected in significantly higher PER value ($P < 0.01$) for the group maintained on conventionally ripened processed cheese (Table 9C). Interestingly, values were markedly higher than those observed for control group receiving 10 percent casein in the diet.

Though, it would appear that the quality of protein from conventionally ripened processed cheese was better than that from accelerated ripened cheese, such influence could be marred in view of the limitations in this procedure. It was reported by Venkata Rao *et al.* (1964) and Hurt *et al.* (1975) that gains in body weight do not solely reflect the magnitude of

TABLE 9. Protein efficiency ratio (PER) for groups receiving experimental diets.

Diet	No. of animals in each group	Average initial weight (g)	Average final weight (g)	Average protein intake in 28 days (g)	Average gain in weight in 28 days (g)	Average PER
Type C	8	36.0	101.8	26.0	65.6	2.51 ±0.02
Type D	8	33.9	113.1	27.2	79.1	2.82 ±0.04
Casein	8	34.3	111.4	29.5	76.5	2.58 ±0.09
C.D.				1.38	4.18	0.107

Type C - Accelerated ripened processed cheese

Type D - Conventionally ripened processed cheese.

TABLE 9A. Analysis of variance of dietary intake of protein in PER experiment.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between groups	2	52.99	26.49	27.89**
Error	21	19.95	0.949	
Total	23	72.93		

** Significant at 1% level

TABLE 9B. Analysis of variance of gain in weight in PER experiment

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between groups	2	817	409	46.64**
Error	21	184	8.76	
Total	23	1000		

** Significant at 1% level

TABLE 9C. Analysis of variance of PER

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between three groups	2	0.4196	0.2098	35.64**
Error	21	0.1235	0.0058	
Total	23	0.5431		

** Significant at 1% level

nitrogen deposition in body carcass, since it also reflects changes in water and fat contents in the body carcass.

Further, it was also pointed out by these workers that protein efficiency ratio determination primarily accounts for the protein utilized for growth and does not take into account the protein required for maintenance of the body. In order to overcome this objection, a group of animals, identical to other three groups in sex, age and body weight was maintained on protein-free diet and losses in body weights were taken to compute net protein ratio (NPR). Data on the same are given in Table 10 to 10C. It was again evident that NPR for Type D cheese was significantly higher than that for Type C cheese ($P < 0.05$).

Nitrogen balance study

In order to overcome objections mentioned earlier, a study was performed on the nitrogen balance and data on the intake of dietary nitrogen, losses of nitrogen in faeces and urine and digestibility coefficient and biological values computed from the same, are presented in Tables 11 to 11F.

It was observed from the table that there was relatively lower faecal excretion of nitrogen in the

TABLE 10. Net protein ratio (NPR) for groups receiving experimental diets.

Diet	No.of animals in each group	Average initial weight (g)	Average wt.after 10 days (G)	Average gain in wt. in 10 days (g)	Average loss in weight on protein free diet (g)	Average protein intake (g)	Average NPR
Type C	8	36.03	55.63	19.6	7.0	7.86	3.40 ±0.06
Type D	8	33.93	55.19	21.26	7.0	7.67	3.69 ±0.08
Casein group	8	34.31	57.19	22.63	7.0	8.53	3.51 ±0.09
C.D.							0.223

Type C - Accelerated ripened processed cheese

Type D - Conventionally ripened processed cheese.

TABLE 10A. Analysis of variance for dietary protein intake in NPR experiment.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between groups	2	3.292	1.646	2.689[NS]
Error	21	12.85	0.612	
Total	23	16.15		

NS Non Significant

TABLE 10B. Analysis of variance for gain in body weight in NPR experiment.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between groups	2	37.65	18.82	2.17[NS]
Error	21	181.6	8.647	
Total	23	219.2		

NS Non Significant

TABLE 10C. Analysis of variance for NPR.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between three groups	2	0.3507	0.1753	3.80*
Error	21	0.9677	0.0460	
Total	23	1.319		

* Significant at 5% level

group receiving diet based on Type D cheese than the quantity excreted by animals maintained on Type C cheese based diet. Such significant difference appeared to have significance in the light of higher nitrogen intake in the former group than the later group and further reflected in significantly higher digestibility coefficient for type D cheese. Further, calculation of data showed slightly higher values of biological value in case of Type D cheese than for Type C. The differences were found to be statistically non-significant.

The values for biological value in the present study compared well with those reported by Steen and Eggum (1968)for conventionally ripened cheddar cheese. However, values reported by Henry and Kon (1946) and Sehgal (1982), were higher as compared to the values obtained in the present study. Such differences in biological value in different studies could be partly explained on the basis of several factors such as initial weight, extent of protein depletion occured during protein-free dietary regime, level of test protein in the diet and the extent of limitation of essential amino acids in test protein which are reported to affect the biological value (Henry and Kon, 1957; and Bodwell, 1976).

TABLE 11. Digestibility coefficient for groups of rats receiving different experimental diet.

Diet based on	No. of animal	Average total N intake (mg)	Average $F_n - F_e$ (mg)	D.C. %
Type C	8	901.95	132.39	85.35* ± 0.41
Type D	8	981.31	100.52	89.76* ± 0.224
C.D.		52.26		1.401

* Mean ± S.E.

Type C - Accelerated ripened processed cheese

Type D - Conventionally ripened processed cheese.

TABLE 11A. Analysis of variance for digestibility coefficient.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between two types of cheese	1	77.70	77.70	88.71**
Error	14	12.26	0.875	
Total	15	89.96		

** Significant at 1% level

TABLE 11B. Analysis of variance for nitrogen intake (I_n) in B.V. experiment.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between three groups	1	25196	25196	10.61**
Error	14	33244	2375	
Total	15	58440		

** Significant at 1% level

TABLE 11C. Analysis of variance for faecal nitrogen ($F_n - F_e$) in B.V. experiment

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between three groups	1	4064	4064	22.37**
Error	14	2543	181.6	
Total	15	6607		

** Significant at 1% level

TABLE 11D. Biological value for groups of rats receiving different experimental diets.

Diet based on	No. of animals	Average N intake (mg)	Average $F_n - F_e$ (mg)	Average $U_n - U_e$ (mg)	B.V. %
Type C	8	901.95	132.39	268.27	65.23[x] ± 1.05
Type D	8	981.32	100.52	296.08	66.38[x] ± 0.72

[x] Mean ± S.E.

Type C - Accelerated ripened processed cheese

Type D - Conventionally ripened processed cheese.

TABLE 11E. Analysis of variance for biological value

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between two types of cheese	1	5.24	5.24	0.806[NS]
Error	14	91.01	6.50	
Total	15	96.25		

[NS] Non Significant

TABLE 11 F. Analysis of variance for urinary nitrogen ($U_n - U_e$) in B.V. experiment

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between three groups	1	3094	3094	3.65[NS]
Error	14	11867	847.7	
Total	15	14962		

[NS] Non Significant

<u>Modified protein efficiency ratio (PER_D)</u>

From the data on changes in body weight obtained during nitrogen balance studies it was observed that when animals were maintained on protein free diet they lost about 12.5 percent of their body weight. According to Venketa Rao <u>et al.</u> (1964) such sensitized animals respond to differences in protein quality better than normal growing animals. When these animals were given diets based on either Type C or Type D cheese and modified protein efficiency ratio (PER_D) calculated, it was observed that PER_D was significantly higher for group receiving Type D cheese than the one receiving Type C. The trend thus noticed, supported the observation made in respect of other parameters, mentioned earlier.

Though estimation of nitrogen balance had a definite advantage over method based on gains in body weight, still it is hypothesized that the balance nitrogen between the intake and excretion, is deposited in the body as carcass nitrogen. In order to confirm this, determination of carcass nitrogen contents, in animals maintained on test protein diets, were made and the net protein utilization value was calculated (Table 12). It was observed that despite identical protein intakes in the diet, carcass nitrogen in the

TABLE 11G. Protein efficiency ratio (PER_D) for groups of rats receiving different experimental diet.

Diet based on	No. of animals	Average initial body weight (g)	Average body weight after 10 days on protein free diet (g)	Average body wt. after 20 days (g)	PER_D
Cheese Type C	8	43.56	35.31	55.87	2.90x ±0.07
Cheese Type D	8	42.43	32.56	57.62	3.24x ±0.07

x Mean ± S.E.

Type C – Accelerated ripened processed cheese

Type D – Conventionally ripened processed cheese.

TABLE 11H. Analysis of variance for PER_D

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between two types of cheese	1	0.469	0.469	11.45**
Error	14	0.573	0.041	
Total	15	1.042		

** Significant at 1% level

case of group maintained on Type D cheese was higher than that in the case of animals receiving Type C cheese diet. Calculation of net protein utilization value as described by Miller and Bender (1955) showed that Type D cheese had significantly higher N.P.U. ($P < 0.01$) than Type C cheese (Table 2A). The value obtained in the present study compared well with net protein utilization values reported by Deodhar and Kiran (1981) and Sehgal (1982). In an another study on different varieties of hard and semihard cheese Poznanski and Siudak (1971), reported NPU values to range between 53 and 65. It was observed that processing treatment does not influence protein quality (Deardon et al., 1945).

From the experiments conducted on biological evaluation of protein quality of cheese preparations, it was observed that processed cheese made from conventionally ripened cheese has a distinct superiority over the one made from the accelerated ripened process. This was clear from better protein efficiency ratio, digestibility coefficient as well net protein utilization values for these two preparations. Despite such superiority, the overall protein quality for processed cheese made from accelerated ripened cheese can not be considered any way low, since values of 2.48 for PER, 65.23 for biological value, 85.35

TABLE 12. Net protein utilization for groups of rats receiving different experimental diets.

Diet based on	No. of animals	Average N intake (mg)	Average N retained (mg)	N.P.U. %
Cheese Type C	8	1328	1075	56.19[x] ± 0.384
Cheese Type D	8	1342	1158	61.45[x] ± 1.266
C.D.			64.18	3.96

[x] Mean ± S.E.

Type C - Processed cheese prepared by conventional method

Type D - Processed cheese prepared by accelerated method

TABLE 12A. Analysis of variance for net protein utilization.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between two types of cheese	1	110.9	110.9	15.81**
Error	14	98.21	7.02	
Total	15	209.1		

** Significant at 1% level

TABLE 12B. Analysis of variance for nitrogen intake in N.P.U. experiment.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between two types of cheese	1	752.28	752.28	0.161 NS
Error	14	65187	4656	
Total	15	65939.28		

NS Non Significant

TABLE 12C. Analysis of variance for nitrogen retention in N.P.U. experiment.

Source of variation	d.f.	S.S.	M.S.S.	'F'
Between two types of cheese	1	27541	27541	7.68*
Error	14	50161	3582	
Total	15	77702		

* Significant at 5% level

percent for digestibility coefficient, 56.188 for net protein utilization were within thenormal range reported by different workers for variety conventionally ripened cheese.

CHAPTER - V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSION

The study was conducted to evaluate the nutrional quality of accelerated ripened cheddar cheese.

The accelerated ripened cheddar cheese (Type B) was prepared by blending two parts of shredded curd with one part of 5.2 percent sterile sodium chloride solution at 45°C and ripening at 30°C for the period of 8 days.

Samples were collected at different intervals namely 0 day, 4th day and 8th day. A comparison was made with cheese which was ripened by conventional manner (Type A).

A comparison of proximate principles showed that the Type B cheese had distinctly higher moisture content which further influence the levels of different constituents. However, when data were expressed on dry matter basis, fat and protein contents were found to be identical. The contents of lactose was higher in Type B cheese. Experiments were also conducted to find changes in the levels of free fatty acid, folic acid, vitamin B_2 and vitamin A.

It was observed that free fatty acid increased progressively as the period of ripening increase. However, the contents in 8 days accelerated ripened cheese were significantly lower than that observed in conventionally ripened cheese.

As regards vitamin contents, levels of folic acid increased markedly and progressively as the duration of ripening increased. The contents in 8 days ripened cheese was found to be significantly higher than present in Type A cheese. As regards vitamin B_2 and vitamin A, when data were expressed on dry matter basis, there were no changes in their contents as ripening progress, likewise values obtained for accelerated ripened and conventionally ripened were also similar.

In the light of its utility, processed cheese samples well made from both types of cheese. Proximate composition as well as protein quality of these preparations were studied.

Again when data were expressed on moisture free basis, appearent differences in composition when data were expressed otherwise on fresh weight basis, were found to be non-significant. As regards protein quality, the biological evaluation conducted using albino rats showed that the processed cheese made from Type A cheese had superior protein quality than the one made from Type B cheese. This was evident from significantly higher values of PER, NPR, DC, PER_D and NPU. However, the difference in respect of biological value was not statistically significant.

CHAPTER - VI

BIBLIOGRAPHY

BIBLIOGRAPHY

Abd-El-Salam, M.H., El-Shibiny, S., El-Bagoury, E., Ayad, E. and Fahmy, N.	1978	J. Dairy Res., 45, 491.
Abd-El-Salam, M.H., Mohamed, A.A., Ayad, E., Fahmy, N. and El-Shibiny, S.	1979	Egypt. J. Dairy Sci., 7, 63.
Acton, G.H.	1977	Australian J. Dairy Technol., 32, 111.
Ahovocava, J.K. and Odavic, R.	1969	J. Chromat., 40, 90
Alexeev, A.	1966	XVII Inter. Dairy Congr. D_2, 451.
Allen, L.A. and Knowles, N.R.	1933-34	J. Dairy Res., 5, 185.
Anagama, Y. and Kauzuya, Y.	1968	Res. Bull. Fac. Agri. Gifu. Univ., 26, 176. Cited: Dairy Sci. Abstr., 31, 4000.
Anonymus	1973	Cultured Dairy Prod. J., 8, 16.
Anonymus	1977	Dairy and Ice Cream Fld. March, 66K.
AOAC	1980	Methods of Analysis. Association of Official Analytical Chemists of America, Washington.
Arsenova, L.V. and Chebotarev, A.I.	1972	Trudy Volog. Mol. Inst., 64 , 65. Cited: Dairy Sci. Abstr., 35, 1969 (1973).
Beadles, Quisenbery, Nakamura, Mitchell	1933	J. Agric. Res., 47 , 947. Cited by Poznanski, S. and Siudak, F. Indian J. Nutr. Dietet., 8, 189. (1971).

Bender, A.E. and Doell, B.H. 1957 Br. J. Nutr., 11, 140.

Benraadt, R.D.R., Borges, S.F., Bussani, R., Festa, E.N. and Mukuno, J.L. 1978 V Inter. Congr. of Food Sci. & Tech.(1978) 134. Cited: Dairy Sci. Abstr., 41, 2836 (1979).

Berger - Gruner, M. 1966 Milchwissenschaft, 21, 222. Cited by Gregory,M.E. J. Dairy Res., 34, 169 (1967).

Bodwell 1976 Evaluation of Proteins for Humans. AVI. Westport.

BSI 1969 British Standard Institution, England BS: 696 part 2.

Carr and Price 1926 Biochem. J., 20, 497.

Cremer, L.K. 1974 Inter. Dairy Congr.,1E,265.

Dahlberg, A.C. and Kosikowsky, F.V. 1947 J. Dairy Sci., 30, 165.

Davide, C.L. and Foley, J. 1979 Laguna, Philippine, Philippine Society of Animal Sci., p 21. Cited: Dairy Sci. Abstr., 43, 4682 (1981).

Davies, A.W. and Moore, T. 1939 Ztschr Vitamin forsh., 9, 254. Cited: Nutr. Abstr. & Rev., 3, 2786 (1939-40).

Davies, D.T. and White, J.C.D. 1962 J. Dairy Res., 29, 285.

Davis, J.G. 1976 Cheese, Vol. III. Manufacturing Methods Published by Chorchill Livingstone, Edinburgh London and New York.

Dearden, D.V., Henry, K.M., Houston, J., Kon, S.K. and Thompson, S.Y. 1945 J. Dairy Res., 14, 100.

Demon, J.C. and Galesloot, Th.E.	1962	Neth. Milk Dairy Dairy J., <u>16</u>, 19.
Deodhar, A.D. and Kiran, D.	1981	J. Food Sci., <u>46</u>, 925.
Dilanyan, Z. Kh. and Sarkisyan, R.A.	1970	Prom. Armenii, <u>10</u>, 104. Cited: Dairy Sci. Abstr., <u>36</u>, 2701(1974).
Dilanyan, Z.Kh., Saakyan, R.V., Sarkisyan, R.A., Sagoyan, A.S., Chuprina, D.F. and Amirkhanyan, R.A.	1975	Biologicheskii.Z. Armenii, <u>27</u> , 70. Cited : Dairy Sci. Abstr., <u>37</u>, 5000.
Doeglas, H.M.G. Huisman, J. and Nater, J. P.	1967	Ned. Tijdschr. Geneesk., <u>111</u>, 1526. Cited: Dairy Sci. Abstr., <u>30</u>, 602 (1968).
Dulley, J.R.	1976	Aust. J. Dairy Tech., <u>31</u>, 143.
Dulley, J.R., Brooks, D.E.J., and Grieve, P.A.	1978	XX Inter. Dairy Congr. <u>E</u> , 485.
Eckhof, Stork	1976	World Atlas of Cheese. Ed. by Adrian Balley, Paddington Press Ltd. U.S.A.
Eggum, B.O. and Jacobsen, I.	1976	J. Sci. Fd. Agric., <u>27</u>, 1190.
Eitenmiller, R.R. and Koehler, P.E.	1974	IV Inter. Congr. of Food Sci. and Tech., <u>9a</u>,210. Cited: Dairy Sci. Abstr., <u>37</u>, 3703(1975)
Emmons, D.B., McGugan, W.A. and Elliott, J.A.	1960	J. Dairy Sci., <u>43</u>, 862.
Evans, A.C.	1918	J. Agri. Res., <u>13</u>, 237.
Evans, A.C., Hastings, E.G. and Hart, E.B.	1914	J. Agri. Res.,<u>2</u>, 167.

Exterkate, F.A.	1979	J. Dairy Res., 46, 473.
F.A.O.	1978	FAO Trade Yearbook. vol. 32, Table 31, Rome. Italy. 1978.
Frater, S.G., Douglas, G. and Buddle, B.	1977	Aust. J. Dairy Tech., 32, 79.
Freed, M.	1966	Methods of Vitamin Assay. Verleg Chemic. Weinheim.
Govaryutkina, S.A., Babushkina, V.A. and Ostroumov, L.A.	1975	In Inteus. Proiz. i. Uluchs. Kach. Natural. Syrov. Brnaw. U.S.SSR. Altaiskii TSNTI (1954). Cited: Dairy Sci. Abstr., 38, 8123 (1976).
Gregory, H.S.	1975	J. Dairy Res., 42, 197.
Guerrant, N.B.	1938	J. Dairy Sci., 21, 69.
Harper, W.J. and Swanson, A.M.	1949	XII Inter. Dairy Congr., 2, 147. Cited: Dairy Sci. Abstr., 12, 81 (1950-51).
Hartman and Dryden	1965	Vitamins in Milk and Milk Products. American Dairy Sci. Assoc. U.S.A.
Henery, K.M. and Kon, S.K.	1946	J. Dairy Res., 14, 330.
Henry, K.M. and Kon, S.K.	1957	Br. J. Nutr., 11, 305.
Hietarata, M. and Antila, M.	1953	Proc. 13th Inter. Dairy Congr., 3, 1456. Cited by Hrtman and Dryden Vitamins in Milk and Milk Products. American Dairy Sci. Assoc. U.S.A.
Higuchi, K. and Peterson, W.H.	1946	J. Dairy Sci., 29, 189.
Hofi, A.A., Mahran, G.A., Abd-El-Salam, M.H. and Rifaat, I.D.	1973	Egypt. J. Dairy Sci., 1, 45.

Hofi, A.A., Mahran, G.A., Farohat, S.M., Ashour, M. and Khorshid, M.A.	1973	Egypt. J. Dairy Sci., 1, 159.
Hofi, A.A., Shehata, A.E., Magdoub, M.N.I, and Fayed, E.D.	1979	In Abstracts of the XII Inter. Congr. of Microbiology. Cited : Dairy Sci. Abstr. 42, 5901 (1980).
Hunter, G.J.E.	1950	J. Dairy Res., 17, 79.
Hurt, H.D., Forsythe, R.H. and Krieger, C.H.	1975	Cited in Protein Nutritional Quality of Foods and Feeds PartIMarcel Dekkes, Inc. New York.
Irvine, O.R., Bryant, L.R., Sprouce, W.H., Jackson, S.H., Crobk, A and Johnstone, W.M.	1945	Sci. Agr., 25 , 817. Cited by Hartman and Dryden. Vitamins in Milk and Milk Products. American Dairy Sci. Assoc. U.S.A. (1965).
I.S.I.	1967	Indian Standard Institute, Delhi. IS: 1664.
Ismail, A.S. and Ahmad, N.S.	1978	Egypt. J. Dairy Sci., 3, 127.
Jacquet, J. and Lenoir, J.	1954	Cited by Vanslyke, L.L. and Price, W.V. Cheese Orange Judd.Publishing Co. Ind. New York. (1952).
Jensen, J.D., Reinbald, G.W., Washam, C.J. and Vedamuthu, E.R.	1975	J. Miilk Food Tech., 38, 78.
Kalmikova, L. and Prodanski, P.	1976	Khran, Prom. 25, 24. Cited: Dairy Sci. Abstr., 39, 718 (1977).
Kalinowski, L., Frackiewicz, E., Janiszewska, L., Pawlik, A., Kikolska, D., Pisaperk, J., Szadkowska, M. and Suitacz-Tomaszewska, J.	1979	United States Patent, 4, 158. Cited: Dairy Sci. Abstr., 42, 2893.

Kelly, C.D.	1932	N.Y.(Geneva) Agr.Expt. Sta. Tech. Bull., 200. Cited by Van Slyke, L.L. and Price, W.V. 'Cheese' Ed. 2nd Orange Judd. Publishing Co., Inc. New York (1952).
Klimovskii, I.	1954	Mol. Prom., 15, 22. Cited: Dairy Sci. Abstr., 16, 627.
Kon, S.K.	1972	Milk and Milk Products in Human Nutrition, F.A.O., Rome.
Kosikowsky, F.V.	1951	J. Dairy Sci., 34, 228.
Kosikowsky, F. V.	1966	Cheese and Fermented Milk Foods, Published by the author.
Kosikowski, F.V. and Iwasaki, T.	1975	J. Dairy Sci., 58, 963.
Kristoffersen, T.	1967	J. Dairy Sci., 50, 279.
Kristoffersen, T. and Falls, W.T.	1965	J. Dairy Sci., 48, 785.
Kristoffersen, T., Mikolajcik, E.M. and Gould, I.A.	1967	J. Dairy Sci., 50, 292.
Labuschagne, J.H. and Nieuwoudt, J.A.	1978	South African Journal of Dairy Technology, 10, 77. Cited: Dairy Sci. Abstr., 41, 72(1979).
Law, B.A. and Wigmore, A.	1982	J. Dairy Res., 49, 137.
Law, B.A., Castanon, M.J. and Sharpe, M.E.	1976	J. Dairy Res., 43, 301.
Lee, J.Y.	1974	Korean J. Animal Sci., 16, 393. Cited: Dairy Sci. Abstr., 39, 70 (1977).

Lowrie, R.J., Lawrence, R.C. and Peberdy, M.F.	1974	N.Z.J. Dairy Sci. and Tech., 9, 116.
M.A.F.F.	1959	Cheese Making Bull. 43. Her Mejesty's Stationery Office, London.
Magdoub, M.N.I., Shehata, A.E., Fayed, E.O. and Hofi, A.A.	1980	XII Intr. Congr. of Microbiology, 174. Cited: Dairy Sci. Abstr., 42, 690.(1980).
Magee, Jr. E.L., Olson, N.F. and Lindsay, R.C.	1979	Uni. of Wasconsin Madison. Cited: J. Dairy Sci. (Suppl I) 62: 65.
Malkki, Y. and Mattson, R.	1981	Food Process Engg. (1979). Cited: Dairy Sci. Abstr., 43, 563 (1981).
Marschke, R.J. and Dulley, J.R.	1978	Aust. J. Dairy Tech., 33, 139.
Marschke, R.J., Nickerson, D.E.J., Jarrett, W.D. and Dulley, J.R.	1980	Aust. J. Dairy Tech., 35, 84.
Menshikov, N.	1966	Inter. Dairy Congr., D_2, 565.
Miller, D.S. and Bender, A.E.	1955	Br. J. Nutr., 9, 382.
Mitchell, H.H.	1948	Biological Utilisation of Proteins in Sahyum, Proteins andAmino Acid in Nutr. New York. Cited by Poznanski, S. and Siudak, F. Indian J. Nutr. and Dietet., 8, 189 (1971).
Mitchell, H.H.	1924	J. Biol. Chem. 58, 387.
Nakanishi, T. and Itoh, M.	1974	XIX Inter. Dairy Congr. 1E, 343. Cited: Dairy Sci. Abstr., 37, 3709 (1975).
Nelson, J.H.	1975	J. Dairy Sci., 58, 1739 .

Nilson, K.M., Vakil, T.R. and Shahani, K.M.	1965	J. Nutr., <u>86</u>, 362.
Nofal, A.A. and Abou-Dawood, A.E.	1975	Res. Bull. Faculty of Agric. Tauta Uni.Egypt. <u>7</u>, 10. Cited: Dairy Sci. Abstr., <u>38</u>, 3146 (1976)
Nieuwoudt, J.A.	1977	South African Journal of Dairy Technology, <u>9</u>, 143. Cited : Dairy Sci. Abstr., <u>40</u>, 6163 (1978).
Ohren, J.A. and Tuchley, S.L.	1965	J. Dairy Sci., <u>48</u>, 765.
Olson, N.F.	1981	J. Dairy Sci., <u>64</u>, 1063.
Polansky, M.M. and Toepfer, E.W.	1969	Journal of Agricultural and Food Chemistry, <u>17</u>, 1394.
Perry, K.D.	1961	J. Dairy Res., <u>28</u>, 221.
Peters, I.I.	1960	Milk Prod. J., <u>51</u>, 8.
Peterson, M. and Johnson, M.J.	1949	J. Dairy Sci., <u>32</u>, 862.
Pettersson, H.E. and Sjostrom, G.	1975	J. Dairy Res., <u>42</u>, 313.
Poznanski, S. and Siudak, F.	1971	Indian J. Nutr. Dietet., <u>8</u>, 189.
Randoin, L. and Causeret, J.	1953	Proc. 13th Inter. Dairy Congr. <u>3</u>, 1372. Cited by Hartman and Dryden. Vitamins in Milk and Milk Products. American Dairy Sci. Assoc. U.S.A.
Rao, B.P., Rao, S.D.T. and Reddy, B.R.	1972	J. of A.O.C.S. <u>49</u>, 338.
Rasic, J. and Panic, B.	1961	Arhiv Polzoprivredene Nautre i Techniku, 14, 94. Cited by Hartman and Dryden. Vitamins in Milk and Milk Products. American Dairy Sci. Assoc. U.S.A.

Rieter, B., Fryer, T.R., Pickering, A., Chapman, H.R., Lawrence, R.C. and Sharpe, M.E.	1967	J. Dairy Res., 34, 257.
Robinson, C.H.	1977	Normal and Therapeutic Nutrition, Ed. 14, Oxford & IBH Publishing Co, New Delhi.
Rose and Gottlieb	1959	Lab. Manual of Milk Products, 624.
Sammis, J.L.	1923	N.Y.Prod. Rev. and Ani. Cry., 57, 147. Cited by Van Slyke and Price. 'Cheese'. 2nd. Edition.
Sammis, J.L. and Germain, Leo.	1929	Butter and Cheese J., 20, 18.
Sehgal, A.	1982	M.Sc.Thesis Kurukshetra Univ., Kurukshetra.
Sellars, R.L.	1981	J. Dairy Sci., 64 , 1070.
Sen, N.P.	1969	J. Fd. Sci., 34, 22.
Shahani, K.M., Hathaway, I.L. and Kelly, P.L.	1962	J. Dairy Sci., 45, 833.
Shaligina, O.M., Surovaseva, V.A. and Yaremenko, M.F.	1974	Kharchova Promislovist Naukovo-virobnichnii Zbirnik p.34. Cited: Dairy Sci. Abstr., 37, 375 (1975).
Shanley, R.M. and Sutherland, B.J.	1975	CSIRO Dairy Res. Rep. No.13 Cited by Sutherland B.J. Aust. Jr. Dairy Tech. 30, 138. (1975).
Sherwood, I.R. and Whitehead, H.R.	1934	J. Dairy Res., 5, 208.
Sheuring, J.J. and Tuckey, S.L.	1947	J. Dairy Sci., 30, 803.
Singh, S.	1968	M.S.Thesis Ohio State Uni.

Singh, R.V. and Kalra, K.K.	1979	Costing of Dairy Products. A Research Report, Dairy Economics Statistics and Management Division, N.D.R.I., Karnal.
Singh, V.K. and Kristoffersch, T.	1969	J. Dairy Sci., 52, 899.
Singh, S. and Kristoffersen, T.	1970	J. Dairy Sci., 53, 533.
Snedecor and Cochron	(1967	Statistical Methods Oxford. London.
Snell, E.E. and Strong, F.N.	1939	Microbiological Assay of Vitamin B Complex Pitman and Sons, Ltd.
Sood, V.K. and Kosikowski, F.V.	1979	J. Dairy Sci., 62, 1865.
Steen, K. and Eggum, B.	1966	Maelk., 81, 479. Cited from Dairy Sci. Abstr., 30, 3660 (1968).
Sullivan, R.A. and Infantino, D.	1975	United States Patent, 3, 859. Cited: Dairy Sci. Abstr., 38, 1429 (1976).
Sutherland, B.J.	1975	Aust. J. Dairy Tech., 30, 138.
Thompson, M.P. and Brower, D.P.	1976	Cult. Dairy Products J., 11, 22.
Todhunter, E.W.	1942	J. Dairy Sci., 25, 1023.
Thompson, M.P., Somkut, G.A. Flanagan, J.F., Benuvengo, M., Brower, D.P. and Steinberg, D.H.	1979	J. Dairy Sci., 62 (Suppl.), 68.
University of Reading	1941	Rep. for 1941, 42 and '45, National Institute for Res. in Dairying.

Van Slyke, L.L. and Price, W.V.	1952	Cheese. Orange Judd Publishing Co. Inc. New York.
Van Slyke, L.L. and Hart, E.B.	1903	N.Y. (Geneva) Agri. Expt. Sta. Bull. 215. Cited by Van Slyke L.L. and Price, W.V. 'Cheese' Orange Judd. Publishing Co. Inc. New York (1952).
Venkat Rao, S., Narayen Rao, N., Swaminathan, N. and Subramanyan, V.	1964	Ind.J.Nutr. Dietet., 1, 42.
Voigt, M.N., Eitenmiller, R.R. Kochler, P.E. and Hamdy, M.K.	1974	J. Milk & Food Tech., 37, 377. Cited: Dairy Sci. Abstr., 37, 927 (1975).
Von Bocklemann, I. and Loidin, L.O.	1974	XIX Inter. Dairy Congr. 1E, 441.
Weaver, J.C.	1977	Diss. Abstr. Inter. B. 38, 2109.
Wong, N.P., Lacroix, D.E., Vestal, J.H. and Alford, J.A.	1977	J. Dairy Sci., 60, 1522.
Zerfiridis, G. and Kristofersen, T.	1970	XVIII Inter. Dairy Congr., IE, 351. Cited: Dairy Sci. Abstr. 32, 4614.
Zielinska, M. and Hiscox, E.R.	1954	J. Dairy Res., 21, 238.