Studies on the Quality of *Peda* and Evaluation of its Physico-Chemical Changes During Storage

A dissertation
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in partial fulfilment of the requirement for the Degree of
Doctor of Philosophy

in

Dairying (Dairy Chemistry)

By

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IN

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This is to certify that the work recorded in the thesis entitled "**Studies on the quality of *peda* and evaluation of its physico-chemical changes during storage**" submitted by **Miss Sumita Das** in partial fulfilment of requirements for the **Degree of Doctor of Philosophy** in **Dairying (Dairy Chemistry)** in the West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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Abbreviations

@	at the rate of
°C	degree centigrade
gm	gram
h	hour
IU	International Unit
kg	kilogram
KV	Kilovolt
M	Molar
Max.	maximum
Min.	minimum
min	minute
mA	millampere
mg	milligram
ml	millilitre
mm	millimeter
mR	microrad
mV	millivolt
μ	micron
μl	microlitre
μm	micrometer
m. eq. of O_2	milli equivalent of oxygen
N	Normality
nm	nanometer
%	Percentage
ppm	parts per million
rpm	revolutions per minute
sec.	Second
v	Volume
w	Weight

Chapter - I

Introduction

Introduction

India has emerged as the largest milk producer in the world with a record annual milk production of 84.5 million tonnes during the period of 2001 – 2002 (Balaraman, 2003) and is predicted to reach the level of 90 million tonnes in 2003 – 2004 (Indian Dairyman, March, 2004). Out of the 53.5% of milk produced, which is converted into various products, the share of indigenous dairy products comes to about 37% (Anon, 1995). Available data indicates that on an average about 6.5% of milk produced in India is utilized for *khoa* production (Alam, 1999). Lack of cooling facilities to keep liquid milk fresh in warm climate lead to the diversion of milk for the preparation of different indigenous milk products with comparatively longer shelf-life. Production and marketing of most of the indigenous dairy products still lies within the hands of local sweetmeat makers or *halwais* who seldom take care of the hygienic standards. This leads to the spoilage of the products as well as public health hazards. Since indigenous dairy products represent higher value added items for milk producers, more systematic approach to process innovation, quality assurance and shelf-life improvement for these products is needed to promote the interest of both the producers and the consumers.

Traditional milk products which are converted from milk in order to preserve the milk solids in a concentrated form for a longer period, play a significant role in the economic, social, religious and nutritional functions of the Indian masses from the time immemorial. *Peda* is a popular *khoa* based indigenous dairy product. The quantity of *peda* produced in India exceeds any other indigenous milk based sweets using *khoa* as raw material (Mahadevan, 1991). The cost of raw materials viz. milk and sugar accounts for only one-third of the selling price of the final product. The manufacture of *peda* is mostly confined

to *halwais* and no uniform manufacturing process is followed, consequently wide variations are observed in the composition of *Peda* (Rajorhia and Srinivasan, 1979). *Peda* is expected to have good shelf-life due to low moisture content and high percentage of sugar. However, it is also susceptible to microbial spoilage due to unhygienic conditions adopted during manufacture and subsequent handling.

1.1 What is *Peda* ?

Peda is a popular indigenous heat desiccated milk product, the base material of which is *khoa*. It is obtained by the dehydration of fresh whole milk of cow, buffalo, goat or their admixture, with addition of cane sugar in it, in an open shallow pan over a low fire with continuous stirring-cum-scraping action with a khunti until it assumes a consistency capable of forming a hard flat surface on spreading. The hard mass is collected in a tray and after setting, it is cut and moulded into desired shapes. It is granular and harder in texture.

No chemical and bacteriological standards have yet been laid down for judging the quality of *peda*. But *khoa* which is used as the base material for its preparation, should follow the following standards (IS : 4883 – 1980).

Total solid (% by mass)	- 60 (min.)
Fat (% by mass, on dry basis)	- 37 (min.)
Total ash (% by mass, on dry basis)	- 6.0 (max.)
Total acidity (% by mass, in terms of lactic acid)	- 0.9 (max.)
Coliform (per gm)	- 90 (max.)
Yeast and mold (per gm)	- 50 (max.)

1.2 Regional Complexion and Varieties

Peda, which is mostly consumed as religious 'Prasad' in temples as well as a mark of jubilation, is extremely popular in central and northern parts of India and to a lesser extent, in the western and eastern regions also. *Peda*s are distributed at ceremonial functions in our society (Patel, 1986).

Numerous varieties of *peda* are available in different parts of our country and the methods of manufacture vary from region to region. Patel (1986) described about different varieties of *peda*. In U.P., Mathura *peda* are small, round in shape and have burnt chocolate flavour and longer keeping quality. Gajarola *peda*s are chocolate coloured and ghee residue is used for preparing the same. In Gujarat and western part of the country, *peda*s are white in colour and made from buffalo milk.

1.3 Nutritional Importance

Due to about five-fold concentration of milk solids, the food and nutritive value of *peda* is considerably high. Fairly large quantities of muscle binding proteins, bone forming minerals and energy giving fat and lactose contribute a lot to its nutritive value. *Peda* also retains most of the fat soluble vitamins like vitamin A and D and some of the water soluble B – vitamins contained in the initial milk.

1.4 Justification of the Present Investigation

Peda available in the market is usually of poor chemical and bacteriological quality due to the high trend of adulteration and the unhygienic processing methods and subsequent handling. For *peda* no legal standards have yet been fixed under the Bureau of Indian Standards (BIS) and Prevention of Food Adulteration (PFA) Act. There is a great need for collecting data on the market quality of *peda* for their quality. Therefore, the present investigation is undertaken to

survey the trade practices of *peda* making and to compare the market and laboratory made samples under controlled conditions, which will provide guideline to BIS and PFA for formulation of legal standards for *peda*. During the present investigation attempt has been made to increase the shelf-life of *peda* by using preservatives.

Chapter - II

Review of Literature

Review of Literature

The interest of the scientists has been shifted towards Indian dairy products in the recent past, because of their complex physico-chemical characteristics and growing international acceptance. The dairy industry is currently going through a phase of consolidation and making the entire business more sustainable. Traditional *khoa* based sweets, particularly *peda* is extremely popular among the milk products and the dairy industry should focus on it because of its tremendous market potential. Though published works on the technology of *peda* is extremely limited, excellent review articles are available on *khoa* manufacture. Rajorhia and Srinivasan (1979), Sangwan and Sharma (1984) and Moulik and Ghatak (1997) reviewed the literature to elucidate the work on *khoa* manufacture. In this present review an endeavour has been made to embrace a wide range of work on *khoa* with a detailed discussion on manufacture, composition, packaging, storage, shelf-life, texture, microstructure and nutritive value.

2.1 Type and Composition of Milk

Khoa has been manufactured from different sources of milk. *Khoa* was prepared from cow milk (Prajapati *et al.*, 1986; Boghra and Mathur, 1990; Sahai *et al.*, 1992; Goyal, 1992; Adhikari *et al.*, 1993; Ray *et al.*, 1999), from buffalo milk (Patel *et al.*, 1985; Goyal, 1992; Patil *et al.*, 1992; Ranganadham and Rajorhia, 1993; Kumar and Pal, 1994; Ray *et al.*, 2000) and from the admixture of cow and buffalo milk in the ratio 30 : 50 (Narain and Singh, 1981; Magadum *et al.*, 1988; Adhikari *et al.*, 1993).

Different workers has standardized the milk to different level of fat and SNF for the manufacture of *khoa*. Standardization of buffalo

milk to 5% fat was suggested by De and Ray (1952) to produce *khoa* of a satisfactory quality. Ranganadham and Rajorhia (1989) standardized milk to 4.0% fat. Milk had been standardized to 4.5% fat and 8.5% SNF for cow milk and 6.0% fat and 9.0% SNF for buffalo milk (Goyal and Srinivasan, 1989). According to Sapre and Deodhar (1991) milk had been standardized to 5.0% fat for the manufacture of *khoa*. Ranganadham and Rajorhia, 1993 standardized cow milk to 3.0, 4.0 and 5.0% fat and 8.5% SNF and buffalo milk to 4.0, 5.0 and 6.0% fat and 9.0% SNF for the manufacture of *khoa*. Milk containing 6.0% fat was used for *khoa* making by Kumar and Pal, 1994. The also recommended the use of concentrated buffalo milk by reverse osmosis technique. However, preconcentrated milk (Boghra and Rajorhia, 1982), dried milk (Boghra and Rajorhia, 1984) and lactose hydrolyzed buffalo milk (Prakash and Sharma, 1984) could be preferably used for the manufacture of *khoa*. Good quality *khoa* can be prepared from cow milk with the incorporation of whey protein concentrate (5%) and keeping the total solids of the product low (Patel *et al.*, 1993).

2.2 Technology of *Peda*

Traditionally *khoa* is used as the base material for the preparation of *peda* and the amount of sugar to be used also vary according to consumers choice and trade practice. But according to Patel (1986), *khoa* mixing method is found unsuitable for *peda* making in bulk manufacturing as *khoa* consistency and texture vary from batch to batch and mixing of sugar also causes problems.

Traditional methods of small scale manufacture had been employed in earlier research studies of *peda* (Patel and Gandhi, 1980). In 1986, Patel described a method for large scale production of *peda*, which was adopted by Rajkot Dairy. About 5 litres of milk was taken in one batch and heated in a karahi on a crude oil combustion furnace. When the milk started to boil, about 450 gm of sugar was

added and the stirring – cum – scrapping was continued until a pasty consistency was obtained. The paste was spread out on the walls of the karahi for cooling. Then the product was removed to a tray and moulded into desired shapes. The final product contained about 13 to 13.5% moisture.

In 1980, Patel and Gandhi reported that similar approach in *peda* making was adopted by the rural milk processing centres in Kutch district run by the Gujarat State Rural Development Corporation. Here in one batch about 6 litre of milk was heated in an open iron vessel on a diesel furnace. At the last stage of *khoa* making 400 gm of sugar was added. After cooling, the product was rolled and shaped in circular balls of about 25 gm each measuring 4 cm in diameter and 1.5 cm in height. In Mathura district of U.P. *khoa* is first cooked to brown colour in ghee and then *peda* is prepared from it by blending sugar and other additives.

In Sugam Dairy, Baroda, Kesar *Peda* is prepared by adopting a large scale mechanized process which involves manufacture of *khoa* using continuous machine, heating *khoa*-sugar mixture in planetary mixture, cooling, mechanical forming of *peda* and packaging (Banerjee, 1997).

De and Srinivasan (1967) standardized the technique of *khoa* manufacture from aged roller dried skim milk powder and white butter. Realizing the drawbacks of small scale *khoa* manufacture, Banerjee *et al.* (1968) developed a prototype machine for continuous production which was further studied by Singh (1970) and Rajorhia and Srinivasan (1975).

The feasibility of utilizing vacuum concentrated milk with 31% TS for manufacture of *khoa* was reported by De and Srinivasan (1968), whereas Boghra and Rajorhia (1982) prepared *khoa* from cow and buffalo milk concentrated to 31% and 41% TS respectively.

Khoa was prepared by Rajorhia *et al.* (1991) in four mechanized systems, namely inclined scraped surface heat exchanger (ISSHE), conical-vat, contherm-convat heat exchanger and roller drier. Finally they concluded that ISSHE was the most suitable for continuous *khoa* making and could be sealed up for industrial production. Later, in 1992 a three stage continuous *khoa* making machine was developed by Christie and Shah, which was further studied by Dodeja *et al.* (1992).

2.3 Yield and / Out Turn

An average yield of 18.32% for cow milk and 21.50% for buffalo milk *khoa* was reported by De and Ray (1952). However, Rajorhia (1971) observed the yield to be 17.5% to 21.0% and 20.5 to 23.4% for cow and buffalo *khoa* respectively depending upon the fat and total solids content in milk. 600 litre of buffalo milk can be converted to 168 kg *peda* (Patel, 1986).

2.4 Chemical Composition of *Khoa*

2.4.1 Market khoa

The chemical composition of *khoa* samples available in the market as reported by various workers had been described in the Table 2.1.

Total solid content of the market *khoa* samples varied from 60.0 to 80.0%. Bhat *et al.* (1948) found maximum value while Iyer *et al.* (1948) reported the minimum value for total solids content of the market *khoa* samples.

Wide variations were observed amongst the market *khoa* samples in respect of fat content. Earlier research workers like Iyer *et al.* (1948); Sharma and Zariwala (1978); Kumar and Srinivasan (1982); Goyal and Srinivasan (1988); Ghatak and Bandyopadhyay (1989);

Arora *et al.* (1991) reported a fat content varied between 17.32 to 37.24% in market *khoa* samples. They further reported that such a wide variation in fat content was obviously due to the differences in fat levels of milk used for the preparation of *khoa* and also the degree of desiccation obtained in each case.

Table 2.1: Chemical composition of market *khoa*

Authors	Source of samples	Chemical constituents (%)				
		Total solids	Fat	Protein	Lactose	Ash
Bhat *et al.* (1948)	Bombay	80.31	29.72	26.68	20.24	3.67
Iyer *et al.* (1948)	All parts of India	65.80	36.60	27.40	30.90	4.40
De and Ray (1952)	CM, BM	74.40	34.80	25.80	34.30	5.20
Dastur and Lakhani (1971)	Poona	74.14	27.24	19.58	-	3.36
Ghodekar *et al.* (1974)	NA	75.91	27.04	18.99	24.80	3.70
Zariwala *et al.* (1974)	Bombay	71.87	27.14	-	17.67	-
Sharma and Zariwala (1978)	Bombay	72.80	24.80	21.60	19.44	-
Narain and Singh (1981)	Varanasi	68.65	37.24	24.76	33.46	4.56
Kumar and Srinivasan (1982)	NA	71.60	24.60	19.00	25.20	3.60
Sharma and Lavanta (1987)	Baraut	71.70	25.80	-	-	-
Goyal and Srinivasan (1988)	NA	69.07 – 77.66	22.00 – 32.19	17.73 – 19.12	23.70 – 25.80	3.60 – 3.72
Ghatak and Bandopadhyay (1989)	Calcutta	73.70	24.30	22.80	20.80	3.1
Arora *et al.* (1991)	All parts of India	77.60	17.32	13.40	16.64	2.53
Gothwal and Bhavadasan (1992)	NA	69.38	-	17.86	26.33	3.69

NA = Not available, CM = Cow Milk, BM = Buffalo milk

Several workers had reported variable results in regard to protein content. The protein content ranged from 13.40 to 27.40% in market *khoa* samples (Bhat *et al.*, 1948; Iyer *et al.*, 1948; Dastur and

Lakhani, 1971; Sharma and Zariwala, 1978; Sharma and Lavanta, 1987; Goyal and Srinivasan, 1988; Arora *et al.*, 1991).

The lactose content also showed fairly wide variations ranging from 16.64 to 34.30% (De and Ray, 1952; Ghodekar *et al.*, 1974; Narain and Singh, 1981; Ghatak and Bandopadhyay, 1989; Arora *et al.*, 1991; Gothwal and Bhavadasan, 1992). The ash content of market *khoa* samples varied from 2.53 to 5.20% (De and Ray, 1952; Dastur and Lakhani, 1971; Kumar and Srinivasan, 1982; Arora *et al.*, 1991; Gothwal and Bhavadasan, 1992). The type of milk used, extent of desiccation and addition of adulterants would lead to such disparity.

Ghodekar *et al.* (1974) and Ghatak and Bandopadhyay, (1989) reported that a considerable number of *khoa* samples were found adulterated with starch. Sharma and Lavanta (1987) examined *khoa* samples from Baraut market and reported the presence of alum (an aluminium compound) resulting in lower acidity values.

2.4.2 Laboratory khoa

Chemical composition of laboratory made *khoa* samples had been studied by several workers who reported wide variations in the moisture, fat, protein, lactose and ash contents (Table 2.2). The average content of total solids (60.00 to 80.80%), fat (21.73 to 45.90%), protein (16.30 to 25.80%), lactose (18.85 to 35.80%) and ash (2.92 to 5.20%) found in the laboratory made *khoa* samples. These results varied considerably with those of the market *khoa* samples (Table 2.1).

Table 2.2: Chemical composition of laboratory made *khoa*

Authors	Source of samples	Chemical constituents (%)				
		Total solids	Fat	Protein	Lactose	Ash
De and Ray (1952)	CM	74.40	34.80	25.80	34.30	5.20
	BM	80.80	45.90	22.10	27.40	4.50
Mani *et al.* (1955)	NA	74.80	25.90	20.10	24.90	4.00
Srinivasan and Anantakrishnan (1964)	CM	74.40	25.90	19.20	25.60	3.70
	BM	80.70	37.10	17.80	22.10	3.70
Rajorhia (1971)	CM	70.05	26.05		-	
	BM	68.80	21.73	-	-	-
Hemavathy and Prabhakar (1973)	NA	73.50	39.11	20.54	28.57	
Kumar *et al.* (1975)	NA	66.13	22.40	19.54	20.29	3.00
Narain and Singh (1981)	CM	76.85	34.14	23.14	33.97	4.84
	BM	74.98	39.44	23.00	32.26	4.28
	MM	75.40	36.20	23.39	35.80	4.56
Kumar and Srinivasan (1982)	CM	69.10	22.00	19.10	24.20	3.70
	BM	77.70	32.20	17.70	23.70	3.70
Patel *et al.* (1985)	BM	70.50	32.28	16.30	18.85	2.92
Sharma and Lavanta (1987)	MM	72.20	27.33	-	-	-
Rajorhia *et al.* (1990)	BM	63.92	37.53	-	-	5.04
Sapre and Deodhar (1991)	BM	64.40	22.30	19.80	21.00	-
Gothwal and Bhavadasan (1992)	CM	74.44	-	19.15	26.64	3.75
	BM	80.38	-	18.11	25.51	3.82

NA = Not available

CM = Cow Milk

BM = Buffalo milk

MM = Mixed milk

Free fat content which contributes to the flavour and texture of the product, was not evaluated in case of market *khoa* samples. However, Ranganadham and Rajorhia (1989) reported that laboratory samples containing about 33% fat and 56% free fat had the best texture quality. Earlier workers reported the free fat content of *khoa* to be 48.57 ± 12.38% (Rajorhia *et al.*, 1990) and 54.54% (Kumar and Pal, 1994). Ranganadham and Rajorhia (1989) also observed that free fat content increased with an increase in fat and TS content in *khoa* and with the type of milk used (cow or buffalo). However, homogenization of milk reduced the content by 50%. Addition of tween – 80 was found to be most effective, followed by glycerol monostearate, in controlling the free fat content in *khoa*.

2.5 Chemical Composition of *Peda*

2.5.1 Market peda

Commercial *peda* samples were collected from different markets all over India (Rastogi *et al.*, 1966; Ghodekar *et al.*, 1974; Sharma and Zariwala, 1978 in Bombay; Vijayakhader and Patel, 1983; Garg and Mandokhot, 1984 and Roy *et al.*, 1998 in greater Calcutta) to analyse for the chemical composition. The fat content of *peda* samples collected from different markets varied from 3.3 to 28.54%, protein content varied from 1.4 to 19.5%; lactose content ranged from 4.0 and 18.6%; sucrose content varied between 13.2 and 61.8%, ash content varied from 1.4 to 3.4% and moisture content ranged between 4.2 to 20.30% (Table 2.3). The wide variation in the chemical composition may be due to the initial quality of milk, quantity of sugar added, the extent of heat desiccation, the storage conditions and the presence of adulterants.

Table 2.3 : Chemical composition of market, factory and laboratory made *peda*

Authors	Source of sample	Constituents (%)					
		Moisture	Fat	Protein	Lactose	Sucrose	Ash
Market							
Rastogi *et al.* (1966)	NA	11.4 – 18.2 (14.08)	13.7 – 21.7 (18.78)	17.01 – 18.7 (17.97)	12.05 – 18.6 (15.89)	38.9 – 47.6 (45.46)	2.02.8 (2.28)
Ghodekar *et al.* (1974)	India	6.8 – 10.8 (8.80)	3.3 – 17.9 (9.70)	6.3 – 11.8 (9.10)	7.3 – 11.7 (9.4)	52.1 – 60.5 (55.3)	1.4 – 3.4 (2.0)
Sharma and Zariwala (1978)	Bombay	4.2 – 14.02	7.0 – 25.0	1.4 – 12.0	4.0 – 18.6	13.2 – 61.8	Not given
Vijayakhader and Patel (1983)	NA	10.8	14.4	11.1	6.5	57.0	1.8
Garg and Mandokhot (1984)	Hissar	11.26 – 20.30 (14.99	10.49 – 28.54 (22.06)	9.46 – 16.89 (13.77)	Not given	Not given	Not given
Dharampal (1997 - 98)	NA	4.20 – 18.20	3.50 – 25.00	1.40 – 19.50	4.0 – 20.60	31.80 – 61.80	1.40 – 3.40
Roy *et al.* (1998)	Greater Calcutta	12.3 – 16.5 (14.73)	9.01 – 17.5 (14.12)	4.05 – 15.7 (11.26)	8.5 – 16.4 (12.96)	44.34 – 50.7 (46.69)	2.0 – 2.9 (2.37)
Factory							
Patel and Gandhi (1980)		13.5	20.0	19.5	16.31	31.8	Not given
Laboratory							
Vijayakhader and Patel (1983)	Cow milk	10.1	12.2	7.7	17.3	44.8	2.1
	Buffalo milk	8.6	14.5	9.9	12.3	48.5	2.3
	Mixed milk	10.0	14.1	10.3	15.2	50.9	2.2
Ray, P.R. (1998)	Cow milk	17.81 – 21.65 (19.25)	21.7 – 23.35 (22.83)	15.35 – 16.10 (15.86)	13.87 – 14.98 (14.67)	27.15 – 30.72 (29.32)	2.31 – 2.58 (2.46)
	Buffalo milk	12.85 – 17.70 (14.65)	29.63 – 33.15 (31.74)	16.21 – 16.96 (16.79)	14.9 – 16.1 (15.83)	19.50 – 24.7 (21.54)	2.67 – 2.89 (2.87)

NA = Not available

Figure in the parenthesis are the average values

Ghodekar *et al.* (1974) found the average value of titratable acidity of the market *peda* samples to be 0.28% in terms of lactic acid.

Several workers reported that a considerable number of *peda* samples were found to be adulterated with starch (Sharma *et al.*, 1969; Ghodekar *et al.*, 1974; Sharma and Zariwala, 1978).

2.5.2 Laboratory and factory made peda

Table 2.3 represents the chemical composition of factory and laboratory made *peda* samples. Patel and Gandhi (1980) gave the chemical composition of factory made *peda* as 13.5% moisture, 20.0% fat, 19.5% protein, 16.31% lactose, 31.8% sucrose and 13.5% moisture.

Vijayakhader and Patel (1983) and Ray, P.R. (1998) studied on the chemical quality of laboratory made *peda* samples and reported that the moisture (8.6 to 21.65%), fat (12.2 to 33.15%), protein (7.7 to 16.96%), lactose (12.3 to 17.3%), sucrose (19.5 to 50.9%) and ash (2.1 to 2.89%) content of those samples varied considerably with those of the market *peda* samples. The average value of free fat content, titratable acidity, perixode value, free fatty acid and hydroxy methyl furfural (HMF) value were found to be 73.61% of total fat, 0.72% in terms of lactic acid, 1.10 m.eq. of O_2/kg of fat, 0.058% oleic acid and 0.378 micro moles/100 gm respectively in case of *peda* samples prepared from cow milk, whereas, the corresponding values in case of buffalo's milk *peda* samples were 74.81, 0.75, 1.13, 0.056 and 0.423 respectively (Ray, P.R., 1998).

2.6 Microbiological Quality of *Peda*

2.6.1 Market peda

The microbiological specifications for *peda* has not been laid down so far. Studies on the microbiological quality of market *peda* samples are summarized in Table 2.4. A number of workers had studied on the microbiological quality of market samples of *peda*.

Gatlewar *et al.* (1970) reported that about 47% of the total sweet meats collected from Bombay market were contaminated with enteropathogenic organisms and about 50% of the *peda* samples were contaminated with *Staphylococcus aureus*. The market *peda* samples evaluated by Kamat and Sulebele (1974) for differential counts showed the presence of mesophilic bacteria, which were largest in number, followed by psychrophilic and thermophilic bacteria. Staphylococci were found in 14 out of 16 samples, of which four were coagulase positive and 64.3% of the strains isolated haemolysed human red blood cells. Other characteristics of the staphylococcal strains, such as horse and rabbit blood haemolyses, gelatin liquefaction, aerobic and anaerobic mannitol fermentation and chromogenesis were also found. Ghodekar *et al.* (1974) isolated several thousands of micrococci, sarcinae, aerobic spore formers, coliforms, streptococci, lactobacilli and staphylococci from each gram of market samples of *peda*. Garg, S.R. (1981) studied on the *peda* samples collected from Hissar market and reported that 98.9% of the total bacterial flora were gram-positive bacteria which included *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Bacillus* , *Corynebacterium*, *Cinobacter*, coliforms and some unidentified species constituted gram-negative flora. The most significant finding of this study was the predominance of *Staphylococcus* and *Bacillus*, since the former was isolated from 75 and the latter from 90% of the samples. The presence of staphylococci was also detected in *peda* samples collected from Allahabad market by Singh *et al.* (1975a); Mysore market by Dwarkanath and Srikanta (1977) and Bombay market by Kakar and Udipi (1997). However, Kudchodkar and Singh (1964) did not find any trace of staphylococci in *peda* samples collected from Karnal market. These observations are significant since outbreak of staphylococcal food poisoning by consumption of *peda* has been recorded by Mandokhot and Chandiramani (1983).

Table 2.4 : Microbiological quality of market *peda*

Authors	Source	SPC per gm.	Coliform per gm	Faecal coliform per gm	Enterococci per gm	Staphylococci per gm	Yeast and/mold per gm	Other pathogens
Kudchodkar and Singh (1964)	Karnal					0		
Sharma *et al.* (1969)	Agra		2×10^4 – 7.68×10^6				$0 - 1.1 \times 10^3$ / $10^2 - 3.4 \times 10^3$	
Ghodekar *et al.* (1974, 1980)	India	2×10^3 – 3×10^5	Present	Present		$40 - 3 \times 10^4$	$30 - 4 \times 10^3$	
Kamat and Sulebele (1974)	Bombay	6×10^2 – 1×10^7	0			$0 - 6.7 \times 10^4$		
Singh *et al.* (1975a)	Allahabad	4.5×10^6	1.89×10^5	-	-	6.2×10^6	450 / 610	-
Singh *et al.* (1975b)	Agra	5×10^3 – 2.67×10^5					3×10^3 – 5.4×10^4	
Dwarkanath and Srikanta (1977)	Mysore	7.6×10^2 – 5×10^3	0 – 460	0		0 – 240*	1.42×10^4 – 5.4×10^4	*Salmonella* absent
Garg, S.R. (1981)	Hissar	1.1×10^3 – 5.6×10^6	1.2×10^3	0 – 50	$0 - 3.2 \times 10^5$	$0 - 3 \times 10^5$	$10 - 3 \times 10^3$	*Salmonella* absent
Roy *et al.* (1998)	Greater Calcutta	23.5×10^5 – 639.3×10^5	10 – 213				300 – 700	

* Coagulase positive *Staphylococcus*

A wide range of yeast and mold counting from 0 to 9.4 x 10^4 cfu/gm were found in the market samples of *peda* as reported by Sharma *et al.* (1969), Singh *et al.* (1975a, 1975b), Dwarkanath and Srikanta (1977), Ghodekar *et al.* (1980), Grag, S.R. (1981) and Roy *et al.* (1998). The presence of coliform were also observed by Sharma *et al.* (1969), Ghodekar *et al.* (1974), Singh *et al.* (1975a), Dwarkanath and Srikanta (1977), Garg, S.R. (1981) and Roy *et al.* (1998).

However, Kamat and Sulebele (1974) could not detect coliform in any of the market *peda* samples they analysed.

2.6.2 Laboratory made peda

There is no literature available on the microbiological quality of laboratory made *peda* samples.

2.7 Packaging of *Peda*

Packaging plays an important role in both handling and storage of a product. Varieties of packaging material had been used by the early researchers for packaging of *peda*. Vijayakhader and Patel (1983) packaged freshly prepared *peda* samples and market sample in three types of polyethylene bags : low density transparent (LD), medium density blue (MD) and high density white opaque (HD) for periods of 14 and 28 days at ambient temperature (25 - 29°C). The changes in fatty acid composition was highest in LD bag followed by MD and HD bag. HD bag offered poorest protection against rise in peroxide value. Increase in moisture content were slight and not significant either between bags or *peda* samples.

Biradar *et al.* (1985) packed *peda* samples in about 250 gm lots in 100 and 300 gauge low density polyethylene pouches (LDPE – 100 and LDPE – 300), heat sealed and kept on open aluminium trays. LDPE packaging reduced weight loss, browning and free fatty acid development and retarded deterioration of flavour, odour and

increased acceptability of *peda* samples for a longer period. Peroxide value was not influenced by LDPE packaging. LDPE – 300 was more effective than LDPE – 100, though, film thickness did not influence the organoleptic attributes significantly.

Peda is usually packed in paper board /boxes having a parchment paper liner or grease proof paper liner (Reddy, 1985). Packaging of *peda* samples in multilayer transparent laminates with oxygen scavenger extended the shelf-life upto 2 months at 37°C, 5 months at ambient temperature and 6 months at 20°C (Kumar *et al.*, 1997). Polypropylene trays covered with transparent coloured MXXT are recommended packages of burfi, *peda* and kalakand (Goyal, 1997-98).

2.8 Storage and Shelf-Life of *Peda*

Peda is expected to have good shelf-life due to low moisture content and high percentage of sugar. The sugar-in-water concentration (sugar ratio) of the finished product should be atleast 60 giving an atmospheric pressure of 140 atmosphere which is sufficient to inhibit bacterial growth. However, *peda* is most susceptible to microbial spoilage due to unhygienic conditions adopted during manufacture and subsequent handling and its surface contamination.

The effect of metals on the shelf-life of *khoa* was studied by Jalil *et al.* (1963). These workers observed that *khoa* prepared in iron pan has minimum while that prepared in stainless steel pan has maximum shelf-life. They suggested that the absolute shelf-life of *khoa* could be enhanced to 15 – 20 days by eliminating the contamination with chelating metals. Boghra (1988) observed that addition of iron (1.25, 2.5 and 5%) and copper (1.0, 1.25 and 1.5%) enhanced the lipolytic and oxidative deterioration of *khoa* during storage at less than 10°C for 14 days. Iron had more pronounced effect than copper.

Reddy and Rajorhia (1990) reported that optimum equilibrium relative humidity (E.R.H) for storage of *peda* was 60% (reached in 12 days), at which point the equilibrium moisture content was 10.25%. A higher E.R.H. encouraged mold growth whilst a lower E.R.H. resulted in a hard and dry product with a tendency to brown discolouration.

The deterioration of *khoa* was attributed to the unhygienic methods of processing and improper handling (Arora *et al.*, 1991) followed by lactose breakdown, proteolysis and lipolysis due to the activities of mixed flora during storage (Ahmad and Ranganathan, 1967; Kumar and Srinivasan, 1983; Patel *et al.*, 1985; Goyal and Srinivasan, 1989). Being aerobil in nature, molds cover the entire surface of the product kept under room temperature producing an abnormal colour, flavour and appearance (Bhat *et al.*, 1948; Rajorhia and Srinivasan, 1979; Patel *et al.*, 1985). The flavour deterioration was principally related to microbial and chemical changes occuring during storage (Rao *et al.*, 1977).

Kumar *et al.* (1977) packed *peda* samples under modified atmospheric packaging MAP – (N_2 : CO_2 :: 80 : 20) and also under normal atmospheric environment with oxygen scavenger. The samples with MAP showed shelf-life of 15 days both at 37°C as well as ambient temperature, and 30 days at 20°C, whereas, control samples irrespective of storage temperature showed mold growth after 7 days of storage.

2.9 Physico-chemical Changes During Storage

During storage, every product undergoes a series of physico-chemical reactions. The post manufacture chemical reactions occurring during storage of *khoa* considerably changed the physico-chemical attributes and render it unfit for consumption. In 1990, Kumar *et al.* developed a shelf-life stimulation model for *khoa* and *khoa* based sweets. It was found to predict the shelf-life using a

multiple linear regression technique by the four variables – fatty acids, thiobarbituric acid value, tyrosine value and reflectance. The physico-chemical attributes that undergoes changes during storage of *khoa* have been reviewed here under.

2.9.1 Titratable acidity

Ghodekar, D.R. (1969) observed that market *khoa* samples developed a flat taste in 7 days during storage at 5 - 7°C but the laboratory made samples remained in good condition upto 14 days. After 14 days, the titratable acidity of the market samples was 0.56% and that of the samples prepared in the laboratory was 0.3%.

Khoa wrapped in parchment paper impregnated with 20% potassium sorbate solution had a satisfactory rise in titratable acidity during storage at 30°C and 5°C (Rao *et al.*, 1977). The titratable acidity of milk increased from 0.155 to 0.596% (lactic acid) during its conversion to *khoa*, which could be attributed to the increase in total solids content in *khoa* as compared to milk (Patel *et al.*, 1985). Several workers reported the changes in acidity during storage of *khoa* with or without additives at room temperature. Laboratory made *khoa* samples were studied on its acidity content during storage at 5°C and 12.32°C with (Ramzan and Raiz-ur-Rahman, 1973; Goyal and Srinivasan, 1989) and without packages (Sharma and Lavanta, 1987).

Ray, P.R. (1998) studied cow and buffalo milk *peda* samples and reported that the net increase in titratable acidity for cow milk *peda* samples were 25% for control, 22.85% for sorbic acid treated samples after 6 days at 35°C. The corresponding values for buffalo milk *peda* samples were 21.33% and 14.21% respectively. In refrigerated condition, there was an increase in titratable acidity of 28.57% and 21.62% after 45 days in case of cow milk *peda* and buffalo milk *peda* respectively.

2.9.2. Peroxide value

There was increase in peroxide value of *khoa*, especially when stored at room temperature as reported by Ramzan and Raiz-ur-Rahaman (1973) and Sachdeva (1980).

Patel *et al.* (1985) suggested a repressive effect of added salts on peroxidation of *khoa* resulting in the increase in peroxide value during storage at room temperature.

Peda prepared from both cow and buffalo milk with 0.02% sorbic acid can be better effectively stored at refrigeration temperature for a period of 45 days without moderate change in its peroxide content (Ray, P.R., 1998).

2.9.3 Free Fatty Acid (FFA)

The degradation of fat was considered to be primarily affected by the growth of yeasts and molds (Goyal and Srinivasan, 1989). However, Patel *et al.* (1985) found the correlation of changes in FFA content with both the SPC and yeast and mold count in *khoa*.

The free fatty acidity of *khoa* treated with additives (Patel *et al.*, 1985) and stored in different packages at 17°C, 8 ± 1°C and - 20°C (Rao *et al.*, 1977) was found to increase in different rates.

According to Goyal and Srinivasan (1989) the mean package value for FFA of *khoa* samples increased from 0.53 to 0.109, 0.117, 0.123 and 0.130 in four different types of packages after 60 days of storage at 4 - 5°C and 100% relative humidity.

Ray, P.R. (1998) worked on cow and buffalo milk *peda* and reported that FFA content of all samples stored at 30°C increased more sharply than that at 5°C irrespective of treatment imposed. The increase in FFA of cow milk *peda* were 39.6% and 32.71% when

stored at 30°C and 5°C respectively. These results in buffalo milk *peda* were 32.83% and 29.85% after 7 days.

4.9.4 Hydroxymethyl furfural (HMF) value

During storage at 5°C, 25°C and 30°C the HMF value increased more in cow milk *khoa* than buffalo milk *khoa*, although the increases were low and negligible at 5°C (Gothwal and Bhavadasan, 1992).

According to Ray, P.R. (1998) the initial HMF value of buffalo milk *peda* were higher than cow milk *peda* probably due to the higher TS content in buffalo milk than in cow milk *peda*. The author also concluded that the browning as a result of storage increased more rapidly in *peda* prepared from cow milk than that from buffalo milk under storage at ambient temperature.

Patel *et al.* (1985) studied the effect of SO_2 in *khoa* as a browning inhibitor.

2.9.5 Tyrosine value

Ahmad and Ranganadham (1969) analysed the market *khoa* samples during storage and reported that the increase in proteolytic breakdown in the samples incubated at 22°C were 94.71 and 217.33% after 4 and 7 days respectively, while that at 37°C were 71.03, 135.37 and 251.74% after 2, 4 and 7 days respectively.

Patel *et al.* (1985) showed that irrespective of the treatments imposed, the proteolysis decreased and then increased with storage period and was significantly higher in control samples than in the treated samples of *khoa* throughout storage suggesting suppression of proteolytic activity by the additives. *Khoa* in packaged condition showed less tyrosine value on storage depending upon the type of packages, treatments undergone and temperature of storage (Rao *et al.*, 1977; Goyal and Srinivasan, 1989).

Changes in tyrosine value was more rapid in the product stored at 30°C than that at 5°C, suggesting that the proteolysis was directly related to the storage temperature (Ray, P.R., 1998). The author also concluded that irrespective of the type of milk used, proteolysis increased with storage period and was higher in control than in the treated samples throughout storage suggesting suppression of proteolytic activity by the preservatives.

4.9.6 Moisture

Narang *et al.* (1969) evaluated moisture content of *khoa* on storage and reported that at room temperature the moisture content increased steadily for a period of 4 weeks and started decreasing thereafter. However, some other workers had reported a continuous decrease in moisture content of *khoa* at both ambient and refrigerated temperatures irrespective of its treatment (Ramzan and Raiz-ur-Rahman, 1973; Sachdeva, 1980; Patel *et al.*, 1985; Sharma and Lavanta, 1987). The rate of decrease in moisture content of *khoa* during storage was less, when packed in different packages and stored at 4 - 5°C and 100% relative humidity (Goyal and Srinivasan, 1989).

Sethna and Bhat (1949) showed that irradiation of *khoa* samples with ultraviolet rays decreased the moisture by 7%, extending the keeping quality to 25 days against 5 – 6 days for untreated samples at 28 - 29°C. However, the authors were not certain whether the improved keeping quality was a result of the bactericidal effects of irradiation or the reduced moisture level.

The shelf-life of *khoa* packed in heat sealed polyethylene bags and containing 70, 80 and 90% total solids were respectively 4, 6, 9 days at 30°C; 7, 8 and 15 days at 22°C and 35, 45 and 60 days at 5°C (Deshmukh *et al.*, 1977). They also reported an increase in keeping quality with increasing total solid content of *khoa*. The studies of Rudreshappa and De (1971) on preservation of *khoa* suggested that

an initial moisture content of 20 – 25% and a temperature of 80 - 90°C at the time of packing in sterilized cans ensures a minimum shelf-life of 14 days at 37 ± 1°C, while packing at 25 - 30°C affected the acceptability adversely.

Ray, P.R. (1998) reported that irrespective of the type of milk used and the treatments imposed, *peda* stored at 5°C had a better shelf-life than that at 30°C. The author also reported that the treated samples showed better results than the control samples. 3.6% and 7.5% moisture decrease for cow milk *peda* and 3.2% and 8.3% moisture decrease for buffalo milk *peda* at 30°C and 5°C respectively was reported by him.

2.10 Addition of Preservatives

Above 55% relative humidity, *khoa* samples tend to develop mold (Goyal and Srinivasan, 1989). But with the advent of technology there are signs that the shelf-life problems can be minimized. Efforts are necessary to a greater extent for improving the keeping quality of *peda* through addition of peservatives.

Bongirwar and Kumata (1967) observed that *khoa* treated with gamma irradiation (^{60}Co source) upto 0.5 Mrad did not induce organoleptic defects.

Jalil *et al.* (1963) also reported satisfactory resistance against mold growth when *khoa* was wrapped in butter paper previously soaked in sodium propionate solution. The spoilage of *khoa* was noticed after the fifth day at room temperature and after 20 days at 5 ± 1°C. Almost similar results were obtained by Rajorhia (1965) who reported the shelf-life of *khoa* wrapped in waxed butter paper to be 7 days at 28 ± 1°C. De and Ray (1953) also studied the shelf-life of *khoa* and reported the results to be 7 – 8 days under room conditions which could be improved slightly by wrapping with butter paper while stored

at 7.5°C. Impregnation of *khoa* wrapping with 30% potassium sorbate solution doubled the shelf-life at 7°C, but NaCl had less effect on keeping quality at 22 - 37°C (Ghodekar *et al.*, 1978). Rao *et al.* (1977) studied the effect of parchment paper, with and without impregnation with 20% potassium sorbate solution as a packaging material for *khoa*. The shelf-life increased from 4 days to 10 days in case of treated package at 30°C and 45% relative humidity and 60 days at 5°C and 84% RH.

However, Rudreshappa and De (1971) found no effect of adding potassium sorbate (0.05% and 0.10% on weight basis) and butylated hydroxy anisole (0.005% and 0.10% on fat basis) into the body of *khoa*. But Ghodekar *et al.* (1978) reported an improvement in shelf-life of the hot treated *khoa* from 2 to 4 days at 37°C, from 2 to 7 days at 22°C and for more than 14 days under refrigeration.

Jha *et al.* (1988) showed that addition of 0.2% to 0.4% potassium sorbate to *khoa* during the last stage of manufacture resulted an increase in the shelf-life from 2 days to 10 - 11 days at 30°C and from 20 days to 40 days at 5°C. They also concluded that the additives helped in inhibiting the bacterial growth and reducing the rate of proteolysis, titratable acidity and free fatty acidity development.

Kalra *et al.* (1973) studied the effect of nisin on *khoa* and reported that incorporation of 100 IU/gm of nisin to canned *khoa* increased the shelf-life by 1 month, 3 weeks and 2 weeks at 10°, 20° and 30°C respectively. During the research work, Jha *et al.* (1977) did not find any effect of adding hisaplin (0.005 - 0.02%) to canned *khoa*. Grewal and Jain (1977) also showed that addition of 0.2% nisin to *khoa*, packed in sorbitol smeared butter paper, helped to maintain the original flavour at both storage temperature upto the third week.

A dose of 0.2 ml formalin (40%) per 25 gm of sample was found to be more effective in preserving *khoa* samples for 5 months at room temperature meant for analytical purposes, as compared with the recommended dose of 0.10 ml of formalin per 25 gm of sample under the PFA rule (Dinakar and Sharma, 1989).

Mukherjee and Mathew (1974) examined the other preservatives viz., alcoholic measures chloride (0.5%), formalin (4%), acetic acid (4%), sodium benzoate (0.05%) and hydrogen per oxide (3%), to be less effective as compared with formalin. Kumar *et al.* (1997) revealed that optimum condition for increased shelf-life (5 – 6 months) adequate for safe commercial exploitation can be obtained by storing *peda* at ambient temperature (18 - 32°C) using high film supplemented with oxygen scavenger packing without showing any perceptible defects in dryness, texture, organoleptic attributes, mould growth and chemical changes.

An attempt was made by Ray *et al.* (1999, 2000) to examine the effect of sorbic acid on the physico-chemical changes in *peda* during storage at 30 ± 1°C in 70% relative humidity (R.H.) and at 7 ± 1°C with 90% R.H. Addition of 0.02% and 0.05% sorbic acid to *peda* samples made from cow milk increased the shelf-life from 6 days to 9 days at 30 ± 1°C and from 16 days to 45 and 60 days at 7 ± 1°C respectively. Buffalo milk *peda* samples treated with 0.02% and 0.05% sorbic acid had shelf-life of 9 days at 30 ± 1°C and 37 and 55 days at 7 ± 1°C respectively.

2.11 Nutritive Value of *Khoa*

The conventional production of a heat concentrated product, *khoa* as well as *peda* involves prolonged application of heat to milk, and therefore, a reduction in the nutritive value may logically be expected. However, the experiments conducted on nutritional value of *khoa* and *peda* indicated that the loss is only marginal when

compared to the nutritive value of milk. Very limited information is available on this aspect.

Since heat treatment denatures proteins, they are unfolded and the gastric enzymes can effectively cause hydrolysis of the denatured protein molecule, resulting in better availability of the nutrients (Mahadevan, 1991). Sapre and Deodhar (1991) reported that net protein utilization and net protein ratio were significantly higher in *khoa* than in milk, which was confirmed by Lily *et al.* (1955) and Balasubramanian *et al.* (1955).

Reports regarding mineral composition of *khoa* are available (Iyer *et al.*, 1948; Narain and Singh, 1981; Patel *et al.*, 1985). Boghra and Mathur (1990) stated that mineral levels increased upto 6 and 7 fold during manufacture of *khoa* from cow milk and buffalo milk respectively. Soluble citrate, sodium, potassium, chlorine, copper and iron levels were maximum at *khoa* formation, while a decrease in soluble calcium, phosphorus and citrate and increase in soluble chlorine, copper and iron were observed at storage for 30 days at less than 10°C.

The loss of Vitamin A during the preparation of *khoa* was 75% and 25% as reported by Shroff *et al.* (1954) and Mani *et al.* (1955) respectively. They also reported a loss of 35% of thiamine and 60% of total riboflavin during desiccation upto 75% total solids. Vitamin levels per 100 gm total solids in *khoa* as studied by Sapre and Deodhar (1988) were as follows: retinol – 0.226 mg; riboflavin – 0.98 mg; vitamin B_6 – 0.13 mg; folic acid – 1.06 mg and ascorbic acid – 8.46 mg. The biological value and digestibility of *khoa* was stated as 69% and 90% respectively (Mahadevan, 1991).

2.12 Texture Profile of *Khoa* and *Peda*

According to Rajorhia *et al.* (1990) the increase in size and hardness of the grain lead to poor body and texture of *khoa*. Smoothness of *khoa* was also adversely affected by the developed acidity. They reported that the hardness of *khoa* was found to be increased from 19.47 mN to 22.85 mN and 28.44 mN due to increase in the acidity upto 0.19% and 0.27% lactic acid respectively. Gumminess and Chewiness of *khoa* was also increased due to the developed acidity in milk and was reduced by neutralization of the same.

Patel *et al.* (1990) have found a positive and significant correlation in total solids and hardness of *peda*. Instron measurement showed that working significantly decreased the hardness and springiness but increased the adhesiveness and cohesiveness. An increase in total solids was also accompanied by a considerable increase in hardness, gumminess and chewiness but a decrease in cohesiveness.

Adhikari *et al.* (1994) also determined the Instron parameters of *khoa* and observed a significant but negative correlation of hardness, cohesiveness, gumminess and chewiness with its moisture content, while the fat had negative correlation with hardness, chewiness and cohesiveness. However, a positive correlation was found between protein content of *khoa* and its textural parameters. Lactose, ash and calcium had a significant role in determining the hardness, chewiness, cohesiveness and gumminess.

Texture profile parameters of *khoa* were significantly influenced by certain compositional factors as observed by Gupta *et al.* (1990). The total solids content was the single most important variable affecting hardness ($r = 0.89$), cohesiveness (0.71), gumminess (0.83) and chewiness (0.75). These instrumental data were correlated with

sensory data, which enabled development of mathematical models aimed at predicting the textural quality (TQ) on the basis of instrumental cohesiveness (C), gumminess (G) and chewiness (ch):

$$TQ = 85.12 - 38.769\ C + 8.111\ G - 1.961\ ch$$

Texture profile analysis also been employed to monitor the post-manufacture hardening of *khoa* especially of the low-moisture type i.e. Pindi (Garg *et al.*, 1989), effect of working or kneading (Patil *et al.*, 1992) and incorporation of whey protein concentrate into cow milk *khoa* (Patel *et al.*, 1993).

Patel *et al.* (1992) characterized market samples of *peda* by means of Instron and noted that hardness was significantly correlated with sensory firmness. Unfortunately, there is hardly any report indicating texture measurement of *peda* using fundamental approach.

2.13 Microstructure of *Khoa*

Patil *et al.* (1992) studied the microstructure of *khoa* in details. *Khoa* has a granular texture consisting of protein granules with several hundred of micrometers in diameter, the granules consist of intact and partially fused casein micelles and non-micellar protein along with condensed milk serum and fat (free as well as globular fat) filling the inter-granular spaces. Large aggregate of lactose crystals developed in the intergranular space in un-worked *khoa* during storage and the sandiness in the stored product was markedly increased. Working of *khoa* reduced the dimensions of protein granules and the inter-granular void spaces and produced large amounts of fat globule membrane fragments liberating free fat. Individual lactose crystals in worked *khoa* stored at 20°C for 48 hours were more uniformly distributed compared to unworked *khoa*. Storage did not increase the sandiness in worked *khoa*.

Conclusion

Peda has a good nutritional and therapeutic attributes. But continuous scientific support is needed to ensure that such indigenous sweetmeat can be prepared on industrial scale by the mechanized processes. Systematic surveys and laboratory support is needed for establishing the sensory, rheological, microbiological and physico-chemical profile of *peda*. This data base and catelogue will be useful for providing scientific basis of further developmental plans.

Chapter - III

Material and Methods

Material and Methods

3.1 Material

Fresh-pooled milk sample was obtained from cow maintained at the herd situated near the institute. Fresh skim milk was obtained from the Students' Dairy of the faculty. Fine crystalline cane sugar was obtained from the local market.

Sodium metabisulphite was purchased from E. Merck (India) Ltd. Nisin was purchased from Aplin and Barrett Ltd. The other reagents used in the study were of analytical grade.

Polypropylene pouches (thickness : 75 μ, size : 4" × 3") of food grade quality and paper box (thickness : 365μ, size : 4" × 4") were purchased from Kolkata market.

Twenty five (25) samples of *peda* were collected aseptically in sterile containers from different markets of Kolkata city and its suburbs. The samples were then transported in an ice box and stored in refrigerator and analysed within 24 h from the time of collection.

3.2 Processing Method

The method of Patel and Gandhi (1980) was adopted with certain modifications for the preparation of *peda*.

3.2.1 Standardization

Whole cow milk was standardized to 4.5% fat and 8.5% SNF with fresh skim milk using Pearson's square method.

3.2.2 Preheating of raw milk

The standardized milk thus obtained was heated in three karahis, each containing a volume of one litre of milk, at about 85°C for 10–15 min. A continuous agitation was done with a khunti, in a circular motion.

3.2.3 Dehydration of milk

The milk was then vigorously stirred until its moisture level reached to about 35%.

3.2.4 Addition of sugar and preservative

Cane sugar was added @ 8% to each lot and stirred well. One lot of concentrated milk was heated further until a pasty consistency obtained and then spread out on the walls of the pan for cooling. The product was removed to a tray and moulded into desired shapes. The final product contained about 13–13.5% moisture level. This was designated as control *peda*.

The remaining two lots were processed in the same way as control except that when their moisture levels reached between 25–30%, potassium metabisulphite and nisin were added respectively @ 1000 ppm on the basis of milk used. The rest process was similar to the process used for the preparation of control *peda*.

3.2.5 Packaging of peda

The *peda* samples were packed in polypropylene pouches and also in paper boxes and kept on open petridishes made of glass without packaging. The pouches were heat sealed. Then they were divided into two lots, one of them kept in a refrigerator at 7 ± 1°C and the other in an incubator at 30 ± 1°C.

3.3 Analytical Methods

3.3.1 Total solids / Moisture

The total solids content was determined by the gravimetric method as described in BIS (1964).

3.3.1.1 Procedure

2 gm of sample (5 ml in case of milk) was taken in a previously weighed dry aluminium dish containing 5 gm of purified sand and 5 ml of distilled water was used for uniform mixing before heating. It was then kept in a hot air oven maintained at 100 ± 1°C for about 2 h to get the content dried and cooled in a desiccator, and weighed. The process of heating, cooling and weighing was repeated until the difference between two consequetive weights was less than 1 mg.

3.3.1.2 Calculation

$$\text{Total solids (TS) percentage} = \frac{100\ W}{W_1}$$

Where, W = Weight in gm of residue after drying

W_1 = Weight in gm of sample taken for the test.

Therefore, percentage of moisture content = 100 - % TS.

3.3.2 Ash

The ash content was determined by the method described in BIS (1961).

3.3.2.1 Procedure

Accurately weighed 3 gm of sample (10 ml in case of milk) was taken in a previously weighed dry silica dish. It was then kept in a muffle furnace maintained at 550 ± 1°C for 5 h until the ash is free from carbon. The dish was removed and cooled in a desiccator and weighed immediately.

3.3.2.2 Calculation

$$\text{Percentage of ash} = \frac{100\ W}{W_1}$$

Where, W = Weight in gm of residue after drying

W_1 = Weight in gm of sample taken for the test.

3.3.3 Total fat

The total fat content was determined by Gerber method as described in BIS (1964) and modified by Arora *et al.* (1991).

3.3.3.1 Reagents

i) Sulphuric acid solution (85% v/v) [90% v/v in case of milk].

ii) Isoamyl alcohol [density 0.803–0.805 gm/ml at 27°C].

3.3.32 Procedure

1 gm of sample (10.75 ml in case of milk) was diluted with 10 ml hot distilled water. Then the content was taken in a milk butynometer, previously containing 10 ml of sulphuric acid solution, by holding the pepette in a slanting manner. Then 1 ml of isoamyl alcohol was poured into the butyrometer to make all the curd dissolved. It was then placed in a water bath maintained at 65 ± 2°C for 5 min followed by centrifugation at 1000–1200 rpm for 5–6 min. The reading of the fat column was taken from the scale of the butyrometer.

3.3.3.3 Calculation

$$\text{Percentage of fat (for sample)} = \frac{11.25 \times BR}{W}$$

Where, BR = Butyrometer reading of the fat column.

W = Weight in gm of sample taken for the test.

Percentage of fat (for milk) = Butyrometer reading of the fat column.

3.3.4 Free fat

The free fat content was estimated by the method of Hall and Hedrick (1975).

3.3.4.1 Reagents

i) Petroleum ether (60 - 80°C)

3.3.4.2 Procedure

5 gm of the sample was taken in a 100 ml dry conical flask. Then 50 ml petroleum ether was added to it and kept aside for 10 min. The mixture was then filtered through an ordinary filter paper and the filtrate was collected in a previously weighed 250 ml dry conical flask. The process was repeated by adding 25 ml petroleum ether and filtering it into the same weighed flask. The total filtrate was then dried on a plate heater and the final weight of the flask was taken.

3.3.4.3 Calculation

Free fat is expressed as percentage of total fat. Therefore,

$$\text{Percentage of free fat in total fat} = \frac{W_2 - W_1}{W} \times 100$$

Where, W = Weight in gm of total fat in 5 gm of sample

W_1 = Weight in gm of empty, dry conical flask

W_2 = Weight in gm of the flask after drying of petroleum ether.

3.3.5 Protein

Protein content was estimated by the semi-micro Kjeldahl's method of Maneffee and Overmann (1940) as modified by Arora *et al.* (1991).

3.3.5.1 Reagents

i) Concentrated sulphuric acid (N_2 free)

ii) Copper sulphate

iii) Potassium sulphate

iv) 50% (w/v) sodium hydroxide solution.

v) Saturated boric acid solution (6 gm/100 ml warm distilled water)

vi) 0.02 (N) hydrochloric acid

vii) Mixed indicator (Methylene blue and methyl red in 1 : 1 ratio)

3.3.5.2 Procedure

0.3 gm of sample (1 ml in case of milk) was taken in a Kjeldahl flask. 8 ml concentrated sulphuric acid and 3 gm of a mixture of copper sulphate and potassium sulphate in the ratio 1 : 50 were added to the flask. It was then digested in fume chamber for about 1 hour until the solution was almost colourless (slightly yellowish). The digested liquid was allowed to cool, diluted with distilled water and the whole content was transferred to a Kjeldahl assembly. Then 25 ml of 50% sodium hydroxide solution was added to it. The content was distilled and the released ammonia vapour was absorbed in 25 ml boric acid solution containing mixed indicator. The green content was titrated with 0.02 (N) hydrochloric acid until the colour changed to grey. A blank experiment was done under identical conditions without taking *peda* or milk sample.

3.3.5.3 Calculation

$$\text{Protein percentage (by weight)} = \frac{0.18 \times (V_2 - V_1)}{W}$$

Where, V_2 = Volume in ml of 0.02 (N) hydrochloric acid used for sample.

V_t = Volume in ml of 0.02 (N) hydrochloric acid used for blank.

W = Weight of sample or milk taken.

3.3.6 Lactose and sucrose

The lactose and sucrose contents were simultaneously determined by the picric acid method as described by Perry and Doan (1950).

3.3.6.1 Reagents

i) Saturated picric acid solution (16 gm in 1000 ml distilled water)

ii) Sodium carbonate solution (25% w/v).

3.3.6.2 Procedure

Accurately weighted 5 gm of sample was taken in a 100 ml volumetric flask and made upto the volume with hot distilled water. The contents were well mixed and 5 ml was transferred (1 ml of milk was directly transferred in case of milk) to another 100 ml flask and made upto the volume with saturated picric acid, mixed and filtered. 2 ml of the filtrate was transferred to a sugar tube containing 1 ml of sodium carbonate solution, mixed and tightly stoppered. 1 ml of the same filtrate was also transferred to a sugar tube containing 1 ml saturated picric acid, placed in the boiling water bath for 5 min, cooled and 1 ml of sodium carbonate solution was added to it (this was not done in case of milk). Both of the tubes (only the first one in case of milk) were then placed in boiling water bath for 20 min along with a blank for each of the tubes containing 2 ml saturated picric acid and 1 ml carbonate for the first and 1 ml of each reagent for the second tube. Distilled water was added to all the tubes to make the volume to 20 ml in each case (not in case of milk). The optical density

of the developed colour was measured at 530 nm in a photoelectric colorimeter using green filter within 20 min of the development of colour.

3.3.6.3 Calculation

$$\text{Lactose percentage (for milk)} = \frac{5X}{W} = \frac{5}{W} \times \left[\frac{(Y + 0.4)}{189.9}\right]$$

Where, W = Weight of milk taken for the test

X = mg of lactose in 2 ml of filtrate

Y = Photoelectric colorimeter reading

$$\text{Lactose percentage (for sample)} = \frac{100\ X_1}{W}$$

$$= \frac{100}{W}\left[\frac{(Y_1 - Y_2 + 0.4)}{189.9}\right]$$

$$\text{And, Sucrose percentage (for sample)} = \frac{200\ X_2}{W}$$

$$= \frac{200}{W}\left[\frac{Y_2 - \left\{\frac{(Y_1 - Y_3)}{246.6}\right\} - 8.9}{246.6}\right]$$

Where, W = Weight of sample taken for the test

X_1 = mg lactose in 2 ml of filtrate

X_2 = mg sucrose in 1 ml of filtrate

Y_1 = Colorimeter reading before inversion of sucrose

Y_2 = Colorimeter reading after inversion of sucrose

Y_3 = Portion of Y_1 reading due to the action of uninverted sugar = $[\{(Y_2-Y_1)/2\} + 25.4/20.2$

3.3.7 Free fatty acid

The free fatty acid content was determined by the method described in BIS (1966).

3.3.7.1 Reagents

i) Ethyl alcohol (95% v/v, specific gravity 0.816)

ii) N/10 sodium hydroxide solution.

iii) Phenolpthalein indicator (0.1% solution in 95% ethyl alcohol)

3.3.7.2 Procedure

Accurately weighed 10 gm of sample was taken in a clean, dry conical flask and 50 ml of neutralized 95% alcohol was added to it. The contents were boiled in a boiling water bath followed by thorough shaking. The mixture was then titrated against 0.1 (N) sodium hydroxide solution, using phenolpthalein indicator, until faint pink colour persists.

3.3.7.3 Calculation

$$\text{Percentage of free fatty acid (by weight)} = 2.82 \times \frac{T}{W}$$

Where, T = Volume in ml of 0.1 (N) NaOH solution required to neutralize the oleic acid in the given sample.

W = Weight in gm of sample taken for the test.

3.3.8 Peroxide value

The peroxide value was determined by the method described in BIS (1981).

3.3.8.1 Reagents

i) Solvent mixture (a mixture of glacial acetic acid and chloroform in the ratio 2 : 1)

ii) Potassium iodide solution (5% w/v in water)

iii) Potassium iodide powder

iv) Sodium thiosulphate solution–0.002 (N)

v) Starch indicator solution (1% w/v in water)

3.3.8.2 Procedure

Accurately weighed 1 gm of sample was taken in a conical flask containing 1 gm powdered potassium iodide. Then 20 ml of solvent mixture was added to it and the contents were allowed to boil on a boiling water bath for 30 seconds, removed and cooled. 20 ml of 5% potassium iodide solution was then added to the flask and titrated against 0.002 (N) sodium thiosulphate solution, using starch as indicator, until straw colour changed to colourless.

3.3.8.3 Calculation

$$\text{Peroxide} = \frac{T}{W}$$

Where, T = Volume in ml of 0.002 (N) sodium thiosulphate solution.

W = Weight in gm of sample taken for the test.

3.3.9 Hydroxymethyl furfural (HMF) value

The total HMF content in the sample was estimated according to the method suggested by Keeney and Bassette (1959).

3.3.9.1 Reagents

i) TCA solution (40% w/v)

ii) Oxalic acid–0.3 (N)

iii) TBA solution–0.05 (M)

3.3.9.2 Procedure

Accurately weighed 1 gm of sample was taken in a 50 ml test tube. Then 5 ml of 0.3 (N) oxalic acid was added, covered and placed in a boiling water bath for 1 hour, after which it was removed and cooled to room temperature. 5 ml of 40% TCA was then added to the tube, mixed and filtered through Whatman No. 42 filter paper. 4 ml of filtrate was transferred to another test tube and 1 ml of 0.05 (M) aqueous solution of TBA was added to it. The tube was placed in a water bath maintained at 40°C for 30 min, removed and cooled to room temperature. The absorbance was measured at 420 nm wave length using a blue filter against a blank prepared in the same way substituting water for sample.

3.3.9.3 Calculation

Total HMF (Free + Potential) =

(Absorbance–0.055) × 87.5 micromoles/100 gm.

3.3.10 Tyrosine value

The tyrosine value was estimated according to the method of Jullis (1973).

3.3.10.1 Reagents

i) Folin–Ciocalteu reagent–0.67 (N)

ii) Solution A(1% copper sulphate in 2% sodium potassium tartarate)

iii) Solution B(2% sodium carbonate in 0.1 (N) sodium hydroxide solution. One part of solution A and 49 parts of solution B were mixed to prepare 50 ml of alkali reagent)

iv) TCA solution (24% w/v)

v) L (-) Tyrosine

3.3.10.2 Procedure

Accurately weighed 1 gm of sample was taken in a test tube and 5 ml of 24% TCA solution was added to it and shaken vigorously. The mixture was then kept aside for 15 min, filtered and 0.2 ml of the filtrate was transferred to another test tube containing 4 ml of alkali reagents. After 15 min, 0.2 ml of 0.67 (N) Folin–Ciocalteu reagent was added rapidly to the mixture, mixed immediately and kept for 45 min for colour development. The developed colour was measured at 650 nm in a photoelectric colorimeter using a red filter and compared with the standard tyrosine curve prepared from L (-) tyrosine solution.

3.3.11 Titratable acidity

The titratable acidity was estimated by the method described in BIS (1981).

3.3.11.1 Reagents

i) 0.1 (N) sodium hydroxide solution.

ii) Phenolpthalein indicator (0.1% solution in 95% ethyl alcohol).

3.3.11.2 Procedure

Accurately weighed 2 gm of sample was taken in a conical flask and 20 ml of hot distilled water was added to it, stirred to obtain a smooth liquid and cooled to room temperature. 2–3 drops of phenolpthalein indicator solution was added followed by 0.1(N)

sodium hydroxide solution, introduced drop by drop from the burette until the colour changed to pink.

3.3.11.3 Calculation

$$\text{Titratable Acidity} = \frac{9\,VN}{W} \text{ (as \% lactic acid, by weight)}$$

Where, V = Volume in ml of 0.1(N) NaOH solution required for titration.

N = Strength in normality of the NaOH used

W = Weight in gm of sample taken for the test.

3.4 Microbiological Analysis

3.4.1 Preparation of samples for analysis

The samples of *peda* was aseptically withdrawn in a sterilized chamber of UV radiation. 11 gm of *peda* sample was weighed and transferred to a sterile mortar and ground with a pestle to obtain a homogenous mass. The sample was then dissolved in sterile 99 ml of dilution blank (1% of sodium chloride solution) and mixed thoroughly. Further dilutions were prepared using 9 ml dilution blank according to the requirements for counting the different types of microorganisms.

3.4.2 Standard plate count

Standard plate count of *peda* samples was done according to the method of BIS (1960).

3.4.2.1 Procedure

After appropriate dilutions, 1 ml diluted sample was transferred into sterilized petridish in duplicate. Then 15 ml of melted and cooled

nutrient agar was poured into each petridish and allowed to solidify. On solidification, petridishes were incubated at 37 ± 1°C for 48 h and results were expressed as colony forming units (cfu) per gm of sample.

3.4.2.2 Composition of nutrient agar (HI-MEDIA)

Ingredients	Quantity (gm /litre)
Peptone	5.0
Yeast extract	1.5
Beef extract	1.5
Sodium chloride	5.0
Agar (Bacteriological Grade)	15.0

Final pH (at 25°C) - 7.4 ± 0.2

3.4.3 Coliform count

Coliform count of *peda* samples was performed as per methods described by BIS (1960).

3.4.3.1 Procedure

After appropriate dilution, 1 ml of diluted sample was transferred into sterile petridish in duplicate. Then about 15 ml of melted and cooled violet red bile agar was poured into each petridish and was allowed to solidify. On solidification, another layer of 10–12 ml of the same media was poured in each petridish. On solidification of this second layer, the petridishes were incubated at 37 ± 1°C for 24–48 h and the results were expressed as colony forming units per gm of sample.

3.4.3.2 Composition of violet red bile agar (HI-MEDIA)

Ingredients	Quantity (gm /litre)
Yeast extract	3.0
Peptone	7.0
Bile salt no. 3	1.5
Lactose	10.0
Sodium chloride	5.0
Agar (Bacteriological Grade)	15.0
Neutral Red	0.03
Crystal violet	0.002

Final pH–7.5 ± 0.02

3.4.4 Yeast and mold count

Yeast and mold count was done according to the method of BIS (1960).

3.4.4.1 Procedure

After appropriate dilution, 1 ml of diluted sample was transferred into petridish in duplicate. Then 15 ml of melted and cooled potato dextrose agar was poured in each petridish and the pH was adjusted to 3.5 using sterile 10% tartaric acid and the agar was then allowed to solidify. On solidification, the petridishes were incubated at 22°C for 3–5 days. The developed yeast and mold colonies were counted and results were expressed as yeast and mold count per gm of the sample.

3.4.4.2 Composition of potato dextrose agar (HI-MEDIA)

Ingredients	Quantity (gm /litre)
Potato–in fusion form	200.0
Dextrose	20.0
Agar (Bacteriological Grade)	15.0

Final pH–5.6 ± 0.2

3.5 Sensory Evaluation

Peda prepared under different experimental conditions and those collected from the market were judged independently by a selected panel of five experienced judges. The products were evaluated in respect of flavour, body and texture, colour and appearance, sweetness and overall acceptability using a 9–point hedonic scale evaluation card specially prepared for this purpose (BIS, 1971).

Hedonic Scale

Score	Numerical	Score subjective
1	9	Extremely liked
2	8	Liked very much
3	7	Liked moderately
4	6	Liked slightly
5	5	Neither liked nor disliked
6	4	Disliked slightly
7	3	Disliked moderately
8	2	Disliked very much
9	1	Disliked extremely

3.6 Statistical Analysis

The data were statistically analysed by the method described by Snedecor and Cochran (1968).

3.7 Electrophoretic Study

Electrophoresis of milk casein and *peda* proteins were carried out according to the method of Kolar and Brunner (1970) excepting that 2 drops of 2-mercaptoethanol was added while preparing the sample.

a) Gel buffer

Boric acid buffer of pH 8.6 was used. It was prepared by dissolving 2.1 gm sodium hydroxide and 13.0 gm boric acid in distilled water and the volume was made upto 1 litre. This buffer was used for both gel preparation and electrophoresis run.

b) Acrylamide solution

47.5 gm acrylamide and 2.5 gm N, N-Methylene bis-Acrylamide were mixed together. 8 gm of the above mixture and 27 gm urea were then dissolved in the boric acid buffer (pH 8.6) and 0.1 ml N, N, N, N Tetramethylethylendiamine (Temed) was then added and the volume was made to 100 ml by the same buffer.

c) Staining and destaining solution

Staining solution was prepared by mixing 2 gm of amido black, 50 ml of glacial acetic acid, 250 ml of methanol and 250 ml of distilled water. For proper mixing magnetic stirrer was used for half an hour and the mixture was filtered through Whatman No. 42 filter paper.

The destaining solution was 7% glacial acetic acid in distilled water.

d) Gel

About 30 ml of bis-acrylamide solution was taken in a beaker and 1 ml of freshly prepared ammonium per sulphate (100 mg in 15 ml of distilled water) was added to it. After proper mixing, the solution was poured in between the two slabs having 1.5 mm gap. A comb of similar thickness was inserted into the solution so that separate lanes were formed for sample application. Then it was left sometimes for complete polymerization of the gel.

e) Sample

40 ml of milk sample was taken in a 100 ml volumetric flask and 40 ml distilled water was added to it. The content was warmed to 35°C. The casein was prepared using 10% acetic acid (pH 4.6). It was then filtered through Whatman No. 42 filter paper and the coagulam was thoroughly washed with water and a solvent mixture of petroleum ether and chloroform (1 : 1) and then dried. Similar procedure was done with *peda* samples.

20 mg of each dried sample of protein was dissolved in 10 ml of sucrose solution (15% in borate buffer) followed by the addition of 2 drops of 2-mercaptoethanol and 1 drop of bromophenol blue solution.

f) Sample application

After the prerun 20 µl of protein solution was applied to the gels using microsyringe.

g) Electrophoretic procedure

The slab containing the gel was fitted into the electrophoretic apparatus and subjected to pre-electrophoresis run to remove all charged particles other than proteins and acetate ions @ 2 mA per sample. Electrophoresis was performed at a constant current of 4 mA

per sample for about 3 h. Electrophoresis was stopped when the tracking dye was about to leave the slab gel.

h) Staining and destaining of the gel

The gel was removed from the slab and was stained for about 10 min by the staining solution in a petridish and then was destained with the destaining solution by several washing till the separated components became prominent.

3.8 Scanning Electron Microscopy Study

Scanning electron microscopy study was carried out by the method laid out by Kalab *et al.* (1988) with certain modifications.

a) Sampling

Peda samples were taken from beneath the surface and cut into small pieces of 1 x 1 x5 mm^3 size with sharp blades.

b) Fixing

To stabilized the protein matrix and to facilitate the removal of soluble constituents the samples were fixed in 2.8% glutaraldehyde solution for 2.5h followed by thorough washing in phosphate buffer (pH 7.3).

This was followed by post-fixation with 2% OsO_4 solution for 2h and washing with 0.135 (M) phosphate buffer. This step was required only for fatty samples to stabilize and fix fat. If milk fat globule membrane is ruptured only unsaturated fats have been reported to be fixed. This step was thus omitted for defatted samples.

c) Dehydration

For removal of the water the aqueous phase was replaced with a cryoprotective media of absolute alcohol by dehydrating with a series

of ethyl alcohol, viz., 50%, 60%, 70%, 80%, 90% and 100% for 30 min each.

c) Defatting

Generally this is followed by defatting with chloroform for 15 min and then returned to absolute alcohol. But for some samples this step was omitted since samples were examined for fat also.

d) Freeze fracturing

The samples were then put into a freeze fracturing tube and fractured with a sharp knife under liquid nitrogen to expose the undamaged interior.

It was then taken back to absolute ethanol of critical point drying at 30°C slightly below atmospheric pressure. Starch, if present may get shrunken here.

e) Mounting

The samples were then fixed on a metal slab with adhesive and dried.

f) Gold coating

The samples were then taken in an Ion coater (model GIKOIB-3, Japan) to coat the sample with a uniform coating (for avoiding artifacts) of gold for rendering the specimen electrically conductive under 0.1 torr vacuum for 5 min. at an ion current of 6 mV. The thickness of gold coating was ≥ 20mm.

g) Scanning electron microscopy

The gold coated samples were then taken under an electron microscope (Hitachi model No-S-405, Japan) and scanned at 20 kV at different magnification for the desired microstructure.

h) Photography

When the desired field has achieved a photographic reversal was done like common photographs and the micrographs were taken on 35 mm film for storage of information and detailed study.

3.9 Measurement of Rheological properties of *peda*

In order to evaluate the rheological properties of *peda* samples in terms of hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness, a Texture Analyser model No. TAHDi (manufactured by Stable Micro System, U.K.) fitted with a 250 kg load-cell was used under two bites linear compression. A cross head pretest speed of 2 mm/sec., test speed of 5 mm/sec; post test speed of 5 mm/sec. and 5 sec. interval between two successive bites were employed for 50% compression / penetration of the original height of samples of *peda*. The compression / penetration study was done using 75 mm diameter compression platen (Probe–P_{75}).

The Texture Analyser having a separate software (Texture Expert) for its operation was run under Windows environments (Microsoft Windows–98). A microprocessor (IBM, Pentium II) has been coupled with Texture Analyser to run the Texture Expert programme and to obtain the various rheological parameters. From the typical Force (gm)–Time (sec) curve thus obtained for double cycled compression the texture profile parameters were measured.

Chapter - IV

Results and Discussion

Results and Discussion

Peda, an indigenous heat desiccated milk product is prepared from cow milk, buffalo milk or a combination thereof. The present investigation was undertaken to evaluate the chemical, microbiological and sensory qualities, the rheological properties and the sub-microstructure of *peda* samples available in Kolkata and its surrounding areas and compared them with those of the products manufactured in the laboratory from standardized milk under controlled conditions. The electrophoretic properties of the *peda* casein were also studied and compared the result with milk casein. Further studies were also undertaken to examine the physico-chemical and microbiological changes that occurred in the laboratory made *peda* with and without preservatives and packaged in different packaging materials, during their storage at 30 ± 1°C in incubator and at 7 ± 1°C in refrigerator respectively. The results obtained in the present investigation had been tabulated and interpreted with the help of suitable illustrations under different headings.

4.1 Standardization of Milk for Manufacture of *Peda*

Since milk has to undergo a long process for production of *peda*, in order to standardize the milk for manufacture of *peda* an innumerable permutation and combination of different parameters are possible. In the present investigation, different combination of fat in milk and sugar percentage were tried. Amongst them the best quality product judged on the basis of organoleptic quality was taken for further study.

4.1.1 Effect of Fat Level in Milk

Milk standardized to 4.0 to 6.0% fat was found satisfactory for the manufacture of *peda* (Ranganadham and Rajorhia, 1989; Sapre and Deodhar, 1991; Ranganadham and Rajorhia, 1993 and Kumar and Pal, 1994). In the present study cow milk was standardized to 3.5, 4.0 and 4.5% fat level by the addition of skim milk in order to investigate their desirability. The results are shown in Table 4.1.

Fat has been found to contribute softness and smoothness in the product. It also influence the flavour of the product to a large extent. On the other hand, the fat content in milk has been found to contribute little influence towards the variation in the yield of *peda* and the recovery of milk solids, both of which has been found to increase with the increase in fat level to small extent in general. *Peda* made from milk with 4.5% fat has been reported to be the best among all by the judges because of their smooth body and rich flavour.

4.1.2 Effect of Sugar Content in Peda

Different sugar percentage in the manufacture of *peda* were tried by several workers. Patel and Gandhi (1980) and Ray *et al.* (1999) use 9% sugar on the basis of milk. However, 7.3% sugar was used by rural milk processing centres in Kutch district of Gujarat. In the present investigation trials were undertaken using 7.0, 8.0 and 9.0% sugar on the basis of milk. The results obtained are shown in the Table 4.1.

The results presented in Table 4.1 revealed that the total solids content in the *peda* samples were increasing along with the increase in sugar content. Sugar contributes towards the body, texture and sweetness of the product. A brown tinge of colour was evident with the highest concentration of sugar. *Peda* prepared with 8.0 % sugar on the basis of milk was preferred by most of the judges.

Table 4.1 : Effect of fat in milk and sugar content on the sensory quality of *peda*

Fat (%)	Sugar (%)	Attributes					
		Total solids (%)	Flavour (Max. 9)	Colour & appearance (Max. 9)	Body & texture (Max. 9)	Sweetness (Max. 9)	Overall acceptability (Max. 9)
3.5	7.0	78.82 ± 0.56	6.9 ± 0.1	6.8 ± 0.09	6.8 ± 0.07	7.6 ± 0.08	7.2 ± 0.10
	8.0	80.92 ± 0.77	7.0 ± 0.06	7.0 ± 0.08	6.9 ± 0.1	7.8 ± 0.04	7.3 ± 0.09
	9.0	84.04 ± 0.48	6.9 ± 0.1	7.2 ± 0.10	7.0 ± 0.1	7.6 ± 0.07	7.3 ± 0.08
4.0	7.0	79.83 ± 0.38	7.1 ± 0.08	6.9 ± 0.04	7.0 ± 0.11	7.6 ± 0.06	7.4 ± 0.09
	8.0	81.07 ± 0.7	7.2 ± 0.08	7.1 ± 0.06	7.2 ± 0.09	7.9 ± 0.04	7.6 ± 0.08
	9.0	84.34 ± 0.54	7.2 ± 0.07	7.2 ± 0.09	7.3 ± 0.08	7.7 ± 0.07	7.5 ± 0.08
4.5	7.0	80.17 ± 0.19	7.3 ± 0.08	7.2 ± 0.08	7.4 ± 0.06	7.7 ± 0.04	7.7 ± 0.07
	8.0	82.64 ± 0.3	7.4 ± 0.09	7.4 ± 0.07	7.5 ± 0.04	8.0 ± 0.04	7.9 ± 0.06
	9.0	84.99 ± 0.3	7.4 ± 0.06	7.3 ± 0.1	7.4 ± 0.08	7.7 ± 0.8	7.5 ± 0.07

Average of 5 trials ± Standard deviation

Table 4.2 : ANOVA on sensory characteristics of *peda* for the effect of fat level in milk and sugar content used

Source of variation	df	Flavour		Colour & appearance		Body & texture		Sweetness		Overall acceptability	
		MS	F	MS	F	MS	F	MS	F	MS	F
Fat	2	0.363	20.872**	0.058	3.34	0.188	7.257**	0.01	0.491	0.288	20.447**
Sugar	2	0.014	0.830	0.307	17.638**	0.099	3.794*	0.347	16.439**	0.103	7.342**
Fat × sugar	4	0.004	0.255	0.053	3.053*	0.029	1.129	0.047	2.228	0.042	2.984*
Error	18	0.017	-	0.017	-	0.026	-	0.021	-	0.014	-

* Significant at 5 % ($P < 0.05$) level

** Highly significant at 1 % ($P < 0.01$) level

The statistical analysis of the sensory scores of *peda* on the effect of fat in milk and sugar content was presented in Table 4.2. For fat content in milk the variations in flavour, body and texture and overall acceptance scores were found highly significant at 1 % ($P < 0.01$), thus confirming a strong contribution of variation in fat levels towards the final desirability of *peda*.

For sugar content, the sensory scores was also analysed for variance (Table 4.2), which revealed that the variations in colour and appearance, sweetness and overall acceptability scores were highly significant at 1 % ($P < 0.01$) level and the body and texture scores was significant at 5 % ($P < 0.05$) level.

The sensory scores was also statistically analysed for the effect of fat and sugar interaction and depicted in the Table 4.2 which showed that the variations in colour and appearance and overall acceptance scores were significant at 5 % ($P < 0.05$) level.

The sensory parameters having significant difference were then tested for C.D. (critical difference) values for determining the

variations of sensory scores amongst different levels of fat in milk and sugar content used during manufacture of *peda*.

Table 4.3 : C.D. amongst means of sensory scores which varied due to change in fat level in milk

Sensory attributes	C.D. value	Mean score		
		F_1	F_2	F_3
Flavour	0.119	6.93^a	7.17^b	7.37^c
Body & texture	0.147	6.90^a	7.17^b	7.43^c
Overall acceptability	0.108	7.27^a	7.50^b	7.67^c

Mean scores having different superscripts (a, b, c) in a row differ significantly at 5 % ($P < 0.05$) level.

F_1 = 3.5% fat in milk

F_2 = 4.0% fat in milk

F_3 = 4.5% fat in milk

Table 4.4 : C.D. amongst means of sensory scores which varied due to change in sugar content used

Sensory attributes	C.D. value	Mean score		
		S_1	S_2	S_3
Colour & appearance	0.119	6.97^a	7.17^b	7.23^b
Body & texture	0.147	7.07^a	7.30^b	7.23^b
Sweetness	0.133	7.63^a	7.90^b	7.67^a
Overall acceptability	0.108	7.40^a	7.58^b	7.37^a

Mean scores having different superscripts (a, b, c) in a row differ significantly at 5 % ($P < 0.05$) level.

S_1 = 7% sugar on the basis of milk

S_2 = 8% sugar on the basis of milk

S_3 = 9% sugar on the basis of milk

To find out the variations among different levels of fat in milk and sugar content used during preparation of *peda*, the sensory scores were statistically analysed and represented in Table 4.3 and 4.4 respectively. The variations in the different fat levels in milk were significant at 5 % ($P < 0.05$) level. Variations were also observed in different sugar levels. Thus on the basis of sensory scores 4.5% fat level in milk and 8 % sugar on the basis of milk were taken for the preparation of *peda* in the laboratory for further study.

4.2 Studies on the Changes Occurring During Transformation of Milk to *Peda*

The basic raw material used for the manufacture of *peda* is milk which is dynamically a balanced mixture of fat, protein, carbohydrate, ash and water co-existing as emulsions, colloidal suspensions and true solution. *Peda* is prepared by alteration of these relationships by heat coagulation whereby some of the components are partially removed and some other changes their forms and the physico-chemical characteristics also changed by the addition of sugar. The composition of milk thus changes a lot from its native array. Investigations were undertaken to evaluate the chemical composition of milk and *peda*.

The chemical composition of milk used for the manufacture of *peda* in the laboratory has been given in the Table 4.5.

Fig. 4.1 : *Different types of peda*

Table 4.5 : Chemical composition of milk

Parameters	Range	Average ± S.D.
Fat (%)	4.49 – 4.51	4.50 ± 0.002
SNF (%)	8.48 – 8.54	8.52 ± 0.01
Protein (%)	3.42 – 3.73	3.60 ± 0.08
Lactose (%)	4.68 – 4.92	4.81 ± 0.06
Ash (%)	0.67 – 0.75	0.71 ± 0.03
Titratable acidity (% lactic acid)	0.130 – 0.153	0.142 ± 0.04

S.D. = Standard Deviation
Average of ten trials

In the raw milk used for the manufacture of *peda* in the laboratory, the fat, SNF, protein, lactose, and ash percentage were found to be 4.50 ± 0.002, 8.52 ± 0.01, 3.60 ± 0.08, 4.81 ± 0.06 and 0.71 ± 0.03 respectively. The average titratable acidity was found to be 0.142 ± 0.04 % in terms of lactic acid.

Goyal and Srinivasan (1989) standardized cow milk to 4.5 % fat and 8.5 % SNF. Milk had also been standardized to 3.0, 4.0 and 5.0 % fat and 8.5 % SNF (Ranganadham and Rajorhia, 1993). However, Sapre and Deodhar (1991) used 5 % fat and Ranganadham and Rajorhia (1981) used 4.0 % fat in milk for the manufacture of khoa.

4.3 Chemical Quality of Laboratory Made and Market *Peda* Samples

4.3.1 Chemical quality of laboratory made peda

The chemical composition of *peda* samples prepared in the laboratory from standardized milk are discussed in the Table 4.6.

The average total solids (T.S.) content in the laboratory made *peda* samples were 83.83 ± 0.45 % (range 82.95 to 84.36 %). The results were in close proximity with the findings of Ray P.R. (1998) who reported the average T.S. content of laboratory cow milk *peda* was 80.75 % and that of buffalo milk *peda* samples was 85.35 %. However, Vijayakhader and Patel (1983) reported much higher value (89.9 % for cow milk *peda*, 91.4 % for buffalo milk *peda* and 90 % for mixed milk *peda*). These variations may be attributed to the different levels of total solids in the initial milk used for the preparation of *peda*.

The fat content of the laboratory made *peda* samples varied from 24.40 to 25.16 % with the average value of 24.90 ± 0.28 %. Ray, P.R. (1998) reported almost similar value for cow milk *peda*. However, Vijayakhader and Patel (1983) reported a lower fat content in the laboratory made *peda* samples. These variations were in quite conformity with the chemical composition of milk used for *peda* manufacture.

Similarly, the average protein, lactose and ash contents of the *peda* samples prepared in the laboratory were found to be 12.98 ± 0.31 %, 15.95 ± 0.18 % and 2.49 ± 0.02 % respectively. Vijayakhader and Patel (1983) and Ray, P.R. (1998), reported more or less similar values for the laboratory made *peda* samples.

The average sucrose content of the final product was 25.77 ± 0.09 % (range 25.36 to 26.10 %). The small range of variation in sucrose level was due to the fact that sugar was added to milk at a fixed rate.

The average titratable acidity of the milk increased from 0.142 % to 0.58 % on its conversion to *peda*. This can be attributed to a six to seven fold increase in total solids in *peda* as compared to milk.

Table 4.6 : Chemical quality of laboratory made *peda* samples

Parameters	Range	Average ± S.D.
Total solids (%)	82.95 – 84.36	83.83 ± 0.45
Fat (%)	24.40 – 25.16	24.90 ± 0.28
Protein (%)	12.56 – 13.37	12.98 ± 0.31
Lactose (%)	15.40 – 16.58	15.95 ± 0.18
Sucrose (%)	25.36 – 26.10	25.77 ± 0.09
Ash (%)	2.45 – 2.55	2.49 ± 0.02
Titratable acidity (% Lactic acid)	0.54 – 0.62	0.58 ± 0.03
Free fat (% of total fat)	74.92 – 76.20	75.43 ± 0.62
Free fatty acid (% oleic acid)	0.054 – 0.070	0.062 ± 0.005
Peroxide value (m. eq. Of O_2/kg fat)	1.10 – 1.12	1.11 ± 0.007
HMF value (Micromoles/100 gm)	0.364 – 0.385	0.376 ± 0.007

S.D. = Standard Deviation

Average of five trials

The average free fat, free fatty acid and peroxide value of the laboratory made *peda* samples were found to be 75.43 ± 0.62 % of total fat, 0.062 ± 0.005 % oleic acid and 1.11 ± 0.008 m. eq. of O_2/kg fat respectively. The average HMF value was found to be 0.376 ± 0.007 micromoles/100 gm. These observations agreed with the findings of Ray, P.R. (1998).

4.3.2 Chemical quality of market peda samples

The overall chemical composition of *peda* samples collected from the market are presented in Table 4.7.

The total solids content of the market *peda* samples showed a wide variation ranging from 83.37 to 89.10 % with the average value of 86.45 ± 1.62 %. The results were in close agreement with those obtained by Rastogi *et al.*, 1966 who reported the value to be 81.8 to 88.6 % and those (79.70 to 88.74 %) obtained by Garg and Mandokhot (1984) in Hissar and 81.5 to 87.7 % by Roy *et al.* (1998) in Greater Calcutta. But the result showed difference with the findings of Ghodekar *et al.* (1974) who found the average total solids content to be 81.20 % and Sharma and Zariwala (1978) who observed much higher value (95.8 %) of total solids content in the market *peda* samples. However, all the *peda* samples conformed to the standards prescribed by ISI (1980) for khoa in respect of T.S. content (60 % min).

The total fat content of the market *peda* samples varied between 7.73 and 18.01 % with the average value of 11.30 ± 2.88 %, which was almost in close proximity with the findings of Roy *et al.* (1998) who reported 9.01 to 17.5 % fat content in market *peda* samples. However, the result showed variation with those (7.0 to 25.0 %) obtained by Sharma and Zariwala (1975). Grag and Mandokhot (1984) found higher fat content (28.54 %) in the market *peda* samples. Out of 25 samples analysed, none conformed to the ISI standard of khoa in respect of fat content (20 % min).

Protein content in market *peda* samples ranged from 4.53 to 14.23 % with the average value of 9.64 ± 2.95 %. This shows similarity with the results obtained by Roy *et al.*, 1998 (from 4.05 to 15.7 %). However, Rastogi *et al.* (1966) obtained much higher value (from 17.01 to 18.7 %) and Sharma and Zariwala (1978) reported much lower value as low as 1.4 %.

Table 4.7 : Chemical quality of *peda* samples collected from market

Parameters	Range	Average ± S.D.
Total solids (%)	83.37 – 89.10	86.45 ± 1.62
Fat (%)	7.73 – 18.01	11.30 ± 2.88
Protein (%)	4.53 – 14.23	9.64 ± 2.95
Lactose (%)	8.05 – 17.81	1303 ± 2.83
Sucrose (%)	41.66 – 59.16	51.77 ± 5.41
Ash (%)	2.14 – 2.98	2.50 ± 0.26
Titratable acidity (% Lactic acid)	0.38 – 0.81	0.62 ± 0.13
Free fat (% of total fat)	43.53 – 67.12	57.62 ± 6.38
Free fatty acid (% oleic acid)	0.062 – 0.087	0.075 ± 0.006
Peroxide value (m. eq. Of O_2/kg fat)	1.11 – 1.17	1.14 ± 0.02
HMF value (Micromoles/ 100 gm)	0.476 – 0.759	0.620 ± 0.09

S.D. = Standard Deviation

Average of twenty five trials

The average lactose content of the market *peda* samples were found to be 13.03 ± 2.83 % (range 8.05 to 17.81 %). The results are in tune with the result reported by Rastogi *et al.* (1966) and Roy *et al.* (1998). However, Ghodekar *et al.* (1974) and Vijayakhader and Patel (1983) found lower lactose content in the market *peda* samples. The ash content was found to have the average value of 2.50 ± 0.26 %.

The sucrose content of the market *peda* samples showed a wide range of variation from 41.66 to 59.16 % with the average value of 51.77 ± 5.41 %. The data on the sucrose content showed similarity with those (52.1 to 60.5 %) obtained by Ghodekar *et al.* (1974) and 44.34 to 50.7 % by Roy *et al.* (1998). Whereas Rastogi *et al.* (1966) reported lower average value (45.46 %). Sharma and Zariwala (1978) observed wide range of variation (13.3 to 61.8 %) of sucrose content in the market *peda* samples. These wide variations in the sucrose content of market *peda* samples may be due to the consumers acceptance in different places of India.

The titratable acidity of the market *peda* samples varied widely from 0.38 to 0.81 % in terms of lactic acid with the average value of 0.62 ± 0.13 %. Few *peda* samples (6) were found to have low acidity values which may be due to the use of additives and neutralized milk for *peda* manufacture. The higher average value in the present investigation seemed to be due to the inferior quality of milk used and unhygienic processing and storage.

The average free fat and free fatty acid content of the market *peda* samples were found to be 57.62 ± 6.38 % of total fat and 0.075 ± 0.006 % oleic acid. The higher free fatty acid content may be attributed primarily to the growth of lipolytic bacteria.

The peroxide value of market *peda* samples varied from 1.11 to 1.17 m. eq. of O_2/kg fat. The HMF values of market *peda* samples were found to vary from 0.476 to 0.759 micromoles/100 gm with the average value of 0.620 ± 0.09. Overheating during manufacture of *peda* might have contributed to higher browning observed in the market samples. Gothwal and Bhavadasan (1992) also found higher HMF value (0.519) in the market khoa samples.

Table 4.8 : t-Test for chemical quality of laboratory made and market *peda* samples

	df	Observed t-value						t critical
		Total solids	Fat	Protein	Lactose	Sucrose	TA	
Laboratory made	4	4.995**	8.874**	2.369*	1.792*	10.211**	1.445	1.734
Market	24	4.995**	8.874**	2.369*	1.792	10.211**	1.445	2.407

* Significant at 5 % (P < 0.05) level

** Highly significant at 1 % (P < 0.01) level

4.4 Microbiological Quality of Laboratory made and Market *Peda* Samples

4.4.1 Microbiological quality of laboratory made peda samples

Though milk has been subjected to severe heat treatment during manufacture of *peda*, the thermoduric organisms survive the high heat treatment. Also the yeast and molds may come from the surrounding atmosphere. The microbiological quality of laboratory made *peda* samples prepared from standardized milk were given in Table 4.9.

The standard plate count of the laboratory made *peda* samples ranged between 7×10^3 and 16×10^3 cfu/gm with the average value of 11.6×10^3 cfu/gm of the sample. The yeast and mold count of the samples varied from 30 to 90 cfu/gm (average 58 cfu/gm).

No coliform was found in any of the samples of *peda* prepared in the laboratory from standardized milk under controlled conditions.

Since no published literature of the laboratory made samples are available, the results can not be compared with any previous result.

4.4.2 Microbiological quality of market peda samples

The microbiological quality of the market *peda* samples were shown in the Table 4.9.

The standard plate count (SPC) of the *peda* samples collected from the market varied from 1.74×10^5 to 43×10^5 cfu/gm of the sample with the average value of 23.6×10^5 cfu/gm. The result showed similarity with the results obtained by Ghodekar *et al.* (1974, 1980) who reported the result varied from 2×10^3 to 3×10^5 cfu/gm and those (5×10^3 to 2.67×10^5 cfu/gm) by Singh *et al.* (1975b) and 23.3×10^5 to 639.3×10^5 cfu/gm by Roy *et al.* (1998). However, the result (6×10^2 to 1×10^7 cfu/gm) reported by Kamat and Sulebele (1974) in Bombay showed a wide range of variation of SPC in the market *peda* samples.

The average coliform count of the market *peda* samples was 120 cfu/gm of the sample (range 20 to 190 cfu/gm). The results were in close proximity with those (0 to 460 cfu/gm) reported by Dwarkanath and Srikanta (1977) and 10 to 213 cfu/gm by Roy *et al.* (1998). But much higher value was obtained by Sharma *et al.* (1969) who reported the value to be 2×10^4 to 7.68×10^6 cfu/gm; Singh *et al.* (1975a) who obtained the count to be 1.89×10^5 cfu/gm and Garg, S.R. (1981) who found the coliform count of the market *peda* samples to be 1.2×10^3 cfu/gm.

The yeast and mold count of the *peda* samples collected from the market varied between 1.5×10^2 and 6×10^2 cfu/gm with the average value of 3.75×10^2 cfu/gm of the sample. The observation agreed with the findings (450 to 610 cfu/gm) of Singh *et al.* (1975a) and 300 to 700 cfu/gm of Roy *et al.* (1998). However, a much higher value was obtained by some other workers (Sharma *et al.*, 1964; Singh

et al., 1975, 1975b; Dwarkanath and Srikanta, 1977 and Garg, S.R., 1981).

Table 4.9 : Microbiological quality of laboratory made and market *peda* samples

Attributes	Laboratory made *Peda**	Market *Peda***
Standard plate count (cfu/gm)	$7 \times 10^3 - 16 \times 10^3$ (11.6×10^3)	$1.74 \times 10^5 - 43 \times 10^5$ (23.6×10^5)
Coliform count (cfu/gm)	Nil	20 – 190 (120)
Yeast and mold count (cfu/gm)	30 – 90 (58)	$1.5 \times 10^2 - 6 \times 10^2$ (3.75×10^2)

* Five samples analysed

** Twenty five samples analysed

Figures in the parenthesis represent the average value.

4.5 Sensory Qualities of Laboratory made and Market *Peda* Samples

4.5.1 Sensory quality of laboratory made peda samples

Table 4.10 depicts that the deviations from the average values of the distinguishing sensory attributes for laboratory made *peda* samples were very low. The deviation was maximum 0.18. This suggested that the *peda* samples prepared in the laboratory were more or less similar in organoleptic quality.

The average overall acceptability score of laboratory made *peda* samples was 7.8 ± 0.07. Hence it can be said that the laboratory made *peda* samples were liked moderately by the judges.

4.5.2 Sensory quality of market peda samples

The results relating to the sensory evaluation of market *peda* samples are presented in the Table 4.10.

The average score of overall acceptability (7.4 ± 0.8) implies that the market *peda* samples were liked moderately by the judges. The deviation from the average score of overall acceptability (0.8) and each sensory attribute except sweetness (0.16) further implies that the organoleptic quality of market *peda* samples varied from place to place.

However, seven samples were liked very much by the judges whereas two samples were found unacceptable by the sensory evaluation.

Table 4.10 : Comparison of sensory evaluation scores of laboratory made and market *peda* samples

Attributes	Score	
	Laboratory made *peda*[a]	Market *Peda*[b]
Flavour (Max. 9)	7.4 ± 0.07	7.2 ± 0.8
Colour and Appearance (Max. 9)	7.2 ± 0.06	7.1 ± 0.9
Body & Texture (Max. 9)	7.3 ± 0.18	7.3 ± 0.7
Sweetness (Max. 9)	7.9 ± 0.07	6.6 ± 0.16
Overall acceptability (Max. 9)	7.8 ± 0.07	7.4 ± 0.8

[a] Average of five samples

[b] Average of twenty five samples

Table 4.11 : t-Test for sensory characteristics of laboratory made and market *peda* samples

	df	Observed t-value					t critical
		Flavour	Colour & appearance	Body & texture	Sweetness	Overall acceptability	
Laboratory made	4	2.215*	0.048	0.236	14.432**	4.162**	1.771
Market	24	2.215*	0.048	0.236	14.432**	4.162*	2.16

* Significant at 5 % (P < 0.05) level

** Highly significant at 1 % (P < 0.01) level

4.6 Rheological Properties of Laboratory Made and Market *Peda* Samples

While discussing about the desirability of *peda* it was observed that they were highly dependent on the body and textural properties which were dependent on human observations. Many a time we have come across to the variation amongst different judges. Hence the rheological properties enumerated by instrumental techniques were used. In the present study, the textural characteristics of *peda* samples prepared from standardized milk in the laboratory were measured with the help of Texture Analyser (Model No. TAHDi, manufactured by Stable Micro System, U.K.) and compared those characteristics with the samples collected from the market. The texture profile thus obtained were depicted in Table 4.12.

4.6.1 Texture profile of laboratory made peda

Table 4.12 showed that the average value of texture profile of laboratory made *peda* samples were hardness 55.10 ± 1.04 kg (range 49.19 to 63.54), fracturability 0.0359 ± 0.002 kg (range 0.0345 to

0.0375), Springiness 0.272 ± 0.002 mm (0.269 to 0.278), Cohesiveness 0.228 ± 0.01 mm (0.201 to 0.264), gumminess 11.18 ± 0.91 kg (9.87 to 14.76) and chewiness 0.0034 ± 0.0002 kg m (0.0026 to 0.0045).

4.6.2 Texture profile of market peda samples

From the Table 4.12, it has also been seen that there was a wide range of variation in the textural properties of the market *peda* samples. The average value of hardness, fracturability, springiness, cohesiveness, gumminess and chewiness of the market *peda* samples were found to be 152.02 ± 34.84 (range 77.08 to 248.15), 20.60 ± 11.38 (5.36 to 39.63), 0.282 ± 0.04 (0.225 to 0.349), 0.19 ± 0.04 (0.073 to 0.398), 43.31 ± 21.13 (5.60 to 98.85) and 0.0121 ± 0.006 (0.0019 to 0.0223) respectively.

From the Table 4.12, it had been found that the hardness, gumminess and chewiness of the market *peda* samples were higher than those of the laboratory made samples and a lower value of cohesiveness in the market samples was also observed. Patel *et al.* (1990) reported that increase in total solids considerably increased hardness, gumminess and chewiness but decreased cohesiveness in khoa samples. So, it can be said that the market samples had higher total solids than the laboratory made samples.

Table 4.12 also revealed that the springiness was lower in laboratory made samples and the adhesiveness of those samples were higher than most of the market samples. Instron measurement as reported by Patel *et al.* (1990) showed that working of khoa significantly decreased the hardness and springiness but increased adhesiveness. So it can be concluded that the laboratory made *peda* samples were finally kneaded or worked as compared to the market *peda* samples.

Table 4.12 : Texture profile of laboratory made and market *Peda* samples

Texture properties	Laboratory made *Peda*[a]		Market *Peda*[b]	
	Range	Average ± S.D.	Range	Average ± S.D.
Hardness (kg.)	49.19 – 63.54	55.10 ± 1.04	77.07 – 248.15	152.02 ± 34.84
Fracturability (kg.)	0.0345 – 0.0375	0.0359 ± 0.002	5.36 – 39.63	20.60 ± 11.38
Springiness (mm)	0.269 – 0.278	0.272 ± 0.002	0.225 – 0.349	0.282 ± 0.04
Cohesiveness (mm)	0.201 – 0.254	0.228 ± 0.011	0.073 – 0.398	0.190 ± 0.04
Gumminess (kg)	9.87 – 14.76	11.18 ± 0.91	5.60 – 98.85	43.31 ± 21.13
Chewiness (kg-m.)	0.0026 – 0.0045	0.0034 ± 0.0002	0.0019 – 0.0223	0.0121 ± 0.006

S.D. = Standard Deviation

[a] Five samples analysed

[b] Ten samples analysed

Table 4.13 : t-Test for texture profile of laboratory made and market *peda* samples

	df	Observed t-value						t critical
		Hard-ness	Fractura-bility	Cohesive-ness	Springi-ness	Gummi-ness	Chewi-ness	
Laboratory made	4	5.866**	5.007**	1.918*	0.481	3.85**	3.82**	1.771
Market	9	5.866**	5.007**	1.918	0.481	3.85*	3.82*	2.16

* Significant at 5 % ($P < 0.05$) level

** Highly significant at 1 % ($P < 0.01$) level

4.7 Submicro Structure of Laboratory Made and Market *Peda* Samples

Characterisation of various food products on the basis of their microstructure forms the backbone of the scientific approach to process development and of quality assurance in modern industrial practices. The current trends, round the globe, favour such studies to facilitate product description/specification for promoting process control and for international trade. Furthermore, the interest of researchers and manufacturers in the structure of various milk products has been growing, as it is recognized that there are definite correlationships between the structure and other physical properties of the products.

The various components of milk undergo complex interaction amongst themselves due to heat treatment and with the added sugar molecules during the process of *peda* manufacture. The investigation was therefore, undertaken to study the submicrostructure of *peda* to determine their topography and internal structure for better understanding of the typical body and texture of this Indian dairy product. In order to achieve a comparative study of the ultrastructural attributes, *peda* made in the laboratory from standardized milk were compared with the samples collected from the market.

4.7.1 Submicrostructure of laboratory made peda samples

Figure 4.2 represents the scanning electron micrograph of laboratorymade *peda* samples at a lower magnification. The bar length as shown in the micrograph represents 50 μm.

It is evident from the Fig. 4.2 that the surface was compact and uneven, obscured with fat. The granules of heat coagulated proteins coalesces together to form the compact protein matrix with numerous small pores. The condensed milk serum and fat filling the

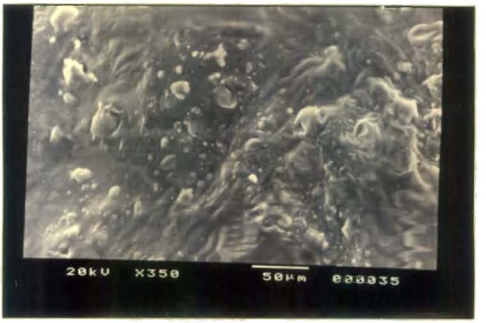


Fig. 4.2 : ***Submicrostructure of laboratory made peda at lower magnification***

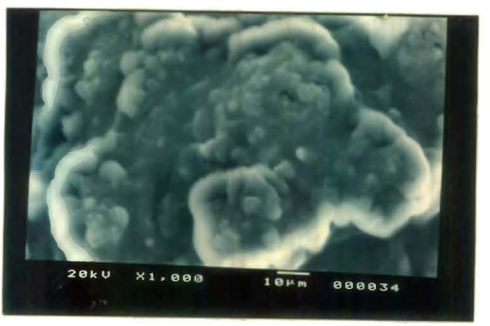


Fig. 4.3 : ***Submicrostructure of laboratory made peda at higher magnification***

intergranular spaces. Some sort of thermal degradation took place in the casein whey protein complexes which ultimately fused with lactose and sugar. Small lactose crystals were found (Fig. 4.2).

Figure 4.3 depicted the same *peda* sample as furnished in figure 4.2 taken at higher magnification. The scale represented a length of 10 μm in this case as written on the figure itself. The coalesced protein agglomerates with thick bridges are clearly seen along with some small lactose crystals and void spaces. The array of the conglomerated mass of protein showed no particular orientation. The heat fusion of protein with lactose and sugar were so strong that not a single casein micelle had restored its subunit size.

4.7.2 Submicrostructure of peda samples from market

Scanning electron micrograph of market *peda* samples reveals somehow a different protein network to that of laboratory samples. Figure 4.5 shows the ultrastructure of market *peda* at lower magnification. It shows a coalesced, ragged protein matrix linked together in a compact thick bridges. Thick thread like micelles are arranged in a regular and folded manner. However, these casein agglomerates fused densely together forming a layer or scale type structure.

Figure 4.4 is the scanning electron micrograph of market *peda* samples at a higher magnification. Here the protein matrix revealed agglomerated uneven protein particles join irregularly to form thick bridges. The agglomerated protein matrix interlinked strongly forming a compact scale or layer type structure. The size of the lactose crystal and fat globules in the inter granular spaces were larger as compared to the laboratory made samples.

Patil *et al.* (1992) also reported the microstructure of khoa consisted of individual granules of heat coagulated proteins with

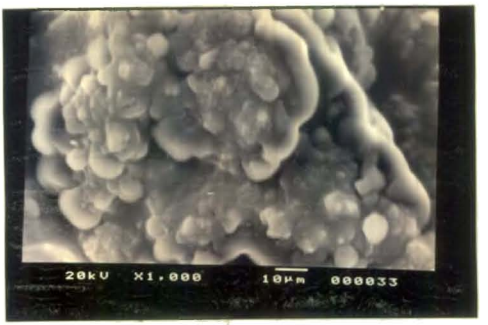


Fig. 4.4 : *Submicrostructure of market peda at higher magnification*

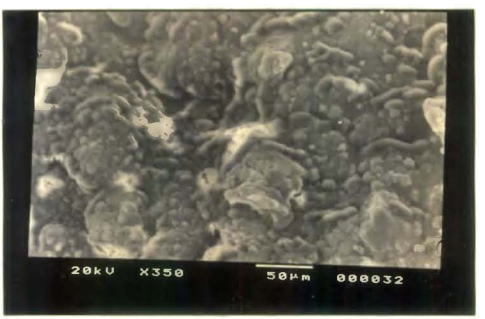


Fig. 4.5 : *Submicrostructure of market peda at lower magnification*

condensed milk serum and fat filling the intergranular spaces. Large aggregates of lactose crystals developed in inter granular spaces in unworked khoa as found in the market *peda* samples in the present investigation.

4.8 Comparison between Laboratory made and Market *Peda* Samples

The total solids content of the market *peda* samples was higher as compared to the laboratory made samples. It may be due to higher sugar percentage and adulterants in the market *peda* samples.

Free fat content determines the texture properties of *peda*. Laboratory made *peda* samples had higher fat content as compared to market *peda*. The sucrose content which was more or less similar in all laboratory samples of *peda* showed higher values in market samples. The values of protein, lactose and ash obtained from market *peda* samples did not show much difference with laboratory made *peda*, so they are not supposed to have influenced any variation in organoleptic quality of the final product to a perceptible extent.

The value of titratable acidity in market *peda* samples was higher than that of the laboratory made samples. This is due to the higher total count in the former than the later one. But few market sample showed lower acidity which was probably due to the addition of neutralizer to the final product.

The total count as well as yeast and mold count was higher in the market *peda* samples. The presence of coliform in the market samples indicated unhygienic trade practices.

The flavour of the laboratory made samples was slightly better than the market samples which is due to high fat content in milk used in the laboratory. The colour and appearance score of the laboratory made samples were slightly higher. However, the average score of body

and texture of the market and laboratory made samples were almost similar.

The higher free fatty acid content in the market *peda* samples indicated that the samples undergo lipolysis to some extent. HMF content was also higher in the market samples which may be attributed to the overheating of milk during manufacture of *peda* as compared to the laboratory made samples.

The laboratory made samples were also finely worked as compared to the unworking of the market *peda* samples. The presence of scale or layer type structure in the scanning electron micrograph of the market *peda* samples indicated overheating of the product.

Thus it can be concluded that the laboratory made *peda* samples were more or less better in almost all aspects than the market *peda* samples.

4.9 Studies on the Poly Acrylamide Gel Electrophoretic (PAGE) Pattern Changes During Transformation of Milk to *Peda*

Peda is prepared from khoa as base material which is basically a heat induced gel. It is prepared by mixing khoa with sugar and then heating until the desired consistency is achieved. It is obvious from the manufacturing techniques that milk proteins, casein in particular, plays a major role in the changes that occurs during preparation of *peda* from milk. Hence the polyacrylamide gel electrophoretic pattern of milk casein and *peda* casein may be different and suggestive to understand the complex changes. Hence, the PAGE pattern of milk proteins and *peda* proteins were studied in the present investigation.

The PAGE pattern of the proteins isolated from milk were compared with the PAGE pattern of the proteins isolated from the corresponding *peda* samples. The electrophoregram is presented in the Fig. 4.6. The electrophoretic pattern revealed the appearance of

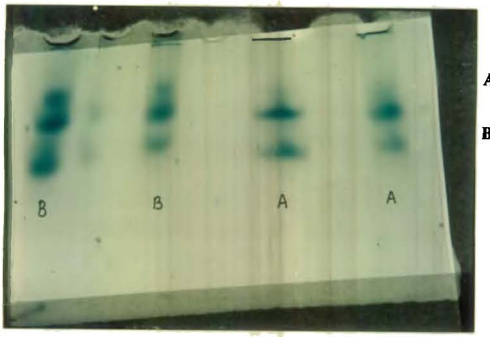


Fig. 4.6 : *PAGE pattern of milk and peda proteins*

two lower molecular weight components in the milk samples. However, the *peda* samples showed prominent band (three) having lower and higher electrophoretic mobility as a result of release of some breakdown product released during high heat treatment of the product.

4.10 Studies on the Spoilage of *Peda* During Storage

For any dairy product, the physico-chemical behaviour during storage is one of the prime interests of the producers and the consumers since it leads towards the termination of the shelf life of the product. In the present study trials were undertaken to investigate the physico-chemical changes of *peda* during its storage under ambient (30 ± 1°C) and refrigerated condition (7 ± 1°C). In this attempt the effect of addition of sodium metabisulphite (T_1) and nisin (T_2) on *peda* samples were examined. Two types of most suitable package viz. paper box and polypropylene pouches were used as packaging material for *peda* and were studied for observing their effectiveness.

It was noted that control *peda* samples kept under ambient temperature were acceptable upto 5 days without packaging (P_0), 9 days in paper box (P_1) and 14 days in polypropylene pouches (P_2), whereas the refrigerated control samples kept well upto 19 days in P_0, 24 days in P_1 and 45 days in P_2. The product treated with sodium metabisulphite kept well at 30 ± 1°C for 9 days in P_0, 14 days in P_1 and 19 days in P_2. Samples treated with nisin were unacceptable after 14 days in P_0, 19 days P_1 and 24 days in P_2 when stored at ambient temperature. Sodium metabisulphite treated samples were no more acceptable after 24, 30 and 58 days in P_0, P_1 and P_2 respectively in refrigerated condition. *Peda* treated with nisin under refrigerated storage were acceptable upto 30, 38 and 66 days in P_0, P_1 and P_2 respectively.

The chemical analysis of the control and treated samples stored at ambient temperature were discontinued after the aforesaid period on the basis of organoleptic quality and the products became brittle. The prolonged storage of the refrigerated samples lead to the hardness and dryness in body, coarse and gritty texture, rancid flavour, bitter taste and discolouration. Hence the chemical analysis was carried out upto the respective day of storage till the product kept well. The sensory evaluation and microbiological analysis were conducted on zero day and on the last respective day the product kept well to correlate the chemical changes with the change in organoleptic quality during storage of *peda*.

4.10.1 Chemical changes

4.10.1.1 Moisture (%)

The changes in moisture content of *peda* samples prepared from standardized milk during storage at 30 ± 1°C and 7 ± 1°C were presented in Table 4.14 and 4.15 respectively. The changes in moisture content of *peda* may be due to the varied biochemical changes in the product during storage. The tables also showed that the decrease in moisture content was gradual during storage for all samples.

At 30 ± 1°C, the net decrease in moisture content in control, T_1 and T_2 samples prepared from standardized milk were 23.84, 20.27 and 18.92% without packaging; 20.54, 19.30 and 20.3% when packed in paper box and 21.45, 20.63 and 20% in polypropylene pouches respectively (Fig. 4.7).

During refrigerated storage the control, T_1 and T_1 sample showed a moisture loss of 23.33, 20.94 and 23.03% in P_0; 21.91, 21.86 and 21.74% in P_1 and 23.23, 24.16 and 24.98% in P_2 respectively at the end of their respective storage days (Fig. 4.8).

Table 4.14 : Change in moisture content (%) of laboratory made *peda* during storage at 30 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	19.67	19.58	19.50	19.67	19.58	19.50	19.67	19.58	19.50
2	17.72	18.12	18.63	17.81	18.10	18.52	17.33	18.14	18.97
5	14.98	16.87	17.01	16.17	17.72	17.83	16.51	17.90	18.05
9		15.61	16.52	15.63	16.95	16.85	16.08	17.26	17.41
14	-	-	15.81		15.80	16.02	15.45	16.57	16.92
19	-	-	-	-	-	15.54	-	15.54	16.35
24	-	-	-	-	-	-	-	-	15.60
30	-	-	-	-	-	-	-	-	

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

Table 4.15 : Change in moisture content (%) of laboratory made *peda* during storage at 7 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	19.67	19.58	19.50	19.67	19.58	19.50	19.67	19.58	19.50
2	18.93	19.00	19.18	18.95	19.08	19.15	19.09	19.20	19.26
5	17.92	18.62	18.25	18.12	18.76	18.70	18.66	18.75	18.90
9	16.87	18.15	17.80	17.69	17.95	18.14	18.12	18.32	18.46
14	15.66	17.61	17.26	16.95	17.33	17.56	17.61	17.91	17.95
19	15.08	16.70	16.90	16.37	16.80	16.92	17.02	17.35	17.50
24	-	15.48	16.35	15.36	16.12	16.54	16.56	16.74	16.93
30	-	-	15.01	-	15.30	15.90	16.00	16.06	16.44
38	-	-	-	-	-	15.26	15.45	15.80	16.00
45	-	-	-	-	-	-	15.10	15.41	15.62
50	-	-	-	-	-	-	-	15.00	15.18
58	-	-	-	-	-	-	-	14.85	14.98
66	-	-	-	-	-	-	-	-	14.63
71	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

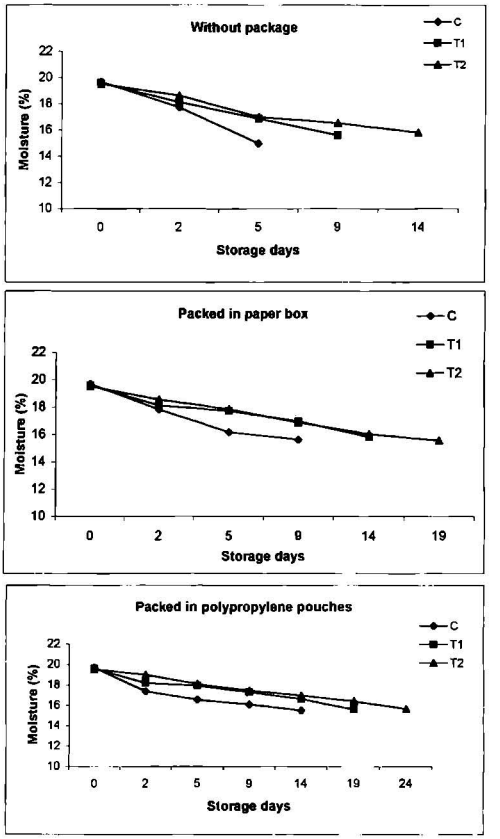


Fig. 4.7 :Change in moisture content of laboratory made *peda* during storage at 30 ± 1°C

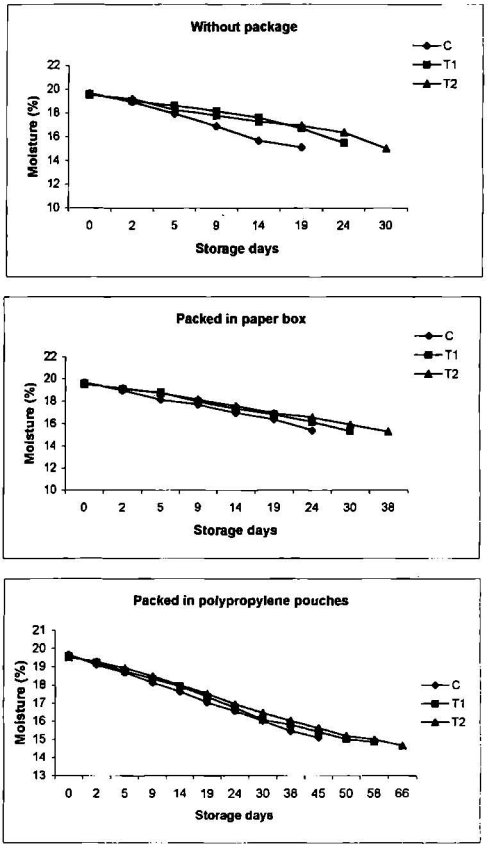


Fig. 4.8 : Change in moisture content of laboratory made *peda* during storage at 7 ± 1°C

The results (Table 4.14 and 4.15) revealed that irrespective of treatments imposed and packaging material used the decrease in moisture content of the *peda* samples stored at 30 ± 1° C was faster than the samples stored at 7 ± 1°C. The treated samples, as expected, showed better results than the control samples as the rate of decrease of moisture content was faster in control samples followed by T_1 and T_2 respectively in the same corresponding period at both 30 ± 1°C and 7 ± 1°C. Also the decrease was slower in polypropylene pouches followed by paper box and without packaging.

This it can be suggested that *peda* treated with nisin and packed in polypropylene pouches can be better effectively stored at 7 ± 1°C for a period of 66 days without moderate change in its moisture content.

4.10.1.2 Titratable Acidity (% lactic acid)

The changes in the titratable acidity (TA) of the *peda* samples during storage at 30 ± 1°C were shown in Table 4.16 and the same at 7 ± 1°C in Table 4.17. The initial TA of control, T_1 and T_2 samples were 0.58, 0.62 and 0.60% respectively. The initial higher TA in T_1 samples over control and T_2 is attributed to the generation of sulphurous acid due to the decomposition of salts during heating.

The net increase in TA in control samples stored under ambient temperature were 34.48, 31.03 and 34.48% in terms of lactic acid in P_0, P_1 and P_2 respectively (Fig. 4.9) whereas in refrigerated control samples the increase in TA were 34.48, 31.67 and 26.15% respectively after their respective storage days (Fig. 4.10). During storage at 30 ± 1°C the T_1 and T_2 samples showed an increase in acidity of 33.87 and 35.00% in P_0; 32.26 and 33.33% in P_1 and 30.64 and 33.33% in P_2 respectively (Fig. 4.9).

Table 4.16 : Change in titratable acidity content of laboratory made *peda* during storage at 30 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	0.58	0.62	0.60	0.58	0.62	0.60	0.58	0.62	0.60
2	0.68	0.66	0.64	0.64	0.65	0.63	0.62	0.63	0.62
5	0.78	0.72	0.70	0.72	0.70	0.67	0.68	0.66	0.65
9		0.83	0.76	0.76	0.75	0.72	0.72	0.68	0.70
14	-	-	0.81	-	0.82	0.77	0.78	0.72	0.75
19	-	-	-	-		0.80	-	0.81	0.78
24	-	-	-	-	-		-	-	0.80
30	-	-	-	-	-	-	-	-	

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

Table 4.17 : Change in titratable acidity (% lactic acid) of laboratory made *peda* during storage at 7 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	0.58	0.62	0.60	0.58	0.62	0.60	0.58	0.62	0.60
2	0.62	0.63	0.61	0.62	0.62	0.61	0.59	0.62	0.60
5	0.66	0.65	0.63	0.65	0.64	0.62	0.62	0.64	0.61
9	0.71	0.69	0.67	0.69	0.68	0.66	0.63	0.66	0.64
14	0.73	0.72	0.70	0.72	0.70	0.70	0.65	0.68	0.66
19	0.78	0.74	0.72	0.74	0.73	0.71	0.68	0.70	0.69
24	-	0.79	0.75	0.79	0.75	0.74	0.70	0.72	0.71
30	-	-	0.80	-	0.79	0.78	0.72	0.73	0.72
38	-	-	-	-	-	0.81	0.74	0.75	0.74
45	-	-	-	-	-	-	0.78	0.76	0.75
50	-	-	-	-	-	-	-	0.77	0.76
58	-	-	-	-	-	-	-	0.80	0.79
66	-	-	-	-	-	-	-	-	0.81
71	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

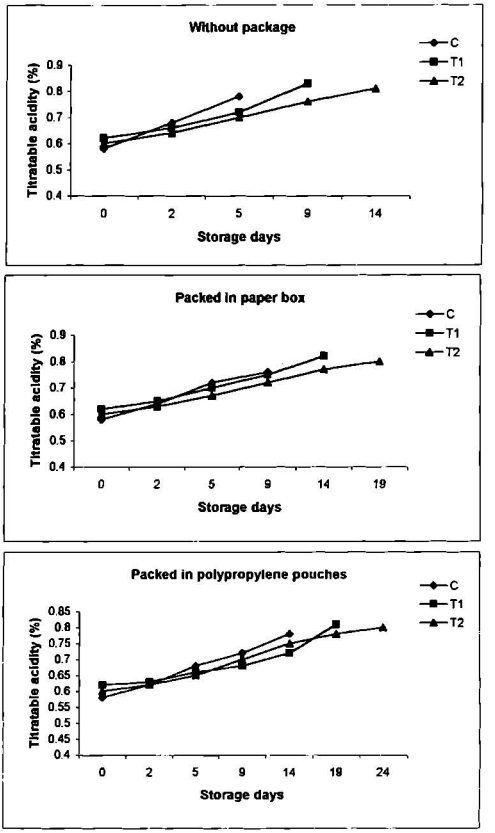


Fig. 4.9 : Change in titratable acidity content of laboratory made *peda* during storage at 30 ± 1°C

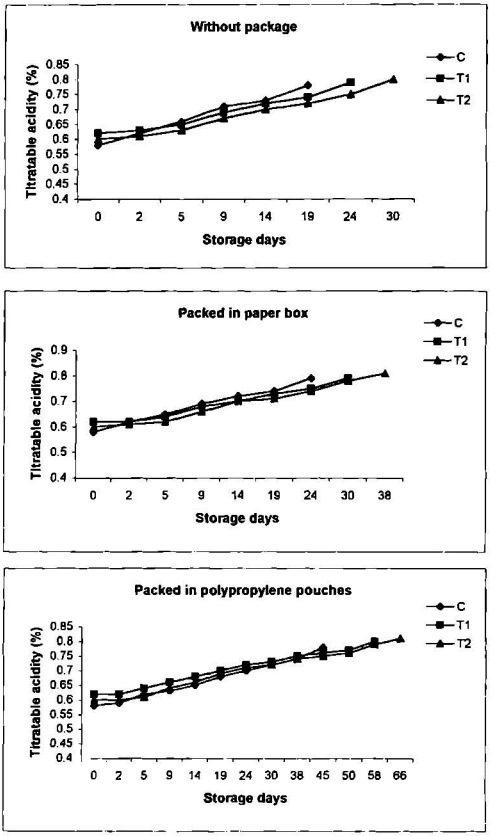


Fig. 4.10 : Change in titratable acidity content of laboratory made *peda* during storage at 7 ± 1°C

Fig. 4.10 represents the net increase in titratable acidity of the T_1 and T_2 samples at refrigerated storage.

The results pointed out that rate of increase in titratable acidity in treated samples of *peda* over control was lower at both storage temperature indicating the inhibitory effect of the preservatives on the microbial activity in the product. The results further revealed that nisin was more effective than sodium metabisulphite as preservative.

Thus it can be concluded that the *peda* samples treated with nisin and packaged in polypropylene pouches can be best stored at refrigerated condition for a period of 66 days without moderate change in its titratable acidity content.

4.10.1.3 Free fatty acid (% Oleic acid)

The changes in the free fatty acid (FFA) content of the *peda* samples prepared from standardized milk during their storage at 30 ± 1°C and 7 ± 1°C were furnished in the tables 4.18 and 4.19 respectively. The results revealed that the FFA content of all samples stored at 30 ± 1°C increased more sharply than that at 7 ± 1°C irrespective of treatment imposed and packaging applied.

The net increase in FFA content of the *peda* samples stored at 30 ± 1°C were 68.96 (C P_0), 58.33 (T_1P_0), 54.84 (T_2P_0), 65.52 (C P_1), 56.67 (T_1P_1), 53.23 (T_2P_1), 62.07 (C P_2), 53.33 (T_1P_2) and 53.23% (T_2P_2) after their respective storage days (Fig. 4.11). In case of refrigerated control, T_1 and T_2 samples there was an increase in FFA content of 65.52, 61.67 and 58.06% without packaging; 63.79, 60.00 and 59.67% in paper box and 62.07, 61.67 and 58.05% in polypropylene pouches respectively (Fig. 4.12).

Table 4.18 : Change in free fatty acid (% oleic acid) of laboratory made *peda* during storage at 30 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	0.058	0.060	0.062	0.058	0.060	0.062	0.058	0.060	0.062
2	0.075	0.069	0.068	0.072	0.068	0.065	0.068	0.067	0.065
5	0.098	0.080	0.073	0.080	0.075	0.072	0.076	0.071	0.068
9	-	0.095	0.087	0.096	0.082	0.81	0.085	0.076	0.074
14	-	-	0.096		0.094	0.088	0.094	0.083	0.081
19		-	-	-	-	0.095		0.092	0.087
24		-	-	-	-	-	-	-	0.095
30	-		-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

Table 4.19 : Change in free fatty acid (% oleic acid) of laboratory made *peda* during storage at 7 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	0.058	0.060	0.062	0.058	0.060	0.062	0.058	0.060	0.062
2	0.065	0.064	0.064	0.064	0.064	0.064	0.065	0.063	0.063
5	0.074	0.072	0.068	0.072	0.070	0.069	0.068	0.065	0.065
9	0.081	0.078	0.071	0.079	0.076	0.074	0.073	0.069	0.068
14	0.090	0.084	0.077	0.085	0.081	0.078	0.076	0.073	0.070
19	0.096	0.089	0.083	0.090	0.088	0.084	0.080	0.077	0.075
24	-	0.097	0.088	0.095	0.092	0.089	0.084	0.081	0.078
30	-	-	0.098	-	0.096	0.095	0.087	0.084	0.082
38	-	-	-	-	-	0.099	0.090	0.087	0.085
45	-	-	-	-	-	-	0.94	0.090	0.087
50	-	-	-	-	-	-	-	0.095	0.090
58	-	-	-	-	-	-	-	0.097	0.092
66	-	-	-	-	-	-	-	-	0.098
71	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

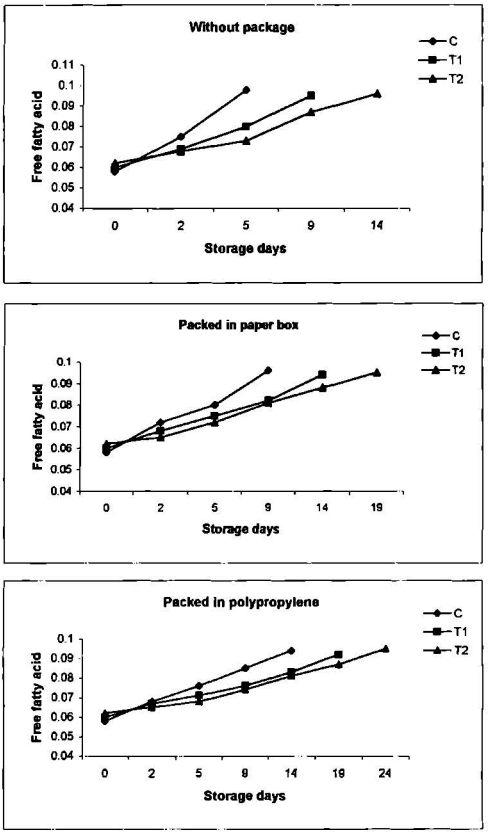


Fig. 4.11 : Change in free fatty acid content (% oleic acid) of laboratory made *peda* during storage at 30 ± 1°C

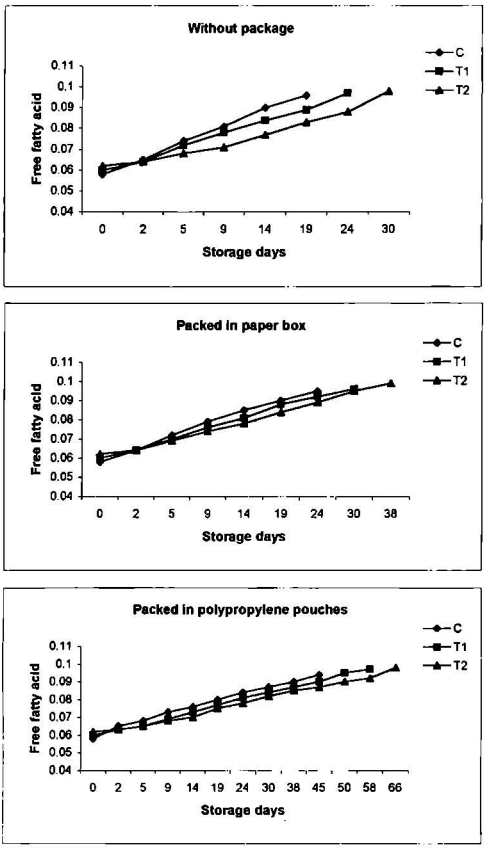


Fig. 4.12 : Change in free fatty acid content (% oleic acid) of laboratory made *peda* during storage at 7 ± 1°C

The results, showed that the rate of increase in FFA content was higher in control samples than in treated samples suggesting that the additives retarded the lipolysis in the product. Polypropylene pouches as packaging material also showed better results than paper box in terms of FFA content.

Thus it can be concluded that the *peda* samples treated with nisin and packaged in polypropylene pouches can be effectively kept under refrigerated storage for a period of 66 days without marginal change in its FFA content.

4.10.1.4 Peroxide value (m. eq. of O_2/kg of fat)

The changes in peroxide value of *peda* samples during their storage at ambient and refrigerated temperatures were illustrated in the Tables 4.20 and 4.21 respectively. The initial peroxide value of control, T_1 and T_2 samples were 1.11, 1.12 and 1.1 respectively. The samples stored at 30 ± 1°C showed the peroxide value of 1.87 (C P_0), 1.83 (T_2P_0), 1.85 (C P_1), 1.84 (T_1P_1), 1.81 (T_2P_1), 1.84 (T_1P_2) and 1.84 (T_2P_2) after their respective storage periods (Table 4.20). In refrigerated storage the T_1 and T_2 samples showed the peroxide value of 1.83 and 1.82 in P_0; 1.81 and 1.82 in P_1 and 1.83 and 1.80 in P_2 respectively at the end of storage (Table 4.21).

During storage at 30 ± 1°C, the net increase in the peroxide value of control, T_1 and T_2 *peda* samples were 68.47, 65.18 and 66.36% in without packaging, 66.67, 64.29 and 64.54% in paper box and 63.06, 64.29 and 64.54% in polypropylene pouches respectively (Fig. 4.13).

Almost similar trend in results was obtained for refrigerated *peda* samples (Fig. 4.14).

Table 4.20 : Change in peroxide value (m.eq. of O_2/kg fat) of laboratory made *peda* during storage at 30 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	1.11	1.12	1.10	1.11	1.12	1.10	1.11	1.12	1.1
2	1.54	1.41	1.37	1.48	1.39	1.33	1.42	1.33	1.28
5	1.87	1.68	1.64	1.69	1.52	1.51	1.57	1.52	1.41
9	-	1.85	1.75	1.85	1.70	1.66	1.67	1.67	1.55
14	-	-	1.83	-	1.84	1.73	1.81	1.76	1.64
19	-	-	-	-	-	1.81	-	1.84	1.74
24	-	-	-	-	-	-	-	-	1.81
30	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

Table 4.21 : Change in peroxide value (m.eq. of O_2/kg fat) of laboratory made *peda* during storage at 7 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	1.11	1.12	1.10	1.11	1.12	1.10	1.11	1.12	1.1
2	1.30	1.30	1.28	1.3	1.25	1.25	1.24	1.2	1.18
5	1.42	1.38	1.36	1.35	1.32	1.30	1.28	1.25	1.2
9	1.56	1.46	1.44	1.42	1.41	1.38	1.36	1.32	1.30
14	1.68	1.61	1.56	1.54	1.5	1.47	1.44	1.48	1.42
19	1.82	1.70	1.68	1.7	1.63	1.54	1.50	1.56	1.48
24	-	1.83	1.76	1.81	1.75	1.63	1.64	1.64	1.57
30	-	-	1.82	-	1.81	1.74	1.7	1.7	1.63
38	-	-	-	-	-	1.82	1.78	1.76	1.68
45	-	-	-	-	-	-	1.81	1.8	1.72
50	-	-	-	-	-	-	-	1.82	1.75
58	-	-	-	-	-	-	-	1.83	1.79
66	-	-	-	-	-	-	-	-	1.81
71	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

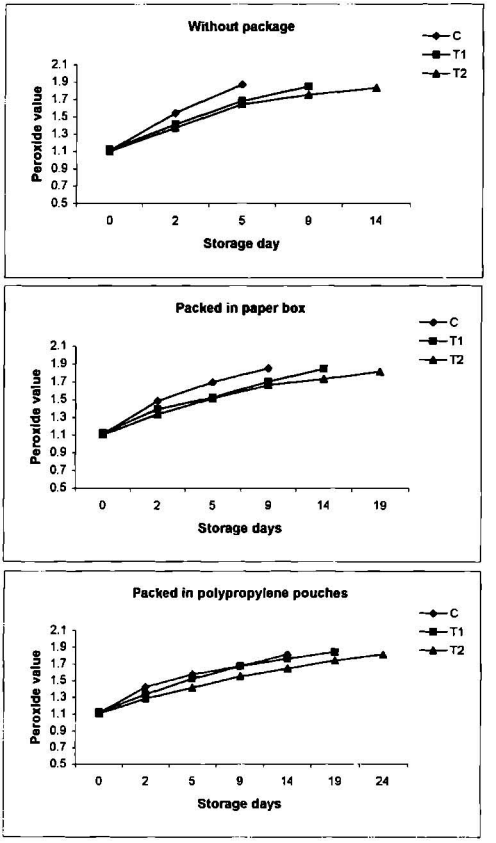


Fig. 4.13 : Change in peroxide value (m.eq. of O_2/gm fat) of laboratory made *peda* during storage at 30 ± 1°C

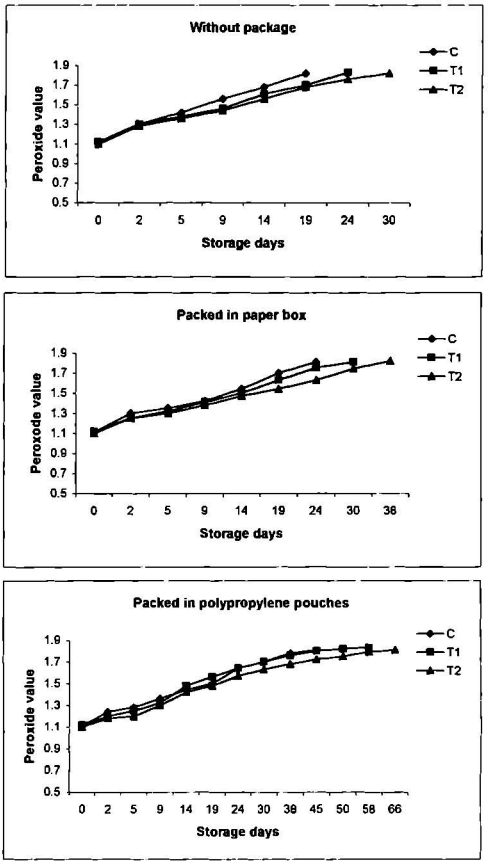


Fig. 4.14 : Change in peroxide value (m.eq. of O_2/gm fat) of laboratory made *peda* during storage at 7 ± 1°C

The results indicated that the rate of increase in peroxide value was higher in control samples than in treated samples suggesting a repressive effect of the added preservatives on peroxidation. From the results it had also been found that storage at 7 ± 1°C produced better results than at 30 ± 1°C and also polypropylene as packaging material was proved to be better than paper box.

Thus it can be concluded that *peda* treated with nisin and packaged in polypropylene pouches can better be effectively stored at 7 ± 1°C for a period of 66 days without moderate change in its peroxide value.

4.10.1.5 Tyrosine content (mg/100 gm)

The changes in tyrosine content of *peda* samples prepared from standardized milk during storage at 30 ± 1°C was represented in Table 4.22 and at 7 ± 1°C in Table 4.23. The initial tyrosine content of the control and treated samples were 21.12, 21.08 and 20.87mg/100 gm respectively. At 30 ± 1°C the maximum tyrosine value was increased in control samples without packaging (43.12) after five days of storage whereas the minimum increase in the same was in T_2 samples in polypropylene pouches (42.06) after 66 days of storage at 7 ± 1°C.

The net increase in tyrosine value of control, T_1 and T_2 samples were 104.17, 103.80 and 103.93% (without packaging), 103.69, 103.04 and 102.44% (in paper box) and 103.12, 102.13 and 101.72% (in polypropylene pouches) respectively during storage at ambient temperature (Fig. 4.15).

Under refrigerated storage the tyrosine content of control, T_1 and T_2 samples were increased 104.07, 102.80 and 101.88% (without packaging), 101.89, 101.99 and 99.71% (in paper box) and 99.72, 99.71 and 98.85% (in polypropylene pouches) respectively at the end of their respective storage days (Fig. 4.16).

Table 4.22 : Change in tyrosine content (mg/100gm) of laboratory made *peda* during storage at 30 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	21.12	21.08	20.87	21.12	21.08	20.8	21.12	21.08	20.87
2	32.85	28.11	27.18	29.85	28.02	26.33	28.31	27.97	27.32
5	43.12	35.53	31.92	36.97	32.16	32.53	32.56	31.62	31.02
9	-	42.96	37.50	43.02	39.54	37.31	37.62	35.31	34.86
14	-	-	42.56	-	42.80	40.10	42.90	38.07	36.31
19	-	-	-	-	-	42.25	-	42.61	39.54
24	-	-	-	-	-		-	-	42.10
30	-		-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

Table 4.23 : Change in tyrosine content (mg/100gm) of laboratory made *peda* during storage at 7 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	21.12	21.08	20.87	21.12	21.08	20.87	21.12	21.08	20.87
2	25.62	25.44	24.92	24.92	24.56	24.56	23.55	23.08	22.56
5	29.11	28.65	28.21	28.12	27.86	27.68	26.32	24.96	24.15
9	36.53	33.82	34.02	35.06	31.80	30.92	29.87	28.15	27.76
14	39.32	37.58	37.11	38.15	34.56	34.15	33.12	30.58	30.45
19	43.10	40.06	39.69	41.24	38.60	37.72	35.58	33.02	32.91
24	-	42.75	40.85	42.64	40.51	39.10	37.36	34.60	34.08
30	-	-	41.96	-	42.58	40.98	39.36	36.02	35.62
38	-	-	-	-	-	41.68	40.64	37.75	37.70
45	-	-	-	-	-	-	42.18	39.40	39.12
50	-	-	-	-	-	-	-	41.02	40.46
58	-	-	-	-	-	-	-	42.10	41.15
66	-	-	-	-	-	-	-	-	41.50
71	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

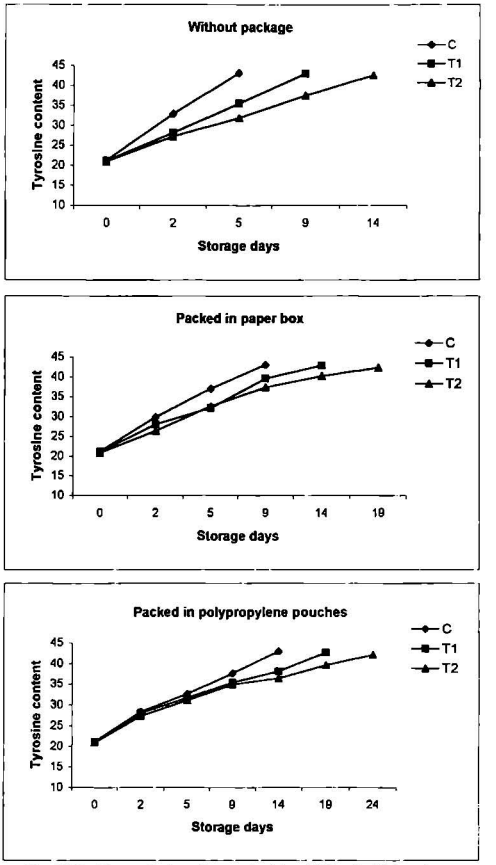


Fig. 4.15 : Change in tyrosine content (mg/100gm) of laboratory made *peda* during storage at 30 ± 1°C

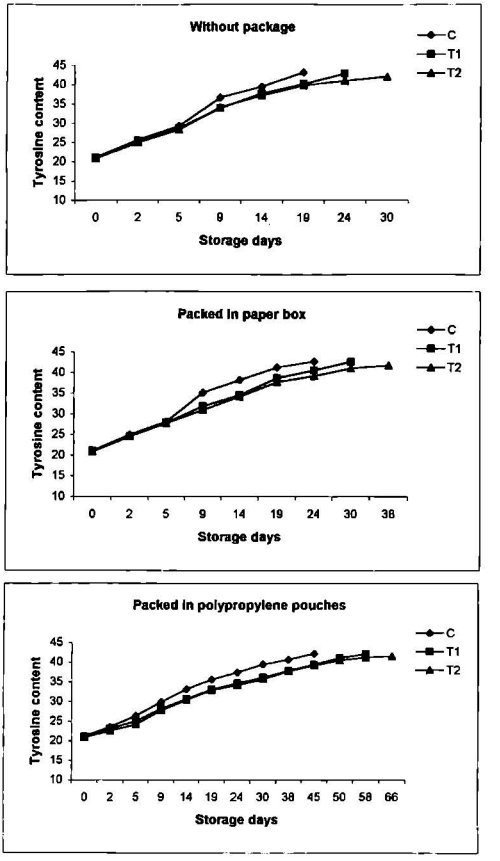


Fig. 4.16 : Change in tyrosine content (mg/100gm) of laboratory made *peda* during storage at 7 ± 1°C

The results revealed that the rate of increase of the tyrosine value of the control samples were higher than the treated ones suggesting suppression of proteolysis by additives. The tyrosine value increased at a faster rate during storage at 30 ± 1°C than at 7 ± 1°C. In this regard polypropylene as packaging material proved to be better than paper box.

Thus it can be inferred that *peda* treated with nisin and packaged in polypropylene pouches can be stored safely at refrigerated temperature for a period of 66 days.

4.10.1.6 HMF content (micromoles/100 gm)

The changes in HMF content of *peda* during storage at different temperatures were illustrated in the Tables 4.24 and 4.25.

The net increase in HMF value of the control samples at 30 ± 1°C was 25.98 (P_0), 23.88 (P_1) and 21.52% (P_2) at the end the 5, 9 and 14 days respectively, whereas the corresponding values of the control samples at 7 ± 1°C were 21.00 (P_0), 19.42 (P_1) and 17.58% (P_2) respectively after 19, 24 and 45 days of storage (Fig. 4.17 and 4.18).

The T_1 samples showed an increase of 21.67 (P_0), 17.75 (P_1) and 16.97% (P_2) in HMF value during its storage at 30 ± 1°C. In the refrigerated T_1 samples the increase in HMF value were 19.70 (P_0), 16.19 (P_1) and 15.67% (P_2) at the end of their respective storage days (Fig. 4.17 and 4.18).

A similar trend but at a lower increase percentage were observed in case of T_2 samples both at ambient and refrigerated storage (Fig. 4.17 and 4.18).

Table 4.24 : Change in HMF value (micromoles/100gm) of laboratory made *peda* during storage at 30 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	0.381	0.383	0.383	0.381	0.383	0.383	0.381	0.383	0.383
2	0.428	0.415	0.405	0.406	0.402	0.402	0.402	0.401	0.398
5	0.480	0.442	0.420	0.432	0.418	0.415	0.427	0.412	0.408
9	-	0.466	0.435	0.472	0.433	0.431	0.442	0.424	0.418
14	-		0.454	-	0.451	0.437	0.463	0.431	0.427
19	-	-	-	-	-	0.448	-	0.448	0.432
24	-	-	-	-	-	-	-		0.439
30	-	-	-	-	-	-	-	-	

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

Table 4.25 : Change in HMF value (micro moles/100gm) of laboratory made *peda* during storage at 7 ± 1°C

Days	Without package			Packed in paper box			Packed in polypropylene pouches		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0	0.381	0.383	0.383	0.381	0.383	0.383	0.381	0.383	0.383
2	0.398	0.398	0.398	0.396	0.395	0.395	0.392	0.390	0.388
5	0.403	0.402	0.402	0.401	0.398	0.399	0.397	0.395	0.394
9	0.424	0.418	0.416	0.420	0.415	0.413	0.414	0.406	0.402
14	0.435	0.432	0.429	0.436	0.425	0.421	0.419	0.415	0.410
19	0.461	0.440	0.436	0.442	0.438	0.432	0.428	0.420	0.414
24	-	0.448	0.442	0.455	0.442	0.437	0.432	0.426	0.419
30	-	-	0.446	-	0.445	0.440	0.439	0.429	0.424
38	-	-	-	-	-	0.442	0.442	0.433	0.427
45	-	-	-	-	-	-	0.448	0.438	0.430
50	-	-	-	-	-	-	-	0.440	0.431
58	-	-	-	-	-	-	-	0.443	0.434
66	-	-	-	-	-	-	-	-	0.437
71	-	-	-	-	-	-	-	-	-

Average of three trials

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated with nisin

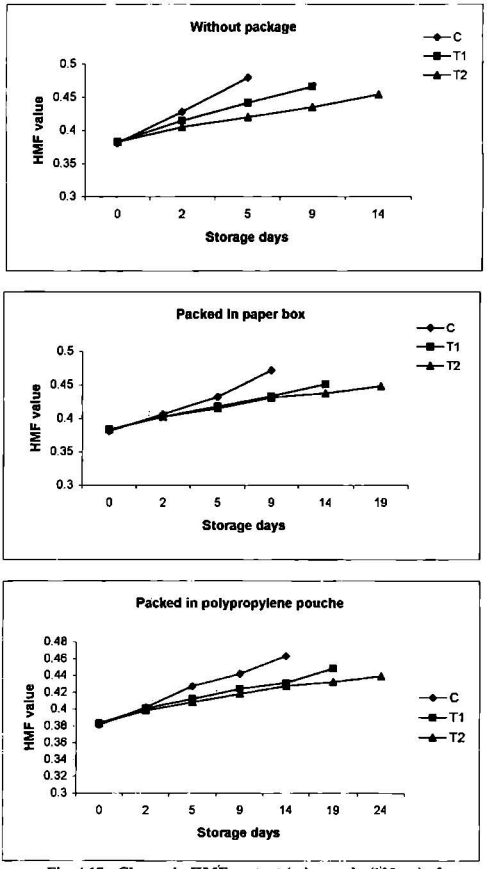
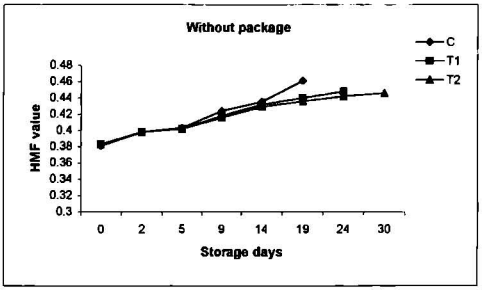
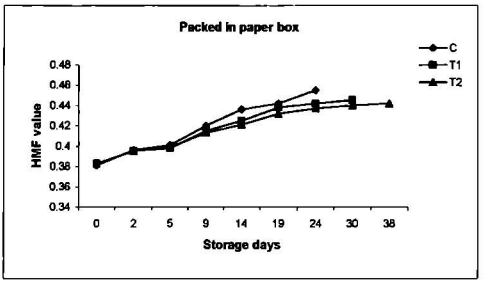


Fig. 4.17 : Change in HMF content (micromoles/100gm) of laboratory made *pedā* during storage at 30 ± 1°C





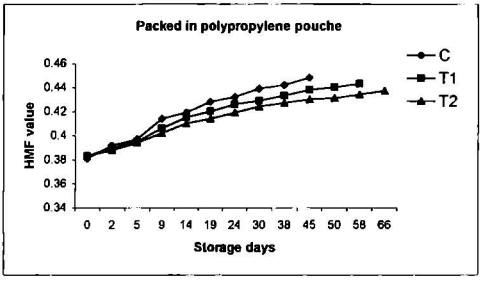


Fig. 4.18 : Change in HMF content (micromoles/100gm) of laboratory made *peda* during storage at 7 ± 1°C

From the above results, it can be said that, the browning as a result of storage was directly related to the storage temperature, as the increase in HMF content was faster when the product stored at 30 ± 1°C than that stored at 7 ± 1°C. Under refrigerated storage the increase in browning index of *peda* was negligible. The results also revealed that the rate of increase in HMF content was higher in control samples followed by sodium metabisulphite treated and nisin treated samples respectively suggested that the addition of the preservatives had a bleaching effect on the product. Patel *et al.* (1985) also observed the inhibitory effect of sulphur dioxide against browning. Products packaged in polypropylene pouches also showed lowering of browning index as compared to paper box and without packaging.

Thus it can be concluded that the *peda* samples prepared from standardized milk and treated with nisin can be better effectively stored at 7 ± 1°C in polypropylene pouches for a period of 66 days without moderate change in its HMF content.

4.10.2 Microbiological Changes

Peda samples in duplicate were subjected to determine the microbiological changes on the first and final day of storage for their standard plate count, coliform count and yeast and mold count. The results relating to microbial changes during storage were depicted in the Table 4.26.

4.10.2.1 Standard plate count

The standard plate count of all the samples increased to a great extent during storage. The increase was maximum in control samples when stored under ambient condition and kept without packaging. The count increased to 37×10^6 cfu/gm from 28×10^3 cfu/gm after 5 days of storage (Table 4.26). However, it was also observed from the

Table that the increase was minimum in T_2 samples stored at refrigeration temperature and packaged in polypropylene pouches (32×10^4 cfu/gm after 66 days storage).

It was obvious from the Table 4.26 that the microorganisms increased at a higher rate at 30 ± 1°C storage than at 7 ± 1°C storage. Also the increase was higher in control samples followed by T_1 and T_2 samples, which revealed the inhibitory effect of preservatives on the micro-organisms. The polypropylene pouches also offered better protection against microbial growth than the paper box as packaging material.

4.10.2.2 Coliform count

No coliform count was found in any of the samples analysed on the initial and final day of storage.

4.10.2.3 Yeast and mold count

Similarly like SPC, the yeast and mold count also increased to a great extent during the storage of the *peda* samples at different temperatures and packaging conditions. The count increased from 35 to 15×10^2 cfu/gm in the samples stored at 30 ± 1°C and kept without packaging after 5 days of storage. Whereas at 7 ± 1°C, the T_2 samples showed an increase in the yeast and mold count from 34 to 8.7×10 cfu/gm when packaged in polypropylene pouches after 66 days of storage.

Thus it can be said that the growth of yeast and molds was faster in 30 ± 1°C as compared to 7 ± 1°C. Also nisin had more inhibitory effect on the yeast and mold count than the sodium metabisulphite. Polypropylene pouches offered some protection towards the growth of yeast and mold followed by paper box.

Table 4.26 : Effect of storage on microbiological quality of laboratory made *peda* at different temperature

Storage tempe-rature	Sensory attributes		C			T_1			T_2		
			P_O	P_1	P_2	P_O	P_1	P_2	P_O	P_1	P_2
30 ± 1°C	Standard plate Count × 10^5 (cfu/gm)	I	0.28	0.28	0.28	0.25	0.25	0.25	0.26	0.26	0.26
		F	370	120	76	118	98	40	106	73	28
	Yeast and mold count × 10^2 (cfu/gm)	I	0.35	0.35	0.35	0.36	0.36	0.36	0.34	0.34	0.34
		F	15 (5)	10 (9)	7 (14)	11 (9)	7 (14)	4 (19)	9 (14)	6 (19)	2 (24)
7 ± 1°C	Standard plate Count × 10^5 (cfu/gm)	I	0.28	0.28	0.28	0.25	0.25	0.25	0.26	0.26	0.26
		F	74	27	8.6	42	19	6.4	34	11	3.2
	Yeast and mold count × 10^2 (cfu/gm)	I	0.35	0.35	0.35	0.36	0.36	0.36	0.34	0.34	0.34
		F	10 (19)	7 (24)	3 (45)	5 (24)	3 (30)	1.6 (58)	3 (30)	1 (38)	0.87 (66)

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated wth nisin, P_0 = Without package, P_1 = Packed in paper box, P_2 = Packed in Polypropylene pouches, I = Initial score, F = Final score

Figures in the parenthesis represent the storage days upto which the product kept well.

In any of the samples coliform cannot detected.

Finally, it can be concluded that the *peda* samples treated with nisin (1000 ppm) and packaged in polypropylene pouches can better be effectively stored under refrigerated condition for a period of 66 days.

4.10.3 Sensory Characteristics

Peda samples in duplicate were subjected to sensory evaluation on the first and final day of storage for their flavour, colour and appearance, body and texture and overall acceptability scores by five experienced judges independently. The changes in the sensory scores of *peda* prepared from standardized milk during storage at different temperature and different packaging condition were presented in Table 4.27.

4.10.3.1 Flavour

Table 4.27 revealed that, the initial flavour scores of control samples were slightly higher than that of treated samples probably due to the presence of some medicinal flavour in the nisin treated samples and slight sulphur dioxide flavour in the sodium metabisulphite treated samples. However, with the increase in storage period, all the samples showed a deceasing tendency in flavour score. The deterioration of flavour was faster at 30 ± 1°C than 7 ± 1°C. It indicated a significant inverse relationship of each of the free fatty acids and peroxide value with the flavour score during storage.

The pleasant flavour of *peda* samples, stored at 30 ± 1°C turned acidic and foul smell developed after 5 (C), 9 (T_1) and 14 (T_2) days in products without packaging, 9 (C), 14 (T_1) and 19 (T_2) days when packaged in paper box and when packed in polypropylene pouches the foul smell developed after 14, 19 and 24 days in the control, T_1 and T_2 samples respectively. The study further revealed that the decline in flavour scores in control and treated samples due to the

production of rancid flavour and bitter taste along with some other off-flavours reached its optimum level after 19 (C P_0), 24 (T_1P_0) 30 (T_2P_0), 24 (C P_1), 30 (T_1P_1), 38 (T_2P_1), 45 (C P_2), 58 (T_1P_2) and 66 (T_2P_2) days during storage at 7 ± 1°C (Table 4.27). *Peda* samples were no longer acceptable after this period.

4.10.3.2 Colour and appearance

Table 4.27 showed that the initial colour and appearance score of control samples was slightly more than that of treated samples which may be due to the bleaching effect of $Na_2S_2O_5$ which imparted whitish yellow colour to the product as against the browned product in control (7.5 for control, 7.3 for T_1 and 7.2 for T_2 samples).

As the storage period increased *peda* stored at ambient condition showed a rapid increase in browning index resulting in lower final colour score than the initial. The colour turned to dull and yellow. However, the refrigerated samples showed less decrease in HMF values with higher colour and appearance scores but at the end prominent white linings were visible on the surface.

4.10.3.3 Body and texture

Table 4.27 depicts that the body and texture score of *peda* samples decreased with the increase in storage period, probably due to the expulsion of moisture and crystallization of sugar during storage. The rate of decrease in body and texture scores were higher in the samples stored at 30 ± 1°C than that at 7 ± 1°C. The body and texture of spoiled *peda* was criticized as sandy, hard body, coarse texture and dry surface towards the end of storage period.

Table 4.27 : Effect of storage on sensory characteristics of laboratory made *peda* at different temperature

Storage temperature	Sensory attributes		C			T_1			T_2		
			P_0	P_1	P_2	P_0	P_1	P_2	P_0	P_1	P_2
30 ± 1°C	Flavour (Max. 9)	I	7.8	7.8	7.8	7.6	7.6	7.6	7.5	7.5	7.5
		F	4.6	4.7	4.8	4.8	4.9	5.1	4.9	5.0	5.2
	Colour and appearance (Max. 9)	I	7.5	7.5	7.5	7.3	7.3	7.3	7.4	7.4	7.4
		F	4.4	4.6	4.8	4.5	4.8	5.0	4.7	4.9	5.1
	Body & Texture (Max. 9)	I	7.4	7.4	7.4	7.3	7.3	7.3	7.3	7.3	7.3
		F	4.4	4.5	4.7	4.6	4.8	4.9	4.7	4.9	5.2
	Overall acceptability (Max. 9)	I	7.8	7.8	7.8	7.6	7.6	7.6	7.6	7.6	7.6
		F	4.6 (5)	4.8 (9)	5.1 (14)	4.7 (9)	4.9 (14)	5.0 (19)	4.9 (14)	5.0 (19)	5.2 (24)
7 ± 1°C	Flavour (Max. 9)	I	7.8	7.8	7.8	7.6	7.6	7.6	7.5	7.5	7.5
		F	4.8	5.0	5.2	5.0	5.1	5.3	5.2	5.3	5.5
	Colour and appearance (Max. 9)	I	7.5	7.5	7.5	7.3	7.3	7.3	7.4	7.4	7.4
		F	4.9	5.2	5.3	5.0	5.3	5.4	5.3	5.4	5.6
	Body & Texture (Max. 9)	I	7.4	7.4	7.4	7.3	7.3	7.3	7.3	7.3	7.3
		F	4.9	5.0	5.2	5.1	5.2	5.3	5.3	5.4	5.6
	Overall acceptability (Max. 9)	I	7.8	7.8	7.8	7.6	7.6	7.6	7.6	7.6	7.6
		F	4.9 (19)	5.0 (24)	5.2 (45)	5.1 (24)	5.2 (30)	5.4 (58)	5.1 (30)	5.4 (38)	5.6 (66)

C = Control, T_1 = Treated with sodium metabisulphite, T_2 = Treated wth nisin, P_0 = Without package, P_1 = Packed in paper box, P_2 = Packed in Polypropylene pouches, I = Initial score, F = Final score

The figures in the parenthesis represent the respective storage days upto which the product kept well.

4.7.10.4 Overall acceptability

Table 4.27 revealed that the rate of decrease in overall acceptability scores were higher in control samples than in treated samples. The table also depicted that irrespective of treatments imposed and packaging conditions applied the rate of decrease in overall acceptability scores of *peda* samples were higher when stored at 30 ± 1°C than that stored at 7 ± 1°C. The final overall acceptability scores of control, T_1 and T_2 samples in P_0, P_1 and P_2 packaging conditions were 4.6 (CP_0), 4.7 (T_1P_0), 4.9 (T_2P_0), 4.8 (CP_1), 4.9 (T_1P_1), 5.0 (T_2P_1), 5.1 (CP_2), 5.0 (T_1P_2) and 5.2 (T_2P_2) after their respective storage days at 30 ± 1°C. Whereas when the samples stored at refrigerated condition the above scores were 4.9 (CP_0), 5.1 (T_1P_0, T_2P_0), 5.0 (CP_1), 5.2 (T_1P_1), 5.4 (T_2P_1), 5.2 (CP_2), 5.4 (T_1P_2) and 5.6 (T_2P_2) respectively.

Hence, from the above discussion, it can be concluded that with the addition of nisin (1000 ppm) and packaged in polypropylene pouches the *peda* samples can be better effectively stored at 7 ± 1°C for a period of 66 days with marginal change in its sensory characteristics.

Nisin is used as a preservative in various food stuffs, which, by their nature, are pasteurized during their production but not fully sterilized, examples of such foods are processed cheese, milks, canned foods including Rasogolla, alcoholic beverages and dairy desserts. Nisin is a heat resistant polypeptide (34 amino acids) bacteriocin that exhibits antimicrobial activity towards a wide range of gram positive vegetative bacteria. Nisin can be successfully used in a number of thermal proceed foods without increasing the risk of bacteriological spoilage. Nisin as preservative can extend the shelf-life of foods and it has no toxic or allergenic effects. Because nisin is completely degraded in the alimentary tract, it can be used safely as a food additive. Nisin appeared to have significant effects of preventing development of

yeasts and molds in the product stored at lower temperature. In 1969, a joint FAO/WHO expert committee accepted nisin as a legal food additive, although it was not until 1988 that it was approved in the USA for use in certain pasteurized cheese spreads. Presently nisin is permitted in at least 46 countries for the inhibition of aerobic spores and clostridia in cheese and canned foods. Recently the use of nisin in paneer and Rasogolla is in final stage approval.

Use of sulphur dioxide in various forms including its neutral or acid salts have been widely followed to retard the microbial and chemical deterioration of food items (Joslyn and Braverman, 1954). The acceptable daily intake of sulphites for adults as established by the United Nation's FAO/WHO is 0.7 mg of equivalent per kg person (Lueck, 1980). Use of metabisulphites in also permitted under Prevention of Food Adulteration Rules (PFA, 1976) in canned Rasogolla but to a limited extent.

So, nisin and sodium metabisulphite can also be added in *peda* to enhance its shelf-life as the present study shows that these two preservatives can check the rise in acidity, liberation of free fatty acids, release of amino acids and formation of peroxides.

Chapter - V

Summary and Conclusion

Summary and Conclusion

The present investigation was undertaken with a view to assess the chemical, microbiological and sensory qualities, the rheological properties and the submicro structure of the *peda* samples sold in Kolkata and its surrounding areas. A total of 25 samples of *peda* were collected from halwais and 5 samples from standardized milk were prepared in the laboratory to draw a comparison between them. The electrophoretic properties of the *peda* casein were also studied and compared with those of milk casein. Storage studies of laboratory made *peda* samples regarding the chemical, microbiological and sensory qualities were also carried out at both ambient and refrigeration temperatures. The effect of addition of preservatives (nisin and sodium metabisulphite @ 1000 ppm on the basis of milk) and also effectiveness of different packaging materials (paper box and polypropylene pouches) were studied. The results obtained in the present investigation have been summarized below:

1. Cow milk standardized to 4.5% fat for the preparation of *peda* was found to be the best on the basis of sensory evaluation of the *peda* samples prepared from it.

2. A sugar content of 8% on the basis of milk for *peda* preparation was preferred by most of the judges of the sensory panel and was taken for *peda* manufacture in the laboratory.

3. The average chemical composition of standardized milk used for the manufacture of *peda* in the laboratory was: fat – 4.50 ± 0.002%, SNF – 8.52 ± 0.01%, protein – 3.60 ± 0.08%, lactose – 4.81 ± 0.06%, ash – 0.71 ± 0.03% and titratable acidity – 0.142 ± 0.04% in terms of lactic acid.

4. *Peda* prepared from standardized milk in the laboratory was assessed in respect of chemical quality. The overall average results found to be : Total solids – 83.83 ± 0.45%, fat – 24.90 ± 0.28%, protein – 12.98 ± 0.31%, lactose – 15.95 ± 0.18%, sucrose – 25.97 ± 0.09%, ash – 2.49 ± 0.02%, titratable acidity – 0.058 ± 0.06% in terms of lactic acid, free fat – 75.43 ± 0.62% of total fat, free fatty acid – 0.062 ± 0.005% oleic acid, peroxide value – 1.11 ± 0.008 m. eq. of O_2/kg fat and HMF value – 0.376 ± 0.007 micromoles / 100 gm.

5. The overall average chemical composition of market *peda* samples were found to be : total solids – 86.45 ± 1.62%, fat – 11.30 ± 2.88%, protein – 9.64 ± 2.95%, lactose – 13.03 ± 2.83%, sucrose – 51.77 ± 5.41, ash – 2.50 ± 0.26%, titratable acidity – 0.62 ± 0.13% lactic acid, free fat – 57.62 ± 6.38% of total fat, free fatty acid – 0.075 ± 0.006% oleic acid, peroxide value – 1.14 ± 0.02 m. eq. of O_2/kg fat and HMF value – 0.620 ± 0.09 micromoles / 100 gm.

6. The overall average microbiological quality of the laboratory made *peda* samples was : SPC – 11.6×10^3 cfu/gm, coliform count – nil and yeast and mold count – 58 cfu/gm.

7. The overall average microbiological quality of the market *peda* samples was : SPC – 23.6×10^5 cfu/gm, coliform count – 120 cfu/gm and yeast and mold count – 3.75×10^2 cfu/gm.

8. The overall average sensory scores of the *peda* samples prepared in the laboratory from standardized milk were : flavour – 7.4 ± 0.07, colour and appearance – 7.2 ± 0.06, body and texture – 7.3 ± 0.18, sweetness – 7.9 ± 0.07 and overall acceptability – 7.8 ± 0.07.

9. The overall average sensory scores of the *peda* samples collected from market: flavour – 7.2 ± 0.8, colour and appearance – 7.1 ± 0.9, body and texture – 7.3 ± 0.7, sweetness – 6.6 ± 0.16 and overall acceptability – 7.4 ± 0.8.

10. Hence it appears that *peda* samples prepared in the laboratory from standardized milk were best in overall quality followed by those collected from local market.

11. The average value of texture profile i.e., hardness, fracturability, springiness, cohesiveness, gumminess and chewiness of the laboratory made *peda* samples were found to be 55.10 ± 1.04 kg, 0.0359 ± 0.002 kg, 0.272 ± 0.002 mm, 0.228 ± 0.011 mm, 11.18 ± 0.91 kg and 0.0034 ± 0.0002 kg m respectively. However, those values for market *peda* samples were found to be 152.02 ± 34.84, 20.60 ± 11.38, 0.282 ± 0.04, 0.19 ± 0.04, 43.31 ± 21.13 and 0.0121 ± 0.006 respectively.

12. From the texture profile of laboratory made and market *peda* samples it seems that the market samples had higher total solids than the laboratory made samples and also the laboratory made *peda* samples were finely worked as compared to market samples.

13. The scanning electron micrograph of the laboratory made *peda* samples showed heat coagulated proteins coalesced together to form the compact protein matrix with small pores filling with milk serum, fat globules and small lactose crystals. Whereas the scanning electron micrograph of market *peda* samples showed coalesced, ragged protein matrix linked together in a compact thick bridges. The agglomerated protein matrix interlinked strongly forming compact scale or layer type structure. The size

of the lactose crystals and fat globules in the inter granular spaces were larger as compared to the laboratory made samples.

14. The polyacrylamide gel electrophoretic (PAGE) pattern of the proteins isolated from milk revealed the appearance of two lower molecular weight components. Whereas, the PAGE pattern of *peda* proteins showed three prominent bands having lower and higher electrophoretic mobility.

15. The chemical quality of the *peda* samples prepared in the laboratory were studied during storage regarding moisture, titratable acidity, free fatty acid, peroxide value, tyrosine value and HMF content. All of the samples showed a gradual increase in all parameters as the storage period increased except moisture, where a decrease in result was observed.

16. The microbiological quality of *peda* samples was studied on the initial and final day of storage regarding standard plate count, coliform count and yeast and mold count. All of the samples showed a considerable increase in number of organisms as the storage period increased except coliform count which was not found in any of the samples.

17. The sensory quality of *peda* samples was studied on the initial and final day of storage regarding its flavour, colour and appearance, body and texture and overall acceptability. All of the samples showed a decrease in the sensory scores as the storage period increased.

18. Nisin (1000 ppm) treated samples, under refrigerated condition, declared unacceptable after 30, 38 and 66 days when kept without packaging, packed in paper box and polypropylene pouches respectively by the panel of judges. Whereas, sodium metabisulphite (1000 ppm) treated samples were found

unacceptable after 24, 30 and 58 days under refrigerated storage when kept without packaging, packed in paper box and polypropylene pouches respectively.

This it can be suggested that out of the two preservatives studied, *peda* prepared with 1000 ppm nisin on the basis of milk and packaged in polypropylene pouches can be better effectively stored at refrigeration temperature without moderate change in its chemical, microbiological and sensory parameters studied.

Chapter - VI

Future Scope of Research

Future Scope of Research

From the investigation carried out on the indigenous dairy product – *peda*, the following research work are recommended to be done in future.

1. Findings of previous researchers regarding the nutritive value of *peda* need to be explored further for their application to the Indian situation.

2. Process innovation is also needed for *peda* manufacture.

3. A parallel marketing system is required to ensure adequate returns and the assurance of a regular market to the producers.

4. Further study is to be carried out in order to increase the shelf-life of *peda*.

5. Investigation is also to be carried out on the effective packaging of *peda* bearing the economical sustainability of the product.

6. The electrophoregram of the casein of milk as well as *peda* can be compared with standard so that the breakdown product released during manufacture can be identified.

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