2. REVIEW OF LITERATURE

India’s traditional dairy products sector is poised for rapid expansion due to the application of modern process and preservation technologies. The raising demand for packaged, fresh dairy products like dahi, paneer, lassi etc is widening the base of the modern dairy sector. The milk production is estimated to be 91.1 million tons in 2004 (Narang, 2004) in which about 45-50 % is converted into a variety of traditional milk products by heat and acid coagulation, heat desiccation and fermentation. Indian traditional products market is estimated to be more than Rs. 6,500.0 crores (Patil, 2004). Punjrat (1995) reported that during manufacture of traditional products, very little attention is paid to sanitation and packaging, which result in high incidence of microbial population as well as large variations in chemical composition, flavor and texture in these products. Furthermore, higher water activity of the product accelerates faster deterioration. Therefore, emphasis should be made on to employ suitable post processing treatments and packaging methods to reduce the microbial load and to improve the shelf life of Indian traditional dairy products.

As the traditional dairy products preparation is labour intensive and the quality of finished products are highly variable in terms of physical, chemical, microbiological and sensory characters, there is an urgent need to produce uniform and high quality products through modernization (Patil, 2002). An estimated one per cent of the country’s total milk production is converted into paneer accounting to annual production of 1, 50,000 tonnes. (Aneja et al., 2002).


2.1. Paneer

2.1.1. Definition
According to Prevention of Food Adulteration Act (1983), paneer has been defined as a product obtained from the cow or buffalo milk or combinations thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 70 % moisture and milk fat content shall not be less than 50 % of the dry matter. The milk fat content of skim milk paneer shall not exceed 13.0 % of the dry matter.

As per the Bureau of Indian Standards (IS: 10984, Part III, 1983), the total plate count should not exceed 5 x 10^5, coliform count not more than 90 and yeast and mould count not more than 250 cfu/g of paneer.

2.1.2. Characteristic

Paneer is obtained through heat/acid coagulation of casein in milk, entrapping fat inside a complex physico-chemical interaction, a part of denatured whey proteins and colloidal salts as well as a part of the soluble milk solids. Typically paneer is marble to light creamy white in appearance. It must have firm and cohesive body with slight sponginess or springiness. The texture should be more compact (close-knit), smooth and velvety. The flavour should be pleasing mild acidic, slight sweet and nutty (Dharm Pal and Gupta, 1985; Patil and Gupta, 1986)

2.1.3. Composition

Paneer contains nearly all the fat, caseins and insoluble salts of milk. It also contains part of the moisture of original milk in which lactose, whey proteins, soluble salts, vitamins and other milk components are present in proportion to their amount. Rao et al. (1992) have reported wide variations in the chemical composition of paneer due to differences in the initial composition and type of milk used. The chemical composition of paneer made from different types of milk as reported by different researchers is given here under:

### Chemical composition of paneer

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Moisture</th>
<th>Total solids</th>
<th>Fat</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Ash</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>56.00</td>
<td>44.00</td>
<td>22.00</td>
<td>18.50</td>
<td>2.10</td>
<td>1.40</td>
<td>Sachdeva et al. (1991)</td>
</tr>
</tbody>
</table>
2.1.4. Standardization of milk

To meet the PFA standards in paneer, milk must be standardized to a fat to S.N.F ratio of 1:1.65 (Sachdeva and Singh, 1988). Vishweshwaraiah and Anantakrishnan (1985) reported that the paneer made from cow milk having less than 4.5 % fat did not meet the PFA standards while Singh and Kanawajia (1988) recorded that a minimum of 5.0 % fat in cow milk is required to fulfill the standards.

2.1.5. Method of manufacture

Although buffalo milk is preferred for manufacture of paneer, methods have been standardized to prepare good quality paneer from cow milk (Singh and Kanawajia, 1988 and Sachdeva et al., 1991). Aneja et al. (2002) have reported a process for industrial scale production, using the available cheese/casein/tofu manufacturing equipments.

2.1.6. Shelf-life study of paneer

2.1.6.1. Physical treatment
2.1.6.1.1. Low temperature storage

Arora and Gupta (1980) studied the effect of low temperature storage of paneer at 10° C, -13° C and -32° C. There was no significant change in the moisture, total nitrogen, non-protein nitrogen (NPN) and pH up to 6 days of storage at 10° C, but the pH decreased significantly on the 7th day. They have also reported that no significant change was observed on the sensory attributes during the 6 days storage at 10° C, however after 7th day it gave putrid odour and became unacceptable. While the sensory quality of frozen paneer remained good up to 90 days after that the body and texture became crumbly and fluffy. Vishweswaraiah (1987) stored paneer at -9° C or -15° C and recorded a shelf-life of 80 days and observed not much difference in the textural and sensory attributes except surface drying of paneer. Kanawjia and Singh (1996) observed that paneer stored at -28° to -30 C had acceptable scores even after 95 days of storage.

2.1.6.1.2. Dehydration

Vishweswaraiah (1987) dried extruded paneer at 75° C for 2 hrs and observed that paneer stored well up to 2 months as against control which had 3 days at 10° C, however the rehydration characteristics of dehydrated paneer (5-9% moisture) was poor and lacked cohesiveness.

2.1.6.1.3. Sterilization

Sachdeva (1983) subjected paneer cubes packed in tin cans to sterilization in autoclave at 15 PSI for 15 min and reported that the paneer kept well for a period of 50 days at ambient temperature, however the product developed mouldy flavour, slight browning and cooked flavour.

2.1.6.1.4. Vacuum Packaging

Sachdeva et al. (1991) reported that the shelf-life of paneer at 6±1° C was 8 days which could be extended to 38 days with vacuum packaging. Kanawajia et al. (1990) observed the efficiency of vacuum packaging in increasing the shelf-life of control samples and the samples dipped for 2 hrs in solutions
containing i) Hydrogen peroxide (0.2%) ii) Delvocid (0.5%) iii) Hydrogen peroxide(0.25%) and iv) Delvocid (0.5%) from 10,12,25 and 35 to 14, 18, 35 and 50 days respectively at refrigerated temperature.

2.1.6.2. Chemical treatment.

2.1.6.2.1. Acidified water

The use of acidulated water at pH 5.5 and 2.5 did not favour extension of shelf-life of paneer at refrigerated temperature and the sample spoiled after 6 days of storage. The deterioration was more for paneer dipped in acidulated water at pH 2.5 than at pH 5.5 (Sachdeva and Singh 1990b).

2.1.6.2.2. Antioxidants

Pawan Kumar and Bector (1991) explored the efficiency of Tertiary Butyl Hydroquinone (TBHQ) and Butylated Hydroxy Anisol (BHA) in enhancement of shelf-life of paneer and reported that the two antioxidants either individually (0.05%) or together (0.05%) were effective in extending the shelf-life of paneer to 20, 7, days and 90 hrs at 5°C, 15°C and 25°C respectively against 10, 4 days an 24 hrs for control sample. They further recorded that there was increase in the acidity, free fatty acid and soluble nitrogen contents, total plate counts, coliforms and yeast and mould counts in control, while antioxidant treated samples showed marked reduction in the microbial counts and thus the glycolytic, lipolytic and proteolytic changes were checked to a greater extent. They also observed rapid deterioration in flavour score of control than antioxidant treated samples and the deterioration was faster for products stored at 25°C.

2.1.6.2.3. Benzoic acid

Modi and Jain (1988) extended the shelf-life of paneer by treatment with benzoic acid at 1200 ppm (0.12%) to 40 and 20 days at refrigerated and room (37°C) temperatures respectively. The inhibitory effect of benzoic acid may be due to its ability to block the oxidation of glucose and pyruvate at the acetate level.

2.1.6.2.4. Brine solution
Sachdeva (1983) suggested dipping of paneer in 5 % brine solution enhanced the shelf-life of paneer to 22 days at refrigerated temperature. Shukla et al. (1984) recorded a shelf-life of 6 days at room temperature when raw paneer was dipped in 18 per cent salt solution for 30 min. Singh et al. (1988) observed that overnight dipping of paneer in brine solution followed by packaging and refrigeration storage could increase the keeping quality up to 12 days. Acidification of brine solution to pH 5.5 had slightly reduced the keeping quality from 22 to 20 days at refrigeration temperature (Sachdeva and Singh, 1990). Makhal (2000) dipped paneer in chilled (4-6°C) brine (4.5 %) for 2 hrs followed by packaging in previously sterilized polyethylene pouches (55 microns thick) using hydrogen peroxide solution (20 %) and recorded a keeping quality of 20 days at 5±1°C.

2.1.6.2.5. Buffered water

Sachdeva and Singh (1990) observed that immersing paneer in buffered solutions (pH 7.5) containing Sodium bicarbonate (2.5 %) and Calcium phosphate (2.5 %) caused deterioration and the sample spoiled on 8th day when stored at refrigeration temperature.

2.1.6.2.6. Chlorinated water

The shelf-life of paneer did not improve when dipped in chlorinated water (35 ppm), rather the treatment had detrimental effect on the flavour score of paneer (Sachdeva and Singh, 1990)

2.1.6.2.7. Delvocid with hydrogen peroxide

The preservative effect of Delvocid (0.5 %) (a non-toxic antibiotic produced by Streptomyces natalensis) in combination with Hydrogen peroxide (0.2 %) was investigated on paneer (Sachdeva, 1983). He reported that the shelf-life of treated paneer was 32 days at 8-10°C, while paneer immersed in Delvocid solution (0.5 %) for 1-2 hrs had deteriorated on 8th day at refrigeration storage.
2.1.6.2.8. **Potassium sorbate**

Thakral *et al.* (1990) reported that paneer containing 0.1 % Potassium sorbate could preserve up to 13, 3 days and 1 day at 7, 22 and 37° C respectively. Further, they indicated incorporating nisin together with potassium sorbate could increase that paneer shelf life. The use of Potassium sorbate (2.0 %) solution as dipping medium did not extend the shelf-life of paneer and the treated paneer kept well only up to 10 days at refrigerated temperature (Sachdeva and Singh, 1990a). They also recorded increase in the acidity, pH, soluble nitrogen and free fatty acid contents in the treated samples and the increase was rapid towards the end of storage.

2.1.6.2.9. **Sorbic acid**

Addition of Sorbic acid to milk (0.10 %) before preparation of paneer and subsequent wrapping of paneer in sorbic acid coated paper (2g/m²) extended the shelf life of product to 6 days at ambient temperature and 36 days at 5° C was achieved by addition of 0.05 per cent sorbic acid in milk (Singh *et al*., 1989).

2.1.6.2.10. **Sorbic acid and irradiation**

Singh *et al.* (1991) studied combined effect of Sorbic acid addition (0.05, 0.10 and 0.15 %) and irradiation treatment (2.5, 5.0 and 7.5 kGy) on shelf-stability of paneer and recorded the combined treatment of 0.01 per cent sorbic acid in milk and irradiation of product at 2.5 kGy preserved the paneer for 30 days at ambient temperature.

2.1.6.3. **Packaging Material and Techniques**

Paneer packed in parchment paper could be stored for 6 days at refrigeration and 16 days in tightly wrapped in heat shrink film (Sachdeva, 1983). Bector *et al.* (1998) investigated different packaging materials like LDPE (200 gauge), opaque MXXQ (300 and 100 gauge) and MST cellulose film (300 gauge) for enhancing the shelf-life of paneer and reported that the MST cellulose film with 300 gauge thickness enhanced the shelf-life of paneer at refrigerated
82
temperature. Vishweshwaraiah and Anantakrishnan (1985) observed that smearing the surface of packaging material with sorbic acid did not enhance the shelf life of paneer but reduced considerably the yeast and mould. They also recorded that the keeping quality of paneer packed in butter paper and polyethylene was smeared with sorbic acid was 2 days at 28° C and 7 days at 5° C.

2.1.6.4. Hurdle Technology

Application of Hurdle Technology for the preservation of Ready-to-Eat paneer curry was investigated (Rao and Patil, 2001). They reported employing more than one preservation parameter such as water activity ($a_w$, pH and Redox-Potential ($E_h$)) and heat treatment and recorded that paneer could be stored well for 30 days at 30° C.

2.1.6.5. Use of GRAS Additives

The role of GRAS additives like cardamom, clove, cinnamon and ginger in preservation of paneer was investigated (Makhal, 2000). These additives were individually added at the time of coagulation to milk. cardamom, clove and cinnamon were added @ 1.0, 1.5 and 2.0 gm and ginger was added @ 5.0, 8.0 and 11.0 gm per kg of milk. Paneer containing cardamom, clove, cinnamon and ginger each for low, medium and high doses showed shelf-life of 28, 32 and 36 days; 24, 28 and 32 days; 24, 28 and 32 days and 32, 36 and 40 days respectively at 5±1° C. Organoleptic and physico-chemical studies during storage revealed that medium dose of four spices were found to be more effective and the treatment with ginger (medium dose) was most effective for preservation of paneer.

2.1.7. Textural studies

2.1.7.1. Raw paneer

Arora and Gupta (1980) evaluated the effect of low temperature on textural quality of paneer prepared from milk containing different fat levels (4, 5 and 6%) using precision penetrometer and reported that there was no significant change in the penetration values up to 6 days of storage at 10° C, while there was a substantial
increase in the penetration values during storage at –13° and –32° C for all paneer samples, implying that the paneer samples became softer due to proteolysis during frozen storage.

Awadhwal and Singh (1985) investigated the force-deformation characteristics of paneer at 15° C using Instron Universal Testing Machine and found that the observed and the predicted forces were in good agreement. The rheological properties such as cohesiveness, springiness and chewiness of conventional buttermilk extended and buttermilk extended low-fat paneer were almost similar to that of control but the hardness and chewiness of control paneer were higher than experimental sample (Dharm Pal and Garg, 1989a). Mudgal (1993) found that the textural characteristics of traditional paneer differed considerably from one batch to another. Hardness of paneer (137g) prepared by centrifugal method was higher than the corresponding value from delayed method (85g) but was lower than that observed from immediate method (151g), however there was no clear cut trend observed in the textural qualities viz., gumminess, springiness and chewiness (Agrawal, 1997). Desai et al., (1991) studied the textural profile of different market samples using Instron Universal Testing Machine and reported that the hardness and chewiness of raw paneer varied significantly among different samples whereas, the variations in the cohesiveness and springiness were not significant. Further they observed that paneer samples with higher moisture showed lower hardness and vice versa.

2.1.7.2 Fried paneer

Generally, raw paneer is deep fat fried before being cooled along with vegetables. Sachdeva and Singh (1987) reported that deep fat frying of paneer had improved the acceptability of paneer. Chawala et al. (1985) recommended 175-185° C as a frying temperature of vegetable oil. Rao and Patil (2001) studied the effect of different “hurdles” viz., water activity, pH and heat treatment on the rheological properties of fried paneer immediately after processing as well as during storage using Instron Universal Testing Machine and reported that the above parameters profoundly influenced the hardness and chewiness during processing and storage of paneer. They further recorded that decrease in water activity and pH increased hardness and chewiness, where as heat treatment decreased them.

2.1.7.3 Cooked paneer
As frying, cooking of fried paneer was also reported to increase its acceptability. Fried paneer was cooked in boiling water having twice as much as the weight of paneer using 1.25-1.5 per cent common salt for 4-5 min (Chawla et al., 1987; Sachdeva and Singh, 1987). Kalab et al. (1988) reported that deep fat frying (175°C for 4.5 min) of paneer led to squeezing of individual protein particles, whereas the cooking of the fried paneer in boiling water with 1.5 per cent salt for 5 min. resulted in partial restoration of the original structure of paneer. Cooking had a tendency to narrow down the textural differences of deep fat fried paneer made from milk with different fat levels. Pant et al. (1993) reported that the springiness of paneer before and after deep-fat frying remained more or less same, while the hardness, chewiness, cohesiveness and increased after frying. Agarawal (1997) reported that frying of raw paneer resulted in 2.15 to 2.33 times increase in hardness, however, cooking led to 0.233 to 0.325 times retention of hardness of fried paneer. The texture profile parameter of market samples of paneer were considerably altered in frying and cooking (Desai et al., 1991). They recorded that the hardness decreased and the springiness increased in paneer after subjecting the fried paneer to cooking and the decrease in the hardness was much more remarkable as compared to the increase in springiness. Further they reported that chewiness also registered appreciable decrease as a result of frying and cooking, while cohesiveness increased only slightly.

2.2. Microwave processing

Microwave used in the food industry for heating are the Industrial, Scientific and Medical (ISM) frequencies 2, 450 MHz or 915 MHz, corresponding to 12 cm or 35 cm in wave length. Microwaves are generated by magnetron that converts electrical energy (60 Hz) into an electromagnetic field with centers of positive and negative charges. Majority of foods contain a substantial proportion of water. The molecular structure of water consists of a negatively charged oxygen atom and positively charged hydrogen atoms and it forms an electrical dipole. When microwave is applied to a food, dipoles in the water attempt to orient themselves to the field. Since the rapidly oscillating electric field changes from positive to negative and back again several million times per second, the dipoles attempt to follow and these rapid reversals create frictional heat. The increase in temperature of water molecule heats surrounding components. (Ohlsson and Bengtsson, 2002). Microwaves are reflected by metals, transmitted by electrically neutral materials such as glass, most plastics, ceramics and
paper, and absorbed by electrically charged materials like several food constituents including water (Mullin, 1995).

Although microwave heating technique was introduced during the sixties, only at the beginning of the 1980s, the possibilities of applying microwave energy were widely recognized. There are advantages evident, such as faster operation, energy saving, higher product quality (retention of nutritional and sensory properties) as in application like enzyme inactivation, pasteurization, sterilization, tempering and drying food products (Fito et al., 2005). Microwave has been successfully applied in the food and dairy industries in processing of fruits and vegetables, cereals, meats, butter, cheese, yoghurt and ice cream mixes to inactivate number of microbes, enzymes and bacteriophages (Young and Jolly, 1990; Schiffman, 1992).

2.2.1 Applications of Microwave processing in dairy industry

2.2.1.1. Pasteurization/ Sterilization

Many studies have been undertaken to increase the shelf-life of milk by Kathleon et al. (1988). Villamiel et al. (1996) showed that microwave pasteurization of milk for 2.5 min resulted in 97.7% reduction of bacteria. Microwave heat treatment of milk for 3, 8 and 10 min completely inactivated \textit{C. jejuni}, \textit{Y. enterocolitica} and \textit{L. monocytogenes}, respectively (Sieber, 1996). Thompson and Thompson (1990) demonstrated that a domestic microwave oven can be used effectively to reduce aerobic plate counts in raw goat’s milk by up to 6-log cycles without impairing the organoleptic quality.

Solid products are usually in-pack pasteurized or sterilized after being packaged, so no metallic materials can be used in packaging when microwaves are used in this process. Due to rapid heating of the product, a better retention of the nutritional properties in comparison with the current technologies can be obtained (Fito et al., 2005). Nevertheless, the heterogeneous heating of the products, depending on the size and shape, which does not ensure that all points of food reach the required temperature to induce microbial death (Buffler, 1992). Microwave application allows pasteurization of glass, plastics etc., which offers a useful tool for package treatment. The food products that best responds to microwave pasteurization treatment are pastry, prepared dishes, soft cheese (Burfoot and James, 1992). The technique has also been tested on
milk (Sierra and Vidal Valverde, 2001 and Valero et al., 2000) and fruit juice (Nikdel et al., 1993).

Fito et al. (2005) reported that the quality of microwave pasteurized milk is superior than that of conventionally pasteurized milk due to the rapid, uniform heating and because of the lack of hot contact surfaces. Assinder (1971) reported that microwave could be used in for sterilization of milk. He demonstrated that a jet of milk could be heated to 200°C in 40 milliseconds, and held for a further 130 milliseconds, before being cooled by turbulent mixing with jets of cold sterile milk. The sterile product was reported to be virtually distinguishable from the starting material and the shelf-life of the treated milk increased substantially. Hamid et al. (1969) reported killing rate of >99.9% after microwave treatment of milk for 12 sec or 65 sec. They further recorded that substantial reduction in bacterial count when batch microwave oven was used than continuous microwave pasteurization of milk which could probably due to poor temperature control. Kenyon et al. (1971) described a system in which food in a plastic package was heated by microwaves in a continuous process. In this system both radio frequency (27.12 MHz) and higher microwave frequency (2450 MHz) energy was used to heat the containers of yoghurt while being conveyed through water. The microwave heated the top 5-10 mm of the yoghurt, while the low radio frequency waves heated the rest of the contents.

2.2.1.2. Thawing /Tempering

Tempering of butter and frozen foods has been the most successful applications of microwave in the dairy industry (George, 1997). Thawing of frozen products has limited applicability in the dairy industry. However, melting of viscous products like fats from bulk containers, offers advantages of more efficient transport (Young and Jolly, 1990). Meredith (1986) reported a microwave system (120 KW at 896 MHz) for tempering butter at Anchor Foods Ltd at Swindon in the UK. The butter blocks are heated from –10°C to 2°C in 5 min. Tempering of 7 tonnes of butter in controlled temperature rooms takes around four days.

2.2.1.3. Cooking

Hussain et al. (1980) used microwaves for cooking Hallum cheese before and after pressing and compared with the conventional process in which the pressed curd is
cooked in the whey. Properties of the microwave-cooked cheese were similar to that of the conventionally cooked cheese.

Tochman et al. (1985) used microwaves for in-pack thermal treatment of cottage cheese and reported that the microbial count reduced immediately after microwave treatment. They also reported that the pH and sensory score cottage decreased, while the microbial counts increased during storage at refrigeration temperature.

2.3. Modified Atmosphere Packaging (MAP)

MAP is the enclosure of food in a package, inside which the atmosphere is modified with respect to Carbon-dioxide, Oxygen, Nitrogen, water vapor and trace gases. This modification is generally achieved using one of the two processes i.e., gas flush packaging or Vacuum Packaging (Farber, 1991).

In 1877 Pasteur and Joubert recorded that Bacillus anthracis could be inactivated by using MAP (Sivertsvik et al., 2002). The modified atmosphere composition has a marked impact on the growth of spoilage micro-organism as well as on pathogens. The anti-microbial effect of CO₂ on micro-organisms has been intensively documented, however, it has been shown recently that only CO₂ levels well above 20 % significantly affect the growth of psychrotrophic pathogens that are relevant to MA packaging (Bennik et al., 1995).

The shelf-life of indigenous milk products is shorter and their transportation to remote places is difficult. Therefore, efforts should be made to increase the shelf life of these products by adopting newer packaging techniques such as modified atmosphere packaging (MAP), active packaging (AP) etc., (Chopra, 1998).

The cooperative dairy sector in India spends nearly Rs.290 crores every year on packaging and the cost of packaging ranges from 2.1 to 24 per cent of the consumers price (Punjrath, 1995). The quality of packaging cannot be compromised. However, to stay ahead of competitors, packaging costs must be competitive. In this sense, modified atmospheric packaging technique is being used increasingly to reduce the rate of spoilage of fresh products and enhance the shelf life. This method is already been used for fresh cheese in the developed countries and is likely to be extended for packaging of paneer and paneer-like products in India.
2.3.1. MAP Gases

2.3.1.1. Carbon dioxide \((CO_2)\) and its effect on growth of microorganisms

Carbon dioxide is one of the important gases used in MAP (Zagory, 1997). Jennifer (1998) observed that \(CO_2\) had bacteriostatic and fungistatic properties. Enfors and Molin (1980) reported that the gram negative bacteria were more sensitive to \(CO_2\) while, the growth rates of lactic acid bacteria were much less affected. Various inhibition actions of \(CO_2\) on bacterial cell have been suggested (Farber, 1991; Daniels et al., 1985; Dixon and Kell, 1989; Davis, 1995) that are displacement of oxygen, penetration of cell membrane leading to changes in intracellular pH, direct changes brought in the physico-chemical properties of cell proteins, direct inhibition of enzymes or decrease in the enzyme activity and alteration of cell membrane, effect on nutrients uptake and absorption. Devlieghere et al. (1998a and 1998b) have demonstrated that the growth inhibition of micro-organisms in modified atmosphere is determined by the concentration of dissolved \(CO_2\) in the product.

2.3.1.2. Nitrogen \((N_2)\) and its effect on growth of microorganisms

The Nitrogen is considered as an inert and filler gas and replaces the Oxygen in the pack thus delay the oxidative rancidity of the product and inhibits the growth of aerobic microbes indirectly (Sahoo and Anjaneyulu, 1995). Sivertsvik et al. (2002) reported that \(N_2\) could be used as an alternative to vacuum packaging to inhibit the aerobic microorganisms.

2.3.1.3. Oxygen \((O_2)\) and its effect on growth of microorganisms

Oxygen is important for cell division and growth of aerobic microorganisms and for respiration of fruits and vegetables, however, the oxidation of unsaturated fatty acids leads to rancidity and spoilage foods (Brody and Marsh, 1997). The use of \(O_2\) in MAP in non-respiring products is normally eliminated or set as low as possible to inhibit the growth of spoilage bacteria, however high levels of \(O_2\) (30\%) are used in red meats to maintain red colour (Gill, 1996)

2.3.2. Application of MAP in Dairy Industry

2.3.2.1. Milk
Rashed et al. (1986) investigated the application of CO$_2$ to raw milk and recorded a standard plate count of $10^3$ cfu/ml after storage of milk for 24 hrs at 7°C. The CO$_2$ could be used to control the growth rate of psychrotrophic bacteria in both raw and pasteurized milk at refrigerated temperature. Hotchkiss et al. (1999) reported that CO$_2$ treated milk could keep well for two months at refrigerated condition. The effect of CO$_2$ incorporation on the keeping quality of milk inoculated with spoilage microorganisms was studied by Hotchkiss and Lee (1996) and reported that the keeping quality could be enhanced by 50-100% and were dependent on flushing of CO$_2$, high barrier package and storage temperature.

Glass et al. (1999) reported that upon carbonation of milk the number of viable organisms declined and also pH of milk reduced from 6.61 to 6.31 during refrigerated storage. Carbonation of milk also decreased pH from 6.61 (control) to 6.15 (1000 ppm CO$_2$) and increased the shelf-life of milk at refrigerated temperature has been reported by (King and Mabbit (1982). Ganguli, (2001) suggested that carbonation would effectively improve the shelf-life of bulk chilled milk without affecting the vitamins such as A and E.

Werner and Hotchkiss (2002) studied the effect of addition of CO$_2$ on the growth of Bacillus cereus spores inoculated in sterile homogenized whole milk at $10^1$ and $10^6$ level per ml and stored at 6.1°C for 35 days and reported that added CO$_2$ reduced the pH of milk from 6.61 to 6.31. They also concluded that the number of viable organisms declined in the sample, had no effect on spoilage and risk of food poisoning.

2.3.2.2. Yoghurt

Karagül-Yuceer et al. (2001) reported that carbonation of yoghurt declined E. coli and L. monocytogenes counts without significantly affecting the growth of typical yoghurt bacterial strains and probiotic organisms in yoghurt. Tamime and Deeth (1980) stated that gas flushing with Carbon dioxide or Nitrogen was a viable alternate preservation method to extend the shelf life of fruit-flavoured yoghurt. Fairbain and Law (1986) recommended the use of carbonation process to improve the shelf-life of yoghurt as the process is cheap, safe and apparently does not have any negative impact on the quality of the product. Flushing of gaseous CO$_2$ into yoghurt to increase the shelf-life has been proposed and patented by Ogden and Inventor (1997).
2.3.2.3. Cottage cheese

Fedio et al. (1994) investigated the effect of MAP on the growth of microorganisms in cottage cheese and reported that samples inoculated with listeria showed growth in packages containing air and 100% Nitrogen but not in packages containing elevated carbon dioxide levels. The growth of pseudomonas and yeast and moulds was observed in samples packaged in air while the growth was slightly suppressed in samples packaged in N₂. The 100% CO₂ environment inhibited the growth of many microorganisms and extended the shelf-life of cottage cheese to 60 days. They recommended that the cottage cheese should be packaged in atmosphere containing high CO₂ level to attain a shelf-life of 28 days.

Bishop and White (1985) reported that shelf-life of cottage cheese in a barrier plastic container held at refrigerated temperature was 10-21 days while Horner (1988) demonstrated that the quality of MA packaged cottage cheese remained well for longer time compared with air packaging. The quality of cottage cheese could be extended up to 28 days by using MAP technique (Maniar et al., 1994). The effect of dissolved CO₂ on gram negative bacteria was investigated and reported that the keeping quality of direct-set cottage cheese packaged in high barrier containers when flushed with 100 % CO₂, 75 % CO₂ and 25 % N₂ remained satisfactory after 28 days, with 100% CO₂ producing best results (Chen and Hotchkiss, 1991).

Chen and Hotchkiss (1993) inoculated Listeria monocytogenes in low fat cottage and packed under CO₂ atmosphere in a polystyrene tube. They stored the sample at 7±1°C and evaluated changes and reported that Listeria monocytogenes increased slowly over the period of 63 days.

Kosikowski and Brown (1973) observed that CO₂ or N₂ flushing suppressed the growth of yeast, moulds and psychrotrophs in cottage cheese for up to 112 days, however the cottage cheese retained excellent flavour and texture for up to 45 days at 4°C. Slight bitterness was apparent in cottage cheese stored for 73 days at 4°C. Mann (1991) reported that significant inhibitory effect of pseudomonas in cottage cheese by bubbling CO₂ through the cheese before packaging.

The dairy products shelf-life could be extended through MAP to control fungal problems. Various fungi respond differently to the altered gaseous environment produced by MAP. The growth of Penicillium roqueforti was not much affected by
lower amounts of O₂ and CO₂ in the package, while that of *Penicillium verrucosum* was significantly reduced under high CO₂ levels (Haasum and Nielsen, 1998).

Mermelstein (1997) advocated the use of CO₂ in the curd dressing for Cottage cheese to improve its shelf-life. Cottage cheese dressing carbonation to 600 -1100 ppm increased the shelf-life of Cottage cheese to 8 weeks as compared to normal shelf-life of 3 weeks in control. Westall and Filternborg (1998) studied the influence of yeast on the spoilage of decorated soft cheese packed in MAP and found that the increase in CO₂ concentration affected the growth of spoilage yeast.

### 2.3.2.4. Mozzarella cheese

Alves *et al.* (1996) studied the stability of sliced mozzarella cheese in MAP. They packaged about 174 g of product in expanded polystyrene tray placed in gas-barrier laminate (EVA/PVDC/EVA) under four different atmosphere (100% CO₂, 100% N₂, 50% CO₂ / 50% N₂ and conventional air). The shelf-life of cheese was 13 days at conventional air package, while the 100% N₂ atmosphere had only 16 days at 7±1°C. A significant shelf-life increases was found under Carbon dioxide atmosphere as compared with air as follows: 63 days (385 % increase) and 45 days ( 246 % increase) for products under 100% CO₂ and 50% CO₂ / 50% N₂ respectively at 7±1°C. They further concluded that the inert atmosphere containing 100 % N₂ was not effective in controlling the microbial deterioration of mozzarella cheese. They recorded that the cheese under 50% CO₂ / 50% N₂ atmosphere showed reduction in the rate of development of aerobic psychrotrophs and yeast and mould, while in case of cheese in the atmosphere of 100% CO₂ the growth of aerobic psychrotrophs, yeast and mould were initially increase and diminished after 6 weeks. Further they reported that atmosphere with higher percentage of CO₂ did not cause undesirable changes in cheese.

Eliot *et al.* (1998) studied the stability of shredded mozzarella cheese under eight modified atmosphere packaging (air, vacuum, CO₂, N₂ and mixtures of CO₂ / N₂ in different proportions) for eight weeks. They reported that the MAP containing CO₂ efficiently stabilized lactic acid and mesophilic organisms, while inhibited staphylococci, yeast and moulds. The psychrotroph grew in all samples but were less numerous in high CO₂ atmosphere. Higher CO₂ concentrations were more effective than N₂ to control mesophills and was effective than vacuum packaging in reducing and inhibiting yeast and moulds. They concluded that CO₂ levels at 75 % and above were
found to be the most appropriate for maintaining microbiological quality and safety of shredded mozzarella cheese during 8 weeks of storage.

2.3.2.5. Whey cheese

Pintado and Malcata (2000) studied the effect of MAP on the microbial ecology in Requeijao cheese (a Portuguese whey cheese) at various storage temperature (4, 12 and 18° C). The MAP constituted 100% CO₂, 100% N₂ and 50% CO₂ and 50% N₂. They reported that the viable numbers of enterococci, staphylococci, yeast and spore-forming bacteria in the sample did not increase much within 15 days when stored at 4° C under 100% CO₂, but those of enterococci increased significantly. They also observed that storage of whey cheese at 4° C under 100% CO₂ atmosphere led to 15 days extension of shelf-life.

2.3.2.6. Quarg cheese

Rosenthal et al. (1991) studied the effect of CO₂ (67.1 % CO₂, 26.3 % N₂ and 6.6 % O₂) on the shelf life of Quarg cheese by monitoring pH, flavour, yeast and mould count. They reported that growth of yeast and mould was slow and increase in the pH value was less for the entire period of storage (67 days at 4°C), while the cheese stored under ambient temperature showed increase in the yeast and mould count as well as pH when observed in cheeses stored under CO₂ enriched atmosphere. The growth of gram-negative bacteria was also inhibited and the flavour of cheese was preserved. They concluded that the CO₂ was found to be bacteriostatic and fungistatic.

2.3.2.7. Cameros cheese

Fandos et al. (2000) evaluated the shelf-life quality of Cameros (fresh cheese made from pasteurized goat’s milk) cheese packed in different gas environments viz., 20%N₂/80%CO₂, 40%N₂/60%CO₂, 50%N₂/50%CO₂, 100%CO₂ and control. They reported that increase in FFA, soluble nitrogen and decrease in pH in control as compared to experimental samples during storage at 3-4° C. Among experimental sample, the 100%CO₂ showed very less changes in the above constituents as compared to rest of the samples. They also reported that the increase in the mesophiles, psychrotrops, enterobacteriaceae and coliforms in the entire sample and the increase were higher in control than experimental samples, while the cheese under 100% had
slow growth rate than rest. The sensory quality of all cheese decreased and the decrease was greater in control than rest of the paneer. The organoleptic quality of cheese under 40%N₂/60%CO₂ and 50%N₂/50%CO₂ was better than cheese under 100%CO₂. The concluded that 40%N₂/60%CO₂ and 50%N₂/50%CO₂ gas environment are the most effective for extending the shelf-life of Cameros cheese and retaining good sensory characteristics.

2.3.2.8. Taleggio cheese

Piergiovanni et al. (1993) studied the storages changes in Taleggio (a soft fresh Italian cheese with moldy smeared surface) cheese packed under 100% N₂, 90% N₂+10%CO₂, 80% N₂+20%CO₂ and 50% N₂+50%CO₂. They reported that a slow rate of increase in the acidity and soluble nitrogen contents during storage of cheese. However, the rate of increase was slower in cheese stored under 50% N₂+50%CO₂ than other cheeses. Further, they recorded minimum organoleptic changes in cheese packed under 50% N₂+50%CO₂ during refrigeration storage.