This is to certify that the

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ULTRAFILTRATION IN SOFT WHITE "DOMIATI" CHEESE MANUFACTURE

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ENAYAT AHMED GOMAA

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Major professor

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ULTRAFILTRATION IN SOFT WHITE "DOMIATI" CHEESE MANUFACTURE

BY

Enayat Ahmed Gomaa

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ABSTRACT

ULTRAFILTRATION IN SOFT WHITE "DOMIATI" CHEESE MANUFACTURE

BY

ENAYAT AHMED GOMAA

The effects of pilot plant scale ultrafiltration (UF) on the retention of milk and whey components were studied. Also, the technical feasibility of ultrafiltration in the manufacture of white soft "Domiati" cheese was studied.

Retentate produced by diafiltration was lower in lactose and ash and higher in protein contents than retentate produced by ultrafiltration. Electrophoretic patterns of protein revealed substantial increase in its α-lactalbumin and β-lactoglobulin. Fat and minerals associated with protein were quantitatively retained in the retentate.

Soft white Domiati cheese was made from whole milk and its ultra/diafiltrated retentate using the Maubois, Mocquot, and Vassal (MMV) and/or conventional technique. Chemical analysis confirmed the retention of more whey proteins in UF-cheese than in conventional cheese. Higher concentrations of free amino acids and free fatty acids were also observed in UF-cheese. Sensory evaluation of the flavor, body/ texture and color revealed that UF-cheese was

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When UF- and conventional cheeses were ripened in pouches for three months at 10°C, a significant increase in solids content, free amino acids and free fatty acids was observed. Texture profile analysis and electrophoretic resolution indicated differences between the two cheeses.

In another experiment retentate was freeze-dried, then reconstituted for making cheese. Cheese was ripened by two methods: (a) in polyethylene-lined aluminum pouches sealed under vacuum, and (b) in 8 % brine in plastic containers at 7°C for eight weeks. Pouch-ripened cheese had higher solids content than cheese ripened in brine solution at $p < 0.05$. The liberation of free fatty acids and free amino acids was significantly increased for both cheeses with greater values for pouch cheese than for brine cheese. The new method of vacuum pouch ripening resulted in improved cheese yields and an acceleration of cheese ripening.

A process for incorporating whey protein concentrate (WPC) into milk to observe its effect on Domiati cheese yield and quality was investigated. Milk was supplemented with WPC on protein basis up to 1:1. Increasing the proportion of WPC resulted in cheese with increasing content of total solids, protein, lactose and decreasing content of fat and coagul- ation time. Maximum cheese yield was obtained with milk mixtures of 1:0.75 protein ratio. Hardness, chewiness and gumminess were significantly high in unsupplemented cheese.

DEDICATION

Dedicated to my family

My husband, Dr. Mohamed Abouzied

My daughter, Amy

My son, Sherif

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The author is grateful to the people and Government of Egypt who supported her during this study.

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Last, but not least, I am forever indebted to my husband, Mohamed, for his love, support, and encouragement thought this long and difficult process.

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INTRODUCTION

Soft white " Domiati" cheese is the most popular pickled cheese variety in Egypt and the Middle East (Abou-Donia 1986). Variants are made in Europe and in many other countries in South America. Domiati closely resembles Greek Feta cheese.

Domiati cheese differs chiefly from other pickled varieties in that it is salted at the very first step in its manufacture; the salt is added directly to the milk.
In Egypt the manufacturing process for Domiati cheese can be summarized as follows: One third of the cow's milk is heated to 80 C, and salt (5-14%) is added to the remainder. The two portions of milk are mixed, renneted and starter is added. Coagulation takes place in 2-3 h at 38 C and the coagulum is ladled into moulds of wood or steel lined with coarse cloth or netting. The drainage time varies from 12-24 h (Abou-Donia, 1986). The cheese is cut into blocks of convenient size and wrapped in waxed paper. The cheese may be consumed fresh. If the cheese is pickled, it is held in a salty whey or brine for 4-6 months in layers in suitable tins completely covered with brine . The tins are soldered and then stored at refrigerator or ambient temperature.

Cheesemakers constantly strive to improve cheese quality while seeking new technologies that offer higher

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production efficiencies. Since the late 1960's, membrane technology has significantly evolved in its range of applications to the dairy industry. Initially, the principal application of membrane technology was the concentration of whey and recovery of whey protein. The development of ultrafiltration (UF) techniques for cheese making has shown great promise (Covacevich and Kosikowski, 1978; Ernstorm et al. 1980 and Kosikowski, 1980). Reduction in labor, energy, enzyme requirements, manufacturing time, and work space may be realized by utilization of UF techniques. By removing water,lactose and minerals through UF, milk may be converted directly to the solids content of certain high moisture cheeses (Anon., 1980; and Maubois and Mocquot, 1975). Soft cheese may be made from the concentrated retentate, and whey proteins, which normally would be lost in the whey, are incorporated into the cheese. The result is increased yield.

The objectives of the present investigation were:

* To study the effects of pilot plant scale UF on the retention of milk, skim milk and whey components.
* To develop a process based on UF technology for making soft white "Domiati" cheese and compare it with cheese made from whole milk by conventional methods.
* To develop a vacuum packaging method for ripening of UF cheese and compare it with the brine method of ripening.
* The feasibility of manufacturing Domiati cheese by supplementing the whole milk with whey protein concentrate (WPC) obtained by UF.

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REVIEW OF LITERATURE

The Concept of Ultrafiltration

Ultrafiltration (UF) is a technique for the concentration of liquids using semipermeable membranes at low temperatures and pressures to separate high molecular weight from low molecular weight components without a phase change. This process is gaining popularity in the food industry and is of particular interest in some segments of the dairy industry, since fluid milk and whey have low solids and high moisture content.

Ultrafiltration membranes are designed to retain solute components, depending on shape and molecular size. The pressure gradient across the membrane forces solvent and smaller species through the pores of the membrane, producing a clear fluid known as filtrate or permeate. Large solute species are retained and recovered as a concentrated retentate (Porter and Michaels, 1970). The nature of the membrane controls the retention of some components and the passage of others during direct ultrafiltration, resulting in a selective concentration (Cheryan, 1986). The membranes often retain only macromolecules or particles

larger than about 0.001-0.02 um (10-200 angstroms) or particles that range from about 500-1,000,000 in molecular weight (Porter and Michaels, 1970 ; Cheryan, 1986).

Industrial ultrafiltration applications of milk and milk products began in 1969 and many large installations now are in operation throughout the world, especially in Europe and the United States (Kosikowski, 1986). During ultrafiltration, milk enters the system under a combination of pressure, temperature and high velocity to maximize the flux rate through the membrane surface. This enables substantial removal of water,lactose, soluble salts, and some nitrogenous materials, which pass through the membrane as permeate. Fat, proteins, and colloidal or insoluble salts are retained in a decreasing pool of milk serum to give a liquid concentrate (retentate) as reported by Richter (1983) and Kosikowski (1986).

Peri et al. (1973) used this principle of membrane dynamics to describe a method for adjusting lactose levels in retentate. This is called diafiltration, and it involves the controlled addition of water to the retentate during UF, either stepwise or in continuous cycle . The effect is a "washing out" of lactose to the levels that are consistent with those of the desired product. The water applied usually is equal the volume of permeate removed.

Successful application of ultrafiltration has been reported for the manufacture of cheese, yogurt, fluid milk drinks and whey protein concentrate (Richter, 1983). Such

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techniques also serve as a replacement for conventional isolation methods, thereby avoiding chemical or physical damage or production of off flavor which may develop in such processes (Glover, 1971; Olsen, 1977; Cheryan, 1983). Ultrafiltration is now an established unit operation for the purification, fractionation and/ or concentration of many liquid food systems, such as fruit juices, alcoholic beverages, vegetable proteins and egg white (Kosikowski, 1986; Nichols and Cheryan, 1981; Nichols and Cheryan, 1982)

Ultrafiltration Membranes

Molecular membranes have evolved rapidly with distinctive features (Horton, 1979; Cooper, 1980 and Lonsdale, 1982). Membranes have a thin surface layer, or skin, where permeation occurs, and most have an open, porous interior or packing to support the surface skin. The chemical nature of the membrane governs compatibility and physical properties to a large extent, while the physical structure of the membrane is governed primarily by the method of preparation (Cheryan, 1986).

Two generations of UF membranes were successively proposed and a third type is appearing. The first generation used in the dairy industry is made of cellulose acetate has now been abandoned because of several disadvantages, including sensitivity to extreme temperature (above 30°C) and pH (limited to pH 3 to 8), sensitivity to

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microorganisms and disinfectants, and poor resistance to chlorine sanitization (Harper, 1979; Cheryan, 1986). As a result, time-consuming cleaning procedures involving the application of proteolytic enzymes were required, with strict limitations set on pH, temperature and chlorine.

The second generation, widely used in contemporary industrial processes, are made from synthetic polymers, mostly polysulfonic or polyolefin derivatives (Maubois, 1980; Kosikowski, 1986). These types of UF membranes are characterized by higher limits of temperature (up to 75°C), wide pH tolerance (2 to 12), wider range of pore size available for UF applications ranging from 1000 up 500,000 in molecular weight cut-off, and good resistance to chlorine sanitization (up to 200 ppm).

The third generation of UF membranes are made from a mineral component (zirconium oxide). This membrane material possesses improved qualities over those of polysulfones, being resistante up to 400°C and tolerant to a wide range of pH, and ability to withstand pressures up to 80 psi (Maubois, 1979, 1980).

Ultrafiltration Configurations

Currently, there are several type of ultrafiltration systems being used in the dairy industry. The most common ones are the plate and frame membrane, the tubular membrane, hollow fiber and the spiral wound membrane. Each of these

systems has some advantages and disadvantages regarding performance, investment and operating costs , dead volume and cleaning efficiency (Maubois, 1980). The spiral-wound system, which was used in this investigation, is one of the most compact and inexpensive designs available today. Membranes are basically flat sheets arranged in parallel to form a narrow slit for fluid flow. In this module, membrane and supporting material are wrapped around a perforated stainless steal pipe and enclosed in a stainless steel housing. Fluid feed material is pumped into one end of the module and flows across the membrane. Permeate, which passes through the membrane, is collected in the center pipe while the retentate exits through the opposite end of the housing. Although membranes are easily changed in this system, some cleaning problems exist (Harper, 1979; Richter, 1983; Cheryan, 1986).

The Mode of Operation

The main modes of operating an ultrafiltration system are designated as single pass, batch and continuous. The single-pass system is designed to concentrate the product to the desired level in a single movement through the membrane system and involves no recycling of the retentate, which is a factor limiting the attainment of high concentrations of the retentate (Kosikowski, 1986). In the batch system, the retentate is continuously recycled to the starting supply tank through the module until the desired

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concentration in the retentate is obtained; meanwhile the permeate is collected in another storage tank. A heat exchanger is included in the recycle loop to control temperature. The batch system is the fastest and the simplest method for concentrating a given amount of material with minimum membrane area.

In a continuous system, the retentate moves through a series of modules and, before entering each module it passes into a loop where an individual pump raises the pressure before the retentate moves through the succeeding modules. Modules can be arranged in series or in parallel. The retentate becomes concentrated to high levels as it moves through the system and is released. Meanwhile, fresh material is supplied to the feed tank (Richter ,1983; Kosikowski, 1986).

Advantages and Limitations of UF Processes

There are numerous advantages of UF processing of milk and whey as reported by Bundgaard et al. (1972) and Cheryan (1986). The most important advantages are: i) increased cheese yield (10-20 %) from a given quantity of milk due to the recovery of whey proteins, ii) less denaturation of protein with minimal loss of functional properties, iii) higher nutritional quality of the products because of the high protein efficiency (PER) of whey proteins, iv) lower energy requirement for heating, cooling and transportation of the products. Considerable savings in

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manufacturing costs are expected, partially as a result of 50 to 80 % reduction in rennet and starter requirements. There is a substantial decrease in the biological oxygen demand (BOD) of the discharged wastes since little or no whey is produced by the UF process. UF also facilitates the application of continuous cheese processing and increases the capacity of the cheese making equipment with minimal air contamination. Despite these advantages, there are limitations to ultrafiltration processing of dairy products such as: i) the ultrafiltration membrane processes are quite limited in their ability to concentrate milk to very high solids content, ii) the decline in flux with time due to membrane fouling from accumulation of macromolecular or colloidal particles on the membrane surface or the precipitation of smaller solutes that are normally permeable in the membrane pores, and iii) the poor cleanability of some modules (Cheryan, 1983).

Application of Ultrafiltration in Dairy Products

Ultrafiltration is now an accepted processing operation throughout the world. The two main applications are the fractionation of cheese whey to produce whey protein concentrate (WPC) and pre-concentration of milk for the manufacture of fresh and ripened cheese.

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Milk

The partition of milk constituents by ultrafiltration or diafiltration has received much attention and research. Maubois et al.(1969) were granted a patent for the use of UF technology in cheese making. The process involves passage of whole or skim milk across a porous membrane. Removal of water and dissolved material is effected by application of pressure. Practically complete retention of fat and protein is achieved during concentration of milk by UF (Ernstrom et al. (1980).

Kosikowski (1986) reported that the composition of retentate and its stability at low temperatures were contributing parameters affecting ultrafiltration processes. The optimization of protein purification has been reported by Peri et al. (1973), who used the principle of membrane dynamics to describe a method of adjusting lactose levels in the retentate by diafiltration. The process involved "bleeding" deionized water into the ultrafiltration holding tank at the same rate permeate was removed. The effect was a "washing out" of lactose to levels that were consistent with those of the desired product. Diafiltration was carried out at constant volume in order to maintain a proper balance between low retentate viscosity and high lactose concentration in the water phase.

Several investigations have addressed the practical limits of UF when used for production of milk retentate. An

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important factor governing the extent to which milk can be concentrated by ultrafiltration is the development of a proteinaceous deposit on the membrane (Glover et al., 1974; Yan et al., 1979). This deposit, sometimes referred to as the "secondary membrane", is responsible for the drop in flux rate. Glover (1985) described the formation of the secondary membrane as concentration polarization. After the milk passes across the membrane under pressure, solids collect on the membrane surface and inhibit filtration. Yan et al. (1979) reported a decrease in flux with increasing concentration of whole milk due to retentate viscosity and development of the secondary membrane, whereas Fenton-May et al. (1972) ascribed this effect to an increase in the protein concentration in the retentate of skim milk. Glover (1985) proposed that high feed velocities during process would produce shear forces that would inhibit the development of the secondary membrane. Electron microscopy and enzymic analysis of the deposit formed on reverse osmosis (RO) membranes revealed a triple gel layer composed primarily of casein (Glover et al. 1974; Brooker, 1974). Closest to the membrane is a thin (11 nm), electron-dense layer which, probably, was deposited at the beginning of RO. The second layer was thicker (10-15 nm) and electron-lucent. The third layer was the thickest (30 nm) and most diffused.

Brule and Fauquant (1965) investigated the effects of acidification of milk and retentate on the levels of soluble

calcium. The amount of soluble calcium increased with decreasing pH. Also, the amount of bound calcium in milk and retentate increased linearly with temperature. Finally they concluded that physicochemical characteristics of the aqueous phase in milk retentate were responsible for the equilibria of colloidal and solubilized mineral salts. Ernstrom et al. (1980) demonstrated that removal of calcium during UF of whole milk could be enhanced by performing this process at pH 5.7. However, decreased flux rate and frequent membrane fouling were the problems associated with acidified milk.

Ultrafilterating whole milk of the dairy farms has received serious attention and was pioneered by Maubois (1979, 1980). It may be desirable to concentrate milk to double or triple concentration at the farm to decrease the number of milk collections and final volume, resulting in potential economies in transportation. The permeate obtained from this procedure, containing lactose, soluble salts, non-protein nitrogen, and vitamins, can be fed to farm animals. The incorporation of UF units into farm milking systems has been described by Slack et al. (1982).

Soft cheese

Industrial scale manufacturing of soft cheese from ultrafiltered milk is currently practiced in Europe and in the United States (Bundgaard et al.,1972; Anon, 1980 and Kosikowski, 1986). The cheese industry has sought to

capitalize on the potential of ultrafiltration to increase product yield and decrease production costs. Traditional cheese making has been defined as a "fractionation process by which fat and casein are concentrated in the curd by the action of the enzyme , while lactose, soluble proteins, minerals and vitamins are lost in the whey fraction ". The yield of curd is typically 9-16 % of the weight of the milk, leaving 84-91 % in the form of whey (Kosikowski, 1986).

The use of ultrafiltration has change this concept somewhat as the retentate (pre-cheese) has the same composition as the final cheese in terms of fat, protein and moisture. Also, whey proteins are incorporated into the cheese rather than drained off with the whey. Mann (1982) discussed the status of the application of ultrafiltration of milk for cheesemaking and referred to several patent applications and current commercial utilization of ultrafiltration processes throughout the world. Ultrafiltration allows for the adjustment of concentration and proportion of principal components of milk to aid in the mechanization of cheese manufacturing. It also increases the recovery of milk components in cheese and controls the selected properties of the cheese. A number of procedures for manufacturing cheese from UF-milk have been developed. Each procedure may influence cheese quality and composition in a characteristic ways (Mann, 1982). Two very basic concepts for making cheese are: first concentrating and adjusting milk to the composition of the cheese to be made

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and, second using traditional cheesemaking techniques in which retentate of varying concentrations were used instead of milk. Maubois and Mocquot (1975) reviewed several methods of soft cheese making by ultrafiltration. An increase in yield is achieved by UF techniques as a result of retaining albumin and globulin when compared to the conventional procedures for processing high moisture cheese. Covacevich and Kosikowski (1977a) and Yan et al.(1979) provided a description of early laboratory scale UF systems and their use in concentrating milk for production of high moisture cheese such as Camembert, and a goat's milk cheese called Feta.

Bacteriological, biochemical, and physico-chemical criteria for these cheese making procedures are reviewed by several investigators. It was suggested that UF of skim milk should proceed for no longer than 5 hr at a UF temperature of 50 to 54°C to preserve milk quality. Ultrafiltration for longer periods at these temperatures may promote bacterial growth and damage proteins. Garnot and Corre (1980) studied the influence of protein content of ultrafiltered milk on the action of milk-clotting enzymes and subsequent clotting times of milk. Reuter et al. (1981) and Green et al. (1981) demonstrated that rates of firming of curd formed from ultrafiltered milk increased in proportion to the extent of concentration of the milk. Green et al. (1984) presented data on whole milk retentate obtained by ultrafiltration which suggested that

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cheeses made by ultrafiltration processes would be comparable to conventionally made cheese with respect to contents of water soluble vitamins. Brule et al.(1974) discussed the mineral content of products from milk concentrated by UF and demonstrated the feasibility of producing a retentate with the necessary mineral content for cheesemaking. The use of UF to concentrate and separate milk constituents has far-reaching effects, especially for soft and semisoft cheese (Setti and Peri, 1976; Kosikowski, 1983 ; Barbar and Bynum, 1984 and Zall, 1984).

Feta cheese. Feta is a high-moisture, white cheese which originated in southeastern Europe and Asia. It is characterized by high salinity and a sharp taste. Ultrafiltration offers an interesting and profitable alternative to the conventional methods of manufacture by increasing the yield by about 25 %, but the structure of the cheese is slightly different (Anon., 1980a). In Denmark, almost all Feta cheese is produced by UF techniques, with nine Danish Feta cheese plants in operation (Anon., 1980b). In the process, whole milk is ultrafiltered and the retentate passed into a holding vat for adjustment of composition to that of the final cheese. Yoghurt culture and calcium chloride are added followed by the addition of rennet and subsequent coagulation. Following drainage of the free whey and when the pH drops to 5.0, blocks are formed, dry-salted and placed in tins. A yield increase of 30% over conventionally produced Feta cheese was claimed

(Anis,1984).

Cottage Cheese. Matthews et al. (1976) developed a procedure for making Cottage cheese from skim milk retentates. Three separate lots of skim milk (9.0 % total solids) were concentrated by UF to total solids contents of 12.2, 12.9, and 13.1 % and made into cheese by conventional method. Yields of Cottage made from the three retentates were similar to that of the control which was made from skim milk. Flavor and texture scores were acceptable although some cheese curd exhibited a tough texture. Covacevich and Kosikowski (1978) studied the feasibility of producing Cottage cheese from retentate concentrated five fold. The final product displayed a good flavor but the curd was gelatin-like and possessed poor absorptive qualities when cream dressing was added. The color and general appearance of the cheese was poor. Diafiltration of the retentate prior to fermentation improved overall cheese quality. Jepsen (1979) has suggested that concentrating the milk to two fold should decrease the required starter culture for making cottage cheese by 50 % and double the capacity of the plant. Also cottage cheese was produced by direct acidification of the retentate through the slow addition of glucono-delta-lactone to pH 4.8. The curd cut easily, cooked well, and had good quality. And the final creamed curd was considered excellent in flavor and texture.

Cream Cheese. Covacevich and Kosikowski (1977b) reported that cream cheese produced from ultrafiltrated milk

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showed excellent shelf-life and smoothness, comparable with standard commercial cream cheese but with much greater hardness of body. Maubois (1979) reported that adjusting the mineral content of UF cheese solved the texture problem.

Ricotta Cheese. Maubois (1979) developed a complete mechanized process for the continuous production of Ricotta cheese which had higher yield and good sensory properties.

Other Soft Cheese. Soft, mold-ripened cheeses such as Camembert have been made successfully using ultrafiltration procedures (Kosikowski 1974, 1986; Maubois, 1979). These cheeses were satisfactory in yield, appearance, flavor and texture.

A Danish blue cheese manufactured at a Danish dairy plant using a UF process was described by Jepsen (1977) and reported to be of sufficiently high quality to justify export. Mahaut and Maubois (1978) reported that blue cheese made from retentate possessing between 4-12% protein exhibited better sensory qualities than the control cheese.

Retentate-Supplemented Milk for Cheesemaking

Alternative ultrafiltration techniques are possible with LCR (low concentrated retentate) where highly concentrated milk or whey retentate is added to cheese milk, after which cheesemaking proceeds in the traditional manner.

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retentate offers potential in cheesemaking for shorter setting time, rapid acid development and reduced rennet requirements. Acid flavor was the only significant defect associated with cheese containing concentrated whey (Richter 1983). Further investigations by several researchers (Kosikowski 1979, 1981; Kealey and Kosikowski 1982; Fernandez and Kosikowski 1983; Massaguer-Roig and Kosikowski 1983) showed that optimum cheese quality is attained with retentate supplemented milk mixtures adjusted to a milk: retentate protein ratio between 1.4:1 and 1.8:1 for Cheddar cheese, Cottage cheese, Mozzarella, and Queso Blanco.

Abrahamsen (1979) investigated the possibility of manufacturing hard and semi-hard rennet cheese of acceptable quality from milk fortified with different amounts of whey protein concentrate. He obtained increased yields of cheese depending on the manufacture procedure. Brown and Ernstrom (1982) studied the benefits of incorporating UF-whey concentrate to cheddar cheese milk and found that such incorporation increased the yield by 4%. The cheese had a higher moisture content and lower fat content than the cheese made by a conventional method. Specific body, texture and flavor characteristics were identified.

Domiati Cheese Made by Ultrafiltration

Domiati cheese, the most popular soft, white-pickled cheese variety in Egypt, is usually consumed either fresh or after a ripening period varying from two to four months.

The cheese is made from fresh or reconstituted whole or skimmed buffalo or bovine milk, or from a mixture of both. However, a specified amount of fat is added if skimmed milk is used for making Domiati cheese. Increasing demand for this cheese conflicts with a market shortage in the fresh milk supply. Several investigations were directed to solve this problem by incorporating the ultrafiltration technique into the manufacturing process. Omar (1987) studied the physical and chemical composition of cheese made from ultrafiltered, reconstituted milk and compared it with conventional cheese produced from recombined milk. According to this study the ultrafiltered milk cheese was characterized by a higher content of moisture, protein and fat in the dry matter, higher buffering capacity and pH than that of the cheese prepared by conventional method. Abd El-Salam et al. (1981) studied the ripening of Domiati cheese made from ultrafiltrated Buffalo skim milk mixed with fresh cream. The cheese stored without brine solution, showed less weight loss as compared to the cheese ripened in brine. Mahmoud et al. (1983) developed a method for making Domiati cheese from UF reconstituted skim milk and lipolyzed recombined cream. Organoleptic scoring revealed that the cheese made with cream which had been treated with an intermediate level of lipase (2 or 5 g/500 g cream), ranked highest in flavor after one and four months of storage. El-Shibiny et al. (1983) investigated the possibility of using vegetable oils in making soft cheese

from UF recombined milk and observed that replacement of milk fat with vegetable oil up to 100 % had no significant effect on the chemical composition or weight loss during storage. A slight oily flavor was observed in fresh cheese, especially when cottonseed oil was employed. During storage, this oily flavor was greatly reduced. Abd El-Salam and El-Shibiny (1982) explored the influence of rennet, rennet substitutes and different starter cultures on the composition and quality of Domiati cheese made from recombined milk which was concentrated by ultrafiltration. They reported that all the cultures used gave the same effect on cheese composition and quality, while rennet affected protein breakdown and weight lose during storage. Ernstrom and Anis (1985) studied the effect of incorporating heated retentates in the production of soft white cheese similar to Domiati varieties made in the Middle East. They reported that this type of cheese can be made from UF whole milk by heating the retentate to at least 180° F for 30 min prior to culturing and setting with controlled pH. Mahmoud and Kosikowski (1979) also studied the ripening of Domiati and Feta cheeses made from ultrafiltrated skim milk. The resulting cheese obtained in higher yield and possessed more flavor than the traditionally ripened cheese. El-Gendy et al.(1983) and Omar (1987) observed that quality Domiati cheese made from ultrafiltrated cows' milk had a uniform and closed texture, good appearance and improved organoleptic properties. The

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data showed that the breakdown of protein with the accumulation of free amino acids and the liberation of volatile free fatty acids were faster in UF-cheese than in cheese made by a conventional method.

Ultrafiltration of Whey

Whey is a highly nutritious by-product of cheese and casein manufacture and it contains about 0.9 % protein, 0.1 % fat, 0.5 % ash and 4.9 % lactose. The whey proteins represent the non-casein proteins as well as the fractions and fragments of the casein which remain soluble when casein has been precipitated enzymatically by rennet or isoelectrically by acid.

Total world cheese whey production is estimated to be about 187.4 billion lb, which would contain about 133.8 million lb of whey proteins (Zall, 1983). Approximately 50 percent of the total whey solids are disposed of by various industrial and municipal waste treatment systems and only five percent used for manufacturing whey protein concentrate (WPC) by ultrafiltration (Morr 1984). Only 50 % of the WPC is processed further (Spangler and Amundson, 1986). Whey contains highly desirable nutritional proteins which also possess good functional properties. The nutritive value of whey is essentially related to its high content of essential amino-acids especially cystine, lysine, leucine, isoleucine and threonine (Hambraeus 1982).

Dehydrated whey contains only 10-12 % protein, but when concentrated by UF and dehydrated, the powder consists of 50 to 80 % protein (Ritcher, 1983). The composition of WPC produced by UF was reported by Morr et al. (1973) and Marshall (1982). Most WPC is dried and is widely used in foods such as cheese supplement, ice cream, bakery products and confectionery applications where the functional properties of the proteins are required and high quality nutritional protein is desired (Richter, 1983). One major advantage of WPC over casein products is the high degree of solubility in acidic food products. This unique property of WPC was used for improving the nutritional quality without adversely altering the sensory characteristics of the food products. UF has emerged as the most appropriate process for industrial manufacture of WPC, and advances are being made in the design and operation of this process (Wesley, 1981). After concentrating whey by UF to 18-22 % protein content, the retentate is pasteurized and spray dried under mild conditions to minimize protein denaturation that would adversely alter protein solubility and functionality. The primary use of UF is to produce WPC that can be marketed more profitably than the conventionally dried whey powder. Whey protein concentrate competes economically with protein ingredients such as non-fat dry milk (NFDM), casein and egg albumin in major product categories. Morr (1984) stated that whey solids in the form of WPC which contain more than 35% whey proteins, with lower lactose and mineral contents,

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are more suitable than dried whey or concentrated whey for replacing NFDM in many food formulations.

MATERIALS AND METHODS

Preparation of Retentate

MILK

Raw, whole cow's milk and skimmilk used for this study were obtained from the Michigan State University Dairy Plant. The milk was pasteurized at 63°C for 30 min. and cooled to 50°C.

WHEY

Sweet whey used for ultrafiltration processing was obtained from manufacture of Cheddar cheese at MSU Dairy Plant. Salted whey was obtained from pilot plant production of Egyptian Domiati cheese in which 7 % (w/w) salt was added to the milk before manufacture. The whey was clarified by centrifugation to remove curd fines, followed by pasteurization at 63°C for 30 min. and cooling to 50°C before ultrafiltration.

Ultrafiltration and Diafiltration

An S-1 ultrafiltration system equipped with an HFK-131, Spiral-wound, polysulfone membrane with 5 m^2 of filtering surface and a nominal molecular weight cut-off of 5,000 Daltons (Koch membrane system, Inc., Wilmington, MA USA)

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was used in the batch mode. An inlet pressure of 50 psi and outlet pressure of 30 psi were used throughout the operation.

Thirty gallon lots of whole milk, skim milk or whey were held in a jacketed, stainless steel vat (100 gallon) which served as the feed tank to the ultrafiltration unit and recycled from the feed tank to the UF membrane, passing through a heat exchanger to maintain isothermality. The operation continued until the retentate reached a desired concentration or the permeate no longer passed through the membrane (very low flux). Once the desired concentration was attained, the retentate was placed in 3 liter plastic containers, and stored at -20°C for later use in cheese manufacture.

During the concentration of whole milk, using diafiltration, the milk was concentrated until 60 % (V/V) of the milk was removed as permeate. At this point, diafiltration was started by introducing deionized water at 50°C to the retentate as described by Vieira et al. (1983). Following diafiltration, an additional recirculation was continued until 80 % of the original milk was removed as permeate, resulting in a retentate possessing a volume concentration ratio (VCR) of 4.5. (Ernstrom et. al., 1980). After processing, the system was cleaned as follow: rinsed with 30 gallons of deionized water at 50^0 C; followed by 20 min recirculation of phosphoric acid (250 mL/25

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gallons of water) and finally rinsed with water follow by circulation of 10 gallons of 200 ppm available chlorine solution. The ultrafiltration unit was sanitized immediately before use with water containing 150 ppm chlorine as recommended by the manufacturer.

To monitor the effect of the ultrafiltration and diafiltration processes on milk and whey composition, retentate and permeate samples were taken at appropriate intervals during the operation. Samples were frozen at -20°C for subsequent chemical analysis. Permeation rate was determined by a measurement of permeate flow (mL/min.) and dividing by the system's active membrane surface area.

Cheese Manufacture From Whole Milk

Conventional Method

Domiati cheese was made from pasteurized cow's milk in 10 gallon stainless steel vats resting in an insulated metal container as outlined by Ibrahim (1963) with minor modification. Milk was divided into two portions and 5 % (w/w) sodium chloride was added to one half. Thawed, frozen concentrated lactic starter (Redi-Set DVS, Chr. Hansen's laboratory, Inc., Milwaukee, WI) was added (0.015 % v/v) to the second (unsalted) portion at 35°C. After ripening for 20 min., the two portions were mixed together and coagulated with 0.025 % single strength microbial rennet (Emporse SF-100, Dairyland Food laboratories, Inc.,

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Waukesha, WI). The smooth gel obtained after 105 min was cut with 1 cm wire knives lengthwise and crosswise and left undisturbed for 15 min. Following the removal of whey, the curds were dipped into round molds 10 cm in diameter and 14 cm high to drain over night at room temperature. Cheeses were sealed under vacuum in polyethylene-lined aluminum pouches, 26 cm x 16.5 cm (Fisher Scientific), using a Multivac, type AGW Vakuum Verpackungs Maschinen. The Cheese was ripened at 7-10^{0}C for 3 months. Representative samples were analyzed at intervals of one month.

Ultrafiltration Method

Liquid pre-cheese retentate was heated to 71.6°C for 16 sec and cooled to 35°C, then inoculated with 0.07 % lactic acid starter, poured into 0.45 Kg cottage cheese containers and ripened for 10 min. Rennet (0.09 %) was added to each container to induce complete coagulation in 8-10 min. A firm cured was obtained with a little whey exudation. Five percent salt was spread on the surface of the cheese which was ripened in polyethylene pouches for 3 months at 7-10°C. Samples were analyzed monthly.

Freeze Dried Retentate

The retentate obtained by ultra/diafiltration of whole milk was spread over aluminum trays (11 1/4 x 7 1/2 x 1 1/2 in) for the freeze-drying experiment. Each tray was sealed with freezer tape and frozen immediately at - 20°F. A

Virtis REPP, Model FFD 42 WS freeze-dryer, with a capacity of 50 lb of water removal per drying run, was employed in these studies. The freeze-dryer was equipped with instrumentation for the control of freeze-drying variables. Automatic controls for condenser and platen temperature, vacuum adjustment, weight system recorder for determination of drying curves, constant recording of absolute pressure and thermocouple connections were controllable with this unit. Operating conditions employed were: 20 microns Hg absolute pressure, platen temperature of 125°F, and condenser temperature of -65°F. Samples were removed when product temperature had reached 85°F. After completion of drying, nitrogen (W/P) was introduced into the vacuum chamber to establish atmospheric pressure. The freeze-dried powder was removed from the tray, screened, put into small, brown-glass jars, tightly sealed and stored at 5°C until used. Freeze dried retentate was reconstituted in warm water (40°C) to the same composition of the liquid pre-cheese, then blended with a mixer (Ultra-Turrax Model SDT 1810, Tekmar Company) for 5 min. Following heat treatment to 71.6°C for 16 sec., the reconstituted retentate was cooled to 35°C and used in the preparation of Domiati cheeses as described in the section on ultrafiltration.

Cheeses were ripened by two method, in 7% brine solution and in vacuum pouches for 2 months at 7-10°C and analyzed at two week intervals.

Cheese from Whey Protein Concentrate Supplemented Whole Milk

Fresh whole milk was pasteurized at 71.6°C for 16 sec and then cooled to 35°C. Concentrated whey protein was obtained by ultrafiltration of whey produced from Domiati cheese manufacture by the conventional method. The concentrated whey was kept frozen at -20°C until used for supplementation of the whole milk for Domiati cheese making. The WPC was heat-treated to 75°C for 30 min with gentle agitation and cooled quickly to 35°C. Whole milk was fortified with the heat-treated WPC to give progressively increasing total protein concentrations ranging from 1:0 (unsupplemented control) to a maximum of 1:2 in the mixture. Domiati cheese was produced by the traditional method from whole milk supplemented with WPC (Figure 1). Whole milk was divided into five equal portions of 5 Kg each. The first portion represented the unsupplemented control while the other four portions were supplemented with WPC. The amount of WPC added to the milk was 25, 50, 75, 100 or 200 % based on the original total milk protein. The mixture was inoculated with 0.15 mL/L of frozen, concentrated lactic starter (Redi-Set DVS, Chr. Hansen's laboratory, Inc., Milwaukee, WI) and held for 20 min. Single strength rennet (SF-100, Dairyland Food Laboratories, Inc., Waukesha, WI) was added at the rate of

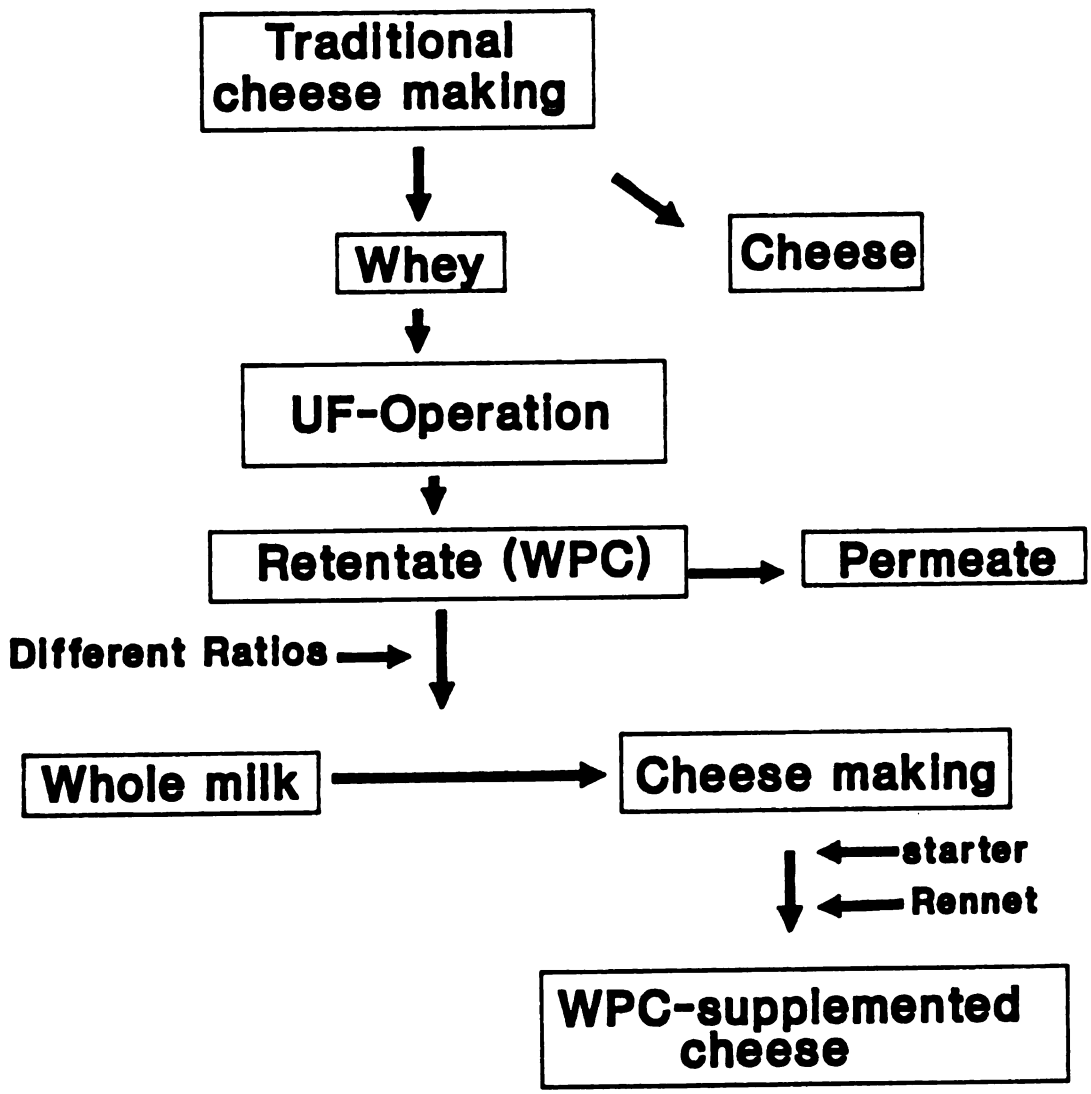

Figure 1. Schematic diagram of Domiati cheese made from WPC-supplemented whole milk.

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0.3 mL/L of mixture. Each batch of cheese curd was weighed at the time of hooping and the yield of cheese was calculated following draining for 48 hr. Each experiment was repeated three times.

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CHEMICAL ANALYSIS

The composition of the milk, whey, retentate, permeate and the cheese produced were determined using the following methods.

pH

Ten grams of cheese were slurred with 40 mL of deionized, distilled water in a Waring semi-micro blender for 2 min. The slurry was immediately transferred to a glass beaker and pH was recorded with an Orion Research Model 301 pH meter. The pH of the fluid samples was obtained directly on the product without dilution.

Total Solids

Total solids of fresh milk, whey, retentate, permeate and cheese were determined by a vacuum oven method at 100°C to constant weight (AOAC, 1984).

Fat

The fat content of milk was measured by the Babcock procedure (AOAC, 1984). Milk and whey retentates, and cheese samples were determined by the modified Babcock procedure (Kosikowski, 1977).

Salt

Determination of salt in cheese,milk retentate,and whey samples was performed by a modified Volhard test described by Kosikowski (1977).

Protein

The micro-Kjeldahl method (AOAC, 1984) was used for the determination of total nitrogen (TN), non-casein nitrogen (NCN), non-protein nitrogen (NPN), and total albumin nitrogen (TAN).

Sample Preparation

For the determination of the reduced nitrogen fractions, a sodium citrate-cheese extract was prepared by weighing 10 g of cheese and blending in a Waring micro-Blender with 40 mL of sodium citrate (0.5 N) and 80 mL of distilled water for 7 min at high speed. The homogenate solution was quantitively transferred to a 200 mL volumetric flask and tempered to 20°C with distilled water as described by Vakaleris and Price (1959). A 10 mL aliquot was taken for determination of TN. An aliquot of 100 mL of sodium citrate cheese extract was adjusted to pH 4.4 by adding 10 mL of 1.14 N HCL and made to 125 mL with distilled water. The mixture was filtered through a Whatman No. 42 filter paper and an accurate portion of the clear filtrate, which contained soluble nitrogen, was used for determining NCN. Twenty mL of a pH 4.4 filtrate was treated with 80 mL of 15 % trichloroacetic acid and filtered through

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Whatman No.42 filter paper (Grippon et al., 1975). The filtrate containing the NPN fraction was assayed by micro-Kjeldahl. Because of the ability of sodium sulfate to precipitate all nitrogen fractions except total albumin and non-protein nitrogen (whey protein), a 20 mL aliquot of the sodium-citrate cheese extract was mixed with 20 mL of a 40% sodium sulfate solution at 45°C and allowed to stand for 30 min and then filtered through Whatman No.4 filter paper. Total albumin nitrogen and non-protein nitrogen were determined in this filtrate (Aschaffenburg, 1959).

Additional nitrogen fractions were derived from the above four fractions determined as follow : Casein nitrogen (CN) was equal to TN minus NCN and the total albumin N (TAN) was equal to (TAN+NPN) minus NPN.

Discontinuous Polyacrylamide Gel Electrophoresis

Sample Preparation

Samples of milk, whey, retentate and cheese were prepared following a procedure described by Ledford et al. (1966) with some modification. Two hundred mg of cheese was dispersed in 0.8 mL of distilled water and 2.0 ml of 7 M urea was added. Samples were warmed to 37°C for 1 hr to effect a layering of fat and centrifuged at 2000 rpm for 10 min at 10°C. The supernatant was dialyzed against 7 M urea at 5°C for 24 hr to remove the salt. Two drops of 2-mercaptoethanol were added to the sample, which was held

for 2 hr at 37°C to visualize kappa-casein by subsequent gel electrophoresis. Ten mL of 1% bromophenol blue and 125 μL sucrose solution were added prior to electrophoresis. All milk and whey samples were dialyzed against distilled water for 48 hr at 5°C. Standards of α-Casein, β-Casein, β-Lactoglobulin and α-Lactalbumin, isolated from bovine milk (Sigma Company ,St. Louis, MO.), were used for identification and comparison with the samples (Swaisgood and Brunner, 1973). A standard was included in each of the electrophoretic run. Procedures for the preparation of solutions and electrophoretic gels are included in Appendix (1).

Electrophoretic and staining procedure

Electrophoresis was carried out in 9% polyacrylamide gels in a vertical, water-cooled Bio-Rad, Model 150A, electrophoretic apparatus according to a method essentially similar to that of Ornstein and Davis (1964). Electrophoresis was performed at 1 mA/tube for the first 10 min and subsequently at a constant current of 3 mA/tube supplied by a MRA Corporation Model M158 Automatic power supply. The gels were removed from the pyrex tubes and stained in Comassie Brilliant Blue R-250 (Sigma) overnight and destained by diffusion for 24 hr in a mixture of acetic acid:methanol:distilled water 4:10:86 (v/v).

Gel Densitometry

Gels were scanned using a Beckman DU spectrophotometer, Model 2400 equipped with a gel scanner Model 2520 and a photometer, Model 252 (Gilford Instrument Laboratories). The system was augmented by a Hewlett-Packard Integrator , Model 3380s. The gels were scanned at a wavelength of 580 nm at a rate of 1.0 cm/min. Results were recorded at a chart speed of 2.0 cm/min. Relative areas of the individual proteins were calculated and recorded from integration signs on the densitograms.

Amino Acid

Picomole quantities of total and free amino acid were determined according to the HPLC method of Cohen et al. (1986). Whey , final retentate (WPC) and extracted cheese were hydrolyzed in 6N HCl at 110 C for 20 hr.

Preparation of Cheese Sample

The samples were prepared by weighing accurately 10 g of cheese, homogenizing in a Waring micro-Blender with 100 mL of redistilled water for 2 min. The slurry was removed and heated to 75°C with agitation for 5 min and cooled to room temperature. Equal volumes of slurred cheese and 24 % TCA were mixed. The resulting solution was allowed to stand 30 min, centrifuged at 2000 rpm for 10 min at 5°C and filtered through Whatman No. 42 filter paper. To remove the residual TCA, the supernatant was washed with an equal

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volume of ethyl ether (1:1), using a separatory funnel, the upper layer was removed. This step was repeated three times. To remove the majority of remaining peptides, the solution was mixed with absolute ethanol (1: 4 v/v) and left undisturbed for 12 hr before centrifuging at 10,000 rpm for 30 min, which was followed by filtration through a Millipore 0.45 um filter (Kosikowski, 1951). An aliquot containing 10 mg of protein was hydrolyzed with 6N HCl at 110°C for 20 hr. Thirty μL of the hydrolysate was used for derivitization with phenylisothiocyanate (PITC) as described in Appendix 2.

Lactose

The lactose content was determined by the phenol-sulfuric acid method of Dubois et al.(1965). One hundred mg of milk , whey ,retentate or cheese sample was made up to 100 ml with distilled water and mixed thoroughly. Two mL of this solution was added with 1 mL of 5 % mixture of redistilled phenol in a test tube. Five mL of concentrated sulfuric acid was added rapidly while the tube was agitated to insure maximum mixing and heat development. The samples were allowed to stand at room temperature for 10 min and the absorbance was read at 490 nm using a Spectronic 2000 (Bausch and Lomb) spectrophotometer. A standard curve for lactose was constructed to cover a range of concentration from 0 to 225 μg.

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Mineral

Minerals analysis was performed using an inductively coupled plasma (ICP) Atomic Emission Spectroscope, Jarrel-Ash Model 955, equipped for simultaneous analysis of 19 elements as described by Braseton et al. (1981).

Sample preparation

Samples were sonicated for one-half hour and one gram aliquot (wet weight) were combined with 2 mL of concentrated nitric acid in a 15 mL screw-capped, Teflon vial (Tuf-Tainer, Pierce Chemical Co.). Samples were incubated at 95-100°C over night, cooled, and quantitively transferred to 10 mL volumetric flasks. Yttrium (Y) was added to give a final concentration of 1 μg/mL as an internal standard (Aldrich Gold Label, 99.999% or better, Aldrich Chemical Co.). Samples were diluted to the final volume with water purified with a four-bowl Milli-Q Water Purification System (Millipore Corp.) and analyzed by inductively coupled plasma (ICP) spectroscopy. Instrumental parameters were optimized and the final operating conditions are given in Appendix 3.

Free Fatty Acids

The method described by Deeth et al. (1983) for the isolation and quantitative determination of free fatty acids (FFA) in milk, retentate and cheese was used. Separation of FFA and lipids was done by placing 5 g of grated cheese or fluid sample in a screw-capped test tube (18 mm X 150 mm)

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containing 25 mL of dimethyl ether, 100 μg of heptanoic acid (C7) and 100 μg heptadecanoic acid (C17) as internal standards and 0.5 mL of 5.5 N H_2SO_4. The mixture was blended thoroughly with a Brinkmann homogenizer (Brinkmann Instruments Co. Westbury, NY) at speed 7 for 30 sec. Ten mL of hexane were used for rinsing the probe twice and combined with the sample extract. Approximately 12.5 g of anhydrous sodium sulfate was added to the test tube containing the sample extract. The test tube was centrifuged at 700 x g for 5 min. (IEC Clinical Centrifuge, Needham Heights, MA). The supernatant was added carefully to a glass chromatographic column (17mm x 265mm) containing 5 g of deactivated neutral alumina (Chromatographic alumina neutral, activity grade 1, Sigma (chemical Co.). The eluate was passed over the column for the second time, followed by washing with 10 mL of hexane:dimethyl ether (1:1, v/v) to remove other soluble materials. The alumina, containing adsorbed FFA, was dried in a vacuum applied to the column outlet for a few seconds, and then transferred to a screw-capped test tube (12mm x 125mm). Five mL of 6% formic acid in Na_2SO_4-dried diisopropyl ether (v/v) were added to the eluted FFA, followed by mixing and centrifugation at 700xg for 5 min. Fatty acids were analyzed with a gas chromatograph (Model 5840A , Hewlett Packard, Avondal, PA) equipped with a flame ionization detector. A glass column (2mmx3mm, ID) packed with 10%

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SP-216-PS ON 100/120 Supelcoport (Supelco, Inc., Bellefonte. PA.) was used for the separation of the fatty acids. The GC oven temperature was held at 100°C for 10 min, then increased at a rate of 3.0°C/min to a final temperature of 190°C and held for 30 min. The injector and detector temperatures were held at 225°C and 275°C, respectively. The flow rate of the nitrogen carrier gas was 25 mL/min.

Identification of the fatty acid was based on comparison of retention times of samples to those of fatty acid standards prepared in diisopropyl ether containing 4% formic acid. Fatty acid distribution for each fraction was based on integration of peak areas (HP-5840A GC Terminal Integrator). To determine the influence of the cheese on FFA recovery, known weights of synthetic FFA mixtures of C:4, C:6, C:8, C:10, C:12, C:14, C:16, C:18:0, C:18:1, C:18:3 were added to cheese sample and extracted as outlined above. Correction factors were obtained for C:4 to C:6 compared to C:7 and for C:10 to C:18:3 compared to C:17. Quantitation method and response factors were the same as those described by Deeth et al. (1983). All solvents and chemical were of analytical grade.

Color

Cheese color was evaluated with a color-difference meter (Hunter Lab, Model D 25-2). The instrument was standardized against a white standard C2-12400.

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Samples were placed in a cup covered with the glass plate and readings were taken in triplicate.

Texture Profile Analysis

Textural properties of cheese such as hardness, cohesiveness and adhesiveness were measured with the Instron Universal Testing Machine, Table Model 4202 (Instron Co., Canton, MA.) following the procedure of Bourne et al. (1967,1968). Fresh and ripened cheeses were tempered at 5°C ±1°C for 12 hr. Samples were cut into 1.5 cm cubes just prior to evaluation. The instrument was operated at room temperature with a crosshead speed of 20 mm/min and a chart speed of 76 cm/min. Full scale load was 50 N, and samples were compressed to 75 % of their initial height. Each sample was compressed twice by the compression load cell. The textural evaluation was done in quadruplicate on each batch of cheese. Areas under the response plot were determined by a Planimeter (Keuffel-Esser). The $A_1$and $A_2(cm^2)$ represent areas under the curves formed during the first and second compression cycles and represent work done during compression. The height of the first peak during the first bite represented the extent of hardness (force) while the distance of the sample under compression during the second bite represented the springiness (cm). Cohesiveness was derived from the ratio of A_2/A_1 whereas the

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gumminess was equal to hardness x cohesiveness and the chewiness was equal to gumminess x springiness. The height of A_3 represented the adhesiveness.

Statistical Analysis

The effect of ultrafiltration process on cheese production were analyzed using the analysis of variance (ANOVA), mean separation, and correlation subprograms of the MSTAT Microcomputer Statistical Program (ver. 4C, 1989). The statistical analyses were performed on the mean values of the duplicates for each replication.

RESULTS AND DISCUSSION

PART-I

ULTRAFILTRATION PARAMETERS STUDY

Introduction

Although the interest in the application of ultrafiltration and diafiltration to food systems continued to increase, yet more information is needed on the changes in composition of whole milk, skim milk and whey before these membrane processes can find wide use in the food industry. The purpose of this part was to study the effects of pilot plant scale ultrafiltration on the retention of milk and whey components, especially the minerals, nitrogen fractions, amino acids, fatty acids, total solids, fat, ash and lactose.

Flux Rate

The permeation rate for whole milk, skim milk and whey was studied using ultrafiltration and diafiltration processes. Flux rate versus the amount of permeate removed is shown in Figures 2 and 3. A gradual decrease in the flux rate of whole milk was observed as the concentration increased through stages of the UF and DF process. Yan et al. (1979) reported a decrease in flux rate with increasing concentration of whole milk. As protein and fat were concentrated in the retentate, the viscosity increased as well as the development of a secondary membrane followed by a decrease in

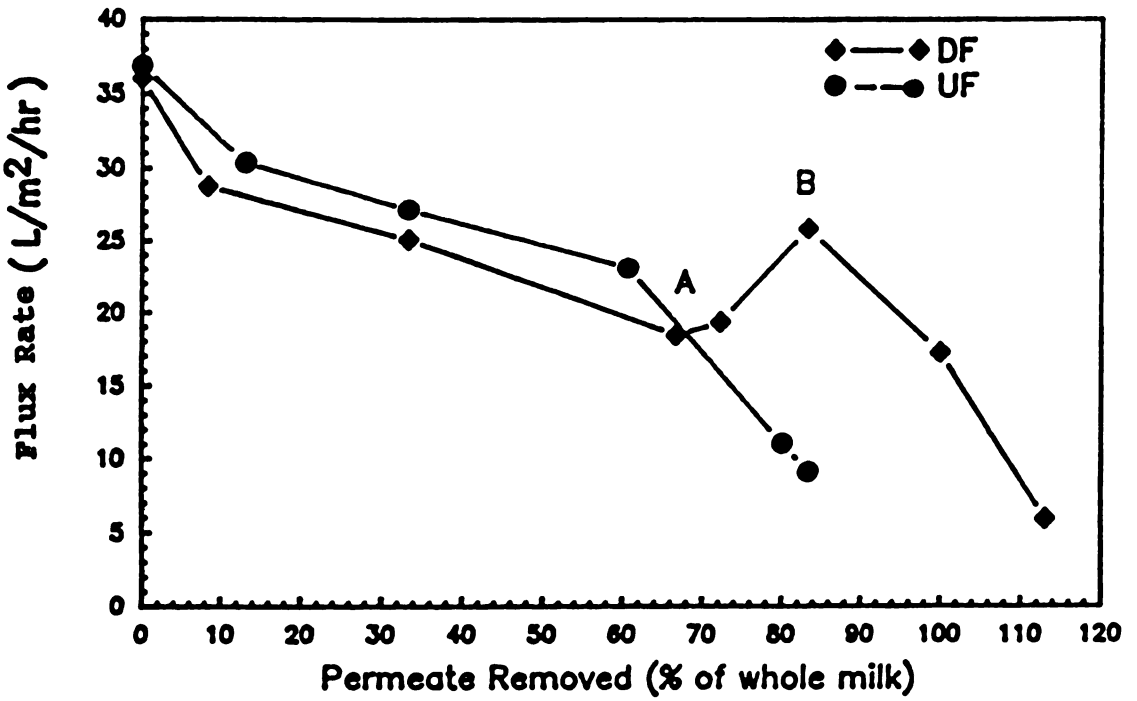
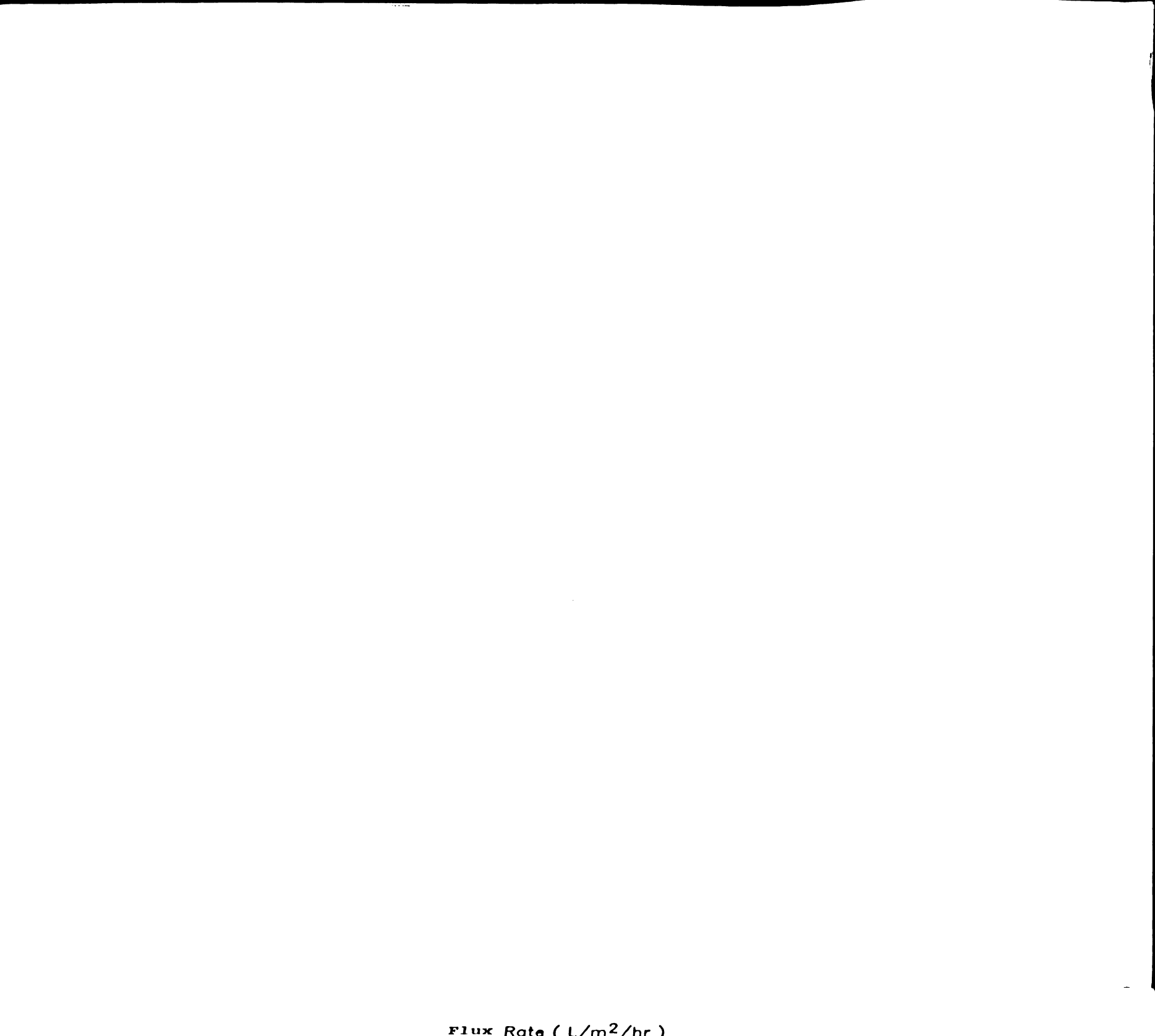


Figure 2. Flux rate of whole milk during ultrafiltration and diafiltration. A and B represent diafiltration start and stop, respectively.

diffusivity . Thus, concentration became a limiting factor of the UF process. Diafiltration was employed to purify the protein to a greater degree and to reduce lactose and ash in the retentate. The data in Figure 2 indicate that during the UF of whole milk the flux decreased from 36.9 to 5.9 $L/m^2/hr$ with the permeate accounting for 80.3% of the milk. The drop in flux represents the practical limitation of UF when used for the production of pre-cheese retentate. The same trend was observed when diafiltration was applied to whole milk (until the point of adding deionized water). The effect of "washing out" the lactose increase the flux from 19 to 25 $L/m^2/hr$, followed by a reduction to 5.9 $L/m^2/hr$. Typical data comparing the permeation rate of whole milk, skim milk and whey during UF, as previously described, are plotted in Figure 3. For all materials, the flux decreased as the process continued. The initial flux for whey, skim milk and whole milk was 78, 50, 36.9 $L/m^2/hr$, respectively. The UF process stopped when the flux for whey, skim and whole milk was reduced to 27, 18.3 and 9.12 $L/m^2/hr$, respectively.

Fenton-May et al. (1971) and Besik et al. (1971) stated that permeation rates are directly proportional to Reynolds number (RE) and inversely proportional to the viscosity of the solution. Consequently, as the concentration increased, the viscosity also increased


Flux Rate (L/m2/hr)

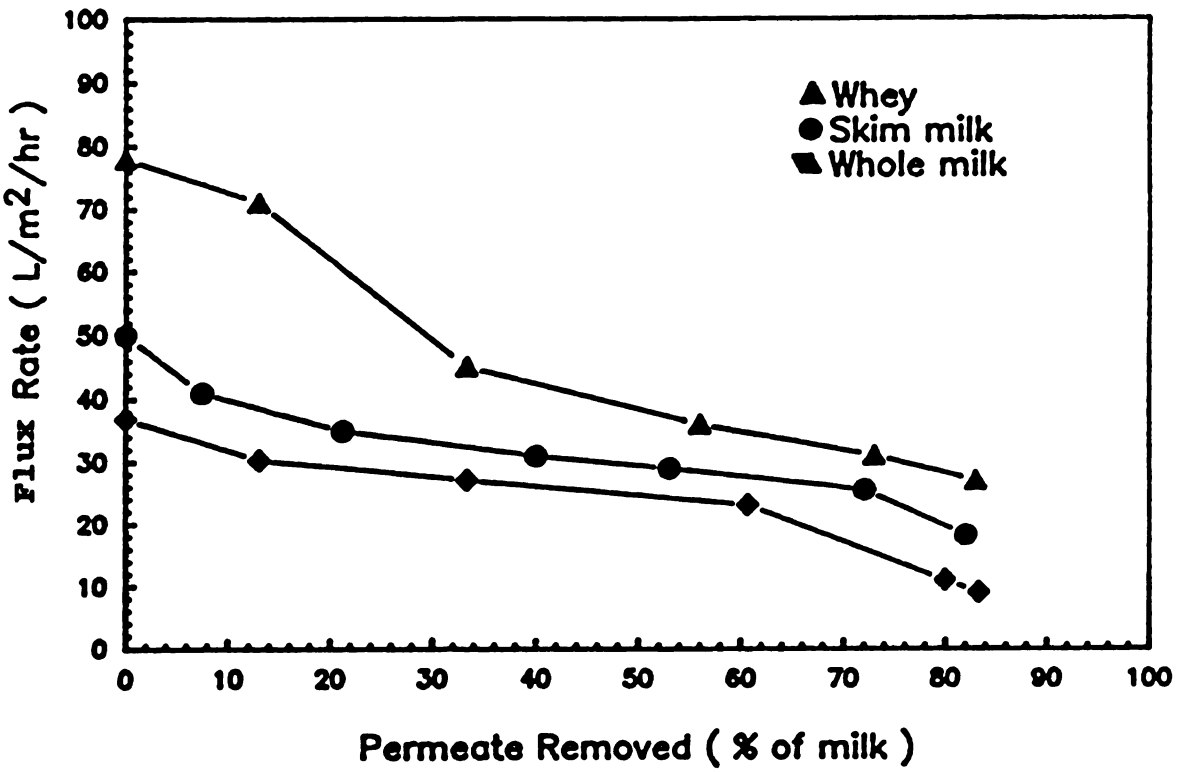


Figure 3. Relationship of flux rate to volume of permeate of whole milk, skim milk and whey obtained during ultrafiltration.

whereas the RE values decreased. Thus, the variation in the flux of whole milk, skimmilk and whey is related to the differences in total solid, protein and fat contents. The permeate flux of UF skimmilk was higher than that for UF whole milk. Peri et al.(1973) reported that the permeation rate during UF of skim milk was inhibited by the increasing concentration of lactose, protein and other solutes. Starting flux for whey was higher than that for skimmilk and whole milk because whey contains less protein and fat (Glover et al., 1978). The type of milk coagulant used in cheese manufacture may influence the subsequent permeate flux. The amount of permeate removed during UF and DF processes is recorded in Tables 4A, 4B, 4C, 4D, appendix 4. The influence of protein and fat concentration on permeation rates during ultrafiltration and reverse osmosis of whey and skim milk is well documented in the literature. Fenton-May et al.(1971) reported that the flux rate was controlled by the thickness of the protein and fat deposit on the membrane surface. Also, the calcium ion concentration, the formation of calcium phosphate gels, and the ionic strength of whey directly influence the ultrafiltration permeation rate of whey.

Material balance

Mass balance was determined for ultrafiltration and diafiltration processes (Table 1). These data illustrates the recovery of total solids and mass from whey, whole milk and skim milk processed by ultrafiltration and diafiltration. Recoveries were not quantitative in all cases and ranged from 94.0 % to 99.4 % for total solids and from 91.7% to 96.0% for the mass. The recovery of total solids from whole milk was higher by ultrafiltration (98.3%) than by diafiltration (94.1 %). These results agreed with the data reported by Covacevich and Kosikowski (1977b) who explained the difference between ultrafiltration and diafiltration on the basis of greater protein purification, and the interactions of flux, RE , protein content and viscosity. Higher retentate viscosities encountered in diafiltration experiments presented difficulties in draining the retentate from the ultrafiltration apparatus. This difficulty resulted in lower recoveries than did direct ultrafiltration. Higher recoveries of total solids and mass were obtained from whey and skim milk than obtained for whole milk by ultrafiltration and or diafiltration.

Table 1. Material balance in concentrated whole milk, skim milk and whey achieved by ultrafiltration and diafiltration

Fluids[a]	Initial feed		Retentate		Permeate		Recovery (%)	
	Wt	TS	Wt	TS	Wt	TS	Wt	TS
*DF[a]	258	31.5	56.6	18.5	260	11.2	91.7	94.1
UF[b]	258	31.7	51.0	19.8	190	11.6	93.4	98.3
UF[c]	221	19.5	50.0	9.8	160	9.6	95.4	99.4
UF[d]	255	16.8	40.0	6.2	205	10.5	96.0	99.2

[a]=DF whole milk, [b]= UF whole milk, [c]= UF skimmilk, [d]= UF whey.

*= Amount of water added during diafiltration.

The data represent average of three determinations.

TS = total solids.

Wt = weight in pounds.

Retentate composition

Gross changes in composition of the retentate of whole milk, skim milk and whey obtained by direct ultrafiltration and diafiltration processes are presented in Figures 4, 5, 6 and 7. Ultrafiltration of whole milk yielded retentate with an average total solid content of 38.4 % , fat 16.5 %, protein 10.5 %, lactose 7.9 % and an ash of 3.5 for three trials after 80.3 % of the original product was removed as permeate (Figure 4). For concentrating protein to a greater degree and reducing lactose and ash in the retentate, diafiltration was employed. The compositional changes of whole milk during the diafiltration process are depicted in (Figure 5). It shows that the retentate had an average composition of 32.6 % total solid , 15.5% fat, 13.65 % protein, 1.9 % lactose and 1.5 % ash for three replicates in which 113 % of permeate was removed. More lactose and salts are removed by diafiltration than by ultrafiltration. Flux limitations were encountered at lower levels of total solids (32.6 %) for diafiltration than for ultrafiltration (38.4%). These results are in agreement with the data reported by Covacevich and Kosikowski (1977b).

The data presented in Table 2 illustrate that the effects of diafiltration as compared to ultrafiltration on the composition of retentate were primarily a reduction in lactose and ash levels from 18.9% and 8.7% to 6.0% and 4.3% (on dry weight basis), respectively, and an increase in

Content (%)

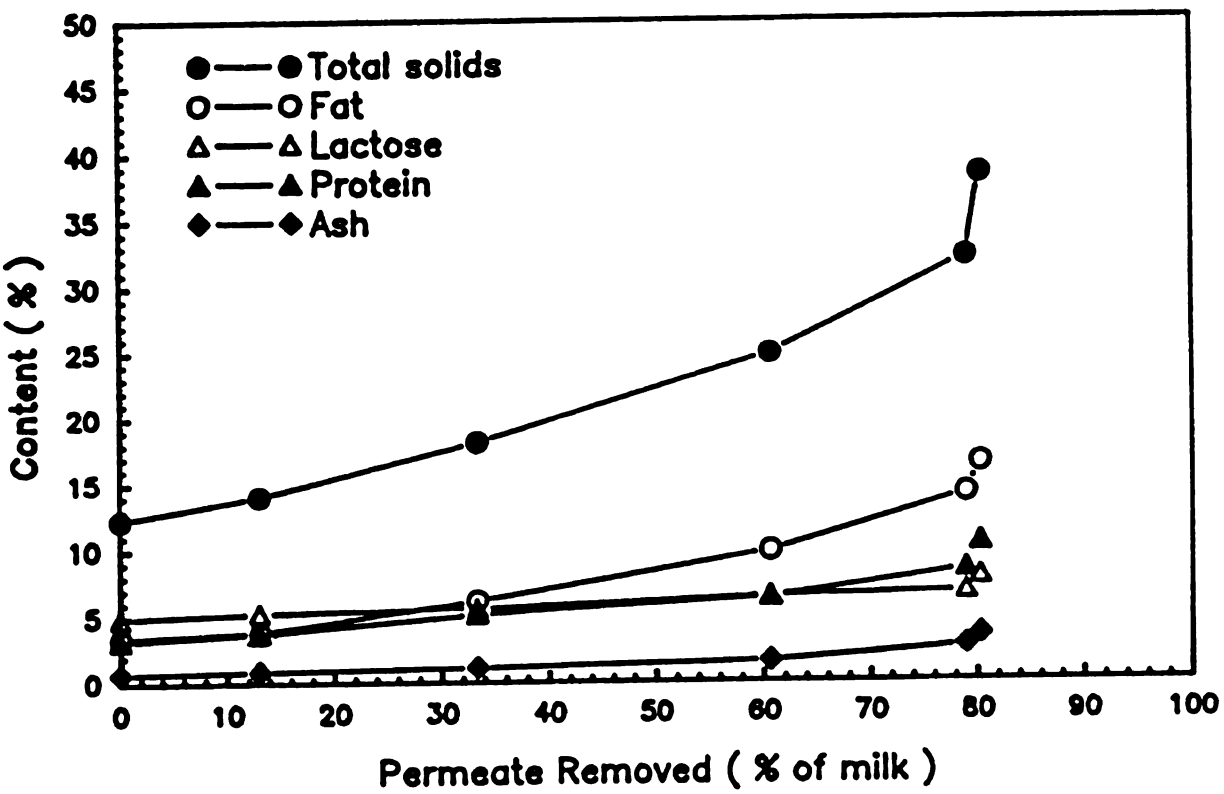


Figure 4. Composition changes in whole milk retentate during ultrafiltration.

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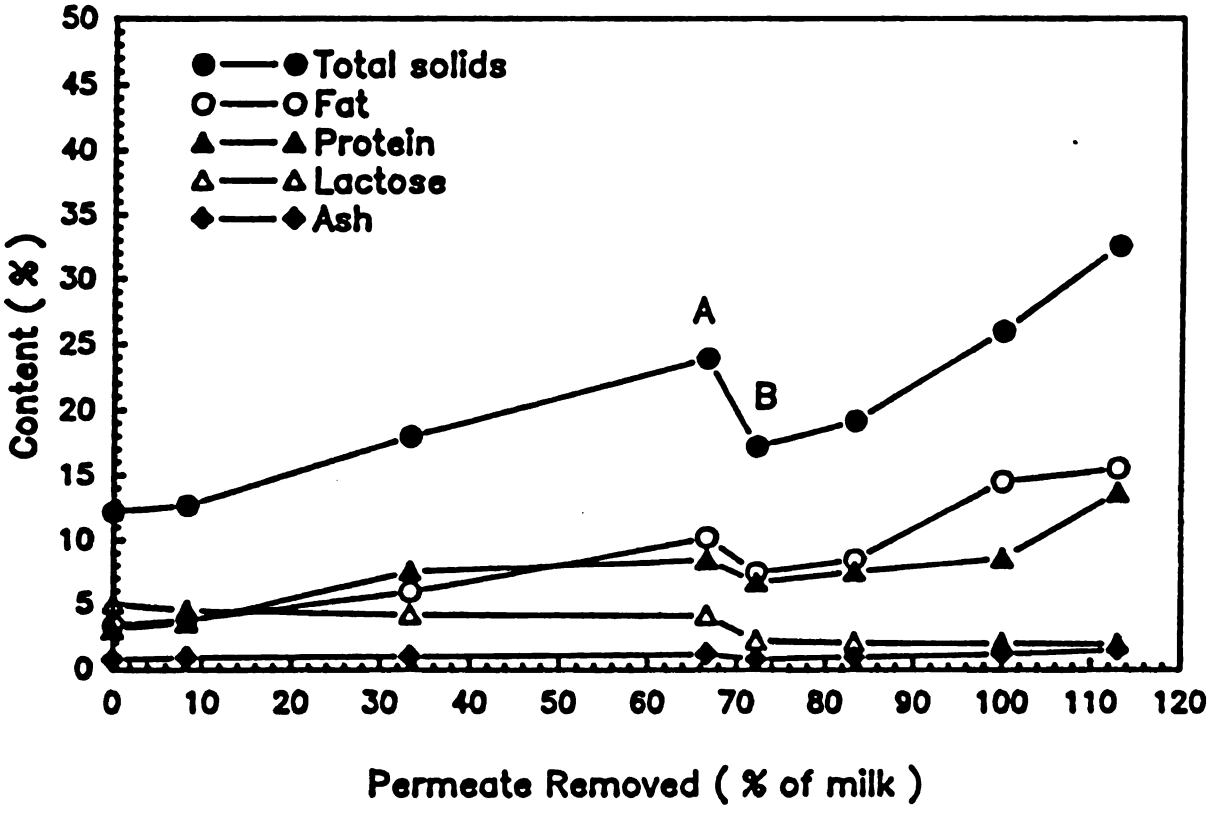

Figure 5. Composition changes in whole milk retentate during diafiltration. A and B represent diafiltration start and stop, respectively.

Table 2. Dry matter composition of whole milk,skimmilk and whey retentate obtained by ultrafiltration or diafiltration

	Content (%)			
Fluids	Protein	Lactose	Ash	Fat
Whole milk (UF)	26.6	18.4	8.7	43.3
Whole milk (DF)	42.0	6.0	4.3	47.4
Skim milk (UF)	51.5	35.5	13.9	ND
Whey (UF)	45.3	44.7	10.0	ND

The data represent average of three determinations.

ND= not detectable.

protein content from 26.6% to 42% (on dry weight basis) in the final retentate. Such differences in composition were also observed by Brown (1986). Figures 6 and 7 depict the change in skim milk and whey retentate induced by the ultrafiltration process. The data reveal a pattern similar to that of ultrafiltrate whole milk using UF (Figure 4). A large increase in individual components was observed when about 82 % of the permeate was removed for producing a whey protein concentrate (WPC) with 15.7 % total solid, 7.1 % protein, 7% lactose and 1.6 % ash and a concentrated skim milk was made with 19.5 % total solid, 10.0 % protein, 6.9 % lactose and 2.6 % ash content. The higher recovery of total solid from whole milk than from skim milk may due to the essentially complete retention of fat (Glover, 1971; Green et al., 1984). Both products showed similar lactose and ash contents and a high degree of protein retention (Table 2), a relationship also noted by Glover (1971), Bundgaard et al.(1972), and Green (1984).

The data presented in Tables 5A, 5B, 5C, 5D (Appendix 5) show the volume concentration ratio (VCR) as determined periodically during ultrafiltration and diafiltration. there was an increase in the VCR of whole milk ,skim milk and whey which was higher for whey than for milk. This observation reflects a greater volume reduction of whey. Also, the ultrafiltration retentate possessed a higher VCR than the diafiltration retentate as a result of greater retention of fat, lactose and ash in the former.

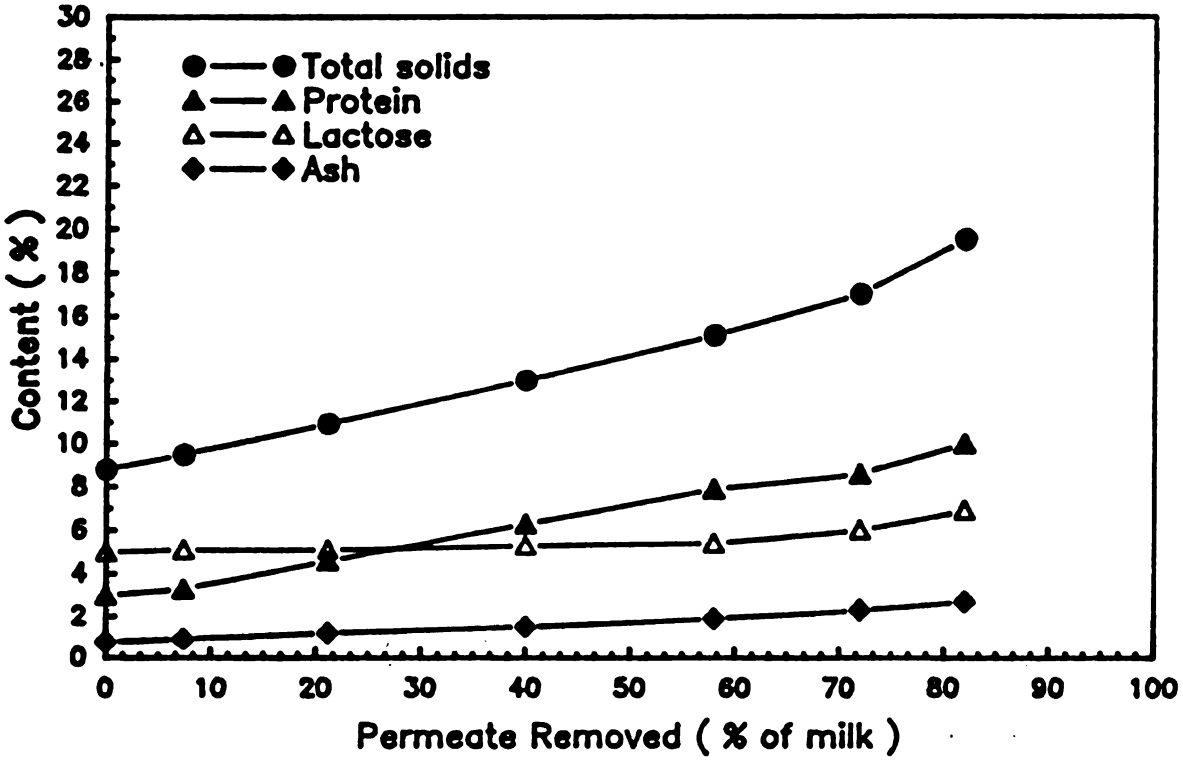

Figure 6. Composition changes in skim milk retentate during ultrafiltration.

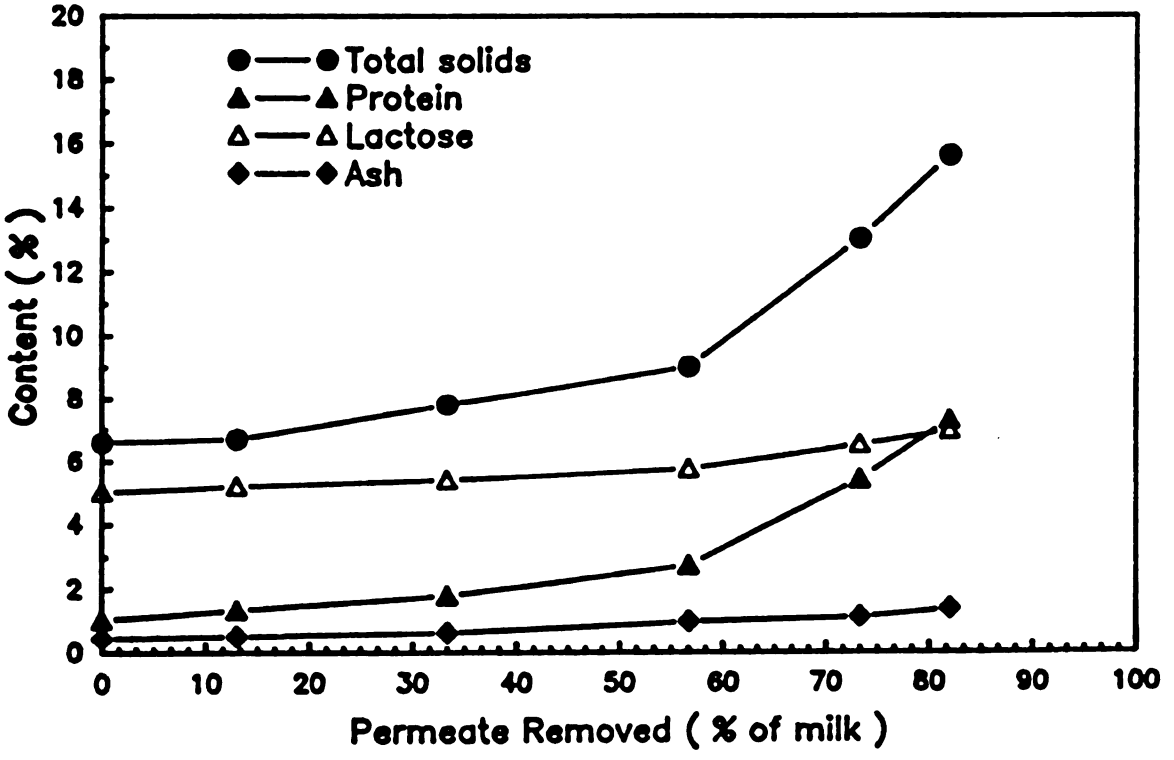

Figure 7. Composition changes in sweet whey retentate during ultrafiltration.

By comparing the VCR of the final retentate obtained by ultrafiltration and diafiltration with the concentration factor (CF) for each constituent (Tables 5A and 5B, Appendix 5), it was found that the fat was equal to or close to the value expected from the amount of permeate removed. These values indicate that fat was completely retained by the membrane which was confirmed by the analysis for fat in the permeate. Conversely, the protein content was less than expected from the amount of permeate removed, indicating that some of the protein passed with the permeate and some were retained in the ultrafiltration unit.

Permeate composition

The effect of ultrafiltration and/or diafiltration on the compositional characteristics of whole milk permeate is indicated by data plotted in Figures 8 and 9. These graphs reveal a complete recovery of fat in the ultrafiltration and diafiltration retentate, confirming the findings of Ernstrom et al. (1980). They have reported that rejection of fat during ultrafiltration was complete while a small loss of nitrogenous fraction occurred in the permeate, consisting primarily of non-protein nitrogen (NPN). Barbano et al. (1988) reported that the NPN content did not increase in the retentate because most of it passed through the membrane, mainly in the form of urea.

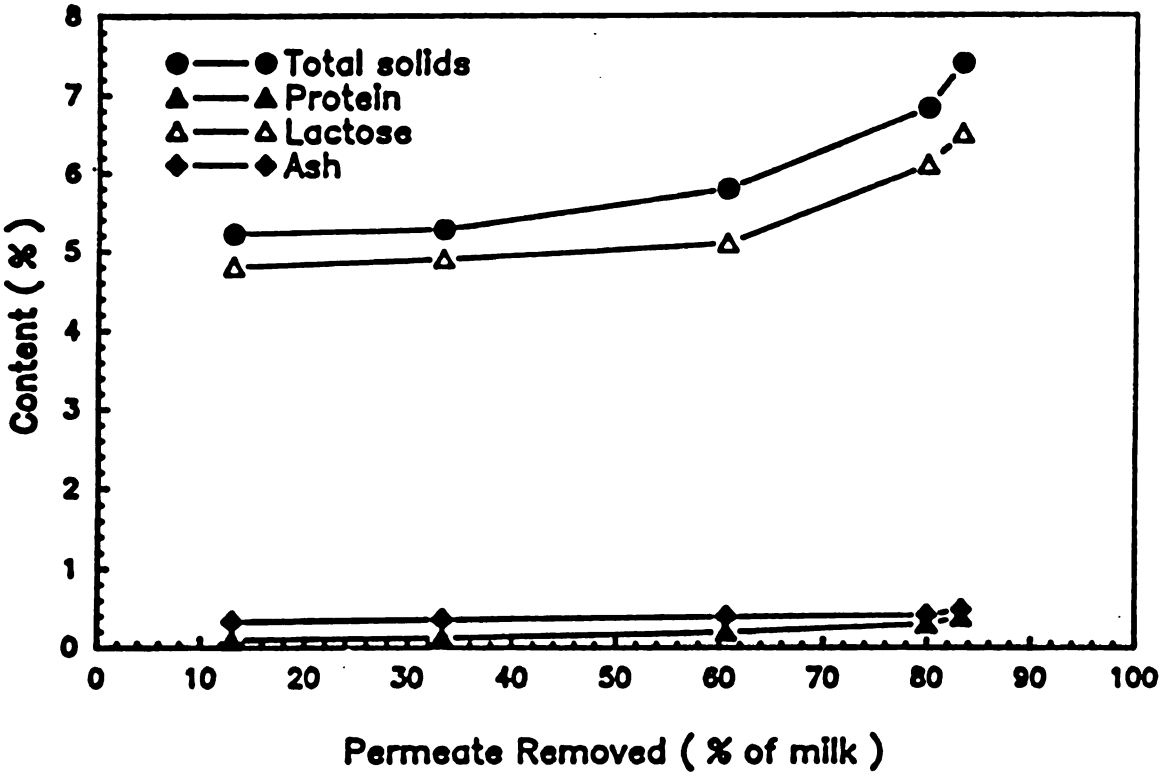

Figure 8. Composition changes in whole milk permeate during ultrafiltration.

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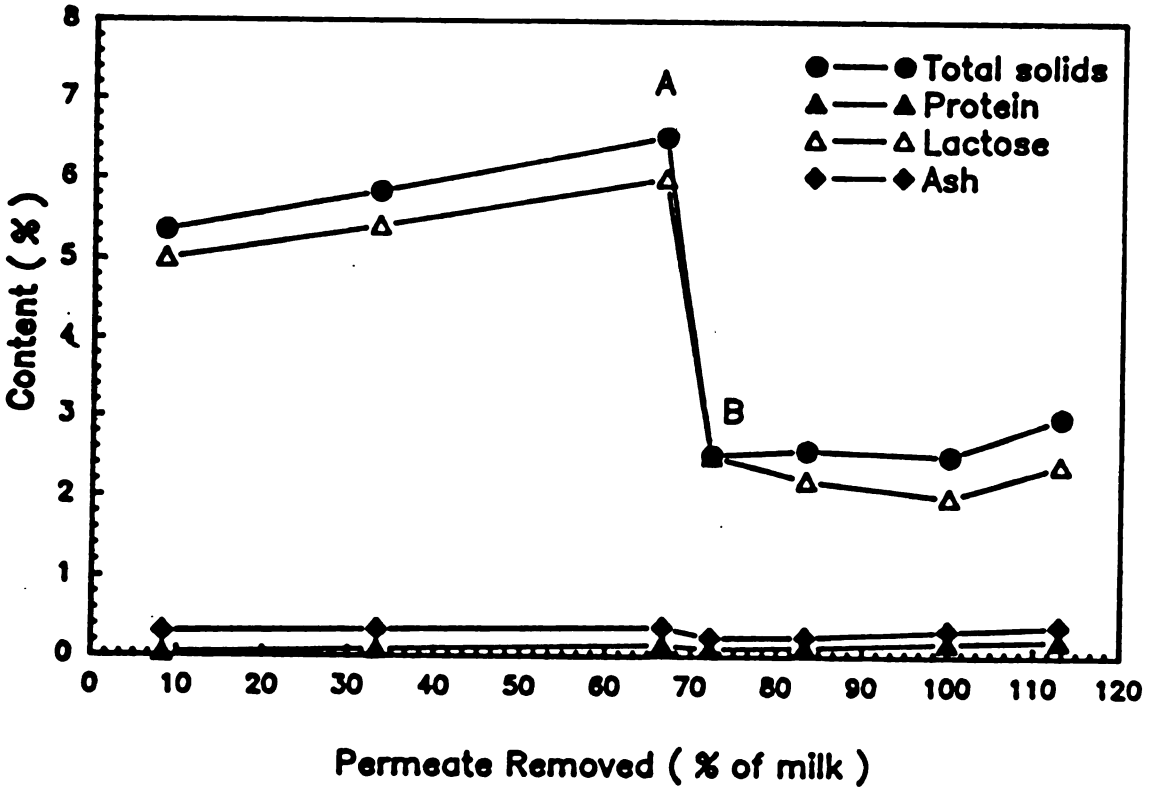

Figure 9. Composition changes in whole milk permeate during diafiltration. A and B represent diafiltration start and stop, respectively.

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Based on the protein analysis and electrophoretic studies of the final permeate, it can be concluded that the nitrogen fraction found in the permeate was composed of NPN and a small amount of α-lactalbumin. This observation agrees with the finding of Barbano et al. (1988), who characterized the ultrafiltrated permeate by SDS-PAGE and found that α-lactalbumin composed 90 % of the permeate protein. Ultrafiltration produced a final whole milk permeate with a composition of 7.4% total solids, 6.5 % lactose, 0.4 % protein and 0.5 % ash. While the composition of diafiltration whole milk permeate consisted of 3% total solid, 2.4 % lactose, 0.2 % protein and 0.4 % ash. As shown in Figures 8 and 9, the high content of permeate constituents was more pronounced with UF than DF due to adding water and lower lactose content. As presented in Tables 6A, 6B, Appendix 6, the average calculation indicated that percentage loss of protein in the permeate was higher (0.22 %) for ultrafiltration than for diafiltration (0.13 %). As stated by Ernstrom et al.(1980) the rate of protein loss varied through the process and appeared to be correlated to the protein concentration in the retentate. Coton (1980) stated that most of the nitrogen content of ultrafiltrated permeate from milk was NPN material normally found in milk. The average permeate composition of skim milk and whey is recorded in Table 3 and 4, respectively. The data indicate that the high level of solids in the permeates consist primarily of lactose and

Table 3. Permeate composition of ultrafiltrated skim milk

Composition (%)					
Total solid	Ash	Lactose	NCN	NPN	α-La
6.00	0.83	4.76	0.35	0.33	0.02

NCN=non-casein nitrogen, NPN=non-protein nitrogen, α-La (α-lactalbumin) equals to NCN-NPN.
The data are the average of three determinations and presented by (%Nx6.38).

Table 4. Permeate composition of ultrafiltrated whey

Composition (%)					
Total solids	Ash	Lactose	NCN	NPN	α-La
5.10	0.34	4.30	0.32	0.24	0.08

NCN=non-casein nitrogen, NPN=non-protein nitrogen, α-La (α-lactalbumin) equal to NCN-NPN.
The data are the average of three determinations and presented by (%Nx6.38).

minerals, accompanied by a small amount of NPN and α-lactalbumin. This incomplete rejection of protein may be due to distribution of pore size in the membrane and/ or the configuration of the protein under these processing conditions. Also, Barbano et al.(1988) attributed the passage of protein through an ultrafiltration membrane to protein molecular weight plus other protein characteristics such as charge, hydrodynamic size, shape and hydrophobic or hydrophilic character. The nature of the foulant material adsorbed on the UF membrane surface may also effect the passage of protein in the membrane.

Nitrogen fraction

The effect of ultrafiltration and diafiltration on the nitrogen distribution of whole milk, skim milk and whey was investigated. The data obtained from the nitrogen analyses are presented in Table 5 through 8. The data reveal a gradual increase in total nitrogen, casein, non-casein and total albumin level in the retentate as a result of UF and FD processing. Most of this increase was due to the retention of casein and total-albumin, especially β-lactoglobulin, with a little increase in the non-protein nitrogen. Matthews et al. (1976) and Glover et al. (1979) reported that the percentage of NPN in milk retentates did not increase with concentration because a portion of the original milk NPN (mainly urea, amino acid and ammonia) freely passed through the membranes.

Table 5. Change in the nitrogen distribution of whole milk during ultrafiltration

Volume Concentration Ratio (VCR)	Nitrogen Fractions[a] (%)				
	TN	CN	NCN	TA	NPN
1.0	3.25	2.49	0.76	0.00	0.15
1.14	4.04	2.90	1.14	0.90	0.16
1.5	4.90	3.50	1.40	1.20	0.16
2.5	7.17	5.48	1.69	1.40	0.16
4.1	9.32	7.10	2.22	1.85	0.17
4.8	10.50	8.00	2.50	2.20	0.18
CF	3.23	3.2	3.3	3.6	1.1

[a]Protein concentrations (%Nx6.38) as the average of three determinations.
CF= Concentration Factor at conclusion of UF is the ratio of final protein in retentate to the protein of original product.
TN, CN, NCN, TA and NPN represent total nitrogen, casein, non-casein nitrogen, total albumin nitrogen and non-protein-nitrogen, respectively.

A comparison between the ultrafiltration and diafiltration processes on the nitrogen distribution of whole milk is presented in Tables 5 and 6. These data indicate that diafiltrated retentates had greater protein concentration than ultrafiltrated retentates. The nitrogen distribution in diafiltration retentate was 13.65 % total nitrogen, 10.35% casein nitrogen, 3.3 % non-casein nitrogen, 2.2% total albumin and 0.17 % non-protein nitrogen as compared to 10.5% , 8.0 % , 2.5 %, 2.2 % and 0.175 %, respectively, in retentates from ultrafiltration . This difference may be attributed to the effect of adding water during the diafiltration process which causes an increase in concentration and purification of the constituent proteins. The protein content decreased upon initiation of diafiltration due to dilution of the retentate. However, it increased during the terminal stages of diafiltration as a result of an increase in the permeation rate and due to the amount of permeate removed (Table 6). When the volume concentration ratio (VCR) was compared to the concentration factor (CF) of the protein fraction, the increase in protein concentration was not as high as anticipated. The same was true in the case of ultrafiltration processed products (Tables 5, 7 - 8). A possible explanation for this discrepancy is the retention of a highly concentrated protein layer on the membrane in the ultrafiltration unit which was not recovered in the retentate. This connection was confirmed qualitatively by

Table 6. Change in the nitrogen distribution of whole milk during diafiltration of whole milk

Volume Concentration Ratio (VCR)	Nitrogen components[a] (%)				
	TN	CN	NCN	TA	NPN
1.0	3.10	2.38	0.72	0.48	0.16
1.09	3.38	2.62	0.76	0.54	0.16
1.5	7.40	5.81	1.59	1.30	0.16
3.0	8.99	7.19	1.80	1.62	0.17
2.0	5.74	4.78	0.96	0.73	0.16
2.66	7.59	5.70	1.89	1.62	0.17
4.0	9.83	7.68	2.23	1.94	0.17
4.5	13.65	10.35	3.30	2.20	0.17
CF	4.4	4.3	4.5	4.5	1.06

[a]Protein concentration(N x 6.38) as average of three determinations.
CF= Concentration Factor at conclusion of UF is the ratio of final protein in retentate to the protein of original product.
TN, CN, NCN, TA and NPN represent total nitrogen, casein, non-casein nitrogen, total albumin,and non protein nitrogen compounds, respectively.

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observing the release of thin sheets of proteinaceous material during the clean up step after the unit was shutdown. The presence of this protein film on the membrane surface caused a decrease in the flux and lower protein retention (Ernstrom et al.,1980). The data in Table 7 show the change in the nitrogen fractions of skim milk during UF. The trend of increasing protein concentration during ultrafiltration, that occurred with whole milk, was observed with skim milk. The final skim milk retentate contained 10 % TN ,7.55 % CN , 2.4 % NCN, 2.0 % TA and 0.17 % NPN. The data in Table 8 relating to the ultrafiltration of whey indicate that the protein concentration of the retentate increased directly with the amount of permeate removed. There is more NCN present in whey than in the milk because of the release of macropeptides by the action of rennet on casein during the manufacture of cheese. There is also a small, variable amount of casein "fines" present in the whey which is concentrated by ultrafiltration /or diafiltration. As the data in Table 8 reveal, the composition of the whey protein concentrate (WPC) was increased from 1.12 % TN ,1.1 % NCN, 0.6 % TA and 0.5 % NPN to 7.0 % , 6.3 %, 3.7 % and 0.6%, respectively. As discussed in the earlier studies with milk, the membrane permits free passage of the NPN fraction, while the TA fraction, α-lactalbumin and β-lactoglobulin are concentrated but not to the same extent. The VCR of whey was higher than the VCR of both whole milk and skim milk due to the

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Table 7. Change of nitrogen distribution in the ultrafiltration skim milk

Volume Concentration Ratio (VCR)	Nitrogen Components[a] (%)				
	TN	CN	NCN	TA	NPN
1.0	3.10	2.40	0.70	0.50	0.15
1.08	3.20	2.40	0.80	0.60	0.15
1.4	4.50	3.50	1.00	0.80	0.15
1.8	6.00	4.70	1.20	1.10	0.16
2.6	7.80	6.10	1.60	1.40	0.16
3.6	8.9	6.8	2.1	1.90	0.16
4.2	10.0	7.60	2.40	2.00	0.17
CF	3.2	3.15	3.5	3.6	1.15

[a]Protein concentration (%Nx6.38) as average of three determinations.
CF= Concentration Factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.
TN, CN, NCN, TA and NPN represent average of total nitrogen, casein, non-casein nitrogen, total albumin and non-protein nitrogen compounds, respectively.

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Table 8. Changes of nitrogen distribution in the ultrafiltration whey

Volume Concentration Ratio (VCR)	Nitrogen components[a] (%)			
	TN	NCN	TA	NPN
1.0	1.12	1.10	0.60	0.50
1.15	1.34	1.29	0.60	0.57
1.5	1.78	1.70	1.00	0.57
2.6	2.60	2.40	1.70	0.57
4.4	5.00	4.60	3.00	0.58
6.2	7.00	6.30	3.70	0.60
CF	6.2	5.7	6.1	1.2

[a]Protein concentration (%Nx6.38) as average of three determination.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.
TN, NCN, TA and NPN represent mean of total protein, non-casein protein, total albumin and non-protein nitrogen compounds, respectively.

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increased quantities of permeate removed. These results demonstrate that ultrafiltration and diafiltration processes can be used to concentrate constituent protein fractions. Diafiltration permits higher levels of protein concentration than does direct ultrafiltration. However, recovery of protein, as estimated from the concentration factor, was not the same as estimated from the volume concentration ratio. This was especially true for ultrafiltration process.

Mineral content

Nineteen mineral elements of whole milk, skim milk and whey and of their respective retentates and permeates were assayed to ascertain the potential contribution of these nutrients to the nutritional value of foods in which they are utilized, especially for milk designated for cheesemaking. The concentration of major and trace minerals in the final retentate and permeate of ultrafiltrated sweet whey is presented in Tables 9 and 10. Higher levels of Ca, P, Mg, Zn, Fe and Cu were observed in the retentate than in the permeate. The remaining minerals showed no definite difference. Since all or a major portion of these elements are associated with proteins they are expected to be retained during the ultrafiltration process. The percent retention of each mineral (Table 9, 10) decreased in the order: Ca 2.3> P 2.1> Mg 1.6> Zn 1.4> K 1.3 > Fe 1.3. When

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Table 9. Major mineral content of sweet whey, retentate and permeate using ultrafiltration[a]

	Ca	Mg	Na	K	P
	mg/100g				
Sweet whey	34.4	6.1	47.8	123	45.4
Retentate	79.2	10.0	53.2	160	95.3
Permeate	41.3	7.1	60.0	157	46.8
CF	2.3	1.6	1.1	1.3	2.1

[a]Average of three determinations.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.

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Table 10. Trace mineral composition of sweet whey, concentrate and permeate using ultrafiltration

	Zn	Fe	Cu	B	Mn	Mo	Ba
				µg/100g			
Sweet whey	19	90	7.2	5.1	1.9	NDA	NDA
Retentate	26	120	19	1.5	1.7	0.36	0.2
Permeate	26	60	6.1	6.4	3.0	3.0	1.9
CF	1.4	1.33	2.6	0.29	0.89	ND	ND

[a]Average of three determinations.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.
ND =not detectable.

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compared with the published data for liquid whey, the values for the major minerals were almost identical with those reported by Wong et al.(1978) and Feeley et al.(1972). However, the values for Cu, Mn, B, Mo, and Ba were somewhat higher than reported by those researchers. This discrepancy could be due to several factors which may influence the mineral content of whey, including the type of cheese being produced, the geographic area, source of milk, stage of lactation and processes employed. The major and trace minerals of whole milk retentate and permeates are tabulated in Tables 11 and 12. Generally, the content of Ca, P, Mg, Na, K, Zn, Fe, Cu and B were higher in the retentates than in the permeates. These results are in agrement with Brule et al.(1974) who stated that calcium increased linearly with CF in milk retentate, and constant in permeate. These minerals have been shown to be partially associated with casein micelles and the fat globule membrane and retained with the protein concentrate. The amount relative to casein usually decreased with increase in the concentration factor, reported by Green et al.(1984), and Walstra and Jenness (1984). The higher the protein concentration, the higher was the colloidal Ca. The data presented in Table 11 and 12 indicate that the minerals concentrated in the retentate were in order of decreasing concentration factors: Ca 4.1; P 3.2; Zn 2.4; Mg 2.1; Fe 1.8. The high retention of Zn and Fe during the ultrafiltration of whole milk was also observed by Fukuwatari et al.

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[a]Aver
CF= C
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Table 11. Major mineral content of fresh whole milk, retentate and permeate by diafiltration

	Ca	Mg	Na	K	P
	mg/100 g				
Fresh	110	9.7	47.9	200	98.6
Retentate	450	20.1	35.6	250	310
Permeate	32.9	6.59	46.15	107	42.4
CF	4.1	2.1	0.74	1.3	3.2

[a]Average of three determinations.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.

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Table 12. Trace mineral composition of whole milk, retentate and permeate by diafiltration

	Zn	Fe	Cu	B	Ba	Mn	Mo
				μg/100g			
Milk	390	50	8.0	17	NDA	5.0	5.8
Retentate	940	90	5.0	6.0	0.6	4.3	3.8
Permeate	410	49	13.0	7.9	ND	0.9	2.0
CF	2.4	1.8	0.63	0.35	0.0	0.86	0.66

[a]Average of three determinations.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.
ND= not detectable.

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Skim

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*Aver
CF= C
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Table 13. Major mineral content of fresh skim milk, retentate and permeate by ultrafiltration

	Ca	Mg	Na	K	P
	mg/100g				
Skim milk	125	10.8	48.1	160	180
Retentate	400	16.6	40.8	300	410
Permeate	53	9.0	40.0	110	85
CF	3.2	1.5	0.84	1.9	2.3

[a]Average of three determinations.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.

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Skim	
Rete	
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[a]Aver
CF= C
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Table 14. Trace mineral composition in fresh skim milk, retentate and permeate by ultrafiltration

	Zn	Fe	Cu	B	Ma
	ug/100g				
Skim milk	420	62	8.2	15.0	3.5
Retentate	840	70	15.0	8.0	4.6
Permeate	390	30	3.1	1.5	0.5
CF	2.0	1.13	1.8	0.5	1.3

[a]Average of three determinations.
CF= Concentration factor at conclusion of UF is ratio of final protein in retentate to the protein of original product.

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(1982). The retention of Na and trace minerals was low in the retentate. Enzymes, including those associated with casein micelles and milk fat globule membrane, contain Mo, Fe, Cu, P and Zn (Webb et al., 1974; Walstra and Jenness, 1984). This Content of the major and trace minerals in the skim milk and their distribution between retentate and permeate were similar to those of whole milk (Table 13 and 14). Since macromolecules were retained during ultrafiltration, high recoveries of associated minerals were expected even though the retention varied a little from one milk to an other. In general all the products showed similar mineral levels as a result of UF processing, with more retention in retentate than permeate.

Electrophoresis

The effect of ultrafiltration on the protein fractions of whole milk, skim milk and whey was studied electrophoretically at the beginning, middle and end of the ultrafiltration run.

Whole Milk

A typical densitometer tracing for separation of protein in the whole milk, its casein (Cn) fraction , and its total albumin (TA) fraction at different stages of the ultrafiltration process are presented in Figures 10, 11 and 12, respectively. Peaks were identified from the protein

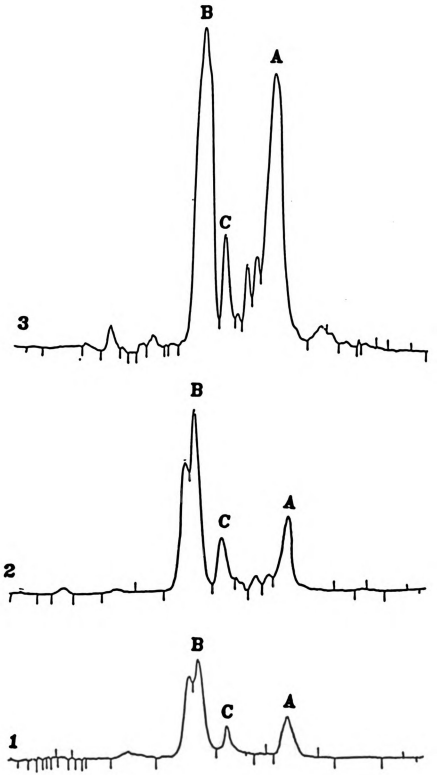

Figure 10. Discontinuous polyacrylamide gel electrophoresis densitograms of whole milk retentate. A; α-casein B; β-lactoglobulin & β-casein, C; α-lactalbumin. Ultrafiltration time: 1= 0.0 min; 2= 30 min; 3= 60 min.

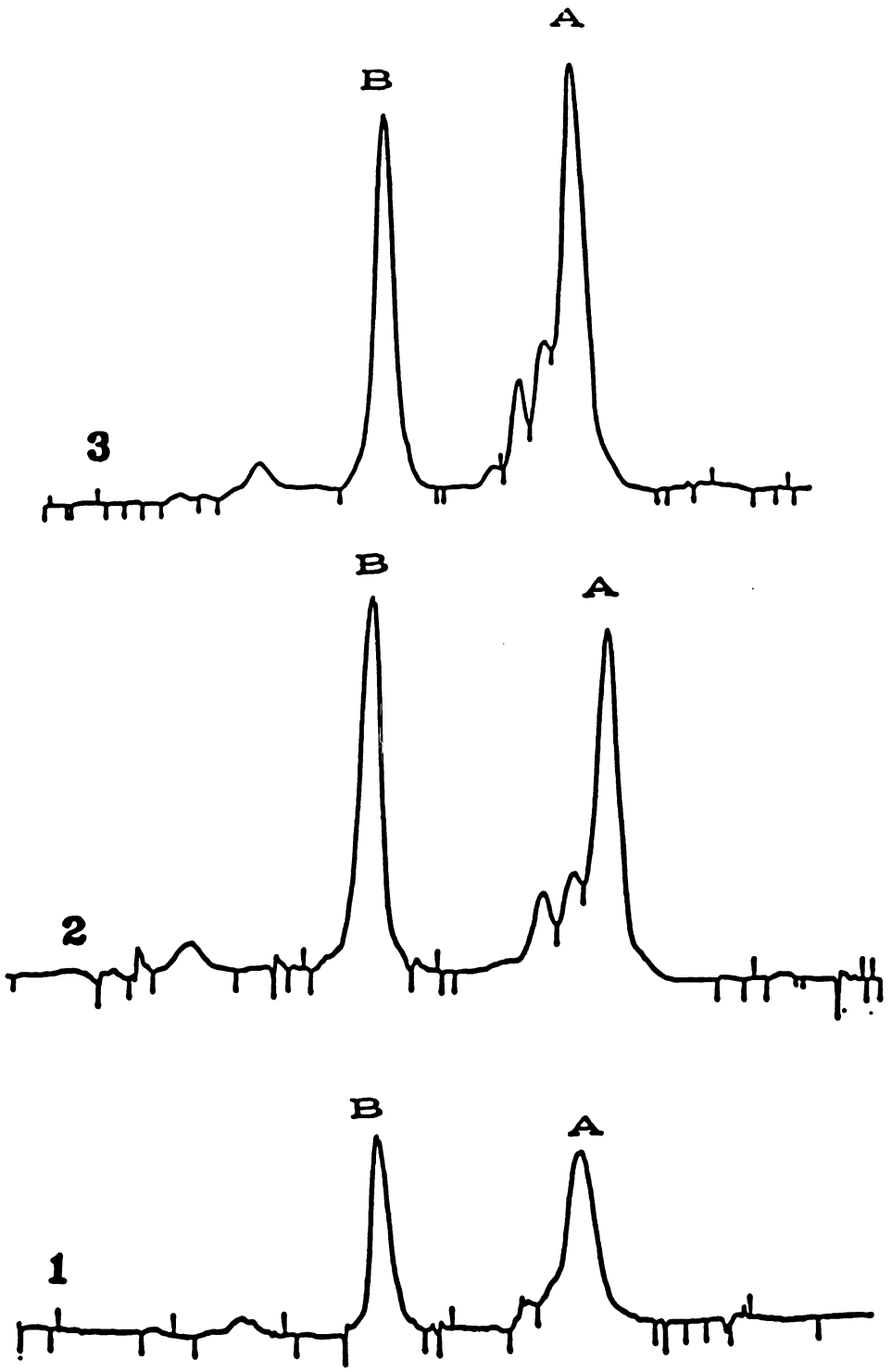

Figure 11. Discontinuous polyacrylamide gel electrophoresis densitograms for casein of whole milk retentate. A; α_{s1}-casein B; β-casein. Ultrafiltration time: 1= 0.0 min; 2= 30 min; 3= 60 min.

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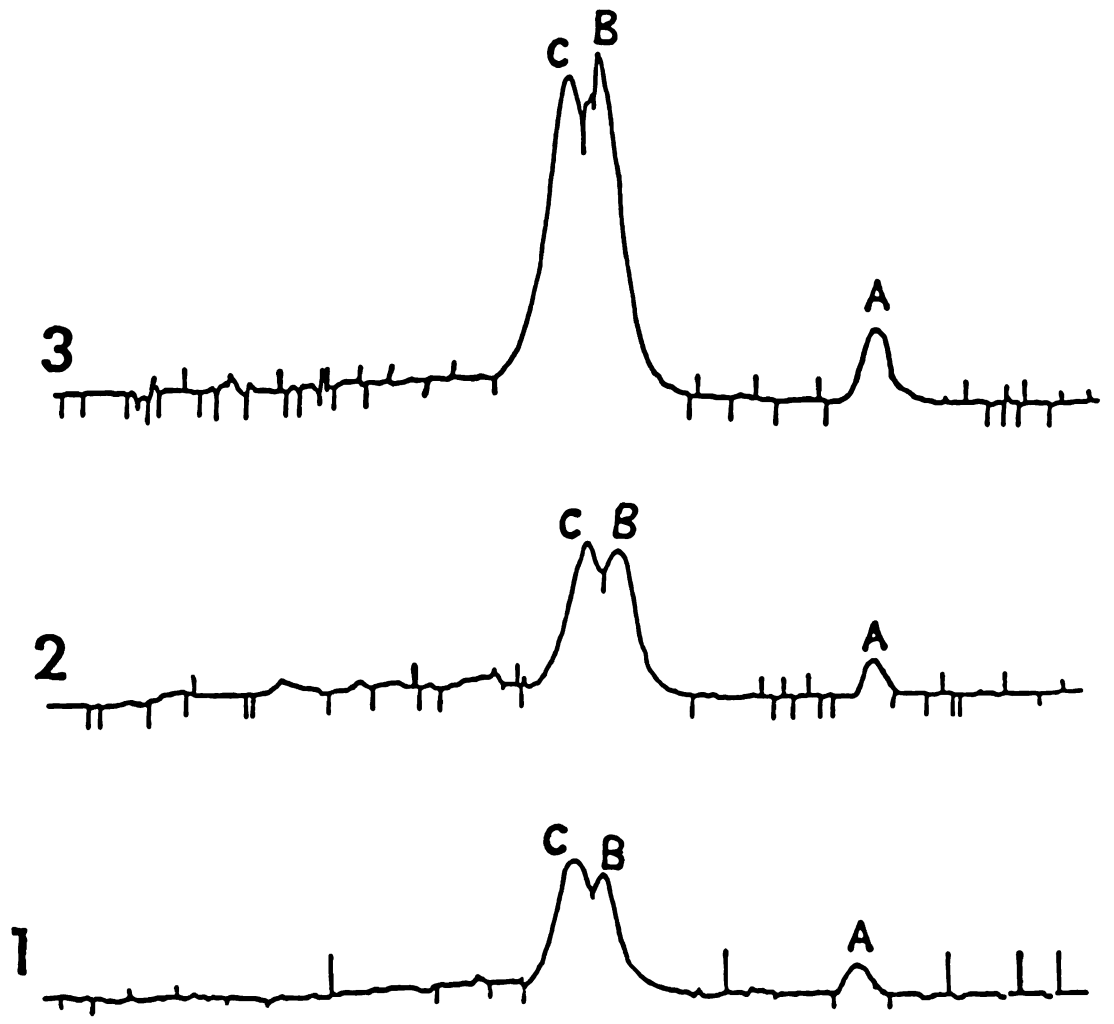

Figure 12. Discontinuous polyacrylamide gel electrophoresis densitograms for total albumin nitrogen of whole milk retentate. A ;α-lactalbumin, B; β-lactoglobulin B, C ;β-lactoglobulin A. Ultrafiltration time: 1= 0.0 min; 2= 30 min; 3= 60 min.

standards of α-casein, β-casein, α-lactalbumin and β-lactoglobulin. The pattern illustrates that there was substantial increase in component peak areas corresponding roughly to the degree of concentration achieved by ultrafiltration. Representative protein profiles for whole milk are shown in Figure 10. Areas under peaks A, α_s-casein (α_sCn); B, β-casein and β-Lactoglobulin (β-Cn and β-Lg) and C, α-lactalbumin (α-La) increased with the duration of ultrafiltration, i.e., corresponding roughly to the extent of concentration (Table 8A, whole milk, Appendix 8). As the minor peaks were not identified, they were not used in the computation. The identified bands from whole milk were similar to that bands which extracted for casein and total albumin nitrogen from same whole milk. Figure 11 represents a typical densitometric tracing of the total albumin nitrogen (TAN) fraction of the whole milk. The B and C peaks represent β-lactoglobulin. In Figure 12, the peak areas under A, B and C were increased with the duration of ultrafiltration. The data in Table 8A for TAN (Appendix 8) reveal that the dominant component of TAN is to β-Lg (A and B), ranging from 77.5% to 83.8 % , whereas α-La ranged from 22.4% to 16.2 %. The ratio between α-La and β-Lg changed with processing time due to the release of α-La through the UF membrane. This relation was confirmed by fractional analysis of the retentate and by the nitrogen determination of the permeate. Figure 12 represent a typical pattern derived from the casein fraction of whole

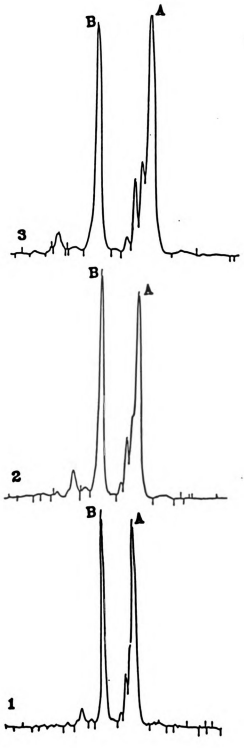

Figure 13. Discontinuous polyacrylamide gel electrophoresis densitograms for casein of skim milk retentate. A; α_{s1}-casein B; β-casein. Ultrafiltration time: 1= 0.0 min; 2= 30 min; 3= 60 min.

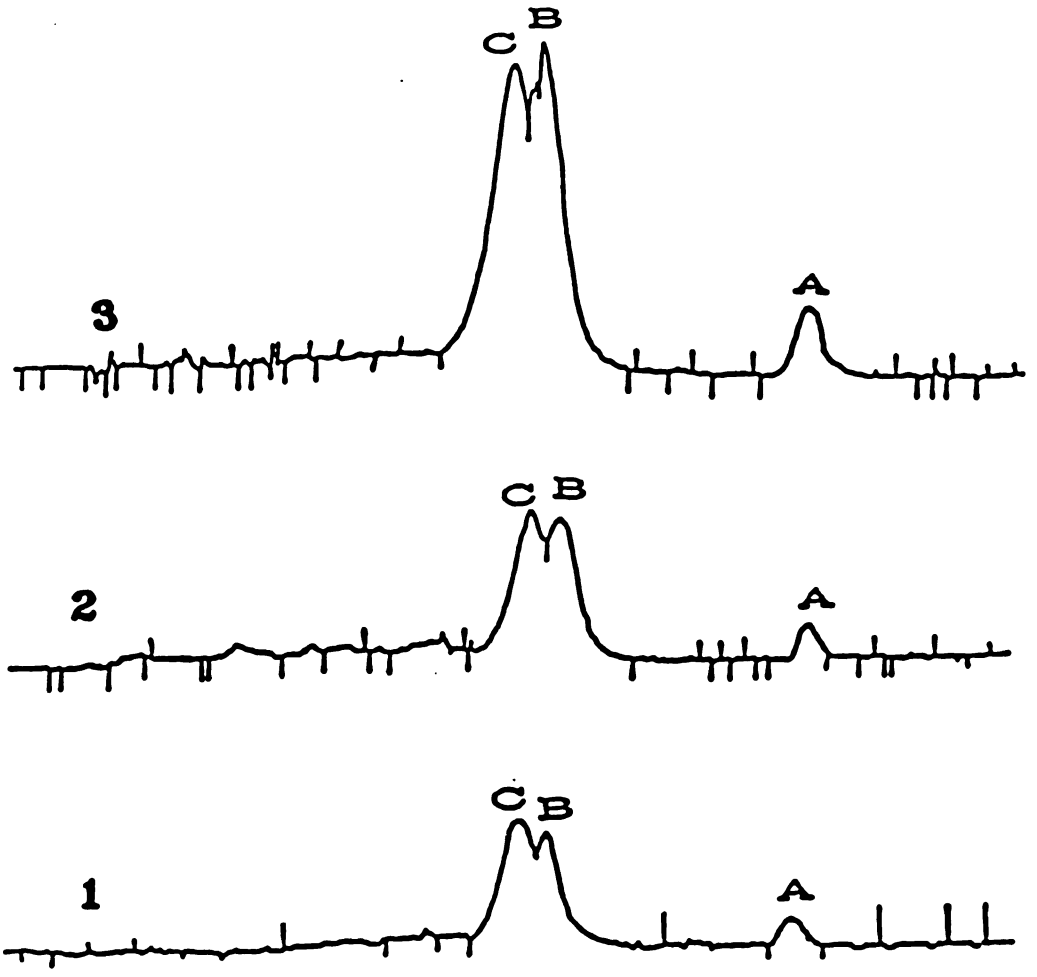

Figure 14. Discontinuous polyacrylamide gel electrophoresis densitograms for total albumin nitrogen of skim milk retentate. A ;α-lactalbumin, B ;β-lactoglobulin B, C ;β-lactoglobulin A. Ultrafiltration time: 1= 0.0 min; 2= 30 min; 3= 60 min.

milk. Areas under peak A, α_s-Cn and B, (β-Cn) indicate a noticeable increase with concentration. Calculations (Table 8A, Appendix 8) indicate that the relative increase in α-Casein and β-Casein was constant throughout the ultrafiltration process. It is also apparent that milk proteins were concentrated during ultrafiltration but with a little loss of α-lactalbumin.

Skim milk

Typical densitometer tracings which illustrate the separation of proteins in the casein and TAN fractions of skim milk and its retentate obtained during UF processing, are presented in Figures 13 and 14. The PAGE pattern of casein and TAN in skim milk and their relative distribution were similar to those observed in the case of whole milk (Table 8B, Appendix 8).

Whey

A typical densitometer tracing for the separation of α-La and β-lg, the principal fraction of (TAN) in the sweet whey, is presented in Figure 15.

A comparison of whey and concentrated whey obtained at different ultrafiltration processing times indicates increasing peak area with concentration. Area under peaks A and B represents the relative amount of α-La and β- Lg, respectively. The larger fraction of TAN was attributed to

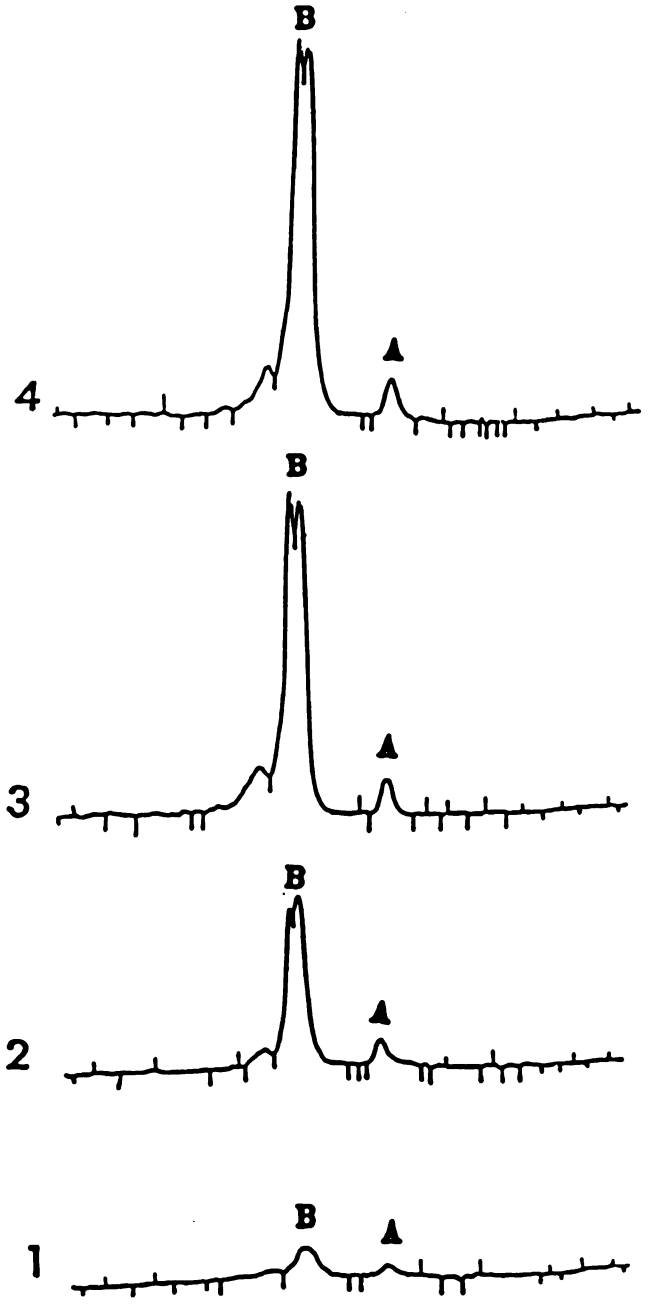

Figure 15. Discontinuous polyacrylamide gel electrophoresis densitograms of whey retentate. A ;α-lactalbumin, B ;β-lactoglobulin A and β-lactoglobulin B. Ultrafiltration time: 1= 0.0 min; 2= 15 min; 3= 30 min; 4 =60 min.

β-Lg, ranging from 76% to 90.2 % , whereas α-La ranged from 24% to 9.8 %. The distribution between both components changed as a result of increased concentration, but not in the same ratio (Table 8C, Appendix 8). These observations indicated that either the ultrafiltration membrane permitted molecules greater than 5,000 Daltons to pass through or the conformational characteristics of α-La (14,200 Daltons) allowed its passage through the membrane. Also, the release of α-La may be due to the age of the membrane used in this process. These results were within same limits previously reported by Lee and Merson (1974) and Barbano et al.(1988).

Total amino acids

Whey protein is a highly nutritious by-product of cheese. The whey represent the non-casein protein as well as the fractions and fragments of the casein which remain soluble when casein have been precipitate. Total amino acid content of sweet whey obtained from a manufacture of Egyptian Domiati cheese and its concentrated protein obtained by ultrafiltration was analyzed by HPLC. The pattern of seventeen amino acid is presented in Table 15. The data revealed a large increase in the total amino acids from 115.8 to 453.36 mg/g of dry matter resulting from ultrafiltration. The increase of individual essential and nonessential amino acid residuals was consistent and

Table 15. Total amino acid contents of sweet whey and whey protein concentrate

Amino Acids	Whey	WPC	Fold Increase
	mg/g (dry weight)		
Essential A.A			
Histidine	2.3	8.2	3.6
Threonine	7.7	33.2	4.3
Valine	8.0	33.6	4.2
Isoleucine	8.8	36.9	4.2
Leucine	13.0	55.6	4.2
Phenylalanine	3.4	17.0	4.1
Lysine	10.8	50.2	4.6
Methionine	3.3	10.5	3.2
Cystine	2.5	6.2	2.5
Nonessential A.A			
Aspartic acid	7.4	25	3.4
Glutamic acid	18.9	69.5	3.6
Serine	5.9	24.3	4.1
Glycine	3.0	12.2	4.0
Arginine	4.5	16.8	3.7
Alanine	8.3	27.0	3.3
proline	4.0	13.7	3.4
Tyrosine	3.8	13.2	3.5
Total A.A	115.8	453.3	3.9

WPC= Whey Protein Concentrate.
The data are averages of three determinations.

ranged from 2.5-fold to 4.6-fold of that present in whey. A low concentration of sulfur amino acids (cystine and methionine) was detected probably due to destruction during the analysis. The results showed that whey protein concentrate (WPC) is a very good source of amino acids reflecting the presence of α-lactalbumin and β-lactoglobulin in the retentate. This observation was previously reported by Wingerd (1971) and Hambraeus (1982) who stated that whey protein concentrate is a superior source of essential and nonessential amino acids and could be an excellent supplement for processed foods.

Fatty acids composition

The separation and quantification of free fatty acids of whole milk and retentate was achieved by GC on a SP-216-PS column. The results are presented in Table 16. The data revealed an increase in the total free fatty acids from 33.7 to 80.1 mg /100 g when whole milk was concentrated by ultrafiltration. The total increase of 2.38-fold in FFA was compatible with the increase in fat content in the retentate (Tables 5 and 6 in appendix 7)

Individual fatty acids increased consistently from 2 to 3-fold except the short chain fatty acids (C:4 and C:6). High temperature (50^0C) used during ultrafiltration may have resulted in the volatilization of short chain fatty acids.

Table 16. Free fatty acid composition of whole milk and retentate

Fatty acid	Milk	Retentate	Concentration factor
	mg/100g		
C4:0	1.5	1.6	1.1
C6:0	1.0	1.6	1.6
C8:0	0.6	1.4	2.0
C10:0	1.2	2.7	2.3
C12:0	1.6	3.5	2.2
C14:0	2.8	7.1	2.5
C16:0	10.4	25.5	2.5
C18:0	3.5	10.0	2.9
C18:1	8.5	21.0	2.5
C18:2	1.5	3.6	2.4
C18:3	1.1	2.2	2.0
Total F.F.A	33.7	80.1	

The data are averages of three determinations

However, adding water during the diafiltration step could also wash out some of the soluble short chain fatty acid.

A typical chromatogram of the fatty acid retention time is presented in Figure 7A (Appendix 7). A recovery study on the procedure and the correction factors for relating the internal standards to the individual fatty acids measured are presented in Table 7A, (Appendix 7). The results for milk fat, correction factor and recovery data agree reasonably well with the results obtained by Deeth et al.(1983).

PART II.

MANUFACTURE OF DOMIATI CHEESE USING CONVENTIONAL AND ULTRAFILTRATION METHODS

Introduction

Egyptian white, soft cheese " Domiati" differs greatly from other cheese varieties by having its milk highly salted before renneting (Fahmi and Sharara, 1950).

Manufacture of Domiati cheese from ultrafiltration retentate, using the concept of Maubois, Mocquot, and Vassal (MMV) in 1969, saved several processing steps normally required in conventional methods. The MMV technique required only 10 min for curd formation, reduced the amount of rennet used, eliminated the cutting and drainage process (12-48 hr) and produced cheese higher in whey protein content.

The objective of this phase of the experiment was to compare fresh Domiati cheese made from ultrafiltration retentate with cheese made from whole milk by a conventional method.

Chemical composition

Fresh whole milk was used to manufacture Domiati cheese using the conventional method as outlined by Mahmmoud (1980). A portion of the same milk was ultrafiltrated to produce the liquid pre-cheese (LPC) used to manufacture Domiati cheese by the MMV technique. The composition of whole milk and liquid pre-cheese is presented in Table 17.

Table 17. The composition of whole milk and liquid pre-cheese used for making Domiati cheese by conventional and ultrafiltration methods

Composition	Whole milk	Liquid pre-cheese
	mg/100g*	
	Mean±SD	Mean±SD
Total solids	12.16±0.61	32.6±0.96
Fat	3.41±0.21	15.5±0.64
Ash	0.71±0.06	1.5±0.11
Lactose	4.75±0.28	1.9±0.24
Protein	3.19±0.22	13.7±1.10
Non-casein N	1.24±0.07	3.3±0.43
Non-protein N	0.25±0.03	0.4±0.05
Total albumin N	0.75±0.09	2.2±0.16
PH	6.60±0.16	6.3±0.11

*Values are the means and standard deviations of three replications.
Protein represented by (% N x 6.38).

The composition of fresh Domiati cheese obtained by the conventional and ultrafiltration methods are presented in Table 18. The data reveal only a slight difference in composition between the cheese produced by the two methods. The moisture was higher in ultrafiltrated milk-derived cheese (UF-cheese) but the difference was not significant at $p< 0.05$. Despite the fact that UF-cheese had a lower total solids content (36.9 vs 38.1 %), it contained a higher total protein content which resulted from the incorporation of whey protein (Covacevich and Kosikowski, 1977b). Cheese produced from ultrafiltrated milk had a pH of 6.1 and acidity of 0.09 % which are significantly different than corresponding values of pH 5.8 and 0.3 % acidity respectively, for conventional cheese. These differences could be attributed to the diafiltration step which reduced the salt and lactose content in the liquid pre-cheese concentrate. The net result is an increase in the buffer capacity of the produced cheese (Green et al., 1981). Salt and ash contents of conventional and UF-cheese are not significantly different at $p< 0.05$ (Table 18).

Nitrogen determinations provided information about differences in protein fractions of fresh Domiati cheese made from ultrafiltrated milk and whole milk, see Table 19. Nitrogen values for total protein, non-casein nitrogen, total albumin nitrogen and soluble nitrogen (SN) fractions of ultrafiltrate-derived cheese were significantly different

Table 18. The composition of fresh Domiati cheese made by conventional and ultrafiltrated methods

Composition	UF-cheese	Conventional cheese
	mg/g	
Moisture	63.10[a]	61.90[a]
Fat	15.75[a]	18.00[b]
Ash	4.88[a]	4.56[a]
Protein	14.78[a]	13.07[b]
Salt	2.87[a]	2.80[a]
PH	6.10[a]	5.80[b]
Acidity	0.09[a]	0.30[b]

[a,b]Means within rows for each components with the same superscripts are not significantly different ($p < 0.05$) Protein represented by (%N x 6.38).

at p< 0.05 from the corresponding fractions of conventional cheese. These results reflect the concentration of all the whey protein in the UF-retentate. The non-casein nitrogen (NCN) fraction of ultrafiltrate-cheese (4.09 %) contained 2.04 % of total albumin nitrogen (TAN), whereas the NCN fraction of conventional cheese (1.47 %) contained about 0.05 % of TAN. The data, also indicate that there was not a highly significant difference of NPN between the two cheeses. Koning et al.(1981) stated that the production of SN in UF-cheese is linearly correlated with the amount of rennet used for the manufacture of the cheese.

The ratio of NCN/TP initially presented in the fresh cheese indicated the obvious difference between the UF-cheese and conventional cheese 27.7% and 11.2 %, respectively. The higher NCN/TP ratio for UF-cheese was attributed to the whey protein retained by the ultrafiltration process which increases the proportion of TAN in NCN from 3.4 % in conventional cheese to 49.8 % in ultrafiltrate-derived cheese. These findings are consistent with the work of Koning et al., (1981).

Free fatty acids are one of the major components which contribute to cheese flavor. A typical reference chromatogram of a mixture of fatty acids, representing the fatty acid profile of milk fat, is shown in Figure 7A. (Appendix 7). The means and standard deviations of free fatty acids in fresh UF-cheese and conventional cheese are presented in Table 20.

Table 19. Comparison of protein fractions (%N x 6.38) between fresh Domiati cheese made by conventional and ultrafiltrated methods

Protein fractions	UF-Cheese	Conventional cheese
	g/100g	
Total Protein	14.78[a]	13.07[b]
Casein	10.69[a]	11.59[b]
Non-casein N	4.09[a]	1.47[b]
Non-protein N	1.50[a]	1.04[b]
Total albumin N	2.04[a]	0.05[b]
Soluble N	2.05[a]	1.42[b]

[a,b]Means within rows for each components with the same superscripts are not significantly different ($p < 0.05$)

Table 20. Free fatty acids of fresh ultrafiltrate- and conventional Domiati cheese

Free fatty acid	Ultrafiltration	Conventional
	mg/100g*	
	Mean±SD	Mean±SD
C4:0	2.1±0.29	3.2±0.66
C6:0	2.2±0.36	2.6±0.33
C8:0	2.0±0.21	2.4±0.10
C10:0	3.4±0.85	1.9±0.73
C12:0	5.2±0.55	3.2±0.66
C14:0	12.3±1.1	10.1±1.3
C16:0	31.0±1.56	25.1±1.41
C18:0	15.5±1.33	10.6±1.15
C18:1	11.0±2.1	19.1±1.25
C18:2	4.0±0.8	2.7±1.8
C18:3	2.0±0.94	1.3±0.80
Total F.F.A	90.5	82.2

*Values are the means and standard deviations of three replications.

A pattern of eleven free fatty acids were identified in fresh UF-cheese and conventional cheese. The total concentration of the free fatty acids was 90.5 mg/100g and 82.2 mg/100g for UF-cheese and conventional cheese, respectively. The higher content of free fatty acids in UF-cheese was due to the higher concentration of the free fatty acids in the Liquid pre-cheese as mentioned in part I. The concentration of total volatile fatty acids (C:4 to C:8) in conventional cheese (8.2 mg \100g) was higher than in UF-cheese (6.3 mg/100g). On the other hand, the amount of non-volatile fatty acids (C:10 to C:18) was higher in UF-cheese (84.2 mg/100g) compared to conventional cheese (74 mg\100g). This decrease of volatile fatty acids in UF-cheese may due to the low amount of these fatty acids in the liquid pre-cheese used.

Free amino acids and very small peptides contribute in part to the cheese flavor. The concentration of free amino acids in the UF-cheese and conventional cheese are presented in Table 21. Similar patterns for sixteen amino acids are found in both cheeses. The data indicate that there is a higher concentration of free amino acids in UF- cheese (262 mg/100g) than in conventional cheese (185 mg/100g). Aspartic acid, lysine, glutamic acids, leucine and proline account for 60.65 % of total free amino acids in UF-cheese, whereas leucine, phenylalanine, lysine, aspartic and,

Table 21. Free amino acids and small peptides of fresh Domiati cheese made by Diafiltration and conventional method

Free amino acids	UF-cheese	Conventional cheese
	mg/100g*	
	Means±SD	Means±SD
Histidine	6±0.4	5±0.8
Threonine	9±1.3	8±1.0
Valine	12±0.9	11±1.0
Isoleucine	18±1.8	15±1.3
Leucine	27±1.5	19±1.1
Phenylalanine	21±1.4	18±1.2
Lysine	33±1.9	19±1.2
Cystine	2±0.8	2±1.5
Methionine	2±0.8	2±1.6
Aspartic acid	34±2.9	19±2.1
Glutamic acid	40±2.7	22±1.8
Serine	10±1.1	9±1.1
Glycine	9±1.0	7±0.9
Arginine	10±1.5	8±0.9
Proline	25±2.5	17±1.2
Tyrosine	4±0.8	4±0.7
Total F.AA	262	185

*Values are the means and standard deviation of three replications.

glutamic acids account for 52 % of the total free amino acids in conventional cheese. The higher concentration of certain free amino acids in UF-cheese is related to the higher concentration of free amino acids in the whey used for making the cheese. This difference in the amino acid profile could contribute to flavor characteristics of UF-cheese and conventional cheeses.

Gel electrophoresis of protein

Densitometry was performed on the gels to quantitate the relative changes in nitrogen fractions of conventional and UF-cheeses. The densitometric pattern are presented in Figure 16. Casein and whey proteins standards were run with the cheese samples to allow the identification of the casein components, α_s-casein and β-Casein, and the major whey protein components, β-Lactoglobulin and α-Lactalbumin. These were identified by comparing of relative mobilities and densitogram characteristics.

The electropherograms of conventional and UF-cheese show the presence of whey protein. In UF-cheese, the α-lactalbumin peak appears as a distinct zone ahead of the β-casein zone. The other whey protein zones (β-Lactoglobulin A and B) overlap with the β-casein zone. This electrophoretic characteristic emphasized the results

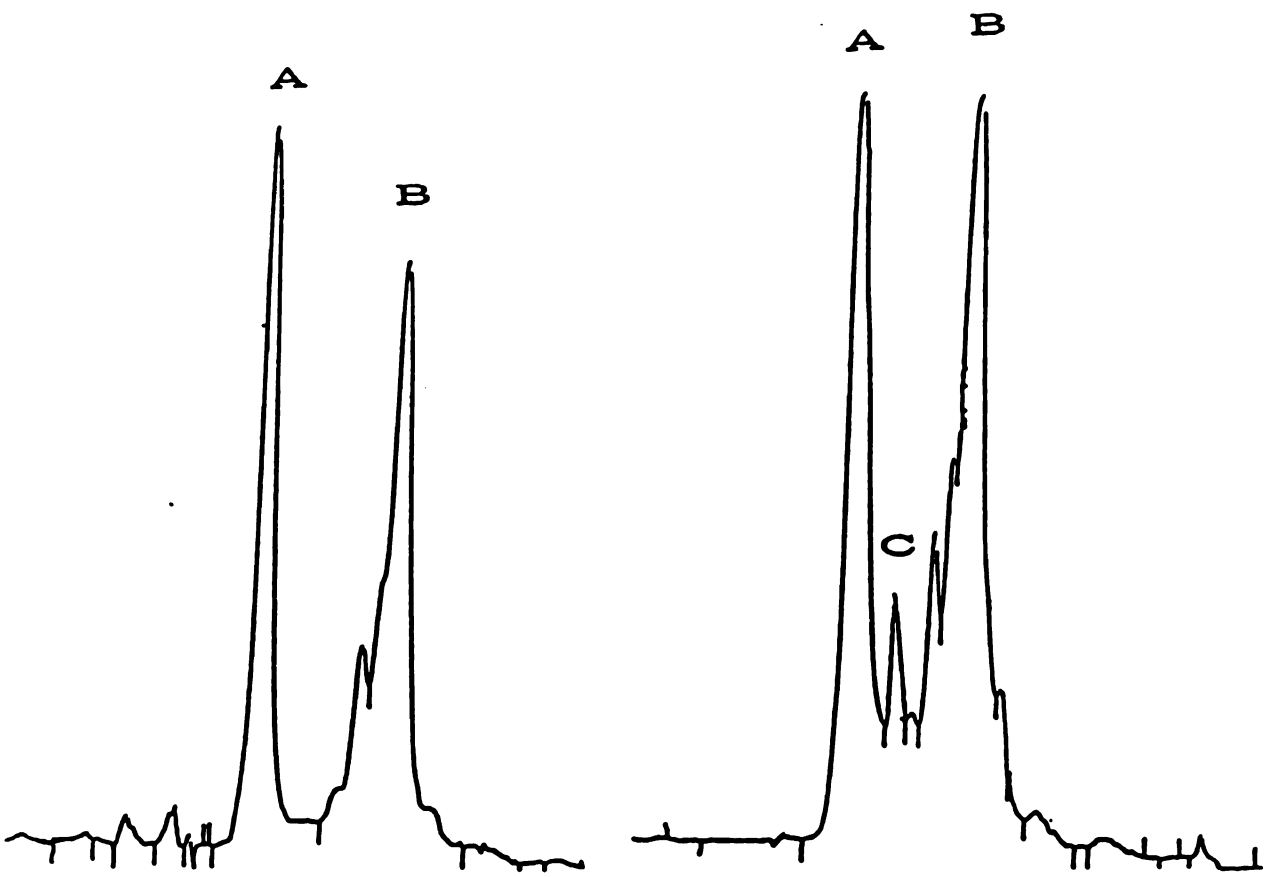

Figure 16. Discontinuous polyacrylamide gel electrophoresis densitograms of conventional and ultrafiltrated fresh Domiati cheese; A, β-casein; B, α_s-casein; C, α-lactalbumin.

obtained by nitrogen fractionation (Table 19) and is in agreement with Koning et al.(1981) and El-Shabrawy (1985).

Organoleptic properties

Sensory evaluation of fresh UF and conventional Domiati cheese were judged for flavor, body, texture, and color by judges familiar with Domiati cheese using cheese score card with maximum scores given for the different attributes of flavor, body/texture, and color. A copy of the score card is provided in Figure 9A (Appendix 9). Scores and comparisons for the conventional and UF-cheese are presented in Table 22. The evaluations indicate that UF-cheese was liked better ($p< 0.05$) when compared to the conventional cheese. Ultrafiltrate-cheese was characterized by a creamy color, a pronounced flavor and a consistently firm texture. The higher concentration of the free fatty acids and free amino acids in ultrafiltrated fresh cheese may explain the higher sensory scores (Tables 20 and 21).

Color

Variables L, a and b were measured for color in ultrafiltrate and conventional cheese and the data are presented in Table 23. Variable (L), whiteness, was 93.3e

Table 22. Organoleptic properties of fresh Domiati cheese using ultrafiltration and conventional methods

Cheeses	Flavor	Body Texture	Color	Total Scores
		Means±SD		
Conventional	25.4±2.9	53.4±1.5	10±0	88.80[a]
Ultrafiltration	27.7±1.4	57.5±1.8	9±0	97.15[b]

[a,b]Total scores that have different superscript differ significantly at $p < 0.05$.
Body and Texture, 60 = Excellent.
Flavor, 30 = Excellent.
Color, 10 = Excellent.

Table 23. Color (L,a,b) of fresh Domiati cheese made by ultrafiltration and conventional methods

Cheeses	L	a	b
Conventional	93.3±0.25	-3.3±0.1	10.0±0.15
Ultrafiltration	83.3±0.2	-3.8±0.2	12.6±0.2

Values are the means and standard deviation of duplicate analyses of three replications.

L= indicates lightness; 100 =perfect white

a= + indicates redness; - indicates greenness; 0 = gray

b= + indicates yellowness; - indicates blue; 0 = gray

for ultrafiltrate cheese compared to 83.3 for conventional cheese. Instrumental value (L) and sensory scoring for color show a high degree of correlation (0.98). Ultrafiltrate cheese possessed a slightly creamer color than the conventional cheese. These results agree with those of Mahmmoud (1980). and El-Gendy et al.(1983).

Texture profile analysis (TPA)

A texture profile, indicating hardness, cohesiveness, adhesiveness, gumminess and elasticity, is offered as a means of helping the food researcher obtain descriptive characteristics of a food (Brandt et al.,1963). Typical force-distance profiles obtained from the conventional and ultrafiltrate fresh cheeses are illustrated in Figure 17. Area and height of the diagram were measured and the data related to mechanical properties are summarized in Table 24. The data reveal that the texture characteristics of ultrafiltrate Domiati cheese are significantly different at $p< 0.05$ from the conventional Domiati cheese. UF-cheese was firmer and more adhesive than conventional cheese. This result was in agreement with Covacevich and Kosikowski (1974) who stated that cream cheese produced by ultrafiltration possessed more hardness, cohesiveness and adhesiveness than conventional cream cheese. This observation may be due to higher retention of calcium and phosphorus associated with the casein micellar complex in

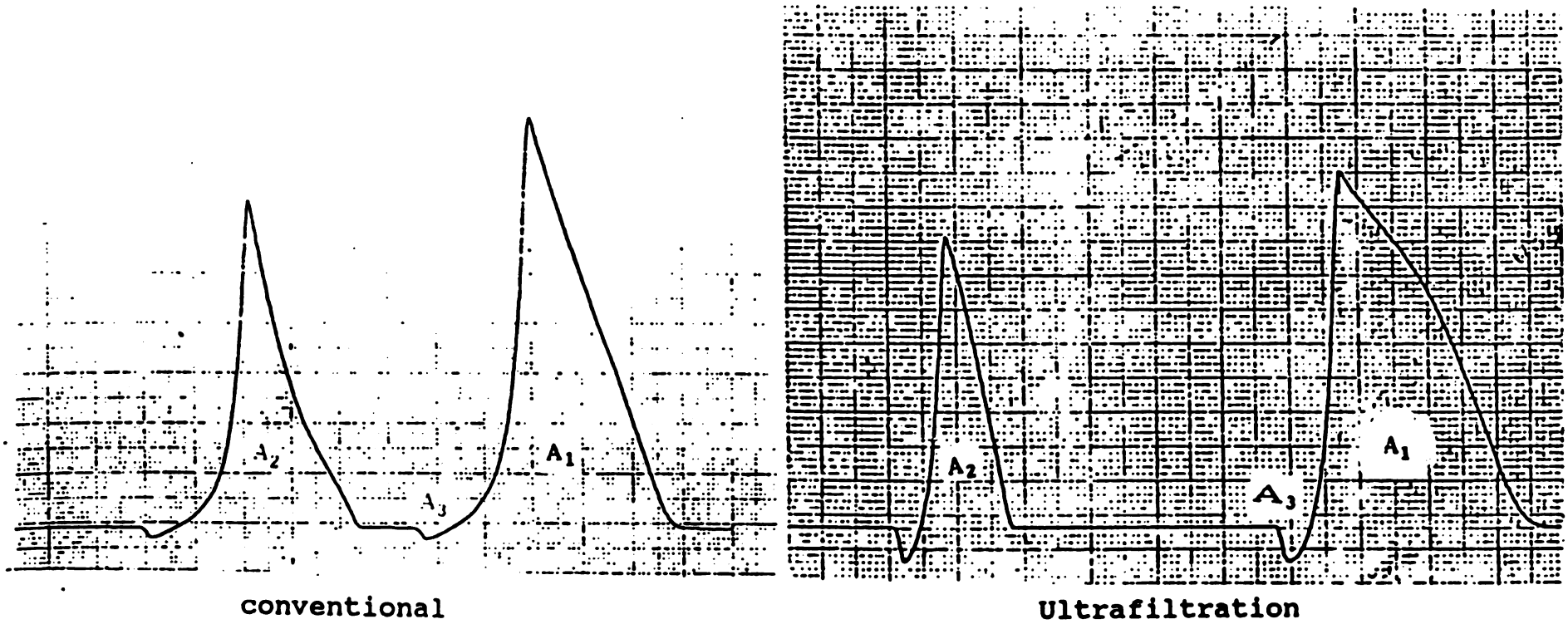

Figure 17. Texture profile curve of conventional and ultrafiltrated fresh Domiati cheese obtained with the Instron Universal Testing Machine. Hardness = height of A_1. Cohesiveness = A_2/A_1. Adhesiveness = A_3.

liquid pre-cheese, which remained in the curd of the UF-cheese, whereas in the conventional method, during coagulation of the casein component, more micellar calcium is solubilized and lost in the whey. Higher levels of calcium affect the hardness of cheese (Lawrence et al., 1983). The rates of firming of curd formed from ultrafiltrated milk increased in proportion to the extent of concentration of the milk used for cheese manufacturing (Reuter et al., 1981 and Green el al., 1981).

The data also, indicate that conventional cheese is characterized by higher chewiness and gumminess than observed in ultrafiltrated cheese. The two cheeses exhibit no difference in texture related to cohesiveness and elasticity. The mechanical measurement of texture was confirmed by the sensory evaluation of the cheeses. And the differences were related to the composition of the cheese as well as the manufacturing process. Green et al. (1981) suggested a possible role of fat in cheese firmness. Reduced fat in the curd would result in a smaller fat-protein interfacial area and an increased separation between fat globules. The capacity of the fat and protein phases of cheese to move in relation to each other would be reduced and would consequently result in a firmer cheese.

Table 24. Texture profile analysis of fresh Domiati cheese made by conventional and ultrafiltration methods

	UF-cheese	Conventional cheese
Hardness	9.00[b]	7.28[a]
Cohesiveness	0.31[a]	0.69[a]
Chewiness	1.36[b]	5.81[a]
Gumminess	1.36[b]	2.91[a]
Elasticity	1.43[a]	2.25[a]
Adhesiveness	0.5	ND

[a,b]Means within rows for each components with the same superscripts are not significantly different ($p < 0.05$)
ND= not detectable.

PART III.

BRINE AND POUCH VACUUM RIPENING OF DOMIATI CHEESE MADE FROM ULTRAFILTRATED MILK

Introduction

Ripening of Domiati cheese is traditionally accomplished by aging or pickling the cheese in salt brine. Weight losses as high as 40 % of fresh curd weight have been reported during pickling of Domiati cheese (El-Shibiny et al., 1973). When Domiati cheese is ripened in brine, the retained whey protein dissolves to a great extent in the brine. Possibly this loss could be avoided by ripening the ultrafiltrate-cheese in vacuum pouch to maintain it's WPC.

The objective of this phase of the experiment was to develop a vacuum packaging method for ripening Domiati cheese and compare it to the conventional method of brine ripening. Fresh Domiati cheese manufactured from ultrafiltered / diafiltered milk by the MMV method, was vacuum packed in polyethylene-lined aluminum pouches and ripened for two months. The experimental cheese was compared to a control cheese packed in brine.

Statistical analysis

To examine the difference between the two methods of ripening and the changes occurring during storage, two analytical designs were followed. First, total solids, fat, protein, ash, lactose, free fatty acids and texture profile

analysis of cheese were analyzed by a one-way analysis of variance. If the F-test proved significant, the Dunnett's procedure was applied to determine the significant difference at $p< 0.05$ and < 0.01 between fresh cheese and 2, 4, 6 and 8 weeks ripened cheese within the same method of ripening. Second, to compare the two methods of ripening at the same time, e.g., 2, 4, 6 and 8 weeks, a split-plot repeated measurement was followed and a T-test was used to differentiate the means at $p< 0.05$ significance (Gill, 1978).

Chemical composition

Whole milk was ultrafiltered / diafiltrated and the final retentate was freeze dried to total solids of 98.99 % and kept in dark brown jars until used for the manufacture of cheese. Retentates were reconstituted in warm water (40^0C) to a composition close to fresh retentates (Table 25). The composition of Domiati cheese made from reconstituted freeze dried retentate (RFDR) is presented in Table 26. These data reveal that fresh cheese made from RFDR exhibited a higher moisture content than fresh cheese produced by conventional methods as cited previously in Part II in this thesis.

The nutritional value of fresh Domiati cheese could be assessed from the amino acid profile of cheese, presented in Table 27. The data reveal that the total essential and

Table 25. The composition of ultrafiltrated freeze-dried whole milk

Moisture %	Fat %	Protein %	Ash %	Lactose %
		Means±SD		
70.2±0.9	14.0±0.61	12.05±0.8	2.18±0.21	1.95±0.35

Values are duplicate analyses of three replications.

Table 26. The composition of fresh Domiati cheese made from Ultrafiltrated whole milk

Moisture %	Fat %	Protein %	Ash %	Lactose %
		Means±SD		
65.07±0.7	14.5±0.6	12.55±0.45	6.01±0.45	1.54±0.16

Values are duplicate analyses of three replications.

Table 27. Total and free amino acid content of fresh Domiati cheese made from ultrafiltrated whole milk

Amino Acids	Total Amino Acids Means±SD	Free Amino Acids Means±SD
	mg/100g*	
Essential A.A		
Histidine	330±19	5±1.00
Threonine	510±21	8±1.15
Valine	830±17	10±1.58
Isoleucine	1080±36	11±1.20
Leucine	710±26	14±1.95
Phenylalanine	590±18	5±0.95
Lysine	1020±35	24±2.33
Cystine	30±8	ND
Methionine	170±10	ND
Nonessential A.A		
Aspartic acid	980±15	25±1.73
Glutamic acid	2490±56	35±2.65
Serine	610±18	9±1.39
Glycine	240±28	7±1.00
Arginine	510±19	8±1.00
Alanine	410±21	8±1.20
Proline	991±65	17±1.22
Tyrosine	640±18	1±0.40
Total A.A	12,141	187

*Values represent the means and standard deviation of three determination.
ND= not detectable.

nonessential amino acids accounts for 5.27 and 6.87 g/100g cheese, respectively. Essential amino acids account for 43 % of the total amino acids in fresh cheese with higher values of isoleucine, lysine followed by valine, phenylalanine and thyronine. The low values for sulfur amino acids, cysteine and methionine, may due to destruction during the analysis as mentioned before.

Domiati cheese can be consumed fresh as well as a ripened cheese, so the characteristics of fresh cheese with respect to free amino acids, free fatty acids, protein fraction, color and texture will be monitored through out the ripening study.

The effect of the ripening period on cheese composition and characteristics was studied. Compositional changes after 2, 4, 6 and 8 weeks of ripening in vacuum pouches are recorded in Table 28. These data demonstrated a statistical difference (at $p< 0.05$ and 0.01) between fresh cheese and the cheese ripened for 2, 4, 6 and 8 weeks. The means of total solids, fat, protein and ash increased from 34.93, 14.5, 12.55, and 6.01 % to 39, 17.23, 13.5 and 6.66 after 1 month and to 42.15, 18.3, 15.3 and 6.96 % after 2 months, respectively. The chemical composition of brine-ripened cheese after 8 weeks are presented in Table 29. Increases in total solids, fat, ash and protein show significant difference (at $p< 0.01$) after 4 and 8 weeks compared to fresh cheese. The increase in protein content was not significant (at $p< 0.05$) prior to the sixth week,

Table 28. Chemical changes of Domiati cheese made from reconstituted, diafiltrated whole milk and ripened in pouches for 8 weeks

Ripening time (Weeks)	Composition (%)			
	Total solids	Fat	Protein	Ash
0	34.93	14.50	12.55	6.01
2	37.64**	16.63*	13.38	6.20
4	39.00**	17.23**	13.50	6.55*
6	40.07**	17.55**	14.60**	6.77*
8	42.15**	18.30**	15.30**	6.96*

* $p < 0.05$ and ** $p < 0.01$) for differences among values of each column compare to zero time.

Standard errors for TS, Fat, protein and Ash are 0.3, 0.28, 0.40, 0.17, respectively.

Table 29. Chemical changes of Domiati cheese made from reconstituted, diafiltrated whole milk and ripened in 8% brine solution for 8 weeks

Ripening time (weeks)	Composition (%)			
	Total solids	Fat	Protein	Ash
0	34.93	14.50	12.55	6.01
2	35.11	15.03**	12.30	6.61**
4	36.00**	15.40**	12.50	7.17**
6	36.95**	15.87**	12.79*	7.56**
8	37.90**	16.20**	13.00*	7.90**

* $p < 0.05$ and ** $p < 0.01$) for differences among values of each column compare to zero time.

Standard errors for Ts, Fat, Protein and Ash are 0.3, 0.27, 0.29, 0.19, respectively.

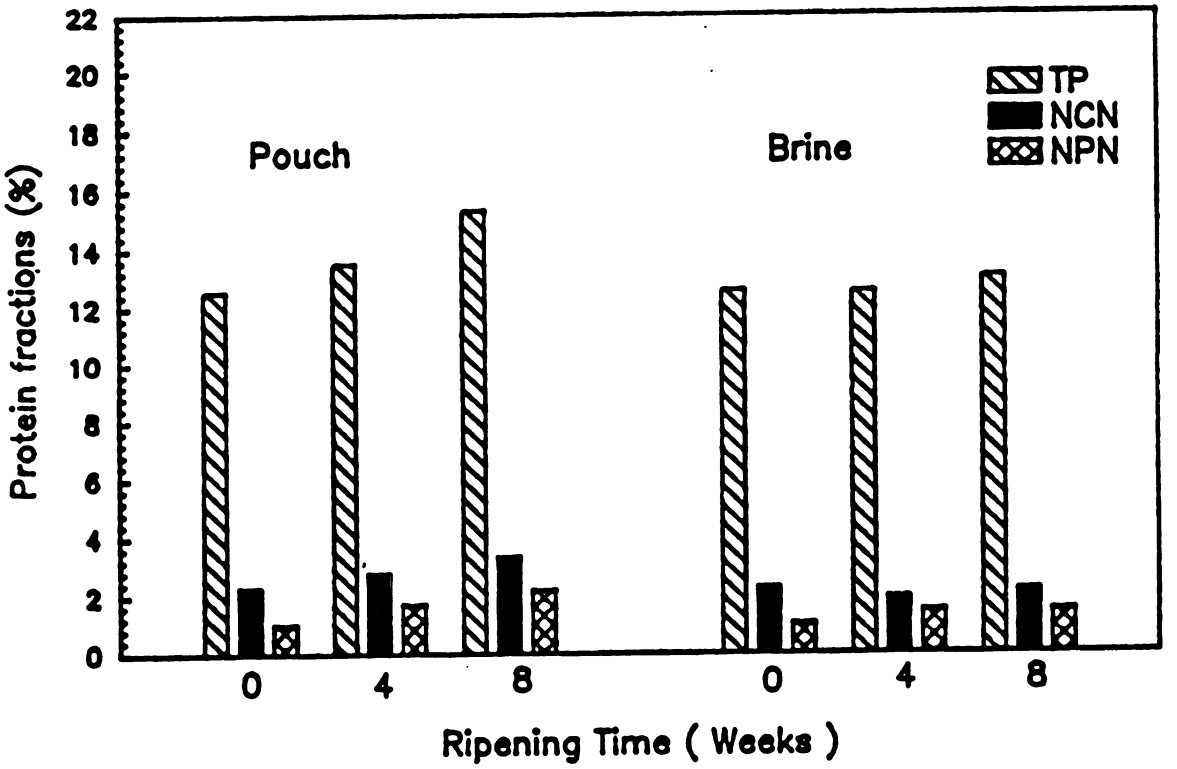

Figure 18. Composition of protein fractions of Domiati cheese made from UF whole milk and ripened in pouches or in 8% brine. TP= total protein, NCN= non-casein nitrogen, NPN= non-protein nitrogen.

while increased significantly after 6 and 8 weeks. Statistical comparisons of cheese ripened in pouches and in brine after 2, 4, 6 and 8 weeks are presented in Table 10A, Appendix 10. The result indicated that cheese ripened in pouches had higher total solids, fat and protein at any time observed than cheeses ripened in brine solution. The observed increases in values reached maximum after 2 months of ripening. These results are in agreement with those obtained by Mahmmoud and Kosikowski (1979) and El-Gendy et al. (1982).

Cheese ripening is a dynamic process during which the proteins undergo considerable decomposition. Schormuller (1968) stated that a complex mixture of decomposition products including proteose, peptones, peptides, amino acids, amines and ammonia is formed during cheese ripening. And the changes in cheese proteins were dependent on the variety and stage of maturity of the cheese. In this study the protein fractions were studied for the two methods of ripening and the mean values plotted in Figure 18. Changes in the protein fractions of pouched cheese were more pronounced than brine-treated cheese at any time of ripening. Total protein (TP), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were 12.55%, 2.31% and 1.02 %, respectively, for fresh and increased significantly ($p< 0.01$) to 15.30%, 3.37% and 2.2%, respectively, for pouched-ripened cheese and to 13%, 2.22% and 1.48%, respectively, for brine ripened cheese (Table 10 B, Appendix 10). The

Table 30. Ripening index* of Domiati cheese ripened in pouches and in 8% brine solution for 8 weeks

Ripening (weeks)	Pouch		8% brine	
	Ripening index			
	NCN/TN	NPN/TN	NCN/TN	NPN/TN
0	18.40	8.12	18.40	8.12
2	19.86	11.33	15.40	9.42
4	20.74	12.74	15.60	10.14
6	21.18	13.01	16.42	10.95
8	22.02	14.37	17.07	11.45

Values represent the means of three replications.
* Ripening indices are: Non casein nitrogen/Total nitrogen and Non protein nitrogen/Total nitrogen.

ratio of NCN/TN has been used to monitor the aging of cheese (Noomen, 1977; El-Salam et.al., 1981; and El-Shabrawy, 1985). The nitrogen-distribution data revealed, as shown in Table 30, that the NCN/TP ratio increased to 22.02 after 2 months in pouches compared to 17.07 for brine-ripened cheese. This difference indicates a higher proteolytic activity in the pouch. The NPN/TP ratio was 8.12 for fresh cheese and increased to 12.74 and 14.37 for pouch comparing to 10.14 and 11.45 for brine after 1 and 2 months, respectively. The ripening index was lower in brine cheese because of increased exudation of cheese serum carrying more soluble N from the cheese (Abd El-Salam and El Shibiny, 1982). Salt content, or salt-in-moisture (SM) of the cheese, could influence hydrolysis of protein during ripening (Lawrence and Gilles 1982; Thomas and Pearce 1981).

Fatty acids

The pattern of the free fatty acid composition in fresh Domiati cheese and the changes occurring during the two methods of ripening are presented in Tables 31 and 32. Eleven free fatty acids were identified in fresh cheeses, totaling to 74.9 mg/100g. The liberation of free fatty acids in cheese ripened in a pouch (Table 31) showed highly significant increases in total FFA with values of 130.4, 173.6 and 226.7 mg/100g after 2, 4 and 8 weeks, respectively. This increase of the FFA due to some lipolysis occurs during the ripening. Cheese ripened in

Table 31. Quantitative changes of free fatty acids in Domiati cheese made from ultra/diafiltrated whole milk ripened in pouches for 8 weeks

Free fatty acids	Ripening Time (Weeks)				
	0	2	4	8	Standard errors
	mg/100g[a]				
C4:0	1.47	2.07	2.99**	4.85**	0.11
C6:0	2.87	3.22	10.12**	12.65**	0.16
C8:0	1.75	4.92**	13.34**	18.40**	0.10
C10:0	2.76	3.36	3.47	7.77**	0.11
C12:0	1.65	1.80	1.92	2.96**	0.07
C14:0	11.50	17.36**	17.87**	20.70**	0.12
C16:0	29.90	35.65**	38.87**	47.60**	0.08
C18:0	11.73	12.42	21.62**	27.69**	0.12
C18:1	5.24	32.15**	41.86**	74.75**	0.17
C18:2	2.64	11.27**	12.24**	17.96**	0.14
C18:3	0.62	3.79**	7.15**	7.36**	0.14
Total	74.3	130.36	173.6	226.68	

Means within a row are significantly different at *, $p < 0.05$; **, 0.01 from fresh (0 time).

Values are the means and standard errors of three replications.

Table 32. Quantitative changes of free fatty acids in Domiati cheese made from ultra/diafiltrated whole milk ripened in 8% brine for 8 weeks

Free fatty acids	Ripening time (Weeks)				
	0	2	4	8	Standard errors
	mg/100g				
C4:0	1.47	1.72	2.28	2.76*	0.14
C6:0	2.87	2.99	8.74**	10.05**	0.13
C8:0	1.75	1.89	3.15*	5.29**	0.11
C10:0	2.76	2.99	3.11	4.14*	0.11
C12:0	3.79	3.59	5.66**	6.83**	0.12
C14:0	11.50	11.96	19.44**	24.61**	0.16
C16:0	29.90	34.66**	40.62**	43.95**	0.19
C18:0	11.73	14.26**	18.17**	20.15**	0.11
C18:1	5.24	30.13**	38.18**	40.94**	0.14
C18:2	2.62	7.15**	7.89**	23.76**	0.16
C18:3	0.62	2.30**	2.99**	5.64**	0.10
Total	74.3	122.8	150.21	188.1	

Sample with the same raw are significantly differ from fresh at * $p < 0.05$; ** 0.01 .

Value are the means and standard errors of three replications.

brine showed increases in free fatty acids to 122.8, 150.2 and 188.1 mg/100g, respectively, for similar times of ripening (Table 31). The percent of volatile fatty acids (C4-C10), which contribute to cheese flavor, was higher (20 % of total) in pouch ripened cheese than in brine-ripened cheese (11.8% of total) after 2 months of ripening. This result is in agreement with the data obtained by Buruiana and El-Senaity (1986) in their study on white soft cheese. Rabie et al.(1984) stated that ripening of Domiati cheese made from dried milk took place at a relatively slow rate compared with cheese made from fresh milk. Generally, long chain free fatty acids were more prevalent than the short chain species in UF-cheese, probably as a result of ultrafiltration process. Analysis of variance for the two methods of ripening showed that pouch-ripening favored the formation of total free fatty acids, especially after two months. This result was confirmed by the flavor evaluation score (Table 34) and is in agreement with Morris (1970), who found that good flavor, color and uniform composition can be obtained in cheese made from pasteurized cow milk and ripened in pouches.

Amino acids

The quantity of free amino acids accumulated during the ripening period was the direct result of liberation of amino acids from casein and a transformation of amino acids already liberated into further decomposition products.

The changes in amino acid composition during ripening of Domiati cheese in pouches and brine are presented in Table 33. The concentration of free amino acids increased from 187 mg/100g in fresh cheese to 397 and 791 mg/100g in brine-ripened and pouch-ripened cheese respectively, after 2 months. These results illustrate the greater breakdown of proteins in pouch-ripened cheese. The low-concentration of free amino acid in brine cheese may be attributed in part to the loss of free amino acids in the brine. This result agrees with El-Sadek and Abd El-Motteleb (1958) who ascribed the lower concentration of free amino acids in more ripened Domiati cheese to the brine solution. Also, free glutamic acids, proline, lysine and leucine, which are mainly responsible for the formation of cheese flavor, were found in greater concentration in pouched cheese compared to brine-ripened cheese. The concentration of glutamic acid, leucine, aspartic acid and lysine represent about half of the total free amino acids present in Domiati cheese (Table 33). This observation is in agreement with that reported by Buruiana and Frag (1983) and Omer et al. (1987) in their study on white soft cheese.

These results indicate that as total free amino acids increase, the flavor of the cheese increase and that a sharp increase in amino acid concentration coincide with optimum flavor and body. The variation in the rate of production of certain amino acids after ripening reaches a certain

Table 33. Free amino acid contents of ripened Domiati cheese made from ultra/diafiltrated whole milk and ripened in pouches and brine solution after 8 weeks

Free Amino Acids	Fresh	Pouch	Brine
		mg/100g	
	Means±SD	Means±SD	Means±SD
Histidine	5±1.00	21±1.9	11±2.0
Threonine	8±1.15	32±2.7	18±2.2
Valine	10±1.58	59±2.6	24±1.9
Isoleucine	11±1.20	41±3.6	22±1.9
Leucine	14±1.95	75±2.6	31±2.3
Phenylalanine	5±0.95	33±1.8	19±1.2
Lysine	24±2.33	99±3.5	44±2.3
Aspartic acid	25±1.73	45±2.2	36±2.1
Glutamic acid	35±2.65	175±4.1	86±3.2
Serine	9±1.39	48±1.3	23±2.1
Glycine	7±1.00	15±1.3	90±0.6
Arginine	8±1.00	24±1.9	15±1.1
Alanine	8±1.20	26±2.1	13±1.2
Proline	17±1.22	89±3.5	42±3.3
Tyrosine	1±0.40	1±0.2	2±0.2
Cystine	ND	3±0.5	1±.05
Methionine	ND	2±0.3	ND
Ammonia	ND	5±0.8	11±1.9
Total F.A.A	187	793	397

Values are the results of three determinations.
ND = not detectable.

point makes it difficult to evaluate the role of free amino acids in the development of typical Domiati cheese flavor.

Organoleptic properties

Using the cheese Score card as a guide (Figure 9A, Appendix 9), fresh and ripened cheese were evaluated for flavor, body /texture and color by experienced judges who were familiar with Domiati cheese; the scores are presented in Table 34. Fresh cheese was scored 80.5. Pouch-ripened cheese was scored 88 and 94 after 4 and 8 weeks, respectively. Cheese ripened in brine for similar periods scored 83.5 and 85.0, respectively.

Vacuum treatment compacts the cheese, produce a close texture and smooth surface, which also aids in preventing mold growth and spoilage (Morr and Richter, 1988).

Color

Color evaluation for fresh and ripened cheeses are presented in Table 35. The changes in color, variable L, a and b, reflected differences in the method of ripening. Variable L (whiteness) was 82.6 in fresh cheese and changed to 79 for pouch compared to 89.3 for brine-ripened cheese after 8 weeks of ripening. Variable b (yellowness) was 11.2 for fresh and changed to 18.74 for pouch compared to 9.5 for brine cheese after 8 weeks of ripening. This result indicates less whiteness and more yellowness in pouch-ripened cheese than brine-ripened cheese. The sensory

Table 34. Organoleptic properties of fresh Domiati cheese made from ultra/diafiltrated whole milk and ripened in pouches and 8% brine solution for 8 weeks

Ripening (Time)	Flavor	Body/Texture	Color	Total
Fresh	24±1.5	47±2	9.5±0.3	80.5
		Pouch		
4 Weeks	29±1	50±1.5	9.0±0.7	88.0
8 Weeks	30±0	56±2.2	8.0±1.0	94.0
		8% Brine		
4 Weeks	25±1.3	49±1.9	9.5±0.5	83.5
8 Weeks	26±2.0	49±2.5	10.0±0.0	85.0

Values are the mean and standard deviation of duplicate analyses of three replications.
Flavor 30 = Excellent.
Color 10 = Excellent.
Body and Texture 60 = Excellent.

Table 35. Color profile of Domiati cheese made from reconstituted freeze dried ultrafiltrated whole milk and ripened in pouches and 8% brine solution for 8 weeks

Ripening time	L	a	b
Fresh	82.65±0.35	-5.65±0.15	11.20±0.21
		Pouch	
4 Weeks	80.33±0.33	-3.90±0.10	14.60±0.08
8 Weeks	79.00±0.30	-2.33±0.14	18.74±0.30
		8% Brine	
4 Weeks	83.50±0.50	-4.90±0.10	10.20±0.45
8 Weeks	89.30±0.40	-1.44±0.04	9.50±0.27

Values are the means and standard deviations of duplicate analyses of three replications.

L = indicates lightness(100 =perfect white)
a = + indicate redness; - indicate greenness; 0 = gray
b = + indicate yellowness; - indicate blue; 0 = gray

evaluation confirmed the result which represented brine-ripening cheese as being whiter than pouch-ripened cheese. Pouched cheese ripened in the absence of salted whey was attractive with a uniform, creamy color. Similar observation were made by Morris (1970), El-Gindy et al.(1983) and Mahmmoud (1980).

Texture profile analysis

The structure and arrangement of the protein (mainly casein) molecules are largely responsible for the textural characteristic of cheese. Also, fat and moisture affect the rheological quality and acceptability of the finished cheese (Fukushima et al., 1965). The mechanical measurements of hardness, cohesiveness, chewiness, gumminess and elasticity for fresh Domiati cheese and cheeses ripened in pouches or in brine are presented in Figures 19 to 23.

Values for hardness, chewiness and gumminess increased significantly (at $p< 0.05$ and $p< 0.01$) from 3.85, 5.09 and 2.21 for fresh cheese, respectively, to 9.85, 12.39 and 6.21 for pouch cheese ripened for 2 months (Table 10C, Appendix 10). Compared to brine ripened cheese, the mean values of hardness and chewiness increased significantly from 3.85 and 5.09, respectively, to 5.63 and 6.04 after 2 months (Table 10D, Appendix 10). For both ripening methods, cohesiveness and elasticity did not show any significant difference ($P< 0.05, 0.01$) at any time of ripening.

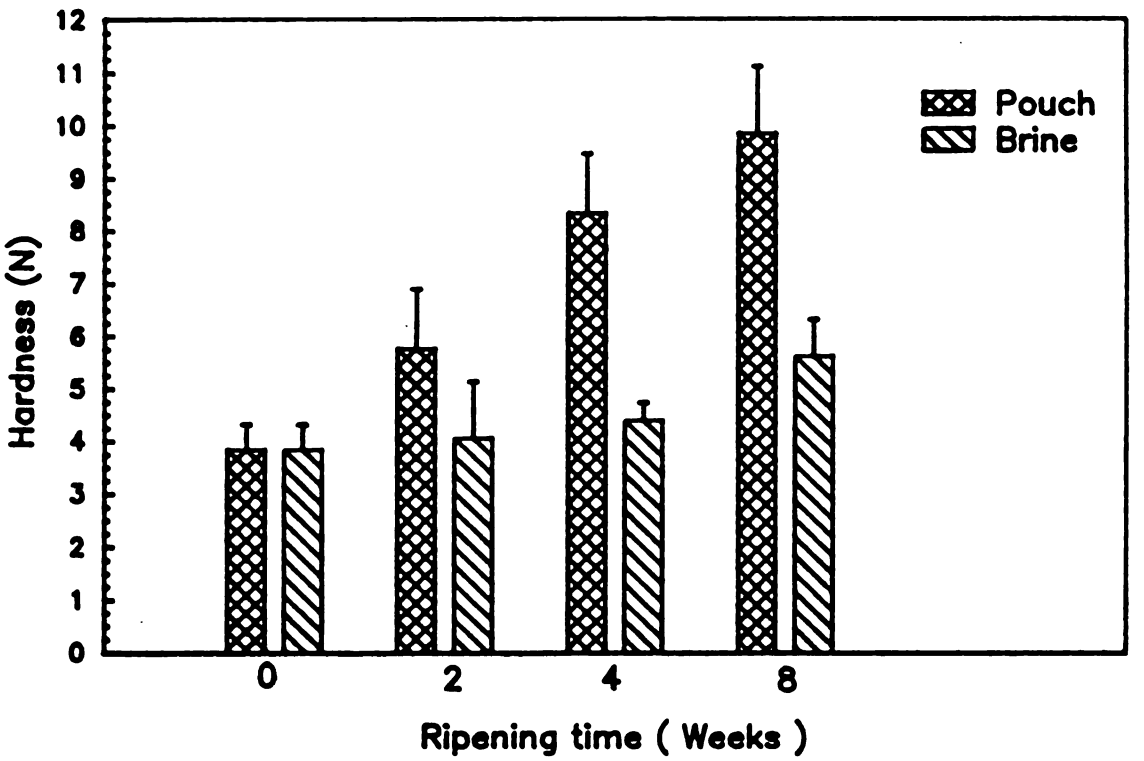

Figure 19. Hardness changes on Domiati cheese made from UF whole milk and ripened in pouches and in 8% brine. N=Newtons.

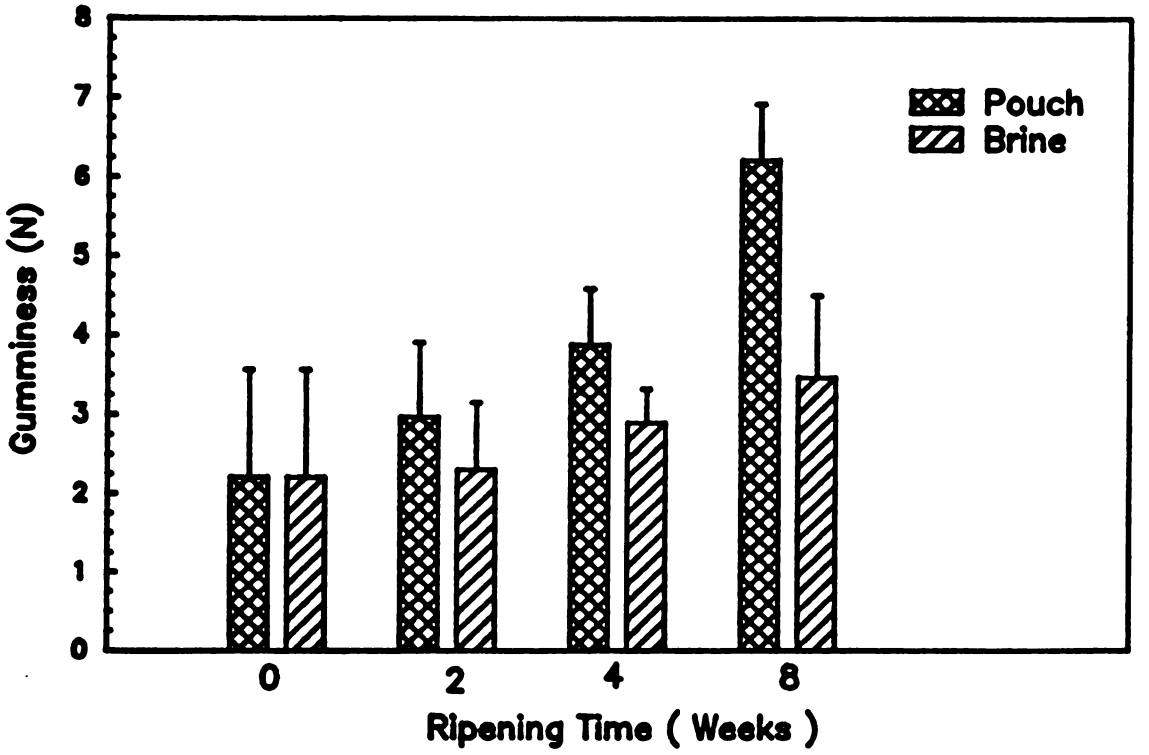

Figure 20. Gumminess changes on Domiati cheese made from UF whole milk and ripened in pouches and in 8% brine. N=Newtons.

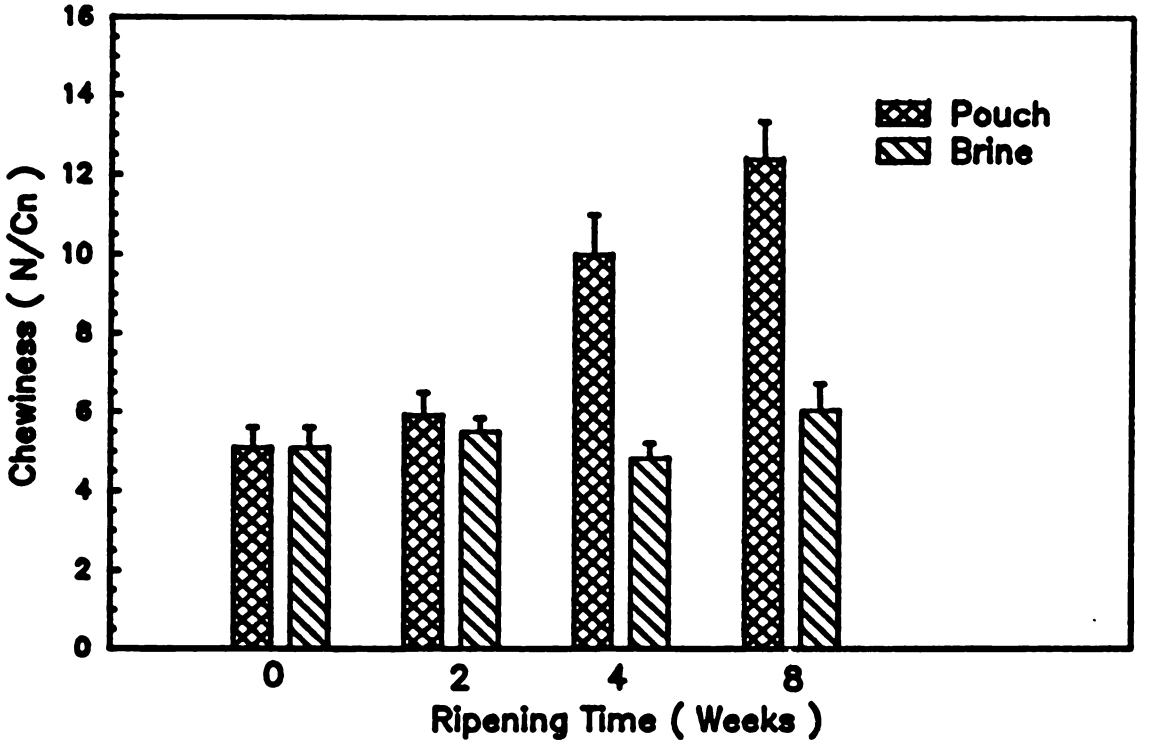

Figure 21. Chewiness changes on Domiati cheese made from UF whole milk and ripened in pouch and in 8% brine. N=Newtons.

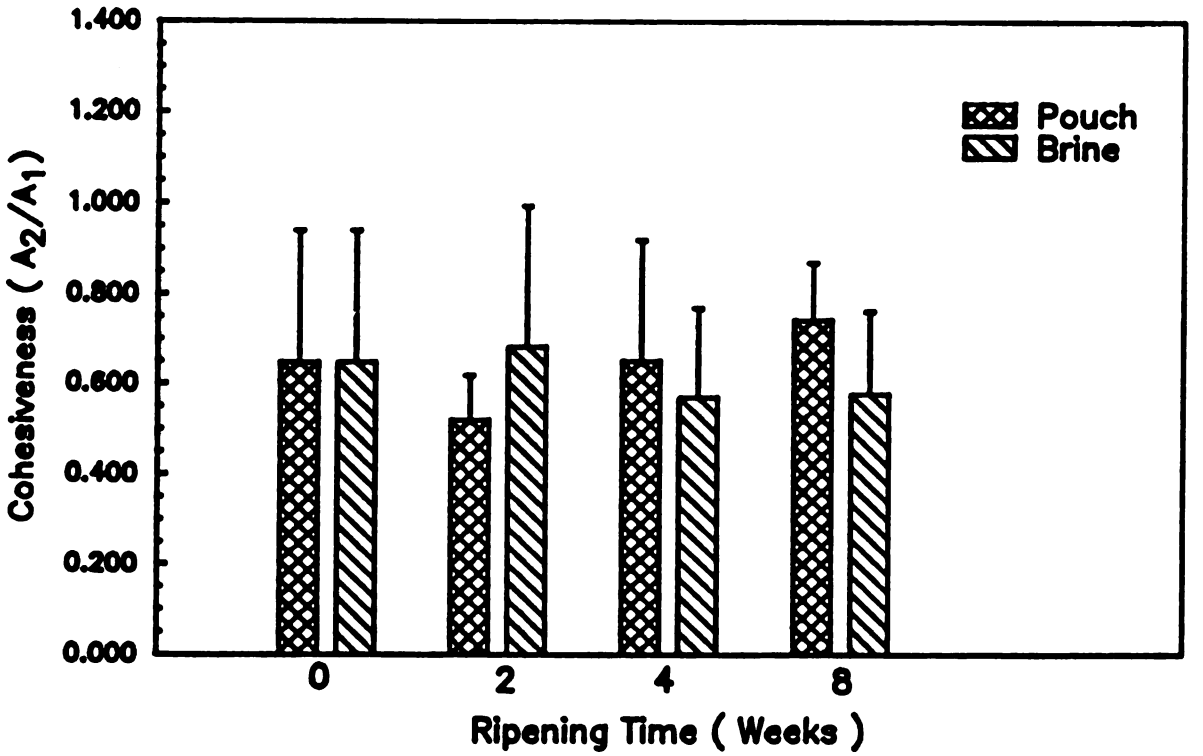

Figure 22. Cohesiveness changes on Domiati cheese made from UF whole milk and ripened in pouches and in 8% brine.

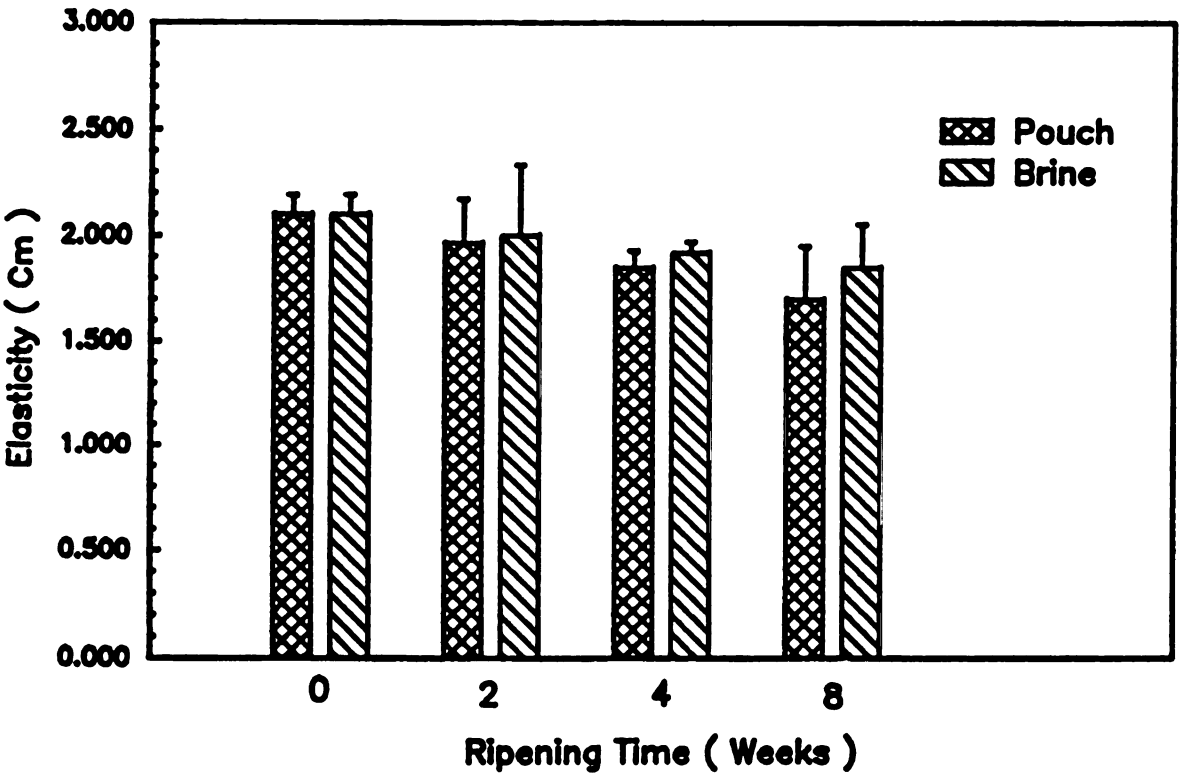

Figure 23. Elasticity changes on Domiati cheese made from UF whole milk and ripened in pouches and in 8% brine.

Chemical and physical changes in cheese during ripening cause the body of freshly made cheese to lose its curdy texture and become soft. This phenomenon is related principally to the breakdown of protein to smaller peptides and the gradual accumulation of amino acids (Ernstrom and Wong, 1974). Lawrence et al.(1983) stated that moisture, pH and casein proteolysis could affect the texture of cheese. To compare the effect of the two methods of ripening on the rheological properties on Domiati cheese, one-way analysis was used and the data are presented in Table 36. At any time of ripening, hardness is significantly higher for pouch-ripened cheese, indicating more firmness. Chewiness and gumminess showed a significant difference after 1 and 2 months with higher values for pouch-ripened cheese. Elasticity and cohesiveness were not significantly different at any stage of the ripening periods.

Whey contents

During the ripening process, the serum exuded from pouch-packed cheese and into the brine solution of brine-ripened cheese were analyzed at 2, 4, 6 and 8 weeks. Fat and protein values of pouch-serum and the brine solution are presented in Table 37. The amount of fat and protein released from pouches cheese were 0.25 % and 3.70 %, respectively, after 2 weeks and increased to 0.39 and 4.19 %, respectively, after 8 weeks. On the other hand, the

Table 36. Texture profile changes during the ripening of Domiati chees in pouches and 8% brine solution for 8 weeks

Ripening time (Weeks)	Hardness		Cohesiveness		Chewiness		Gumminess		Elasticit	
	Pouch	Brine	Pouch	Brine	Pouch	Brine	Pouch	Brine	Pouch	Brin
2	5.77[a]	4.07[b]	0.52[a]	0.68[a]	6.02[a]	5.20[a]	2.91[a]	2.30[a]	2.07[a]	1.97[a]
4	8.33[a]	4.40[b]	0.65[a]	0.57[a]	8.73[a]	5.87[b]	3.88[a]	2.90[b]	2.00[a]	2.12[a]
8	9.85[a]	5.62[b]	0.74[a]	0.57[a]	12.89[a]	7.86[b]	6.21[a]	3.47[b]	2.00[a]	2.21[a]

[a,b]Means within rows for each components with the same superscripts are not significantly different ($p < 0.05$) at ripening time.

amount of fat and protein in the brine solution was 0.13 and 0.52 %, respectively, after 2 weeks and increased to 0.23 and 1.16 %, respectively, after 8 weeks. The observed increase in fat and protein in the serum and brine did not change significantly during ripening (Table 10E) Appendix 10. Comparison of the above components in the serum and brine obtained from both cheeses during ripening show that there was a significant difference at $p < 0.05$ at any time of ripening (Table 37). Because the amount of exuded serum from pouch cheese was very small compared to the volume of the brine solution, the actual loss of the fat and protein in the whey of both cheeses was calculated in relation to the volume produced throughout the ripening process. The data in Table 10F, Appendix 10, indicate that the loss of components was higher in the brine solution than in the pouch-serum. These results are in agreement with those stated by Mahmmoud and Kosikowski (1980).

Domiati cheese produced from ultrafiltrated whole milk and ripened in a pouch-pack represents a suitable means of packaging eight ounce (225 gm) portions immediately following manufacture. This allows the presentation of Domiati cheese in a convenient, self-service pack and retailed in a form which overcomes the disadvantage of bulk-dispensing in brine and maintains the cheese " in the natural state " until the package is opened for consumption. Good flavor, texture and color and uniform composition can be attained with ripening the cheese in a pouch.

Table 37. Fat and protein content of serum and brine obtained from Domiati cheese made from ultrafiltrated whole milk ripened in pouches and 8 % brine solution for 8 weeks

Ripening time (week)	Fat (%)		Protein (%)	
	Pouch	Brine	Pouch	Brine
2	0.25[a]	0.13[a]	3.70[a]	0.52[b]
4	0.36[a]	0.15[b]	3.99[a]	0.79[b]
6	0.33[a]	0.18[b]	4.00[a]	0.88[b]
8	0.39[a]	0.23[b]	4.19[a]	1.16[b]

[a,b]Means within rows for each components with the same superscripts are not significantly different ($p < 0.05$) at the same ripening time.

PART IV

COMPARISON BETWEEN CONVENTIONAL AND ULTRAFILTRATED DOMIATI CHEESE DURING RIPENING IN POUCHES

Introduction

Egyptian white, Domiati cheese is marketed mostly as pickled, ripened cheese. The objective of this experiment was to determine the feasibility of using a new ripening method (vacuum pouch) and its effect on the quality of ultrafiltrate-derived and conventional cheese made from concentrated liquid pre-cheese and whole milk, respectively.

Chemical composition

Domiati cheese was manufactured by two methods: namely, by the MMV method from liquid pre-cheese, and by the conventional methods. After ripening for three months at 7-10°C under vacuum in polyethylene-lined aluminum pouches, compositional changes were studied. These data are presented in Figure 24 and 25 and demonstrate that both conventional and ultrafiltrate-derived cheese (UF-cheese) follow similar changes in composition during the ripening process. Total solids, protein and fat of ultrafiltrate-derived cheese significantly increased from 36.9 %, 14.78 % and 15.75 %, respectively, to 38.9 %, 15.46 % and 18.0 % after 1 month and 42.4 %, 17.68 % and 18.69 % after 3 months, respectively (Figure 24). Conventional cheese increased from 38.1, 13.07 and 18.0 % for total solids, protein and fat, respectively, for fresh cheese to 39.1,

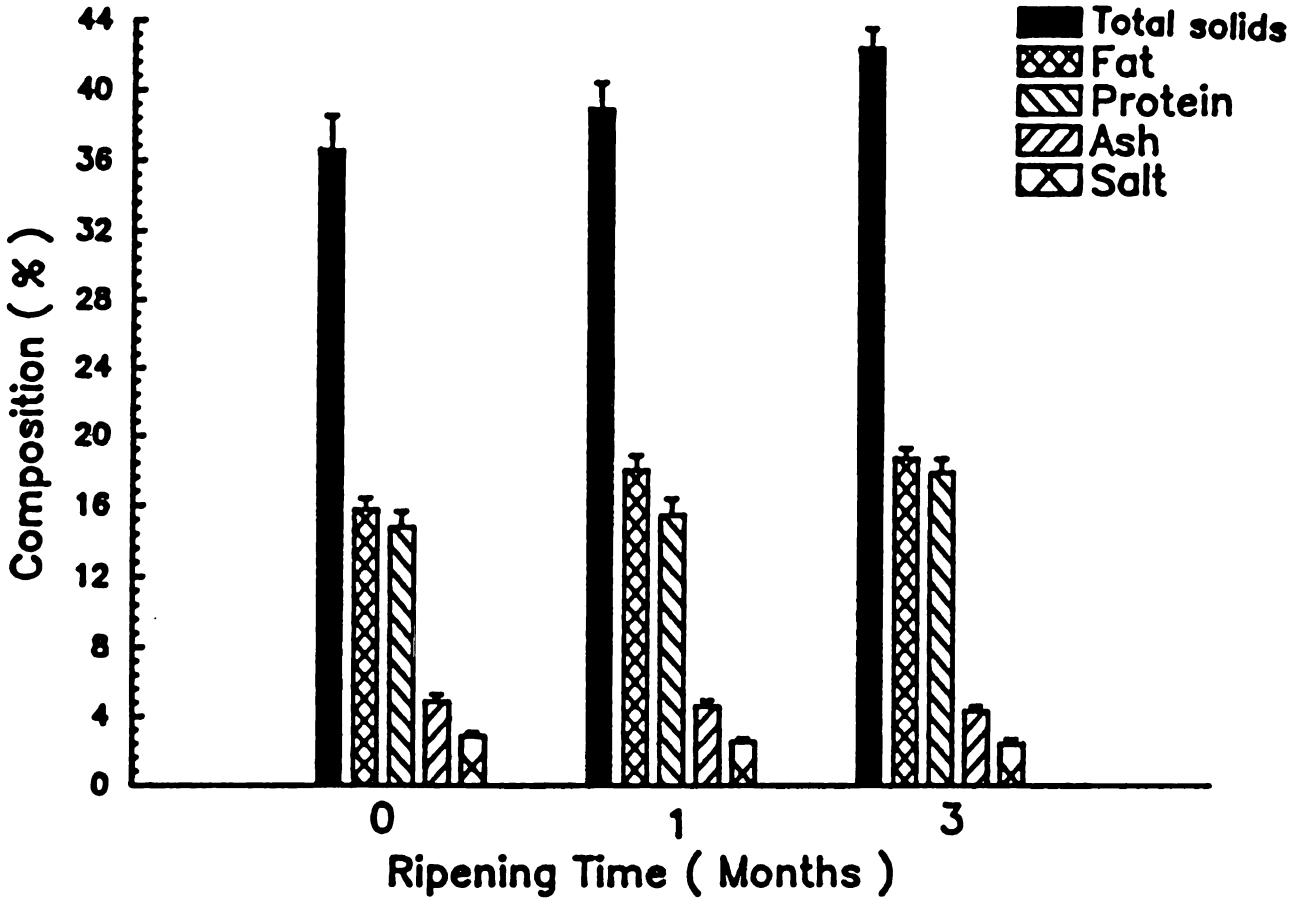

Figure 24. Composition changes of Domiati cheese made by the ultrafiltration method and ripened for three months in pouches.

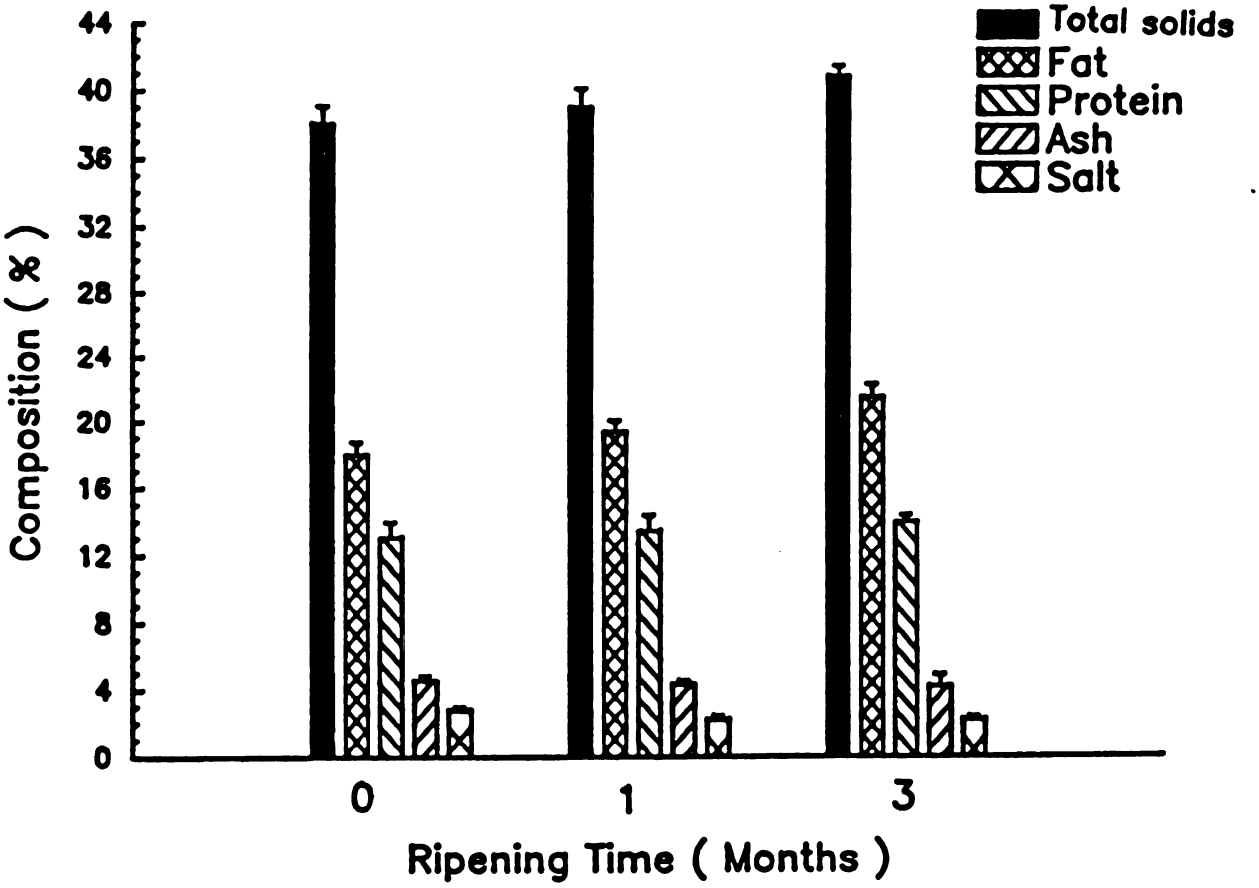

Figure 25. Composition changes of Domiati cheese made by the conventional method and ripened for three months in pouches.

13.45 % and 19.4 % after one month ripening and to 40.93 %, 14.36 and 21.5 % after 3 months ripening, Figure 25. Compositional changes between the conventional and UF-cheese after one and three months of ripening in vacuum packs are presented in Table 38. The data reveal that, after one month of ripening ,there was no significant difference in total solids between conventional and ultrafiltrate-derived cheese ($p < 0.01$), whereas there were significant differences for protein, fat, ash and salt, with higher values for the UF-cheese. After three months of ripening, UF-cheese was significantly higher in protein and fat than conventional cheese. No difference was noted in total solids, ash and salt between the ultrafiltrate and conventional pouch-ripened cheese. During a similar ripening period, salt and ash decreased significantly in both cheeses. However, the decrease in salt concentration was not significant in UF-cheese compared to conventional cheese.

The effect of ripening time on conventional and UF-cheese was calculated, indicating that the percentage increases in total solids, protein and fat in 3 months old UF-cheese were 14.9, 20.8 and 18.7 %, respectively, compared to the fresh cheese. Similarly, increases of 7.4, 9.8 and 19.4 % for TS, protein and fat, respectively, were recorded for conventional cheese after 3 months. Also, ash and salt decreased by 11.4 and 16.3 %, respectively, in UF-cheese and 7.8 and 21.4 % in conventional cheese after the same period

Table 38. Chemical changes of ultrafiltrate-derived and conventional Domiati cheese ripened for 1 and 3 months in pouches

Composition	Ripening Period			
	1 Month		3 Months	
	UF	Conventional	UF	Conventional
	(%)			
Total solids	38.90[a]	39.10[a]	42.40[a]	40.93[a]
Protein	15.46[a]	13.45[b]	17.86[a]	14.36[b]
Fat	18.00[a]	19.40[b]	18.69[a]	21.50[b]
Ash	4.60[a]	4.32[b]	4.32[a]	4.20[a]
Salt	2.52[a]	2.20[b]	2.40[a]	2.20[a]

Means within a row for the same period of time having different superscripts are significantly different at ($p < 0.01$).

of ripening. Changes in the nitrogen distribution in Domiati cheeses made by conventional and UF methods and ripened in pouches under vacuum were assessed (Table 39). Three ripening indices were derived from the nitrogen fractions of cheese and were used to follow the ripening process. Apparent ripening extension Index (ARE) represents the ratio of non- casein nitrogen (NCN) to total nitrogen (TN), accounting for total proteolytic activity. Actual ripening extension (RE) represents the ratio of soluble nitrogen (SN) to (TN), which corrects for the effects of initial (TAN) level in UF-cheese. Ripening depth index (RD) represent the ratio of NPN to TN which accounts for the amino-peptidase activity of starter bacteria in the cheese (Wolfschoom, 1983). The changes noted in ARE, RE and RD values in conventional and UF-cheese after a ripening period of three months at 7-10 °C are recorded in Figure 26. The ARE index increased to 43.7 % for UF-cheese compared to 22.7 % for conventional cheese indicating more proteolytic activity in the UF cheese. The RE index was 29.1 % for UF-cheese and 18.6 % for conventional cheese. The data in Table 11A, Appendix 11, indicate that Uf-cheese was higher in ripening index than conventional cheese during the same. period of time.

Free fatty acids

The extent of flavor developed during cheese ripening was assessed by measuring the concentration of free fatty

Table 39. Changes in protein fractions (%Nx6.38) during ripening of conventional and UF- Domiati cheeses in pouches

Protein fractions	Ripening Period			
	1 Month		3 Months	
	UF	Conventional	UF	Conventional
Total protein	15.46[a]	13.45[b]	17.86[a]	14.36[b]
Non-casein N	5.40[a]	1.89[b]	7.80[a]	3.26[b]
Casein N	10.06[a]	11.55[b]	10.06[a]	11.09[b]
Non-protein N	2.40[a]	1.31[b]	3.10[a]	1.83[b]
Total albumin N	2.60[a]	0.58[b]	2.60[a]	0.58[b]
Soluble N	2.80[a]	1.49[b]	5.20[a]	2.68[b]

[a,b]Means within the same row that has different superscript are significantly different at ($p < 0.01$).

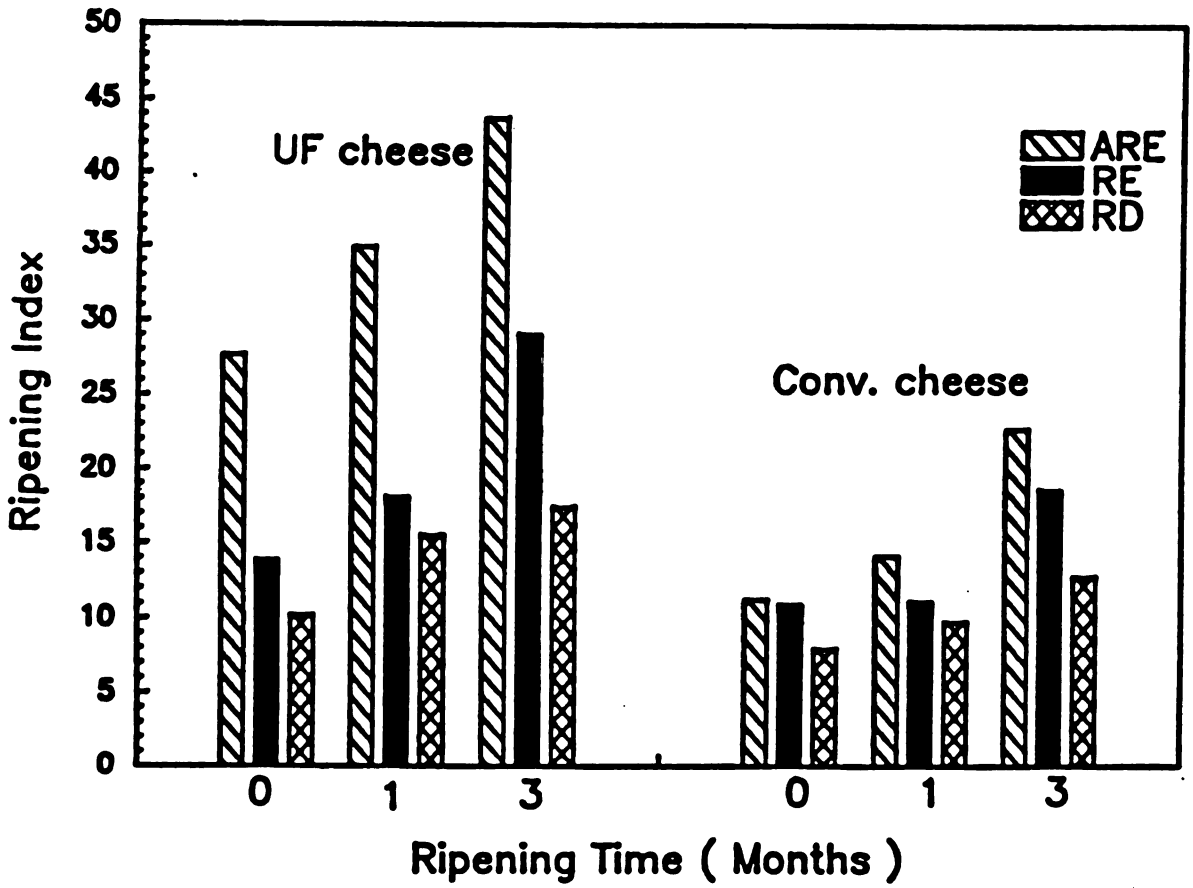

Figure 26. Ripening index of Domiati cheese made by conventional and UF methods and ripened for three months in pouches. ARE= apparent ripening extention, RE= actual ripening extention, RD= ripening depth index.

Table 40. Free fatty acids of fresh and ripened UF Domiati cheese for three months in pouches

Free fatty acids	Ripening time		
	Fresh	1 Month	3 Months
	mg / 100 g		
	Mean±SD	Mean±SD	Mean±SD
C4:0	2.1±0.19	4.5±0.26	10.1±0.84
C6:0	2.2±0.66	2.3±0.71	10.3±0.73
C8:0	2.0±0.21	2.8±0.33	9.0±0.71
C10:0	3.4±0.85	6.9±0.79	23.9±0.83
C12:0	5.2±0.43	10.3±0.85	24.9±0.90
C14:0	12.3±1.10	25.0±1.30	57.1±1.45
C16:0	31.0±1.36	58.0±1.76	104.0±1.86
C18:0	15.5±1.33	37.0±1.29	70.4±1.73
C18:1	11.0±2.10	37.8±2.30	95.0±2.15
C18:2	4.0±2.00	15.0±2.40	40.0±2.70
C18:3	2.0±0.94	5.2±0.76	8.0±0.90
Total F.FA	90.7	204.8	452.7

Values are the means and standard deviations of three replications.

Table 41. Free fatty acids of fresh and ripened conventional Domiati cheese for three months in pouches

Free fatty acids	Ripening Period		
	Fresh	1 Month	3 Months
	mg/100 g		
	Mean±SD	Mean±SD	Mean±SD
C4:0	3.2±0.26	5.7±0.45	12.0±0.83
C6:0	2.6±0.13	4.3±0.36	11.5±0.56
C8:0	2.4±0.08	3.8±0.23	10.5±0.78
C10:0	1.9±0.23	5.0±0.46	19.6±0.83
C12:0	3.2±0.46	8.9±0.58	14.3±1.30
C14:0	10.1±1.30	19.8±1.56	40.6±1.77
C16:0	25.1±1.21	54.7±1.15	98.7±1.45
C18:0	10.6±1.15	21.9±1.21	55.4±1.96
C18:1	19.1±1.05	27.8±1.22	55.6±1.55
C18:2	2.7±1.80	13.9±1.79	30.8±1.37
C18:3	1.3±0.83	3.6±0.93	7.0±1.39
Total F.F.A	82.2	169.4	356.0

Values are the means and standard deviations of three replications.

monitored during the ripening of conventional and ultrafiltrated cheese is presented in Tables 40 and 41. UF-cheese which was ripened for three months possessed higher concentration of liberated fatty acids (452.7 mg\100g cheese) than conventional cheese (356 mg\100g cheese) after 3 months of ripening. The percentage increase in free fatty acids for the UF-cheese was 400 % compared to the fresh cheese. Free fatty acids of conventional cheese increased by 333 % compared to its fresh counterpart. These results are in agreement with those reported by Omar (1987). Conventional cheese possessed a more pronounced flavor than UF-cheese, probably as a result of the higher concentration of short chain fatty acids.

Free amino acids

The extent of proteolysis during cheese ripening was ascertained by measuring the concentration of free amino acids released. The concentration of amino acids liberated from both UF-derived and conventional cheeses were substantially increased as a result of ripening. A summary of free amino acids released from the conventional and UF-cheese after three months of ripening is presented in Table 42. Both UF-cheese and conventional cheese were substantially higher in total free amino acids than their fresh counterpart. After three months of ripening, the increase in amino acids liberated from UF-cheese were more

Table 42. Free amino acids of Domiati cheese made by ultrafiltration and conventional methods and ripened for three months in pouches

Free amino acids	ultrafiltration	Conventional
	mg/100g Cheese	
Essential A.A		
Histidine	35±0.6	15±0.8
Threonine	50±0.8	17±1.0
Valine	69±1.0	34±0.9
Isoleucine	66±1.1	22±1.2
Leucine	90±2.0	40±0.7
Phenylalanine	63±0.9	28±1.1
Lysine	115±1.5	69±2.0
Methionine	9±0.95	7±1.3
Cystine	10±2.00	7±1.9
Non essential A.A		
Aspartic acid	104±1.0	35±0.79
Glutamic acid	230±2.3	65±0.59
Serine	50±0.8	12±1.3
Glycine	29±0.71	12±1.0
Arginine	44±0.90	15±0.49
Proline	106±1.2	25±1.6
Tyrosine	42±0.79	12±0.99
Total F.A.A	1112	415

Values are the means and standard deviation of three replications.

acids. The percentages of free amino acids observed in UF-chesse was more than double that observed in conventional cheese. In UF-cheese, lysine, aspartic acid, glutamic acids and proline accounted for 50 % of the total free amino acids, increasing the most after 3 months of ripening. As compared to conventional cheese, valine,leucine, lysine, aspartic and glutamic acids accounted for 58 % of the total.

The remaining residues increased slightly throughout the ripening period. The relative proportions of individual free amino acids remained essentially constant throughout the ripening period for both cheeses as was reported by Jarret et al.(1982).

The general increase in free amino acids during ripening can be explained on the basis of the higher proteolytic action of microbial enzymes which produce a range of peptidases (Mou et al., 1975).

Electrophoretic Analysis

The proteolytic activity in ripened Domiati cheese was examined using discontinuous polyacrylamide gel electrophoresis (PAGE). The densitograms of the main nitrogen fractions of fresh conventional and UF-derived cheeses and those pouch-ripened for three months at 7-10°C are illustrated in Figure 27. Ripened cheese showed different electrophoretic patterns. UF-cheese demonstrated that extensive protein degradation occurred, particularly in the α_{s1}-casein zone with many minor bands which migrated

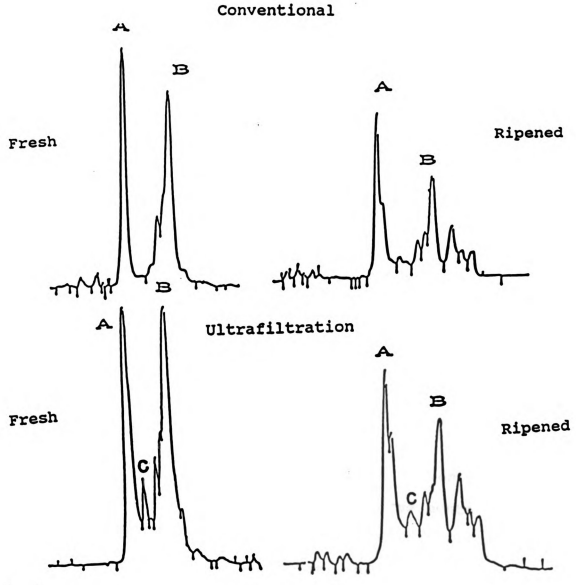

Figure 27. Discontinuous polyacrylamide gel electrophoresis densitograms of fresh and ripened (3 months) conventional and ultrafiltrated Domiati cheese; A, β-casein; B, α_s-casein; C, α-lactalbumin.

rapidly. The cleavage of α_{s1}-casein mostly due to rennet activity (Olson 1982). Beta-casein was more resistant to degradation which is in agreement with results from other studies (Green et al., 1981; Koning et al., 1981; and El-Shabrawy, 1985). The proteolytic breakdown of β-casein are strongly retarded by the presence of salt (Fox and Walley 1971).

Cheese Quality

Cheese is ripened by the action of the rennet and microorganisms during the cheese making and cheese ripening. Degradation of casein during ripening affects the texture, flavor and taste of cheese. Harper (1959) reported the relationship between the physicochemical changes and taste of cheese during ripening.

The color and textural qualities of conventional and UF-Domiati cheese during ripening for three months at 7-10°C were studied. A typical texture profile analysis of conventional and ripened UF Domiati cheese, obtained by the Instron Universal Machine, is presented in Figure 28. In cheese ripening, primary and secondary activities result in the accumulation of lactic acid, free fatty acids and free amino acids. The mechanical measurements of hardness, cohesiveness and gumminess are presented in Figure 29. As the data reveal, both UF and conventional cheeses show the same trend of greater hardness, cohesiveness and gumminess after one month, then decrease after three months of

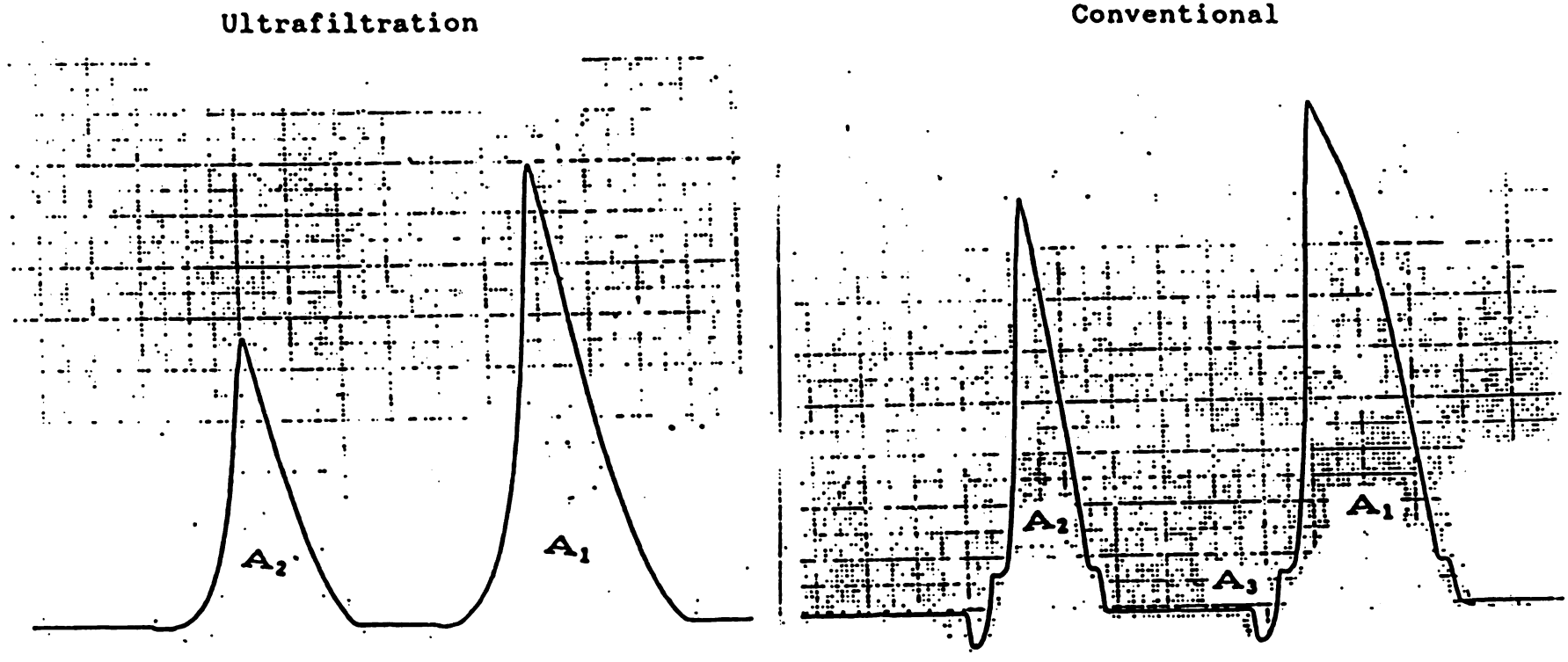

Figure 28. Texture profile curve of conventional and ultrafiltrated ripened Domiati cheese obtained with the Instron Universal Testing Machine. Hardness= height of A_1, Cohesiveness= A_2/A_1, and Adhesiveness= A_3.

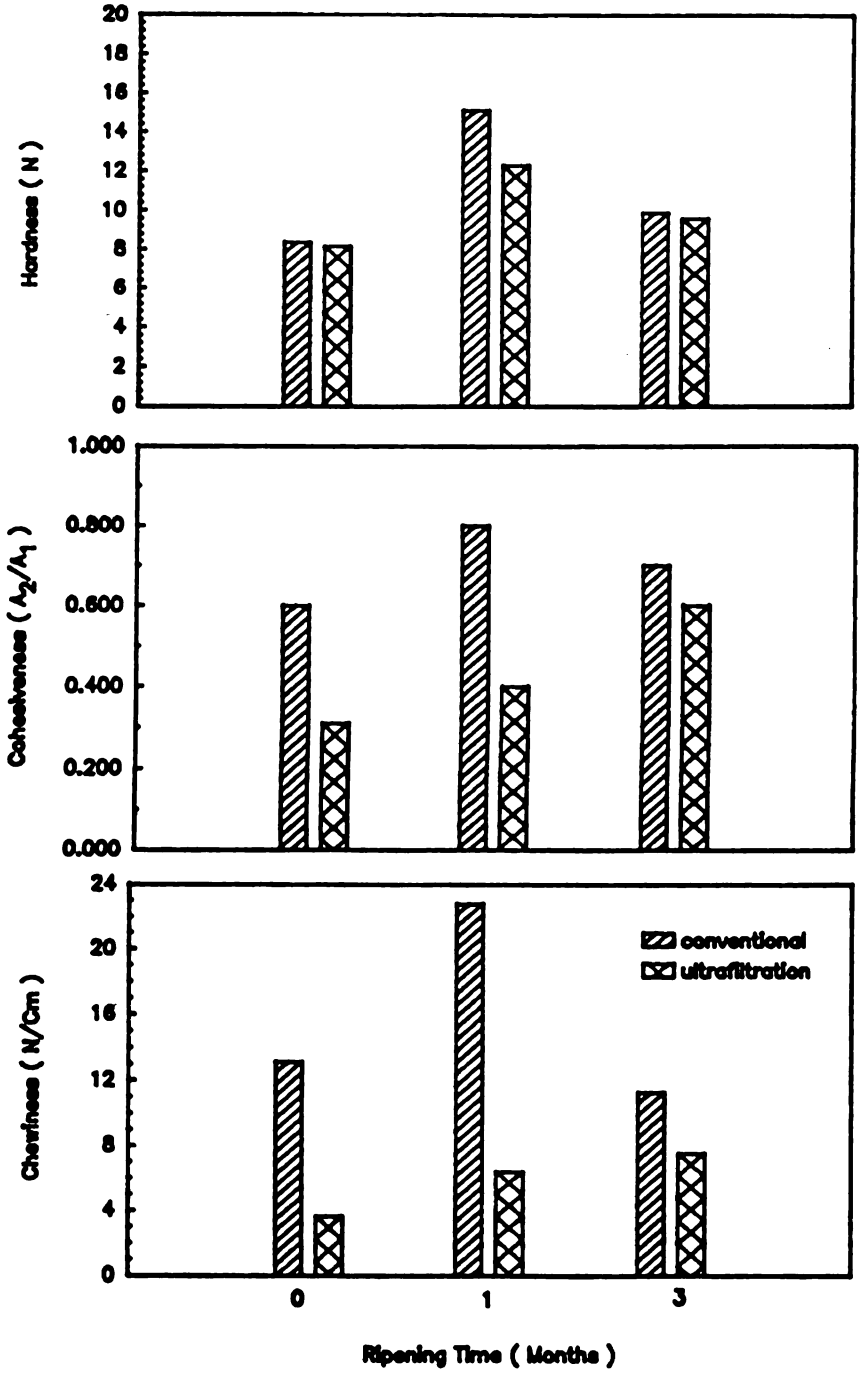

Figure 29. Hardness, cohesiveness and chewiness of Domiati cheese made by conventional and ultrafiltrated methods and ripened for 3 months in pouches. N= Newtons. A_1 and A_2 represent peaks area.

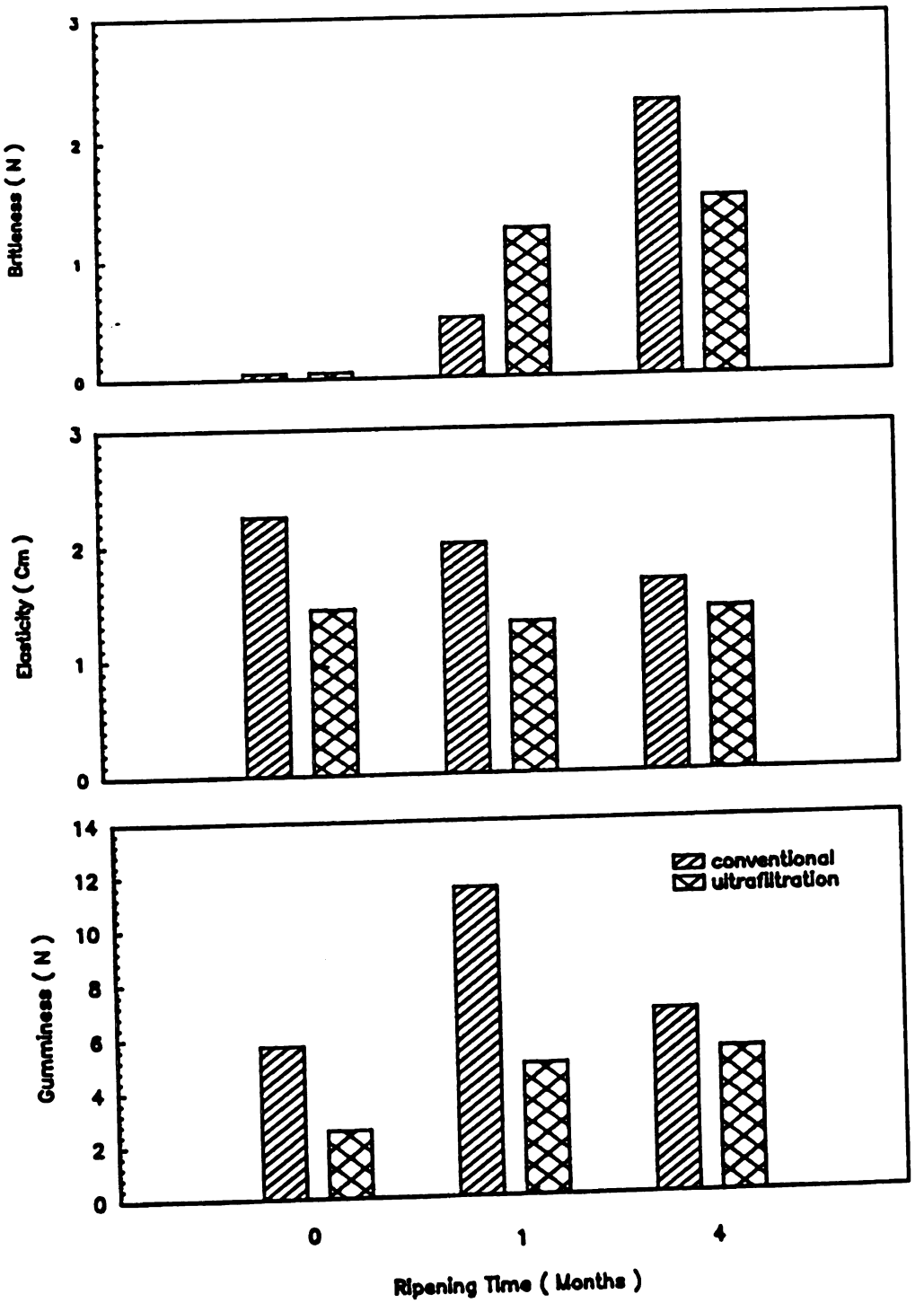

Figure 30. Brittleness, elasticity and gumminess of Domiati cheese made by conventional and ultrafiltrated methods and ripened for 3 months in pouch.

ripening. These changes are due to the chemical and physical changes which cause the body and texture of the freshly made cheese to lose its firm, tough curdy texture and to become softer. Elasticity, gumminess and brittleness of ripened Domiati cheese are plotted in Figure 30. Data indicate that cheese made by the conventional method possesses greater elasticity than UF-derived cheese. In both cheeses, young cheese is more elastic than older cheese. As the data show in Table 11B. (Appendix11), fresh conventional and UF-cheese have less brittleness than 1 and 3 month of ripening.

The changes in the color of ripened Domiati cheese made by the two methods were measured and the data are presented in Figure 31. Fresh ultrafiltrated Domiati cheese has creamer color than conventional cheese. During the cheese ripening, both cheeses have creamer color as the data reveal in Table 11C (Appendix 11).

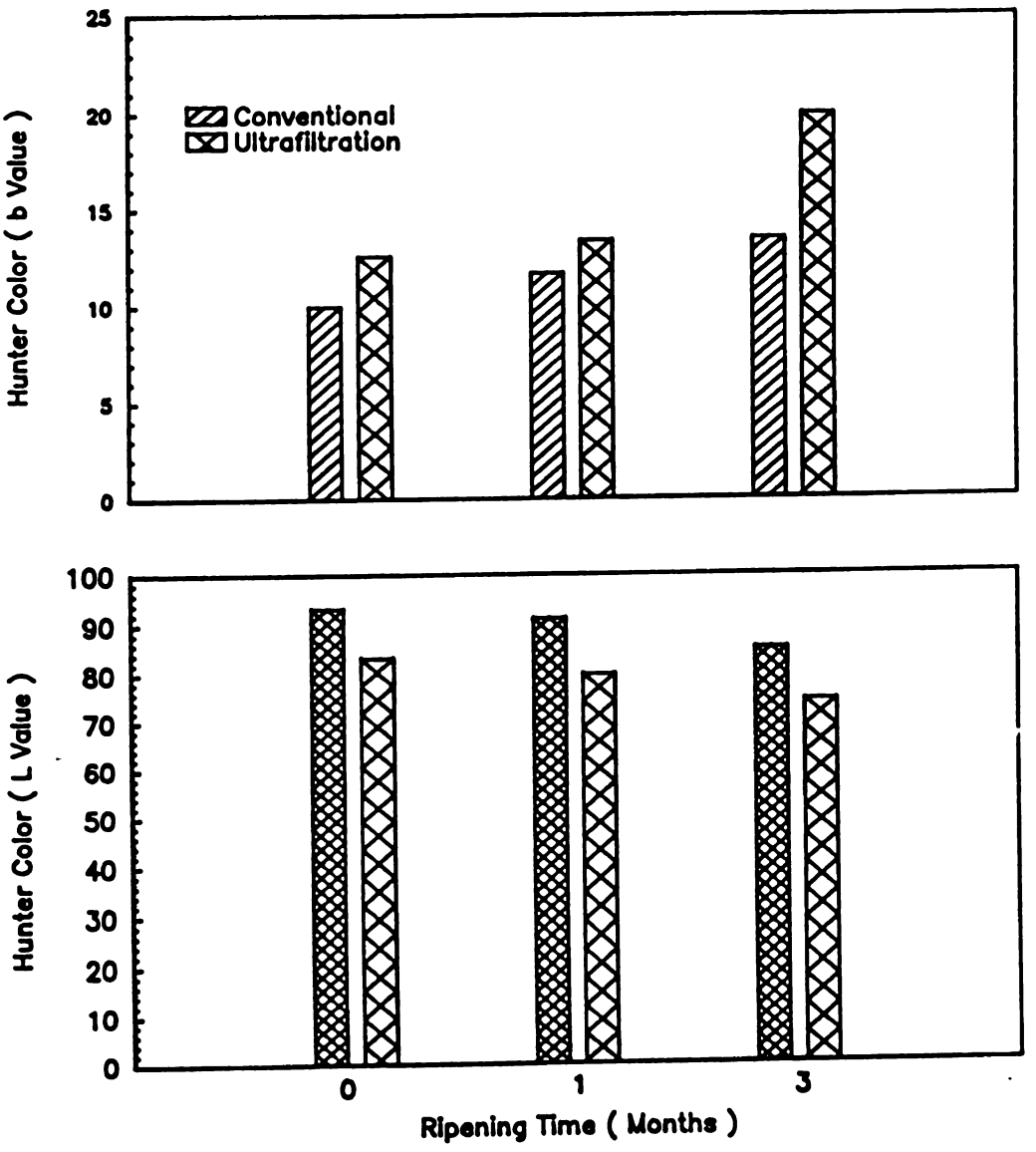

Figure 31. Color changes of fresh and ripened Domiati cheese made by conventional and ultrafiltration methods.

PART V

DOMIATI CHEESE FROM WHEY PROTEIN CONCENTRATE-SUPPLEMENTED MILK

Introduction

Domiati cheese is the most popular soft cheese in Egypt. Increasing demand for this type of cheese conflicts with market shortage of fresh milk. A recent development in the manufacturing of milk protein products that offers excellent potential is the ultrafiltration process by which whey protein can be concentrated, added to milk and coprecipitated with casein by rennet and heat. Whey proteins concentrate (WPC), in an undenaturated complex, contains all of the proteins components in a highly functional form. Many investigations have shown that this process improved cheese yield, body and nutritional value. Additionally, utilization of whey proteins in this way is of great interest, when considering the saving of natural resources and solving the problems associated with pollution.

This modification has two major advantages for cheese production in Egypt: First, to produce a cheese with an inexpensive but balanced protein content, and second, to find a new outlet for salted whey produced during the manufacturing of conventional Domiati cheese.

This part of the study was carried out to investigate a modification of the conventional method of manufacturing Domiati cheese by supplementing the whole milk with whey

protein concentrate obtained by ultrafiltration and to evaluate changes in yield, quality and nutritional value of Domiati cheese produced by the new process. Component losses would also be assessed.

Composition of milk and WPC mixtures

Domiati cheese was manufactured by the traditional procedure as outlined by Mahmmoud (1980). Whey protein concentrate (WPC) was produced by ultrafiltration of Domiati cheese whey, as outlined in part 1, to a final composition of 20.08 % total solids and 8.38 % protein and used as a supplement to whole milk in individual vats (Table 43). The addition of WPC to milk on a protein basis ranged from 1:0 for unsupplemented control up to 1:2 for maximal supplementation.

The chemical composition of the mixtures used for making Domiati cheese is presented in Table 44. The data reveal that there was a significant increase in total solids and total protein content up to 28.1% and 10.1 %, respectively, as the supplementation of the WPC ratio increased up to 1:2. This relationship indicates a cumulative effect of whey concentrate addition on protein content.

Coagulation Time

A study of the rennet-induced coagulation time of cheese milk indicated that the addition of WPC at a 1:1

Table 43. Composition of whole milk and whey protein concentrate used for supplementing the milk

	Composition (%)					
	Total solids	Protein	Fat	Lactose	Ash	Salt
Whole Milk	12.18	3.50	3.3	4.80	0.58	0.16
Whey Protein Concentrate	20.08	8.38	N.D.	5.85	3.89	2.29

Data are the means of three determinations.
N.D.= not determined.

Table 44. Total solids and protein contents of WPC-supplemented whole milk mixtures used for making Domiati cheese

Whey protein concentrate-supplemented whole milk		
Added protein ratio	Total solids (%)	Total protein (%)
1:0.0	12.2	3.5
1:0.25	12.9	3.8
1:0.5	13.4	4.0
1:0.75	14.0	4.6
1:1	14.4	4.9
1:2	15.7	5.7

Data are the average of three replications.

Table 45. Coagulation time of Domiati cheese made from whole milk supplemented with different ratios of whey protein concentrate

Milk/ WPC Protein Ratio				
Coagulation Time (Min)				
1:0	1:0.25	1:0.5	1:0.75	1:1
74.7±2	32.0±1.5	25.3±1.3	23.0±1.7	21.3±3

Data are means and standard deviations of three replications.

ratio was most appropriate for yielding a firm curd. Addition of WPC at a still higher concentration ratio up to 1:2, resulted in no coagulation of the mixture at all. Coagulation times for the various WPC-supplemented milks are presented in Table 45. Coagulation time decreased from 74.7 min for unsupplemented milk to 21.3 min for whole milk supplemented with 1:1 protein ratio. These results are in agreement with those of Rodney et al. (1982) who found that setting time decreased as the supplementation ratio increased. Residual coagulating enzyme in sweet whey may account for this behavior (Holmes et al., 1977). Recovery of clotting enzyme from whey by ultrafiltration for reuse in cheese making was investigated by Castiglioni (1969).

Cheese Yields

The actual yield and yield efficiencies of Domiati cheese produced from whole milk supplemented at various levels with WPC are presented in Figure 32. All cheeses were weighed after draining for 48 hr and the yield calculated. The weight of added WPC was included as part of the milk in calculating yields of experimental cheese. The yield of cheese increased with increasing ratios of protein concentrate added. The significant increase ($p < 0.05$) in actual yield (direct weight) of cheese from 17.5 Kg cheese/100 Kg for unsupplemented milk to 24.8 Kg cheese/ 100 Kg mixture for the 1:0.75 supplementation ratio indicates a strong potential for improving cheese making

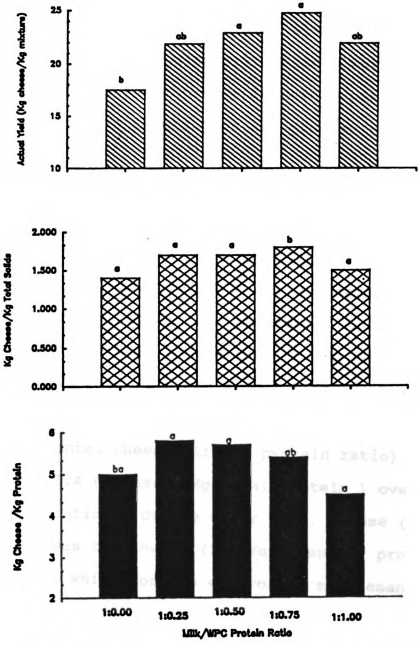

Figure 32. Yields and yield efficiencies of Domiati cheese made from whey protein concentrate-supplemented whole milk. [a,b,c]Means with the same letters are not significantly different at P< 0.05.

efficiency and energy conservation. These results are in agreement with Abrahamsen's (1979), who investigated the possibility of manufacturing semi-hard rennet cheese of acceptable quality from milk fortified with different amounts of WPC by means of ultrafiltration. Walstra and Jenness (1984) stated the increase in cheese yield was due to casein-whey protein interaction and greater retention of moisture.

Cheese yield efficiency is defined as kilograms of cheese obtained per kilogram of total solids or protein utilized (Van Slyke, 1979). As the data reveal in Table 12A, (Appendix 12), the yield efficiency changed with the supplementation ratio, being 1.43 Kg cheese/ Kg total solids for unsupplemented control cheese and 1.09 Kg cheese/Kg total solids for cheese produced from maximally supplemented (1:1) milk. When yield efficiency was based on the total protein, supplemented cheese (1:0.25 protein ratio) was the highest yield (5.4 Kg cheese/Kg total protein) over the entire supplementation. On the other hand, cheese (1:1 protein ratio) was the lowest (3.4 Kg cheese/Kg protein). yield efficiency, which compare control to supplemented cheese based on unit of protein and total solids, were significantly different at $p< 0.05$. The amounts of cheese solids and protein produced per kilogram total solid and protein in the control and supplemented cheese were of the same order as reported by Kosikowski et al.(1984).

Table 46. Composition of Domiati cheeses made from whey protein concentrate-supplementing whole milk using rennet coagulation

Added Protein ratio	Cheese Composition (%)				
	Total solids	Protein	Lactose	Fat	Ash
1:0.0	44.9^{c}	16.8^{bc}	2.4^{c}	24.0^{a}	1.6^{b}
1:0.25	46.3^{b}	18.4^{ab}	3.0^{bc}	23.0^{a}	1.9^{b}
1:0.5	47.2^{b}	19.0^{ab}	3.5^{ab}	22.0^{a}	2.7^{ab}
1:0.75	45.5^{a}	17.6^{bc}	3.7^{ab}	21.0^{ab}	3.2^{a}
1:1	39.5^{d}	14.0^{c}	3.8^{a}	18.5^{b}	3.2^{a}

^{a,b,c,d}Means with the same letter are not significant at $P< 0.05$.

Cheese Composition

The composition of Domiati cheeses produced from whole milk supplemented with various level of WPC was determined and presented in Table 46. As the data reveal, there were significant changes in the characteristics of the experimental cheeses compared to the control (unsupplemented) cheese. A significant increase in the total solids of supplemented cheese at $p< 0.05$ was observed as the level of supplementation was increased up to 1:0.75 protein ratio, then rapidly decreased when a higher protein supplementation ratio (1:1) was employed.

The increase in the total solids and total protein in the WPC-supplemented cheese was correlated with the increase in total solids and total protein at various levels of WPC-supplemented mixtures (Tables 44 and 46). The calculated results indicated that up to 1:0.5 protein/protein ratio high correlation coefficients, $r= 0.98$ and 0.96 were obtained between total solids of the produced cheese and the total solids and total protein of supplemented-mixtures. On the other hand, lower correlations of 0.95 and 0.92 were obtained when the total protein of the produced cheese were correlated to the total solids and total protein in the WPC-supplemented mixtures. At supplementation ratios of 1:0.75 and 1:1, no positive correlation was observed.

There were significant increases in lactose and ash content in the cheese as the protein supplementation ratio increased up to 1:1. On the other hand, percentage of fat

decreased significantly ($p < 0.05$) as supplementation was increased. These result are in agreement with El-Shibiny et al.(1973) who added heat precipitated whey protein to the milk for making Domiati cheese. The results for protein and fat content showed the same trend as observed by Brown et al.(1982) who produced cheddar cheese from WPC-supplemented milk and by Nes (1980) who manufactured low-fat Gouda-type cheese by addition of WPC to the milk.

Protein fractions of Domiati cheese made from WPC-supplemented whole milk were studied and the data presented in Figure 33. The data in Table 12B (Appendix 12) revealed no significant differences at $p < 0.01$ between the amount of casein in the experimental and control (unsupplemented) cheeses except in the case of 1:1 supplemented ratio. The amount of non-casein protein (NCN) significantly increased with increasing supplementation up to 1:0.75 protein ratio. The amount of whey protein (WP) increased significantly from 0.71% for unsupplemented cheese to 2.4 % and 2.0 % for supplementation ratio of 1:0.5 and 1:0.75, respectively. These results are consistent with the findings of Abrahamsen (1979). There was a high correlation (0.83) between the amount of protein in the milk mixtures and the protein present in the cheese for supplementation ratios up to 1:0.5 protein ratio.

The nutritional quality of the cheeses was indicated by the amount of essential and non-essential amino acids

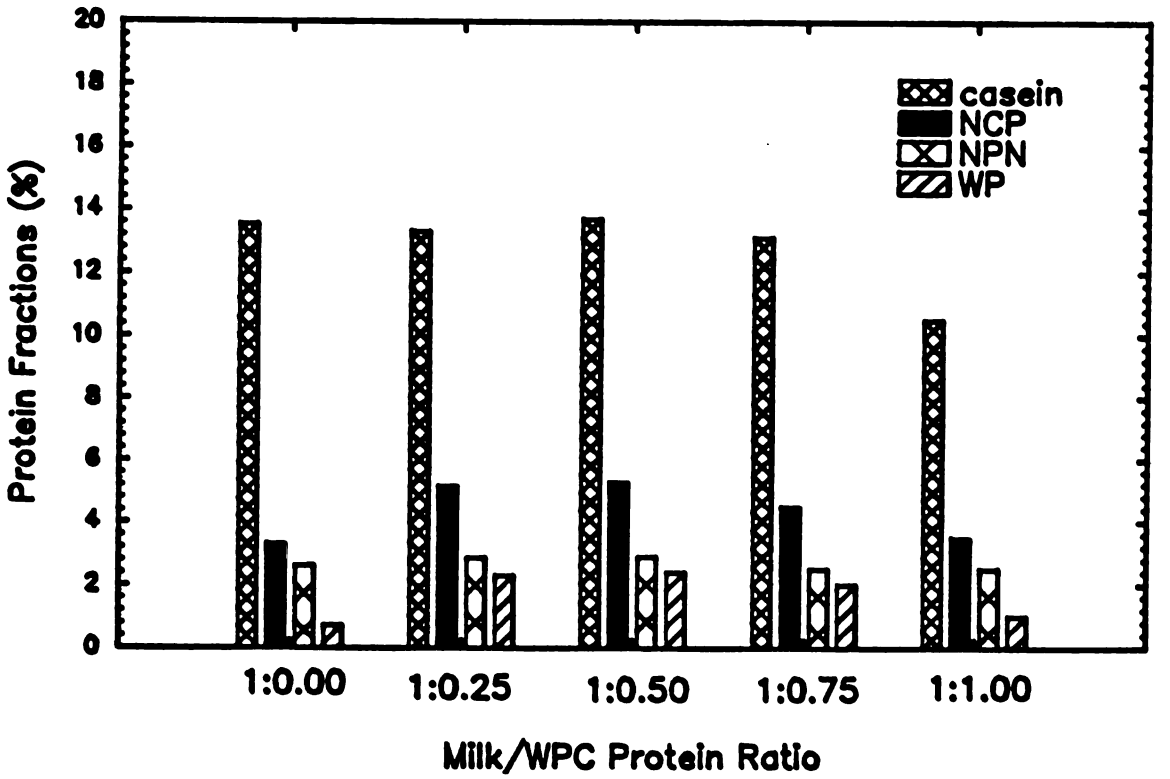

Figure 33. Protein fractions of Domiati cheese made from whey protein concentrate-supplemented whole milk. NCN= non-casein nitrogen, NPN= non-protein nitrogen, and WP= whey protein.

Table 47. Quantitative changes of total amino acids of Domiati cheese made from whey protein concentrate-supplemented whole milk

Amino Acids	Milk/WPC Protein Ratio				
	Control	1:0.25	1:0.5	1:0.75	1:1
	(g/100g)				
Asp	0.96	1.10	1.00	1.00	0.60
Glu	2.10	2.20	2.00	1.90	1.70
Ser	0.93	1.00	0.95	0.95	0.70
Gly	0.43	0.46	0.50	0.50	0.40
His	0.35	0.44	0.50	0.55	0.40
Arg	1.85	1.75	1.50	1.45	1.23
Thr	0.71	0.94	1.10	1.12	0.80
Ala	0.64	0.73	0.73	0.68	0.66
Pro	1.71	1.32	1.20	1.00	1.00
Tyr	1.14	1.00	0.98	0.80	0.80
Val	1.45	1.49	1.52	1.48	1.40
Meth	0.43	0.80	1.10	1.00	0.40
Cys	0.20	0.49	0.65	0.60	0.20
Ile	0.65	0.84	1.00	0.80	0.40
Leu	1.08	1.53	1.70	1.50	0.90
Phe	0.60	0.55	0.45	0.40	0.40
Lys	1.30	1.60	1.85	1.50	0.90
Total A.A	16.53	18.34	18.73	17.23	12.89

The data are the average of three determinations.

Table 48. Quantitative changes of free fatty acids of fresh Domiati cheese made from whey protein concentrate-supplemented whole milk

Free Fatty	Milk/WPC Protein Ratio				
	Control	1:0.25	1:0.5	1:0.75	1:1
	mg/100g				
C4:0	3.9	3.5	3.1	2.8	2.5
C6:0	4.1	3.7	3.5	3.3	3.1
C8:0	5.1	4.7	4.3	3.9	3.0
C10:0	4.4	3.8	3.7	3.4	3.1
C12:0	4.6	4.2	3.6	3.5	3.0
C14:0	11.7	9.3	8.6	8.5	8.2
C16:0	34.1	24.6	23.8	22.0	21.0
C18:0	10.2	9.3	8.7	7.7	6.9
C18:1	28.1	25.0	20.5	19.0	17.6
C18:2	5.2	4.6	3.6	3.4	3.1
C18:3	1.8	1.7	1.5	1.1	0.6
Total FFA	113.14	93.9	84.72	78.56	72.32

The data are the average of three determinations.

presented. Total amino acids found in the experimental Domiati cheese are summarized in Table 47. Generally, the total quantity of amino acids increased with increasing addition of whey protein to the milk up to 1:0.75 protein ratio. The highest increase of essential amino acids was 28.2 % for 1:0.25 protein ratio and 45.79 % for the 1:0.5 protein ratio when compared to the control.

Fresh control and Domiati cheese made from whole milk supplemented with WPC were analyzed for free fatty acids. The results are presented in Table 48. Control cheese contained a higher concentration of free fatty acids than cheese made from whey-supplemented milk. All the fatty acids showed a similar decreasing pattern, as supplementation was increased. Total free fatty acids were 113.14 mg/100g for the unsupplemented cheese, decreasing to 93.9, 84.72, 78.56 and 72.32 mg/100g for supplementation ratios of 1:0.25, 1:0.5, 1:0.75 and 1:1 protein ratio, respectively. These results show a trend similar to fat content decrease in the WPC-supplemented cheeses (Table 46).

Cheese Quality

UF-whey, which possesses protein in an undenaturated form, was used as a supplement to cheese milk to improve cheese quality. Sensory evaluations of whey-supplemented cheese for flavor, texture/body, and color are presented

Table 49. Organoleptic properties of fresh Domiati cheese made from whey protein concentrate-supplemented whole milk

Properties	Milk/WPC Protein Ratio				
	Control	1:0.25	1:0.5	1:0.75	1:1
Flavor	28.5	29.0	29.7	21.7	18.0
Texture/Body	56.0	57.0	59.0	42.0	32.0
Color	10.0	9.5	9.1	8.0	6.7
Total	94.5	95.5	97.8	71.7	56.7

Body and Texture, 60 = Excellent.
Flavor, 30 = Excellent.
Color, 10 = Excellent.

in Table 49. The cheese produced from whole milk supplemented with WPC to a ratio of 1:0.5 attained the highest quality score (97.8). On the other hand, quality scores decreased to 71.7 and 56.7 for supplementation of 1:0.75 and 1:1 protein ratio, respectively. The most prominent defects in the WPC-supplemented whole milk cheese were a slight bitter flavor, weak and pasty body, and a creamier color in the high supplementation ratio cheeses.

Color

The color parameters L, a, and b were measured in WPC-supplemented cheese and are presented in Figure 34. Whiteness, variable L, showed a significant difference at $p<0.05$ between the different supplementation ratios of fresh cheese. It was 96.8 for control cheeses and decreased with increasing supplementation ratios, to 90.0, 84.8, 80.1 and 74.0 for ratios of 1:0.25, 1:0.5, 1:0.75, and 1:1, respectively. The correlation of variable L with the sensory color was highly significant ($r = 0.96$). Supplemented cheese show slightly creamer color as the addition of whey protein concentrate was increased.

While greenness (-a) and yellowness (b) showed significant increase with increasing supplementation as presented in Table 12C (Appendix 12).

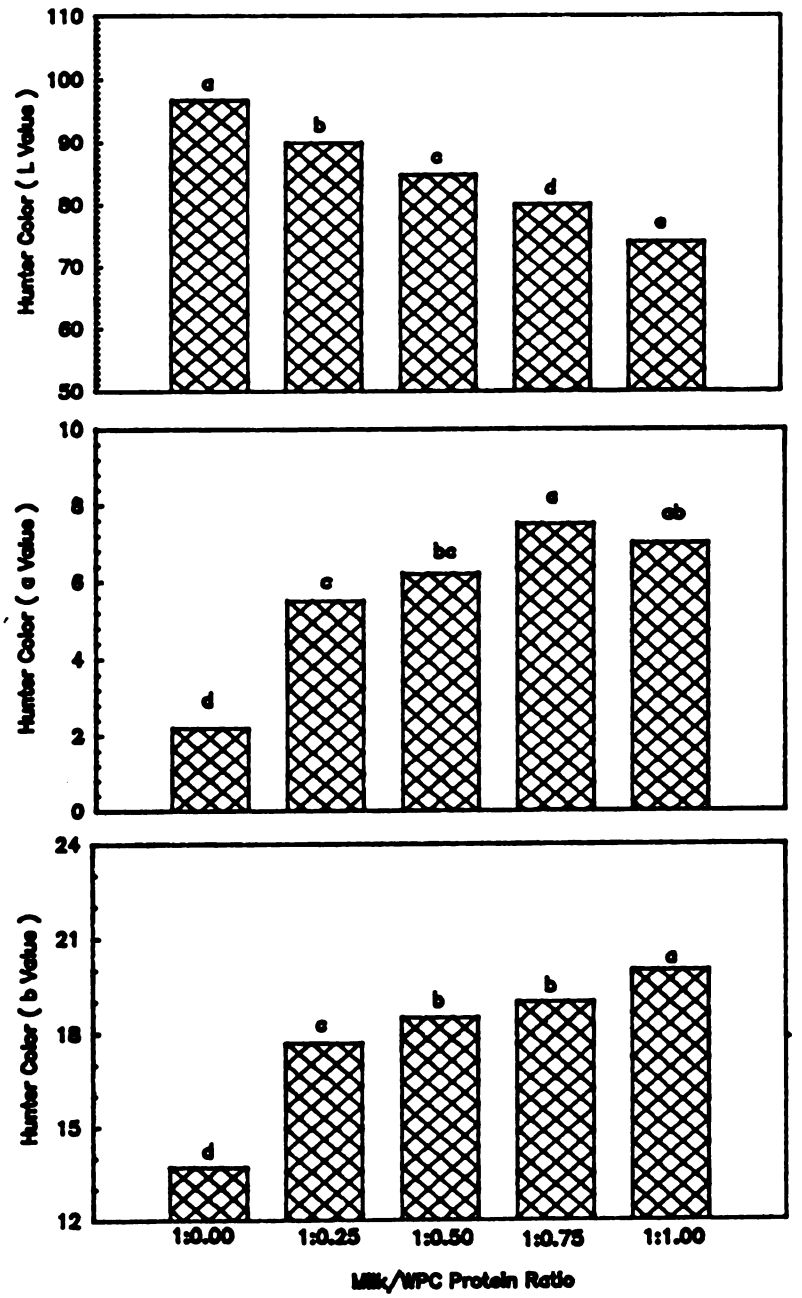

Figure 34. Color changes of Domiati cheese made from whey protein concentrate-supplemented whole milk.
[a,b,c,d,e]Means with the same letters are not significantly different at P< 0.05.

Texture Profile Analysis (TPA)

A texture profile method for hardness, cohesiveness, adhesiveness, gumminess and elasticity was proposed by Brandt et al. (1963) as a means of aiding the food researcher to obtain descriptive characteristics of food. Typical force distance profiles obtained from the control cheese and cheese made from whole milk supplemented with WPC at various levels are illustrated in Figure 35.

Cheese samples were tested for their mechanical properties by a compression test at 80 % deformation. The data indicate that there were significant difference in the area and height of the first and second bite of control cheese and the cheese made from milk supplemented with WPC. The calculated data concerning hardness, cohesiveness, chewiness, gumminess, adhesiveness and elasticity are summarized in Figures 36, 37 and 38. It can be seen that, there were significant differences ($p < 0.05$) between Domiati cheese produced from milk only (control) and cheese produced from milk supplemented with WPC at various levels. As the supplementation ratio increased, the firmness of the cheese decreased and became less firm. These results were consistent with the findings of Abrahamsen (1979) and Brown (1982) who stated that the higher moisture in WPC-supplemented cheese could account for the slight increase in pastiness and decrease in curdiness. A slight resistance of the curd during the press step,

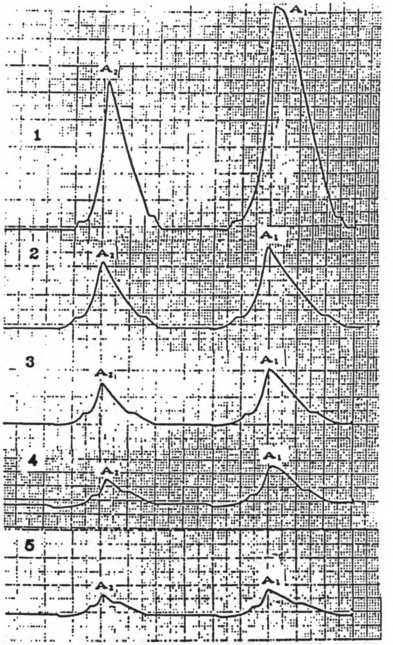

Figure 35. Texture profile curves of Domiati cheese made from whey protein concentrate-supplemented whole milk. Curves obtained with the Instron Universal Testing Machine. Hardness= height of A_1, Cohesiveness= A_2/A_1, and Adhesiveness= A_3. Milk/ WPC protein ratio: 1=control, 2=1:0.25, 3=1:0.5, 4=1:0.75 and 5=1:1.

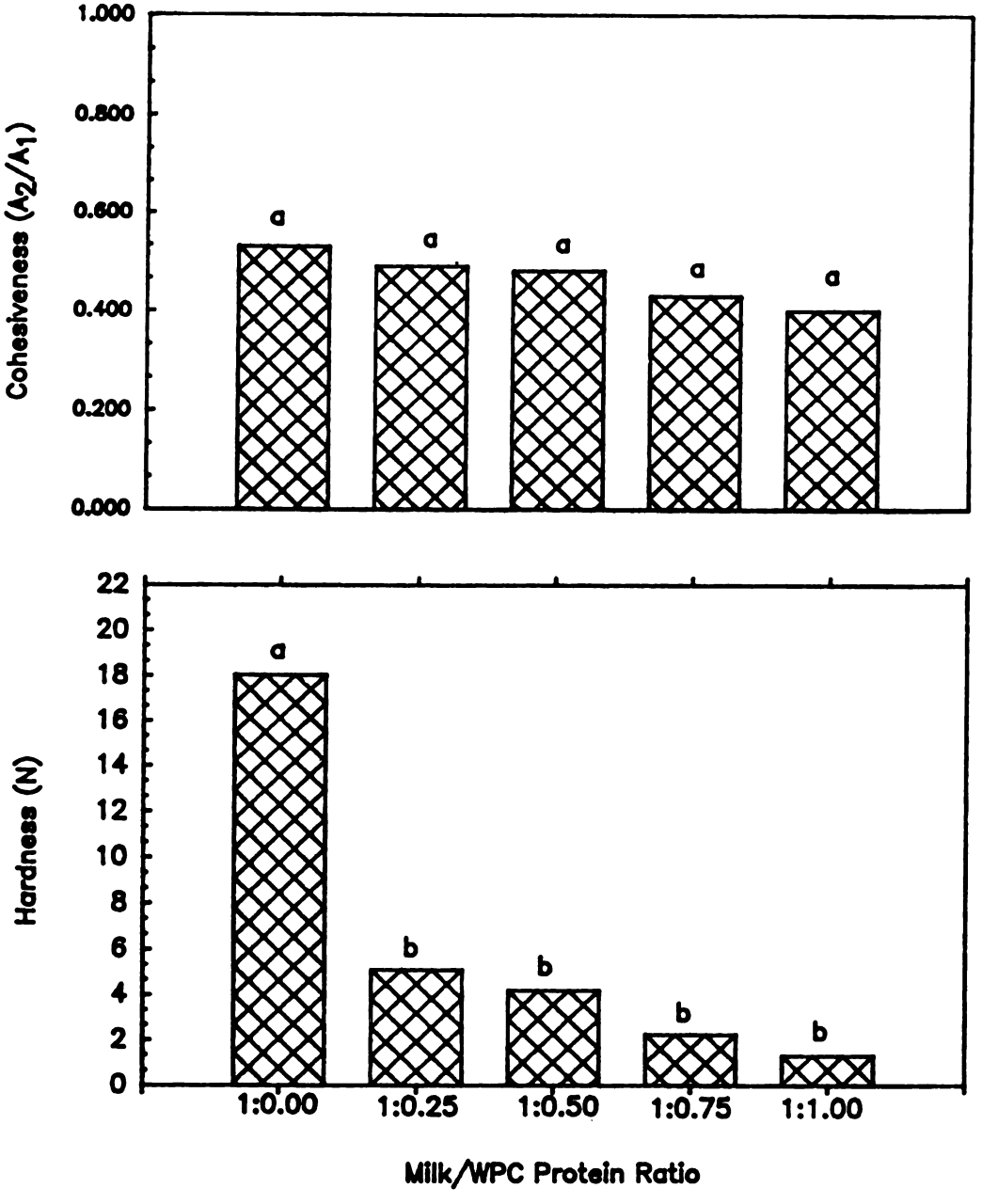

Figure 36. Hardness and cohesiveness of Domiati cheese made from whey protein concentrate-supplemented whole milk. [a,b]Means with the same letters are not significantly different at P< 0.05. N= Newtons.

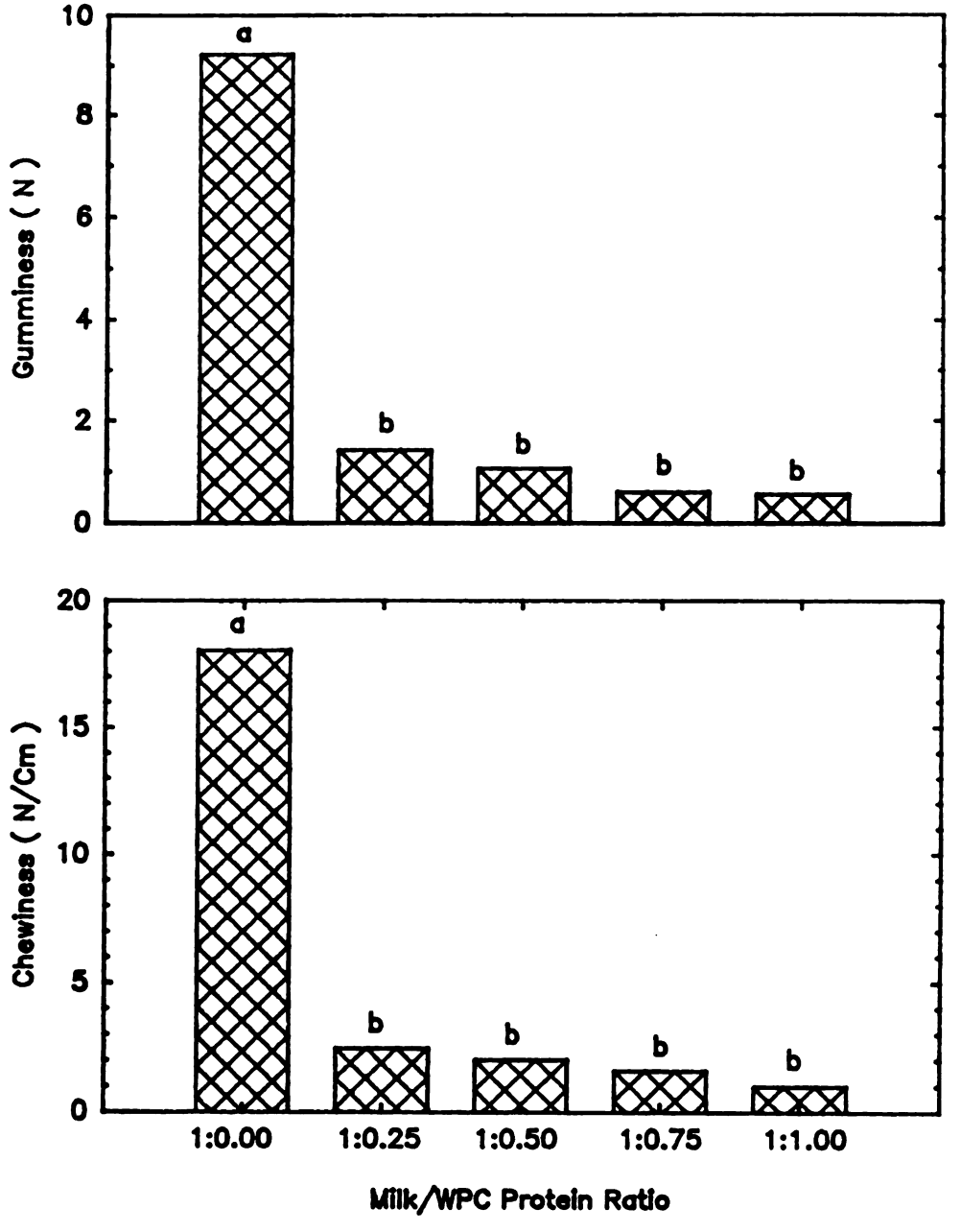

Figure 37. Chewiness and gumminess of Domiati cheese made from whey protein concentrate supplemented whole milk. [a,b]Means with the same letters are not significantly different at P< 0.05. N= Newtons.

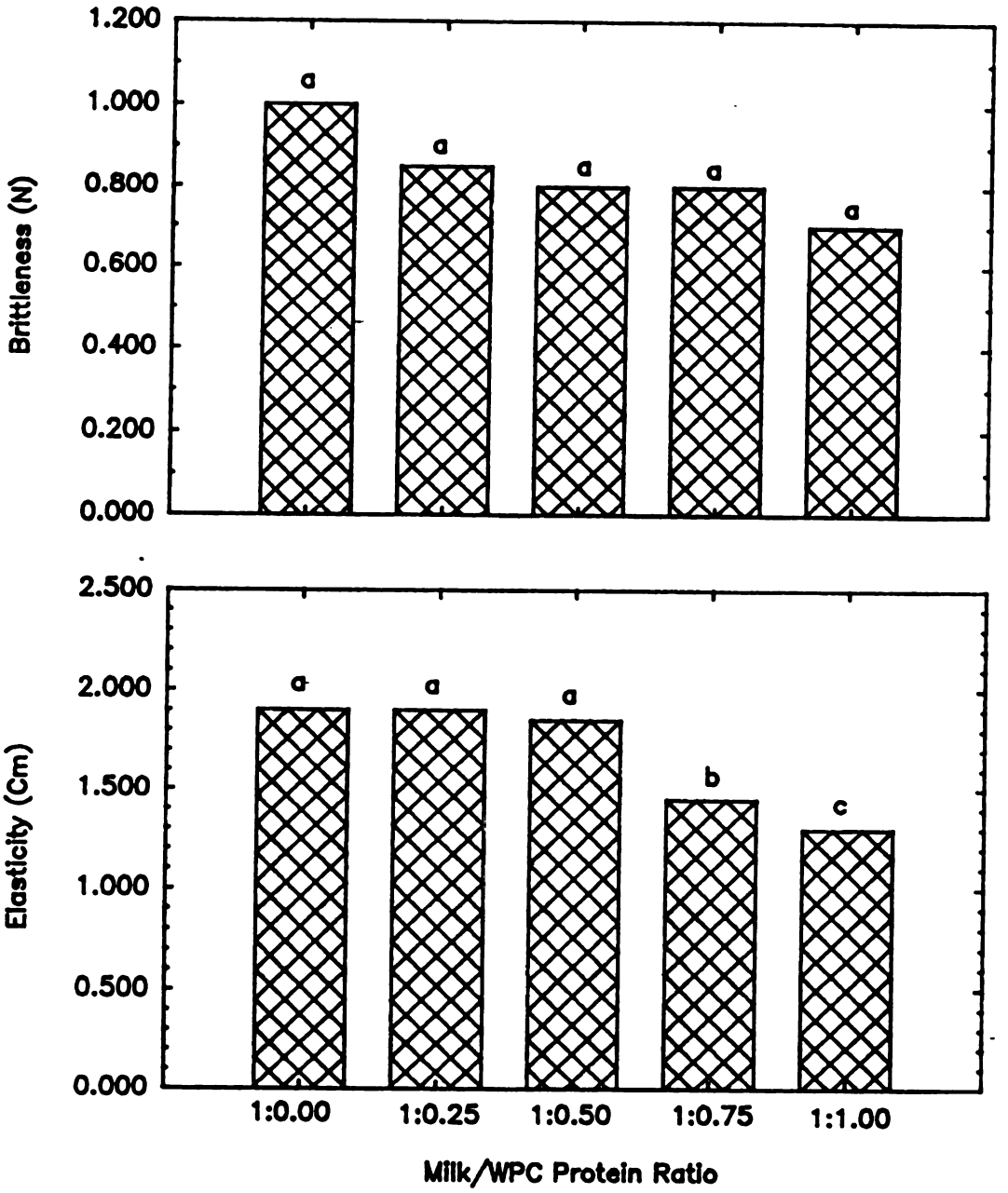

Figure 38. Elasticity and brittleness of Domiati cheese made from whet protein concentrate-supplemented whole milk. [a,b,c]Means with the same letters are not significantly different at P< 0.01. N= Newtons.

presumably an to effect of the whey proteins, resulted in a tendency toward more open and crumbly cheese.

Electrophoresis

The proteins of the cheeses made in this work, were subjected to electrophoresis. The densitograms resulting from scanning the electrophoretic gels of control and WPC-supplemented cheeses are illustrated in Figure 39. To aid in the identification of protein components in the electrophoretic pattern of cheese, known protein fractions were subjected to Disc PAGE at the same time. The main visible protein bands were identified as α- and β-casein, α-lactalbumin and β-lactoglobulin. Comparing the pattern of control cheese and WPC-supplemented cheese revealed a decrease in the relative peak areas of α-casein and β-casein as the addition of WPC increased. On the other hand, The peak area of α-lactalbumin increased as the proportion of WPC added to the whole milk increased. The peak area of β-lactoglobulin also increased with rising supplementation. The mobilities of β-casein and β-lactoglobulin are similar. Thus, the overlap of β-lactoglobulin and β-casein account for the increased peak area as a result of the accumulation of WPC. Comparison of PAG patterns from various WPC-supplemented cheeses revealed substantial differences in the number of bands in each of individual zone. These result is due to the interaction of whey protein and casein micelles

when heated together, they complex with each other primarily through intermolecular S-S bonds between β-lactoglobulin and k-casein (Smits and Van Brouwershaven 1980).

Whey Components

The mean composition of whey obtained at the end of the draining step during the manufacturing of control Domiati cheese and WPC-supplemented cheese with various levels of whey protein supplementation was studied and the data are presented in Figure 40. As the supplementation ratio increased in the whole milk mixtures, the loss of total solids, protein and ash in the drained whey increased. As the data reveal, total solids ranged from 6.37 % (unsupplemented control), to 9.0 % (maximal supplementation). Protein in the drained whey ranged from 1.1 to 3.2 %, and ash from 0.39 to 1.55 %, respectively.

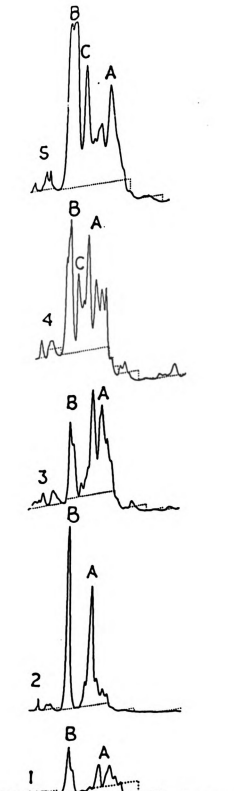

Figure 39. Discontinuous polyacrylamide gel electrophoresis densitograms of Domiati cheese made from WPC supplemented whole milk. A= α-casein B= β-lactoglobulin & β-casein, C= α-lactalbumin. Milk/ WPC protein ratio: 1=control, 2=1:0.25, 3=1:0.5, 4=1:0.75 and 5=1:1.

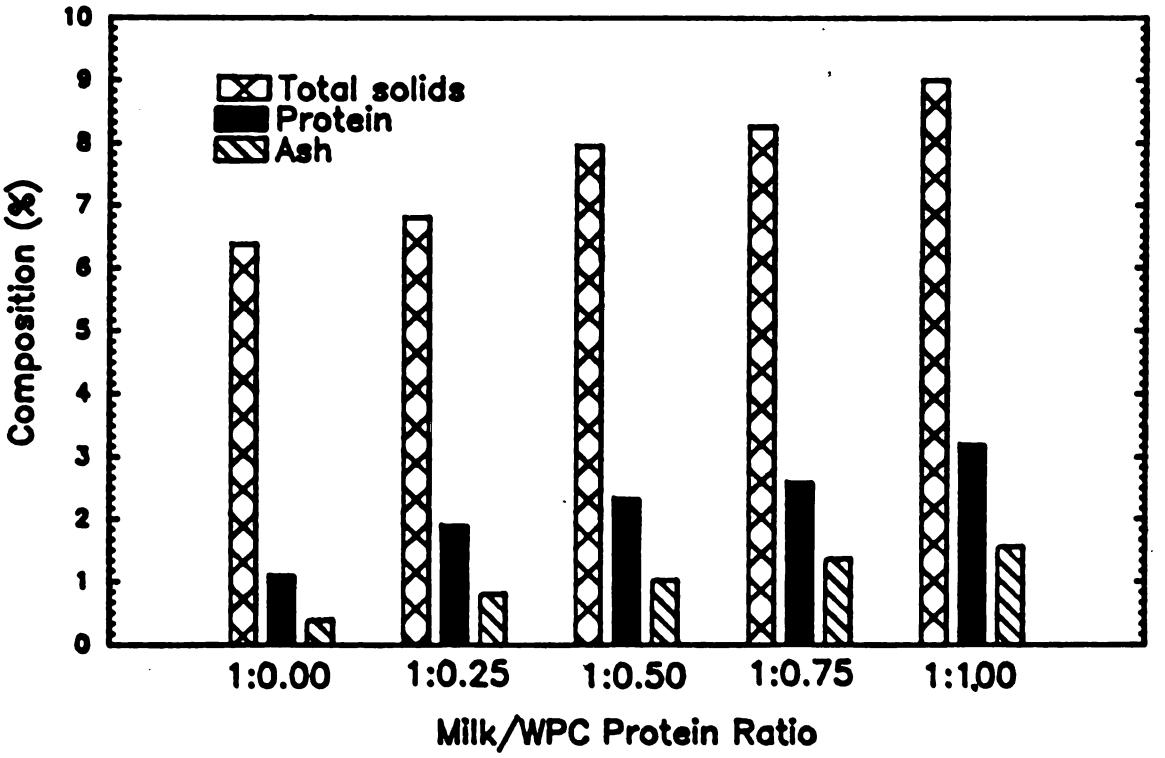

Figure 40. Components of whey from Domiati cheese made from WPC supplemented whole milk.

CONCLUSIONS

Ultrafiltration technology is widely accepted as a preconcentrating step in cheese making and used commercially for manufacturing many cheese varieties all over the world. UF can be envisioned as a routine process in many dairy plants of the future.

The technical feasibility of direct ultrafiltration in the manufacture of Egyptian soft-white "Domiati" cheese has been successfully demonstrated.

A spiral wound membrane with a cut off of 5,000 Daltons was used to concentrate and fractionate whole milk, skim milk and sweet whey using ultrafiltration (UF)and diafiltration (DF)processes.

Retentate produced by DF was higher in protein whereas the retentate formed by UF contained more total solids. Electrophoretic studies showed that β-lactoglobulin was the major whey protein in UF and DF retentates. Of the 19 minerals analyzed, Ca, Mg, K , P, Cu and Fe were higher in the retentate than in the permeates from whole milk, skim milk and whey. Complete retention of the fat phase was obtained by UF and DF. The free fatty acid (FFA) content, with the exception of some short-chain ones, increased with the degree of concentration by UF and DF. Whey protein

concentrate was richer in essential amino acids than that of total milk protein or casein. Also, the permeation rate decreased as the degree of concentration increased during UF and DF.

Fresh Egyptian soft-white "Domiati" cheese made from ultra/diafiltration-whole milk retentate, using the MMV technique, was compared to fresh cheese made from whole milk produced conventionally. Nitrogen values and fractions representing total protein, non-casein nitrogen, total albumin and soluble nitrogen of UF-derived cheese were higher ($p<0.05$) than the corresponding fractions of conventionally prepared cheese. Electrophoretic patterns of protein extracted from fresh UF-cheese confirmed the presence of a higher content of α-lactalbumin and β-lactoglobulin than found in conventional cheese. There was a higher concentration of free fatty acids and free amino acids in UF-cheese than in conventional cheese. Texture characteristics of fresh UF and conventional Domiati cheese showed significant differences ($p<0.05$) with regard to hardness, cohesiveness, chewiness, gumminess, adhesiveness and elasticity. The UF-cheese was harder and more adhesive than conventional cheese, whereas conventional cheese was characterized by more pronounced chewiness and gumminess than UF-cheese. The sensory evaluation of flavor, body/texture and color by a panel of judges also indicated that fresh UF-cheese was more uniform and creamy than conventional cheese.

Also, retentate produced by UF/DF whole milk was freeze-dried and stored. Dried retentate was reconstituted with warm water, for manufacturing "Domiati" cheese, utilizing the MMV method which gave a curd in 10 min. Cheese was ripened by two methods: i) packed in polyethylene-lined aluminum pouches sealed under vacuum and, ii) in plastic containers with 8% brine. The packed cheeses were ripened at 7°C for eight weeks.

Vacuum treatment produced cheese with close texture and smooth surface, which aid in preventing mold growth and spoilage.

Pouch and brine ripened cheeses (4, 6 and 8 weeks) showed significant increases in total solids, fat, ash and protein ($p < 0.05$) as compared to fresh cheese. Statistical comparisons of the two ripening methods at 2, 4, 6 and 8 weeks of ripening showed that pouch-cheese had more protein, fat, sugar and ash than cheese ripened in brine.
The increase in the non-casein nitrogen, non-protein nitrogen and ripening index of pouch cheese was more pronounced than in brine cheese throughout the ripening period. After two months of ripening, the liberation of free fatty acids as well as free amino acids was higher in pouch cheese than in brine cheese.

These differences between the two ripening procedures were related mostly to hardness, cohesiveness and gumminess of the cheese, which were significantly higher for pouch-ripened cheese than brine-ripened cheese as indicated from

the sensory evaluation scores.

Pouch-cheese ripened in the absence of brine were generally attractive, uniform and creamy in color, firm in body, possessing a waxy, buttery-smooth texture and a pleasant flavor. This new method for ripening Domiati cheese in vacuum pouches resulted in improved cheese yields and an acceleration of cheese ripening. Finally, the efficiency of the ripening methods was monitored by measuring the actual loss of fat and protein in the serum produced from pouch- and brine- ripened cheese.

Vacuum pouch-ripened cheese produced by ultrafiltrate and conventional procedures and ripened for three months at 10°C showed significant differences in gross composition with higher values for UF-cheese. After three months of ripening, the increase in ripening indices, free amino acids and free fatty acids was more pronounced in UF-cheese than in conventional cheese. Electrophoretic patterns of the two cheeses confirmed that protein degradation occurred more extensively in UF-cheese than in conventional cheese.

Color and textural qualities in ripened UF and conventional cheese showed a significant difference ($p<0.05$) as compared to their fresh counterpart. Both cheeses increased in hardness, cohesiveness and gumminess after one month, followed by a decrease after three months of ripening. While young cheese exhibited more elasticity than aged cheese, the conventional cheese possessed greater elasticity than the UF-cheese.

The technical feasibility of using ultrafiltration indirectly in the manufacture of Domiati cheese through supplementation of whey protein concentrate (20 % total solids and 8.38 % protein) has been successfully demonstrated.

Small vat trials were conducted on Domiati cheese made from cheese milks supplemented with whey protein concentrate at protein/protein ratio 1:0 1:0.25, 1:0.5, 1:0.75, 1:1 and 1:2 to determine critical processing parameters. The addition of WPC to the whole milk produced a significant increase ($P<0.05$) in total solids and protein, from 12.5% and 3.5% up to 28.1 and 10.1 % respectively. Reduction in coagulation time was observed as supplementation level with WPC increased with a demonstrated dramatic increase in yield ($p<0.05$). There was also a significant increase in total solids, lactose and ash, non-casein protein and whey protein as the protein supplementation increased up to 1:0.75. On the other hand, the percentage of fat decreased significantly as supplementation was increased. The electrophoretic patterns of WPC-supplemented cheese indicated a decrease in the peak areas corresponding to α-casein and β-casein, when compared to those of control cheese. On the other hand, α-lactalbumin and β-lactoglobulin in WPC-supplemented cheese increased with the amount of WPC added. There was an increase in essential and nonessential amino acids as the addition of WPC increased. Whey protein concentrate-supplemented cheese with 1:0.25 and

1:0.5 protein ratios attained the highest scores for flavor, texture/body and color. However these scores decreased as the supplementation level was increased to higher levels. Whiteness value (L) indicated a significant decrease in color, while greenness and yellowness (-a) and (b) showed significant increases with supplementation. The mechanical properties of cheese revealed that there were significant differences ($P<0.05$) between Domiati cheese produced from unsupplemented milk and that produced with various levels of supplementation. In regard to whey drained during cheese manufacture, as the level of WPC increased in the milk mixtures, the loss of total solids, protein and ash in the whey increased from 6.37%, 1.1% and 0.39% to 9%, 3.2% and 1.55%, respectively.

FUTURE RESEARCH

The investigation into the manufacture of Domiati cheese by supplementing the whole milk with WPC obtained by ultrafiltration raised questions which merit further study:

1- Run experiments on the ripening of Domiati cheese made from WPC-supplemented whole milk.

2- In order to minimize losses in the cheese whey, the following should be tried:

a) Acid coagulation of the WPC-supplemented whole milk, without rennet. Acid could be added as glocono-delta-lactone or developed by lactic acid starters.

b) Acid and heat coagulation. Since heat may destroy the ripening microorganisms and enzymes, either lipase or protease should be added to the curd.

3- To gain all the benefit of ultrafiltration of milk and whey. Another experiment should be carried out in which WPC would be added to whole milk preconcentrated by ultrafiltration (milk retentate).

4- Further studies need to be conducted to investigate these different processes comparatively from the economic standpoint.

APPENDICES

Appendix (1)

Electrophoresis solutions

1- Running gel buffer,pH 8.9,0.380 M Tris HCL as prepared by dissolving 4.6018 g Tris (hydroxymethyl) aminomethane in about 95.0 mL distilled water; 42.0 g of urea were added to make the buffer 7 M. The pH was adjusted to 8.9 with concentrated HCL and the volume was made to 100.0 mL with distilled water.

2- Stacking gel buffer,pH 6.7, 0.062 M Tris-HCL,7 M urea , was prepared by dissolving 0.7508 g of Tris in about 95.0 mL distilled water ;42.0 g of urea were added to make the buffer 7 M. The pH was adjusted to 6.7 with concentrated HCL and volume made to 100.0 mI with distilled water. 3- Electrode buffer,pH 8.3,.046 M Tris glycine,was prepared by dissolving 16.71 g of Tris in about 2100 mL distilled water. The pH was adjusted to 8.3 with 2 M glycine solution and the volume was made to 3000 mL with distilled water.

4-Running gel solution, 25 %(w/v) acrylamide solution, was prepared by dissolving 24.83 g of acrylamide monomer and 0.64 g of NN- Methylenebisacrylamide (BIS) in 75 mL of the running gel buffer and making it to 100 mL with the same buffer. This provided a stock solution with 25 % total acrylamide.

5-Stacking gel solution,6.25 % (w/v) acrylamide solution ,

was prepared by dissolving 5 g of acrylamide monomer and 1.25 g of BIS in 75mL of the stacking gel buffer and making it to 100 mL with the same buffer.

6-Ammonium persulfate solution ,5%(w/v) 7 M urea,was prepared by adding 0.625 g ammonium persulfate and 5.25 g urea in 12.5 mL of distilled water. The solution was prepared every two days.

7- N,N,N,N- tetramethylethylene diamine (TEMED).

8-Bromophenol blue ,1% (w/v) solution was made using stacking gel buffer.

9-Saturated sucrose solution was made using gel buffer.

10-Staining solution :it contained 25 % (v/v) isopropanol,10 %(w/v) acetic acid and 0.05 % (w/v) Comassie brilliant blue R-250 in distilled water.

11 - Destaining solution: 5 % (v/v) acetic acid and 10 % (v/v) Isopropanol in distilled water.

Gel Preparation

1-The dry tubes were marked with a felt-tip pen at distances 10.0 and 11.6 cm from the bottom.

2-The bottom of each tube was fitted with a small square of parafilm. Tubes were then placed in a leveled rack.

3-A gel solution of the desired concentration (9%) was prepared by combining 9 mL of running gel solution and 15.7 mL running gel buffer to give a final volume of 24.7 mL.

4- To this gel solution , 20 uL of TEMED and 0.3 mL of

ammonium persulfate solution were added.

5-The gel solution was transferred to the glass tubes with a syringe fitted with an 18 gauge needle. Each tube was filled to the 10.0 cm mark , carefully overlayered with distilled water,and allowed to polymerize overnight.

6-After polymerization of the running gel,the water layer was removed and the top of the running gel was rinsed with stacking gel buffer. The buffer was removed from the running gel.

7-The stacking gel was prepared by mixing 5.0 mL of the stacking gel solution with 1 g sucrose. The volume of the solution was made to 10.0 mL with stacking gel buffer; 40 uL of ammonium persulfate and 10 ul of TEMED were added.

8-Each tube was filled to the 11.6 cm mark with stacking gel, overlayered with water ,and allowed to polymerized for one hr.

APPENDIX (2)

Amino Acid Analysis

Procedure for derivatives of amino acids

30 uL of standard and hydrolyzed amino acid samples were placed in a reaction vial, 10 uL of internal standard was added to each vial. Put the vials into the Millipore reaction vessel and connect to work station, slowly turn on vacuum and allow to dry completely. 20 uL of redrying solution consisting of ethanol:Na acetate:triethylamine (2:2:1) was added with gentle shaking and installed into the work station until dry.

Phenylthiocarbamate (PTC)-amino acids were formed by adding 20 uL of fresh derivative reagent consisting of ethanol: triethylamine (TEA): water: phenylisothiocyanate (PITC) 7:1:1:1 to the dried samples and incubated for 20 min. at room temperature to complete the reaction between the free amino acid and the PITC. Vials was washed with 10 uL of methanol; methanol: H_2o (50:50); methanol in sequential order and dried to drive off excess PITC. 200 uL of diluent was added to each sample, pipetted into volume restriction inserts. Fifteen to 30 uL in volume were analyzed using a reverse phase HPLC model ALC 204 Liquid chromatograph (Water Assoc.) which consisted of two solvent delivery systems, model M440, and a sample auto-injector, Model M710B. Amino acids were separated by a 15 cm x 3.9 mm

pico-Tag analytical column. The solvent system consisted of (A) an aqueous buffer and (B) 60% acetonitrile in water. The buffer was 0.14 M sodium acetate containing 0.5 ml TEA/L of Na acetate solution.

APPENDIX (3)

Mineral Analysis

Operating Parameters for Mineral Analysis by Atomic Absorption.

Argon Gas Flow:	Coolant:	18	L/min.
	Auxiliary:	1	L/min.
Sample:		0.5	L/min.
R.F. Power:	Incident:	1.1	KW
	Reflected:	< 5	W
Integration Time:	Two 4 sec observations with one 4 sec background correction.		
Observation Height:	15 mm above top work coil.		
Sample Uptake:	Peristaltic pump at 1.5 ml/min.		

Wavelengths (A^{o})

Ca	3706.00	Na	3302.98
Fe	2599.40	P	2149.14
Pb	2203.53	Mg	2790.79
Zn	2138.56	K	4044.14
Mn	2576.10	TI	1908.64
AI	2020.30	Hg	1942.27
Cu	3082.15	Cd	2288.02
Se	1960.26	Cr	3247.54
Co	2286.16		

Appendix (4)

Table 4A. Rate and volume of permeate derived from whole milk by diafiltration process

Permeate removed (% of milk)	Concentration factor	Permeate Flux L/m^2 /hr
0.0	1.0	36.1
8.3	1.1	27.8
33.3	1.5	22.8
66.7	3.0	18.5
72.2	2.0	19.4
83.3	2.7	25.9
100.0	4.0	17.2
113.0	4.5	5.9

The data represent average of three determinations.

Table 4B. Rate and volume of permeate derived from whole milk by ultrafiltration

Permeate removed (% of milk)	Concentration factor	Permeate Flux L/m^2 /hr
0.0	1.0	36.9
13.0	1.1	30.4
33.3	1.5	27.2
66.7	2.5	23.2
79.9	4.1	11.1
80.3	4.8	9.1

The data represent average of three determinations.

Table 4C. Rate and volume of permeate derived from Skim milk by ultrafiltration

Permeate removed (% of milk)	Concentration factor	permeate flux ($L/m^2/hr$)
0.0	1.0	50.0
7.4	1.1	41.0
21.1	1.3	35.0
40.0	1.7	31.0
58.0	2.4	29.0
72.0	3.6	25.5
82.0	4.2	18.3

Values are the average of three determinations.

Table 4D. Rate and volume of permeate derived from whey by ultrafiltration

Permeate removed (% of milk)	Concentration rate factor	Flux (permeate ($L/m^2/hr$)
0.0	1.0	78.0
13.0	1.2	71.0
33.3	1.5	45.0
56.0	2.6	36.0
73.0	3.7	31.0
83.0	6.0	27.0

The data represent the average of three determinations.

Appendix (5)

Table 5A. Change in retentate composition during diafiltration of whole milk

Permeate removed (% of milk)	VCR*	Composition (%)				
		Total solids	Ash	Protein	Lactose	Fat
		g/100 g				
0.0	1.0	12.2	0.8	3.1	5.0	3.4
8.3	1.1	12.7	0.9	3.6	4.5	3.8
33.3	1.5	18.0	0.9	7.6	4.2	6.0
66.7	3.0	23.9	1.2	8.5	4.1	10.2
72.2	2.0	17.2	0.8	6.8	2.2	7.5
83.3	2.7	19.2	0.9	7.6	2.1	8.5
100.0	4.0	26.0	1.2	8.6	2.0	14.5
113.0	4.5	32.6	1.5	13.6	1.9	15.5
(CF)		2.7	2.0	4.47	0.39	4.5

[a]volume concentration ratio represent the percent weight of the retentate to the original milk weight.

Values are the average of three determinations.

Table 5B. Change in retentate composition during ultrafiltration of whole milk

Permeate removed (% of milk)	VCR[a]	Composition (%)				
		Total	Ash	protein	Lactose	Fat
0.0	1.0	12.3	0.7	3.3	4.9	3.4
13.0	1.1	14.1	0.9	3.8	5.3	3.9
33.3	1.5	18.2	1.1	5.2	5.7	6.2
60.7	2.5	24.9	1.5	6.4	6.5	9.9
78.9	4.1	32.2	2.7	8.5	6.8	14.2
80.3	4.8	38.4	3.5	10.5	7.9	16.5
(CF)		3.12	4.7	3.23	1.6	4.8

[a]volume concentration ratio represent the percent weight of the retentate to the original milk weight.

The data represent average of three determinations.

Table 5C. Change in retentate composition during ultrafiltration of skim milk

permeate removed (% ofmilk)	VCR*	Composition (%)			
		Total solids	Ash	Protein	Lactose
0.0	1.0	8.8	0.8	3.0	5.0
7.4	1.1	9.5	0.9	3.3	5.1
21.1	1.3	10.9	1.2	4.6	5.1
40.0	1.7	13.0	1.5	6.3	5.3
58.0	2.4	15.1	1.9	7.9	5.4
72.0	3.6	17.0	2.3	8.6	6.0
82.0	4.2	19.5	2.6	10.0	6.9
(CF)		2.2	3.4	3.33	1.4

*volume concentration ratio represent the percent weight of the retentate to the original milk weight.

Values are the average of three determination.

Table 5D. Changes in retentate composition during ultrafiltration of whey

Permeate removed (%of milk)	VCR[a]	Composition (%)			
		Total solids	Protein	Ash	Lactose
0.00	1.00	6.6	1.1	0.5	5.0
13.0	1.15	6.7	1.3	0.5	5.2
33.3	1.50	7.8	1.8	0.6	5.4
56.7	2.60	9.0	2.7	0.9	5.7
73.3	4.10	13.0	5.4	1.1	6.5
83.0	6.00	15.7	7.1	1.6	7.0
(CF)		2.4	6.1	3.4	1.4

*volume concentration ratio represent the percent weight of the retentate to the original milk weight.

Values are the average of three determination.

Appendix (6)

Table 6A. Changes in permeate composition during ultrafiltration of whole milk

Permeate removed (% of milk)	Composition (%)			
	Total	Lactose	Protein	Ash
13.03	5.22	4.8	0.09	0.33
33.32	5.28	4.9	0.12	0.36
60.66	5.8	5.1	0.2	0.4
79.97	6.83	6.1	0.31	0.42
80.3	7.4	6.5	0.4	0.49
Average	6.1	5.48	0.224	0.4

Values are the average of three determinations.
Protein expressed by (N X 6.38).

Table 6B. Changes in permeate composition during diafiltration of whole milk

Permeate removed (% of milk)	Composition (%)			
	Total solids	Protein	Lactose	Ash
8.3	5.35	0.04	5.0	0.31
33.3	5.83	0.09	5.4	0.34
66.6	6.52	0.15	6.0	0.37
72.2	2.85	0.10	2.5	0.24
83.3	2.58	0.12	2.2	0.25
100.0	2.51	0.18	1.99	0.33
113.0	3.00	0.21	2.40	0.39
Average	4.10	0.127	3.64	0.318

The data is average of three determinations.
Protein expressed by (N x 6.38).

Appendix (7)

Table 7A. Percent recovery and response factor of individual fatty acids

Fatty Acids	Concentrations		Recovery (%)	Response Factor[b]
	Added	Recovered		
	mg/g			
C4:0	55	44.6	81	1.17±0.6
C6:0	43	42.7	99	0.96±0.4
C7:0				1.0[b]
C8:0	41	41.94	102	1.03±0.5
C10:0	43	44.9	104	1.04±0.2
C12:0	68	69.2	102	0.91±0.3
C14:0	113	115.2	102	0.95±0.4
C16:0	131	136.1	104	1.03±o.3
C17:0				1:0[b]
C18:0	91	91.9	101	1.05±0.5
C18:1	200	209.9	105	0.99±0.6
C18:2	50	49.3	99	0.98±0.8
C18:3	50	46.9	94	0.89±1.0

Means and standard deviations of three replications.
[b]Assigned.

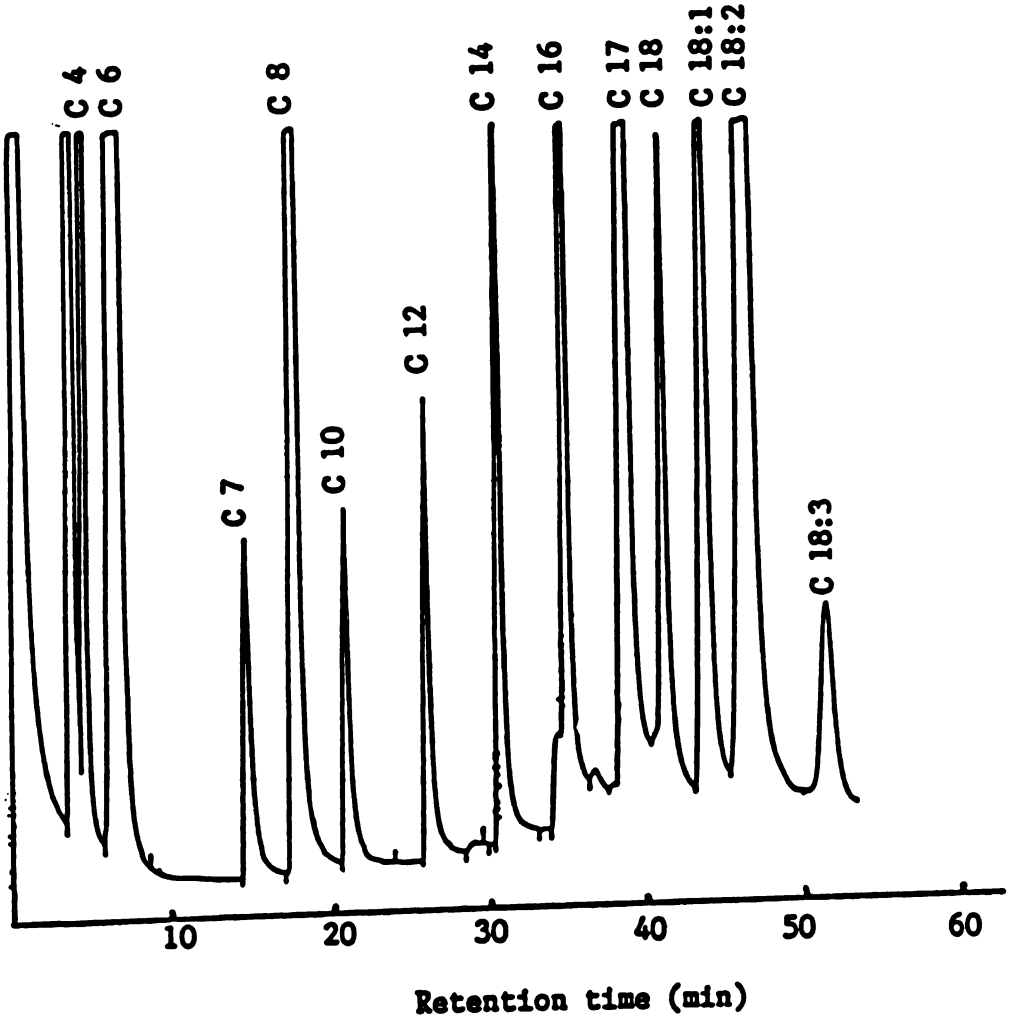

Figure 7A. Typical gas chromatogram of the standard free fatty acids (on 10 % SP-216-PS on 100/120 Supelcoport).

Appendix (8)

Table 8A. Peak areas and their distribution in whole milk,casein and TAN during ultrafiltration process

Time[a]	Peak area			Distribution[b]		
	(α-Cn)	(α-La)	(β-Cn+β-Lg)	(α-Cn)	(α-La)	(β-Cn+β-Lg)
			Milk			
1	301680	24464	222695	55.0%	4.46%	40.6%
2	483315	26267	365988	55.2 %	3.0%	41.8%
3	1418943	74301	1086653	55.0%	2.88%	42.12%
	β-Cn		α-Cn	β-Cn		α-Cn
			Casein			
1	207813		295323	41%		59%
2	427849		608372	41%		59%
3	783635		1189398	41%		59%
	β-Lg		α-La	β-Lg		α-La
			Total albumin nitrogen			
1	96501		28000	77.5%		22.4%
2	327163		70607	82.2%		17.8%
3	521659		100846	83.8%		16.2%

[a]Time :1-original, 2-half time run, 3-final run.
[b]Percent of total areas.
α-Cn= α-Casein, β-Cn= β-Casein, α-La= α-Lactalbumin and β-Lg= β-Lactoglobulin.

Table 8B. Peaks area and their distribution in skim milk during ultrafiltration[a]

Time[a]	Peak area		Distribution[b]	
	α-Cn	β-Cn	α-Cn	β-Cn
		Casein		
1	607807	395145	60.4%	39.6%
2	738168	477410	60.7%	39.3%
3	1714774	1014318	62.8%	37.2%
	β-Lg	α-La	α-Lg	α-La
		Total albumin nitrogen		
1	68702	21565	76.1%	23.89%
2	206316	48081	81.1%	18.9%
3	257319	55341	82.3%	17.7%

[a]Time :1-original, 2-half time run, 3-final run.
[b]Percent of total areas.

Table 8C. Peak areas and their distribution in whey during ultrafiltration process

Time[a]	Peak area		Distribution[b]	
	β-Lg	α-La	β-Lg	α-La
1	26711	8435	76.0%	24.0%
2	383697	40899	87.4%	12.6%
3	515196	61568	88.7%	10.6%
4	616845	67019	90.2%	9.80%

[a]Times: 1= original, 2= 15 min, 3= 30 min and 4= 60 min.
[b]Percent of total area.

Appendix (9)

DOMIATI CHEESE SCORE CARD

Name _____ **Sample #** _____ **Date** _____

Flavor _____

Excellent Very Good Average Lacks Flavor

Body _____

Very Hard Firm Good Weak Very Weak

Texture _____

Very Smooth Smooth Sl.Mealy Very Mealy

Color _____

Excessive Good Lacks Color

Figure 9A. Domiati Cheese Score Card used to evaluate the quality of Domiati cheese.

Appendix (10)

Table 10A. Comparison of compositional changes of Domiati cheese ripened in pouches and in 8% brine solution for 8 weeks

Time week	Total solids		Fat		Lactose		Protein		Ash	
	Pouch	Brine	Pouch	Brine	Pouch	Brine	Pouch	Brine	Pouch	Brine
2	37.64[a]	35.11[a]	16.63[a]	15.03[a]	1.4[a]	1.17[b]	13.24[a]	12.30[b]	6.20[a]	7.09[b]
4	39.00[a]	36.00[b]	17.23[a]	14.40[b]	1.3[a]	0.87[b]	13.5[a]	12.50[b]	6.55[a]	7.46[b]
6	40.07[a]	36.94[b]	17.55[a]	14.66[a]	1.2[a]	0.68[b]	14.6[a]	12.79[b]	6.77[a]	7.33[b]
8	42.15[a]	37.90[b]	18.30[a]	14.60[b]	1.03[a]	0.63[b]	15.3[a]	13.00[b]	6.96[a]	7.35[a]

[a,b]Means within rows for each components with the same superscripts are not significantly different ($p < 0.05$) at ripening time.
Values are the mean of duplicate analyses of three replications.

Table 10B. Nitrogen fractions of Domiati cheese made from reconstituted ultrafiltrated whole milk ripened in pouches and 8% brine solution for 8 weeks.

Ripening time (Week)	Pouch			8% brine		
	Nitrogen fraction					
	TP	NCN	NPN	TP	NCN	NPN
0	12.55	2.31	1.02	12.55	2.31	1.02
2	13.24	2.63	1.50	12.30	1.89	1.15
4	13.50	2.80	1.72	12.50	1.95	1.48
6	14.60**	3.05	1.90	12.79	2.10	1.40
8	15.30**	3.37*	2.20	13.00	2.22	1.48

Means within a column are significantly different at *, $p < 0.05$ and **, $P < 0.01$ from fresh (0 time). Standard error for TP, NCN, NPN are 0.4, 0.3 and 0.32 for pouches cheese; 0.22, 0.17 and 0.3 for cheese ripened in 8 % brine.

Table 10C. Texture profile analysis of Domiati cheese made from reconstituted ultrafiltrated whole milk and ripened in pouches for 8 weeks

	Ripening Time (Weeks)				Standard error
	0	2	4	8	
			Means		
Hardness	3.85	5.77*	8.33**	9.85**	0.44
Cohesiveness	0.63	0.52	0.65	0.74	0.10
Chewiness	5.09	5.91*	10.0**	12.39**	0.38
Gumminess	2.21	2.92	3.88*	6.21**	0.38
Elasticity	2.10	1.97	1.85	1.70	0.11

Sample with the same row are significantly differ at * p, <0.05 and **, p<0.01 from fresh (0 time).
Values are means and standard errors of three replications.

Table 10D. Texture profile analysis of Domiati cheese made from reconstituted ultrafiltrated whole milk and ripened in brine solution at for 8 weeks

	Ripening Time (Weeks)				Standard error
	0	2	4	8	
			Means		
Hardness	3.85	4.07	4.40	5.63**	0.32
Cohesiveness	0.63	0.68	0.57	0.58	0.12
Chewiness	5.09	5.54	4.82	6.04**	0.37
Gumminess	2.21	2.30	2.90	3.47	0.40
Elasticity	2.10	2.00	1.92	1.85	0.11

Sample with the same raw are significantly differ at * $p < 0.05$ and **, $p < 0.01$ from fresh (0 time).
Values are means and standard errors of three replications.

Table 10E. Components of whey of Domiati cheese made from ultrafiltrated whole milk ripened in pouches and 8 % brine solution for 8 weeks

Ripening time (week)	Composition[a] (%)			
	Pouch		8% brine	
	Fat	Protein	Fat	Protein
2	0.25[a]	3.70[a]	0.13[a]	0.52[a]
4	0.36[a]	3.99[a]	0.15[a]	0.79[a]
6	0.33[a]	4.00[a]	0.18[a]	0.88[a]
8	0.39[a]	4.19[a]	0.23[a]	1.16[a]

[a]Means within columns for each components with the same superscripts are not significantly different ($p < 0.05$) at ripening time.
Values are the mean of duplicate analyses of three replications.
Standard error for Fat, protein are 0.05, 0.49 for pouches ripening and 0.03, 0.21 for 8 % brine ripening.

Table 10F . Calculated true loss of protein and fat from Domiati cheese during ripening in pouches and brine solution

Ripening time (week)	Weight (%)			
	Pouch		Brine	
	Protein	Fat	Protein	Fat
2	1.12	0.10	2.10	0.25
4	1.59	0.12	2.37	0.45
6	1.70	0.16	3.10	0.69
8	2.00	0.25	3.48	0.89

Values are the mean of duplicate analyses of three replications.

Appendix 11

Table 11A. Ripening index of fresh and ripened conventional and ultrafiltrated Domiati cheese for three months in pouches

	Ultrafiltrated			Conventional		
	Ripening period (Months)					
	Fresh	1	3	Fresh	1	3
ARE	27.7	34.9	43.7	11.2	14.1	22.7
RE	13.9	18.1	29.1	10.9	11.1	18.6
RD	10.2	15.5	17.4	7.9	9.7	12.7

Values are the mean of duplicate analyses of three replications. ARE= Apparent Ripening Extension, RE= Actual Ripening Extension and RD= Ripening Depth Index.

Table 11B. Texture profile analysis of fresh and ripened UF- and conventional Domiati cheese

Properties	Conventional	Ultrafiltration
Fresh	**Means ±SD**	
Hardness (N)	8.4±1.82	8.2±1.3
Cohesiveness ($A_2\backslash A_1$)	0.7±0.23	0.3±0.03
Chewiness (N\Cn)	13.1±6.14	3.6±0.15
Gumminess (N)	5.9±2.70	2.5±0.02
Elasticity (Cn)	2.3±0.12	1.4±0.2
Brittleness (N)	0.1	0.05
1 Month	**Means ±SD**	
Hardness (N)	15.1±0.75	12.3±0.6
Cohesiveness ($A_2\backslash A_1$)	0.8±0.14	0.4±0.06
Chewiness (N\Cn)	22.9±4.1	6.4±0.6
Gumminess (N)	11.5±6.9	4.9±0.5
Elasticity (Cn)	2.0±0.0	1.2±0.0
Brittleness (N)	0.5	1.3±0.2
3 Months	**Means ±SD**	
Hardness (N)	9.9±0.4	9.6±0.8
Cohesiveness ($A_2\backslash A_1$)	0.7±0.08	0.6±0.2
Chewiness (N\Cn)	11.3±2.6	7.5±1.8
Gumminess (N)	6.8±0.4	5.4±0.7
Elasticity (Cn)	1.7±0.5	1.4±0.05
Brittleness (N)	2.3±0.0	1.5±0.2

Values are means and standard deviations of duplicate of three replications.
N= force (Newtons), $A_2\backslash A_1$= areas of second\ first peak.

Table 11C. Color changes of fresh conventional and ultrafiltrated Domiati cheese and ripened for three months in pouches

Conventional Cheese			Ultrafiltration Cheese		
L	a	b	L	a	b
		Fresh			
93.3±0.25	-3.3±.1	10.0±0.15	83.3±0.2	-3.8±0.2	12.6±0.2
		1 Month			
91.2±0.3	-3.5±0.1	11.7±0.2	79.9±0.9	-3.1±0.2	13.4±0.4
		3 Months			
85.1±0.3	-1.5±0.2	13.5±0.3	74.4±0.1	-2.0±0.4	20.0±0.4

Values are the means and standard deviations of duplicate analyses of three replications.

Appendix (12)

Table 12A. Yields and yield efficiencies of Domiati cheese made from whey protein concentrate-supplemented whole milk

Added protein ratio	Yield Kg per 100 kg supplemented mixture	Kg Cheese per kg total solids	Kg Cheese per kg protein
1:0	17.50[b]	1.4[a]	5.0[bc]
1:0.25	21.90[a]	1.7[a]	5.8[a]
1:0.5	22.94[a]	1.7[a]	5.7[a]
1:0.75	24.80[a]	1.8[b]	5.4[ab]
1: 1	22.00[a]	1.5[a]	4.5[c]

[a,ab,b,bc,c]Means within column with the same superscripts are not significantly different ($p < 0.05$).
Values are the mean of duplicate analyses of three replications.

Table 12B. Protein fraction of Domiati cheeses made from whey protein concentrate-supplementing whole milk

Added protein ratio	Protein Fraction				Protein Distribution			
	CN	NCN	NPN	WP	CN/TN	NCN/TN	NPN/TN	WP/TN
1: 0	13.52	3.32[a]	2.61[a]	0.71[a]	80.2	19.7	15.5	4.2
1:.25	13.27	5.17[c]	2.87[b]	2.3[c]	72.0	28.0	15.5	12.5
1:.5	13.70	5.30[c]	2.90[b]	2.4[c]	72.1	27.9	15.2	15.3
1:.75	13.10	4.50[b]	2.50[a]	2.0[c]	74.4	25.5	14.2	11.3
1:1	10.50	3.50[a]	2.50[a]	1.0[b]	75.0	25.0	17.8	7.1

[a,b,c]Means within column with the same superscripts are not significantly different ($p < 0.05$).
CN, NCN,NPN and WP expressed casein, non-casein nitrogen, Non-protein nitrogen and whey protein compounds, respectively.

Table 12C. Color changes of fresh Domiati cheese made from whey protein concentrate-supplemented whole milk

Color	Added Protein Ratio				
	Control	1:0.25	1:0.5	1:0.75	1:1
L	96.75[a]	90.0[b]	84.83[c]	80.06[d]	74.00[e]
a	-2.2[d]	-5.5[c]	-6.2[bc]	-7.5[a]	-7.0[ab]
b	13.7[d]	17.7[c]	18.5[b]	19.0[b]	20.0[a]

[a,b,c,d,e]Means within column with the same superscripts are not significantly different ($p < 0.05$).
L = indicates lightness(100 =perfect white)

a = + indicate redness; - indicate greenness; 0 = gray

b = + indicate yellowness; - indicate blue; 0 = gray

Table 12D. Texture profile analysis Domiati cheese made from whey protein concentrate-supplemented whole milk obtained with Instron Universal Testing Machine.

Texture Profile Analysis	Added Protein Ratio				
	Control	1:0.25	1:0.5	1:0.75	1:1
Hardness	18.05[a]	4.10[b]	3.20[b]	2.30[b]	1.27[b]
Cohesiveness	0.53[a]	0.40[a]	0.43[a]	0.48[a]	0.49[a]
Chewiness	18.03[a]	2.47[b]	2.06[b]	1.60[b]	1.00[b]
Gumminess	9.49[a]	1.36[b]	1.06[b]	0.90[b]	0.60[b]
Elasticity	1.90[a]	1.90[a]	1.85[a]	1.45[b]	1.30[c]
Brittleness	1.00[a]	0.85[a]	0.80[a]	0.80[a]	0.70[a]

[a,b,c,d]Means within row with the same superscripts are not significantly different ($p < 0.05$).
Values are the mean of duplicate analyses of three replications.

Table 12E. Components in whey from Domiati cheese made from whey protein concentrate-supplemented whole milk

Drained Whey Composition	Added Protein Ratio				
	Control	1:.25	1:.5	1:.75	1:1
			(%)		
Total solids	6.77^{c}	7.15^{c}	8.10^{b}	8.51^{b}	9.45^{a}
Protein	1.15^{e}	2.03^{d}	2.43^{c}	2.65^{b}	3.30^{a}
Ash	0.43^{b}	0.85^{ab}	1.07^{ab}	1.81^{ab}	1.91^{a}

^{a,b,c,d,e}Means within row for each component with the same superscripts are not significantly different ($p < 0.05$). Values are the mean of duplicate analyses of three replications.

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