CHARACTERIZATION OF ULTRA-PASTEURIZED (UP) FLUID MILK: MICROBIAL, SENSORY AND CHEMICAL PROPERTIES

A Thesis

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by

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ABSTRACT

The microbial, sensory and chemical characteristics: vitamins A and D, Tyrosine Value (TV) and Acid Degree Values (ADV), of 2% Ultra Pasteurized (UP) fluid milk from 2 plants in New York State (NYS) were studied over 10 week periods. Four batches of 5 randomly selected samples were obtained from each of the UP processing plants at approximately 3 months intervals. The samples were stored at 7+/-1°C and analyzed during the first, fourth, seventh and tenth weeks of post processing storage. Vitamin A and D contents were determined using HPLC, Tyrosine Value as measured by Juffs' method and ADV using the modified Copper Soap method. Raw skim and whole milk used as raw materials for 2% UP were analyzed in the first week for the same chemical characteristics as the 2% UP milk.

Microbial analysis on the raw milk samples included Standard Plate Counts (SPC), Psychrotrophic Plate counts (PPC), Coliform Counts, Gram-negative counts and Laboratory Pasteurized Counts (LPC). The 2% UP samples were examined for SPC, PPC and Heat Resistant Spore Formers (HRSF).

The microbial quality of the raw milk varied among batches and factories but in all cases, there was no growth on plates inoculated with the 2% UP milk samples over the 10 weeks of storage. This implies that the processing conditions adequately destroyed the microbes present in the raw milk and no post-processing contamination

occurred. The 2% UP milk was judged by a panel of 10 trained panelists as fair (mean score 7.0-7.5) throughout the 10 weeks.

An increase (30 to 50%) was observed in the Tyrosine Values of the 2% UP milk over the 10 week period. The Tyrosine Values encountered (65-350 μg/ml) however, were below the levels at which flavor would be affected (1500μg/ml). Similar trends (increase with time over the 10 week period) were observed in the ADV but still the ADVs observed (0.5 - 0.7 meq FFA/liter) were below the levels at which rancidity could be detected (1.0 - 1.5 meq/liter).
During the first week, Vitamin A and D levels were within the legal fortification requirements of 2000-3000 IU/Qt and 400 IU/Qt. respectively. An increase was observed at weeks 4, 7, and 10 possibly due to interference from degradation products associated with the vitamins. In the tenth week the vitamin A and D levels returned to approximately that observed in the first week, possibly as the degradation products separated out and away from the vitamins.

BIOGRAPHICAL SKETCH

Dorothy Ndawula Nakimbugwe was born in Luwero district, Uganda on September 22, 1968. She spent her early childhood in Luwero before moving with her family to Gaba Uganda. Dorothy graduated from Namagunga college in November 1988 and joined Makerere University in October 1989. She received her B. Sc. Food science in January 1994 and joined Cornell University in August 1994 to pursue M.S. Food science. Dorothy graduated from Cornell University in August 1996 soon after which she returned to Uganda to pursue a career in research and teaching.

Eri baganda bange benenyumirizaamu

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INTRODUCTION

The beverage market is highly competitive, requiring constant monitoring by producers in order to keep their products competitive. Fluid milk, as one of the many beverages on the market, is currently faced with increasing competition from soft drinks, ready-to-drink teas and fruit juices. As compared to the other beverages, milk and milk-based beverages are under-represented in key distribution channels such as convenience stores, vending machines and food service businesses (Coates, 1996). Such outlets generally require products with longer shelf-life than the 10 to 22-day shelf-life offered by conventionally pasteurized fluid milk.

The conventional and major retail outlet for milk in the U.S. has been the supermarket, where milk sales have remained flat at about $18 billion over the period 1993 to 1995 (Coates, 1996). In the same period, soft drink sales in supermarkets, which account for only 33% of total soft drink sales, have increased by about $3 billion. The bulk of soft drink sales occur from vending machines, convenience stores and food service outlets. There is a need to increase the availability of milk and milk-based products in retail outlets other than supermarkets by developing more stable Extended Shelf-life (ESL) milk products which can better meet vending and food service requirements.

The short shelf-life of conventionally pasteurized milk makes it unsuitable for retail outlets that require more stable products. The short shelf-life also poses other marketing and distribution problems which become even more severe when the milk has to be transported over long distances to the consumers. By the time the milk gets to the retail

outlets, it may be near the end of its shelf-life, yet consumers are always seeking to buy the most fresh product. The short shelf-life, therefore, makes frequent deliveries necessary so as to have fresh supplies, which in turn increases marketing and distribution costs.

Fluid milk processing definitions

Three major processing methods currently dominate the U.S. fluid milk industry. The first and most commonly used is conventional pasteurization. In a Low Temperature Short Time processing (LTST) method, the milk is heated at not less than 62.8 °C (145 °F) and not more than 65.6 ° C (150 °F) for at least 30 minutes. In a High Temperature Short Time processing (HTST) method the milk is heated at not less than 71.7 °C (161°F) for at least 15 seconds (Komorowski and Early, 1992). The purpose of pasteurization is to destroy pathogenic microorganisms in the milk to ensure safety of the product.

Ultra-High Temperature (UHT) processing involves minimum holding temperatures of 137.8°C (or 280°F) for at least 6.5 seconds (Dunkley and Stevenson, 1987). UHT products are commercially sterile. All viable microorganisms in the raw product are destroyed, the products are aseptically packaged and stable for up to 2 years at ambient temperatures.

The third and most recently introduced method of heat processing for milk is Ultra-Pasteurization (UP). UP products have not been clearly defined but broadly include all milk processed at temperatures above those used for pasteurization. Unlike UHT products, UP products are not necessarily aseptically packaged and therefore, require refrigerated storage. Cousin (1982) defined UP products as those

receiving a heat treatment of 280°F (137.8 ° C) or above for at least two seconds followed by refrigerated storage. Blake et al. (1995) also included products receiving heat treatments between 100 and 145 °C (121 and 293 °F) with an expected refrigerated shelf-life of 15 to 30 days.

UHT processing greatly extends the shelf-life of milk but UHT milk is generally less acceptable to American consumers than conventionally pasteurized milk (Blake et al., 1995). Reasons for the poor acceptance of UHT milk include presence of a highly cooked flavor which consumers find unacceptable and a long un-refrigerated shelf-life which they associate with less fresh products. UP processing results in more acceptable products than UHT milk, with a less pronounced cooked flavor (Blake et al., 1995). In addition, UP products require refrigerated storage. This makes them comparable to conventionally pasteurized milk so that although they are coded for extended shelf-life, they may still be perceived as fresh and, therefore, more acceptable than UHT.

Other off-flavor concerns, besides cooked flavor, in UP milk are those that originate from heat resistant proteolytic and lipolytic enzymes produced by psychrotrophic bacteria in raw milk. The bacteria do not survive pasteurization (Suhren, 1989) but their thermoduric enzymes do (Mottar, 1989; Champagne et al., 1994). Lipolytic enzymes hydrolyze lipids into free fatty acids which give the milk a rancid favor. Similarly, proteolytic enzymes hydrolyze proteins into amino acids and peptides which have bitter flavors (Champagne et al., 1994). The products of enzyme hydrolysis may be minimal in the product immediately after

processing but they build up with time and may contribute to reduced sensory acceptability (Dunkley and Stevenson, 1987).

UP products are not always sterile. Post-processing contamination may occur when processed milk comes into contact with contaminated bodies or surfaces (Cousin, 1982). The long shelf-life of UP products allows contaminating bacteria time to grow, causing potential health hazards and product loss.

Milk is an important component of diets, especially for children. It was selected to be a carrier for vitamins A and D, which are essential nutrients in human diets. It is therefore, essential to ensure that milk can be relied on as a source of these vitamins. This is not always the case. Possible causes of fortification inconsistencies are inaccurate addition, vitamin destruction during heat processing (processing losses) and storage losses due to degradation with time. There is currently no agreement among researchers on the effect of UHT processing on vitamins A and D (Renken and Warthesen, 1993).

Very little published research currently exists on the microbial, sensory and nutritional properties of Ultra-Pasteurized (UP) products and how they change with time. As such, there is a lack of information concerning the quality and safety of UP products, especially among consumers. Establishing that the quality of UP does not deteriorate during its extended shelf-life could greatly increase its use by consumers who want the benefits of a more stable product such as less frequent shopping.

LITERATURE REVIEW

Trends in the dairy industry

The dairy industry in the US has experienced periods of high and low production since the 1950's. Some of these changes are related to dairy policies, prices of inputs such as feeds and the market price of milk. Changes in milk production and product consumption affect the structure of the dairy industry in terms of the number, size, locations and operations of milk processing and handling plants. In NY State, there has been a decrease in the numbers of all types of dairy plants over the years 1984-1994 (Table 1). Dairy plants are classified into 3 major types;

1. Processing or pasteurizing plants, defined as those that primarily package fluid milk.
2. Manufacturing plants, which predominantly make milk products other than fluid milk.
3. Transfer plants which assemble milk for bulk shipment to other locations.

There is an increasing need for extended storage of raw milk on the farm, in transport and in the dairy plant as well as extended storage of processed milk in the plant, during distribution and in the retail outlets (Cousin, 1982). Reasons for this trend include: Reduction in plant numbers (see Table 1) has contributed to the need to transport raw milk over longer distances from the farms to the processing plants. The increased distance from the farm to the plant have in some cases resulted in changes in pick-up schedules especially for the

lower transport costs by transporting in bulk. The resulting holding of raw milk at refrigeration temperatures for longer periods of time prior to processing may allow significant growth of psychrotrophic microorganisms (Cousin, 1982) with resultant production of proteolytic and lipolytic enzymes. The quality of ESL products processed from such milk may suffer. Reduction in numbers of processing plants also inevitably leads to changes in the market structure to include wider distribution areas for a given plant and longer refrigerated holding for processed milk.

Overall, the extended storage of both raw and processed milk has resulted in new quality problems for the dairy industry related to growth and metabolic activities of psychrotrophic bacteria. A significant change in the milk retail industry is the emergence of fast food restaurants as new retail outlets for milk. These outlets require products of a longer shelf-life. UP milk meets this requirement and is, therefore, the form in which milk sales are taking place.

Trends in milk production

At the production level, changes are taking place throughout the U.S., in the sizes of dairy herds, production efficiency and total milk production. Table 2 shows that NY State has maintained its position as the third largest producer of fluid milk in the U.S. with the third highest number of milk cows. The total number of cows throughout the country and in NY State has been decreasing over the years but production per cow has been increasing slightly in both instances thus maintaining a nearly constant annual milk production level.

Trends in milk consumption

Over the past decade, the per capita consumption of milk in the U. S. has declined by 6% mainly because of decreasing milk consumption by older people (Anonymous, 1993). These changes are reflected by a corresponding decrease in total fluid milk sales in the NY-NJ milk marketing area, which have also declined on a per capita basis. Sales declined by 3.3% in 1992-1993 and another 0.2% in 1993-1994 (Pearce, 1994).

Table 1. Number of dairy plants in NY State by type (1984-94).

Year	Type of plant			Total
	Processing	Manufacturing	Transfer	
1984	94	67	15	176
1985	86	70	15	171
1986	81	64	15	157
1987	73	63	15	146
1988	61	62	12	133
1989	54	60	10	124
1990	47	59	10	115
1991	45	54	10	109
1992	48	55	9	112
1993	44	54	8	106
1994	44	54	7	105

Source: Huff et al., 1993.

Table 2. Numbers of milk cows and the production of milk by state (for the 4 highest milk producers) and totals for the United States 1991-94.

Year	State	Number of milk cows (1,000 head)	Production of milk	
			lb. per cow	**Total Million lb.**
1991	Wisconsin	1,681	14,140	**23,770**
	California	1,155	18,534	**21,407**
	New York	**745**	**15,005**	**11,179**
	Minnesota	681	14,354	**9,775**
	U.S.	9,826	15,031	**147,697**
1992	Wisconsin	1,618	14,737	**23,844**
	California	1,180	18,722	**22,092**
	New York	**735**	**15,724**	**11,557**
	Minnesota	653	15,096	**9,858**
	U.S.	9,688	15,574	**150,885**
1993	**New York**	**748**	**15,274**	**11,425**
1994	California	1,235	20,258	**25,019**
	Wisconsin	1,494	15,001	**22,412**
	New York	**718**	**15,905**	**11,420**
	Pennsylvania	639	16,009	**10,320**
	U.S.	9,525	16,128	**153,622**

Source: Agricultural statistics service, 1995.

Table 3. Number of cows, production per cow and total milk production in NY State, 1983-1993.

Year	Average No. of cows (Thousands)	Production per cow (pounds)	Total milk production (Million pounds)
1983	928	12, 552	11, 648
1984	904	12, 658	11, 443
1985	914	12, 836	11, 732
1986	894	13, 107	11, 718
1987	822	13, 916	11, 439
1988	794	14, 413	11, 444
1989	776	14, 267	11, 071
1990	768	14, 410	11, 067
1991	756	14, 787	11, 179
1992	749	15, 430	11, 557
1993	748	15, 274	11, 425

Huff et al., 1993.

There is a need to boost dairy products sales to enable them to effectively compete with other beverages. One way to achieve this is to make dairy products available at distribution points such as vending machines and convenience stores. Currently, longer shelf-life beverages

such as sodas and bottled teas dominate these outlets (Kosman, 1995).

Present status of the NY State dairy industry

The total number of milk cows in NY State decreased between the years 1985 and 1994 (see Table 3). This is a consequence the reduction in the number of dairy farmers (see Table 4). Productivity, however, as measured by the annual milk production per cow has been increasing over the years, maintaining total milk production at about 11 million pounds a year.

Table 4. Number of dairy farmers NY State 1983-1993.

Year	No. of dairy farmers
1983	15, 316
1984	14, 937
1985	14, 580
1986	13, 933
1987	13, 071
1988	12, 390
1989	11, 671
1990	11, 272
1991	10, 893
1992	10, 625
1993	10, 265

Huff et al., 1993.

Utilization of raw farm milk in NY State

Table 5. Utilization of farm milk, NY State (% of total farm production)

Year	1989	1990	1991	1992	1993
Used on farms	1.9	2.0	1.9	1.9	1.9
Sold by farmers at retail	0.3	0.3	0.3	0.3	0.2
Sold by farmers to processors	97.8	97.8	97.8	97.9	97.9

Huff et al., 1993.

The bulk of raw farm milk is sold by farmers to processors and manufacturers (see Table 5) whose major product is fluid milk. In 1994, the total milk receipts for producer Class I milk and milk and from other sources in the NY-NJ milk marketing area totaled approximately 11.5 billion pounds (Pearce, 1994). Of this:

- About 43% was used in the manufacture of Class I fluid milk (whole, 2%, 1% and skim milk, flavored milk, milk drinks and buttermilk).
- About 17% was used in the manufacture of class II milk products (half and half, light cream, heavy cream, yogurt, ricotta cheese, cottage cheese, frozen desserts, candy products, eggnog and miscellaneous products).
- About 35% went to production of class III milk products (cream cheese, American cheese, Mozzarella and Provolone cheese, other cheeses, evaporated condensed milk, powders of whole milk, lactose and whey and butter).

•About 4% went into production of class III-A milk products (skim milk powder for human consumption).

Challenges to the NY State dairy industry

The fluid milk industry countrywide is faced with increasing competition from other cold beverages (Tripican, 1995). Consumption of fluid milk in NY State has steadily declined as reflected in a 1.1 million pound decrease in sales of over the period 1983 to 1993 (Huff et al., 1993). Some reasons for declining milk consumption include poor visual appeal of skim milk, under representation of fluid milk in retail outlets other than supermarkets, use of unattractive packages and limited product variety (Tripican, 1995).

Milk availability

According to Coates (1996), milk processors sell 72% of their milk to supermarkets, where milk sales have remained flat at around $18 billion for the past three years. In comparison, soft drink sales in supermarkets have increased from $ 17.5 to $ 21.3 billion over the same three years. Supermarkets account for only 33% of soft drink sales.

Only 2% of total fluid milk sales take place in convenience stores, as compared to 11% for soft drinks and 65% of ready to drink teas and juices. Milk can only be purchased in about 3% of the estimated 3 million cold beverage vending machines. These statistics suggest poor milk availability, which may contribute to poor sales and decreasing consumption levels.

The UP and UHT processes

UP and UHT processing can be achieved using either direct or indirect heating systems.

Direct heating

In a direct heat exchange system, milk at 7°C (45 °F) or less is first pumped through an indirect heat exchanger to raise the temperature to about 80°C (176 °F) followed by steam injection directly into the product to raise the temperature to 135-150 °C (275-302 °F).

The milk is held at this temperature for about 4 seconds, followed by evaporative cooling to about 80°C (176 °F) and is then packaged (Komorowski and Early, 1992). Direct heating has the following advantages over indirect heating (Komorowski and Early, 1992):

1. Faster rates of heating and cooling which minimize product exposure to heat and the accompanying loss of nutrients.
2. Direct heating reduces fouling of the heat exchangers, thus increasing the efficiency of the heat exchangers and reducing the necessity for frequent cleaning.
3. Because the product does not come in direct contact with a surface hotter than itself, and because of rapid rates of heating and cooling, the products are less cooked and the flavor is better than that of indirectly processed products.

The major drawback of direct heating is the necessary extra step needed to remove water that condenses into the milk when hot steam is injected. There are other disadvantages of direct heating systems (Burton, 1981).

1. There is a larger initial investment compared to that of an indirect system.
2. Since products come into direct contact with the steam, contamination can easily occur if the steam is not absolutely pure and free from odor, off-flavors and boiler chemicals.
3. The whole system, including the homogenizer, must operate aseptically.
4. Because of the above two reasons, the equipment needs more critical maintenance than a comparable indirect steam system.
5. The direct heating system is more technically complex. For example, it includes a vacuum chamber to remove excess water that gets into the product during steam injection.
6. Direct heating systems have higher energy requirements than indirect systems.

Indirect heating

In the indirect heating method, the steam or hot water does not come into contact with the milk but flows through tubes or plates, thus eliminating introduction of water into the milk. The tubes or plates are corrugated to give high turbulence and ensure rapid heating and cooling and reduce product fouling. The milk is passed through a heat exchanger and preheated to approximately 150°F (66 °C), then heated to 212 °F (100°C) by a second heat exchanger. The milk then goes to a sterilizer where it is heated to 285°F (142°C).

The sterile milk is then partially cooled by heat exchangers before moving through coolers to reduce its temperature even further to 60 to

70 °F (16-21 °C) (Burton, 1979). In the heat exchanger, the sterilized milk, which is separated by the metal wall of the heat exchanger, flows in the opposite direction from the incoming milk. Heat is transferred from hot, sterilized milk to the cold, raw milk, thus reducing both the energy required to heat the raw milk and to cool the sterilized milk. Energy consumption has been estimated to be half that of the direct method.

Other advantages of the indirect heating system over the direct system are (Burton, 1981):

1. It has lower initial investment cost.
2. It requires less frequent equipment maintenance.
3. It is technically less complex since water does not get introduced into the product.
4. It eliminates the possibility of flavors and odors being introduced into the milk with steam.
5. It offers a higher degree of flexibility in that it is not limited to processing milk alone but can process a variety of products such as fruit juices.

The major disadvantages of the indirect system are;

1. Milk protein is readily deposited on the heat exchanger surfaces. This causes loss of efficiency in the system and creates the need for frequent cleaning. The system must be shut down for cleaning every 8 or 9 hours.
2. The products have a more cooked flavor than those processed by the direct systems.

In both direct and indirect heating systems, after the milk has been

sterilized and cooled, it is passed through a homogenizer to break the fat globules and to prevent fat separation in the finished product.

Milk packaging systems

The processing is followed by packaging which may be aseptic, as in UHT, or non-aseptic, as in UP. In aseptic packaging, the packaging material or container is sterilized prior to filling and sealing in a sterile environment. Both the packaging material and seals are sufficient to prevent recontamination of the milk during storage and transportation (Komorowski and Early, 1992). Aseptically packaged products are commercially sterile, stable at ambient temperatures and generally have a long shelf-life, as long as 2 years for UHT (Harwalkar, 1982). Non aseptically packaged products are not commercially sterile. They have a shorter refrigerated shelf-life, 2 to 3 weeks for conventionally pasteurized milk and 30 to 60 days for ESL milk.

An example of an Ultra-Pasteurization process

(The DASI system using direct, free-falling steam heating, Ultra Dairy, personal communication). The process starts with sterilization of the equipment using a mixture of water and culinary steam under pressure. The steam-water mixture is circulated through the DASI system and sterilization is achieved when a constant temperature of 280°F (139 °C) or more is maintained in the system for 30 minutes. The raw milk is then introduced into the balance tank and pumped by a centrifugal pump to a plate exchanger, where it is pre-heated to 170 °F (77.3 °C). The pre-heated milk is then pumped through a positive pump into the steam infuser which consists of a steam chamber and four

distribution tubes with slits. The milk flows through the pipes and through the slits to form thin, laminar, free-falling films (about 1/16 inch thick) through a pressurized steam environment at 50 to 60 lbs.

At the bottom of the steam infuser is the holding tube. The milk, which at this time is at 280°F (139 °C) or higher, flows into the holding tube which is designed to allow 4 seconds of pasteurization. At the end of the holding tube, the milk goes through an orifice into the flash chamber where it is cooled to about 160°F (71.7°C). The water that was added during steam sterilization (up to 13%) is removed. The milk is then pumped by a centrifugal pump into the homogenizer. The homogenizer has two components. The first operates at 2, 000 psi and the second at 500 psi.

After homogenization, the milk is cooled first with water in the first cooling section then with glycol to about 36 °F (2 °C) in the second. The milk then flows into one of the aseptic surge tanks. If at any time during the processing the product loses level or temperature, the system automatically goes into a "divert mode", the aseptic tanks are sealed off with sterile air from steam sterilized air filters. The entire DASI system is then flushed, cleaned and re-sterilized in a 2.5 hour process.

Packaging is done using an Evergreen EQ-4 packaging machine with a non aseptic filler for filling gable-top containers ranging in size from 8 to 32 ounces. The packaging process consists of several stages:

Carton formation

↓

Spraying cartons with hydrogen peroxide

↓

Heating cartons to kill bacteria and drive off the hydrogen peroxide

↓

Filling cartons with product

↓

Sealing

↓

Coding

↓

Packaging cartons into cardboard boxes

↓

Palletizing

↓

Placing into cooler

Figure 1. Stages in non aseptic packaging of milk

Aseptic packaging

A description of the general mode of operation for aseptic packaging machines is as follows (von Bocklemann, 1979). The packaging material is composed of the following layers, from the outside to the inside: polyethylene plastic coating, paper, polyethylene, plastic coating, aluminum foil, polyethylene and plastic coating. The packaging

material is unwound and moved upwards in the filling machine to reach a sterilizing bath of hydrogen peroxide on top of the machine. Before the packaging material reaches the hydrogen peroxide bath, a longitudinal plastic reinforcement strip is heat sealed to one edge of the material web. A film of hydrogen peroxide is then applied to the packaging material contact surface as it passes through the sterile bath. A pair of squeeze rollers removes surplus hydrogen peroxide, which runs back into the sterile bath. The material then passes over a bending roller at the top of the machine and finds its way back downwards and is formed into a tube. Just prior to longitudinal sealing, the product is admitted by way of a filling pipe that extends down through the center of the packaging material tube. The tube heater, a spiral, electrically charged heating element, is placed around the filling pipe. After being sealed longitudinally, the packaging material is heated while passing the tube heater.

The filling pipe extends below the level of the product. The flow of the product is regulated and controlled by a butterfly valve at the outlet of the filling pipe, which in turn is regulated by a float. Transverse seams are made at regular intervals below the level of the product. To seal transversely, the product has to be squeezed away from the sealing zone. This is done by closing sealing jaws, applying pressure and then heat. Individual units are cut at a rate of about one pack per second. The pouches thus formed are fed into a final folder where they assume a brick shape by having flaps sealed down to the sides and the bottom of the package (von Bockelman, 1979). The filling machine is sterilized every time the DASI system and aseptic tanks are sterilized.

Problems of Ultra-High Temperature processed milk

UHT treatment is a sterilization process and the products are commercially sterile, free from all microorganisms capable of growing in the product under normal storage conditions, (Komorowski and Early, 1992). The heat treatment at ultra high temperatures for very short times causes extensive bacterial destruction with minimal damage to food flavor, nutrients and color compared to traditional methods for food sterilization.

Still, in comparison to less severe heat treatments such as pasteurization, UHT treatments are generally associated with cooked flavors and cause greater nutrient destruction. Other problems of UHT include activities of thermoduric microbial enzymes also resulting in quality deterioration. The lipolytic and proteolytic enzymes hydrolyze milk fats and proteins, resulting in off-flavors and smells in the products.

1. Impairment of nutritional quality in UHT products

The extent of loss of heat labile nutrients is less with use of direct than with indirect heat and also less with reduced holding times. UHT treatments, in general, and UP processing, in particular, cause minimal nutrient losses during processing because of the short holding times. The losses, however, can become significant during storage depending on the stability of the nutrient, storage time and conditions, and the product considered (Dunkley and Stevenson, 1987).

i. Effect of UHT treatment on vitamin A

Reduced fat and skim milk are fortified with Vitamin A (2000 to 3000 IU/Qt.) to ensure a constant and guaranteed source of vitamin A in the food supply. The US Food and Nutrition board selected milk as an ideal product for vitamin A fortification because it provides about 10% of the American consumer's food energy and does not create an imbalance of the essential nutrients (Fellman et al., 1991). The amount actually nutritionally available to the consumer, however, still depends on vitamin stability in a given product.

The effects of processing and storage on the stability of Vitamin A in UHT products have been widely studied. Ford et al. (1969) and McCarthy et al. (1985) found no significant loss of Vitamin A in whole milk during processing or storage for 90 days at room temperature. Burton and others (1970) repeated Ford's experiments under standardized conditions and found no decrease in Vitamin A due to processing. In both cases, the processing conditions were between 138 and 145 °C (280-293°C) for 3 to 4 seconds and in all cases samples were packaged in Tetra Pak or Tetra Brik cartons.

Regarding storage stability, McCarthy et al. (1985) reported that a combination of time, temperature, initial vitamin A concentration and dissolved oxygen all appeared to have effects. Stability in pasteurized whole and skim milk was studied by Cox et al. (1957) who found less vitamin A degradation in whole than skim milk, suggesting that natural anti-oxidants such as tocopherol in the milk fat may improve vitamin stability.

Thompson and Erdody (1974) showed that vitamin A added as a supplement to whole milk was more susceptible to photo-degradation than native vitamin A. Results of their studies also showed that a higher percent fat in milk slows down vitamin A degradation and that storage time has the greatest influence on the degradation of vitamin A.

ii. Effect of UHT on vitamin D

Fluid milk is fortified with vitamin D3 to meet US federal requirements of 400 IU/Qt. This vitamin addition fortifies the almost negligible naturally occurring concentrations of vitamin D in milk and has led to the expectation that milk is a good source of vitamin D (Renken and Warthesen, 1993). Levels of vitamins D, however, are not always in agreement with the label claims (Tanner et al., 1988; Renken and Warthesen, 1993). Possible causes include inconsistent fortification and subsequent vitamin degradation.

Research findings on the stability of Vitamin D vary. They were summarized by Renken and Warthesen (1993) as unstable to oxidation, light and acid (Cremin and Power, 1985) unstable to oxidation and light but stable to acid and alkali (Kutsky, 1981) and remarkably stable in the presence of light, heat and oxygen (Kreutler, 1980).

2. Flavor changes in UHT products

The desirable characteristics of processed milk include having the typical appearance of milk, being free from extraneous matter, and having a clean, slightly sweet taste with no abnormal taints or odors and no aftertaste (Komorowsky and Early, 1992). Flavor is the one property that most seriously limits acceptability of UHT milk products

(Dunkley and Stevenson, 1987). Bandler and Barnard (1984) suggest the most serious off-flavors in milk to be rancidity resulting from hydrolysis of milk fat by lipases and light and/or metallic oxidized flavors. Unclean and microbial flavors, which generally occur due to growth of psychrotrophic microorganisms on dirty utensils, are also important.

Because of the seriousness of the effects of rancid flavors on milk quality, a sensitive and rapid method was developed to determine the degree of lipolysis by measuring the levels of free fatty acids in milk. The modified copper soap solvent extraction method (Shipe et al., 1980) is used to estimate free fatty acids by converting them to copper soaps which are then extracted and reacted with a color compound. The procedure, however, extracts less of the short chain fatty acids (C4-C12) which are responsible for rancid off-flavor in milk than the long chain fatty acids (Duncan and Christen, 1991). The method, therefore, is a more useful tool for determining the incidence of lipolysis than rancidity, and should be coupled with sensory analysis.

Milk can also develop off flavors during processing. These flavors are commonly known as "cooked" and are also described as sulfurous, rich, caramelized, sweet, scorched (Heer et al., 1995) and cabbagy (Nursten, 1995). Cooked flavor is caused particularly by hydrogen sulfide, which is formed by thermal degradation of β-lactoglobulin and proteins from the fat globule membrane.

During storage, cooked flavor decreases in intensity, while defects such as stale flavors become evident. After prolonged storage, the stale flavors limit the acceptability of the product (Blake et al., 1995).

Typically, flavor acceptability of UHT products increases during the first few weeks of storage as the volatile compounds decompose and intensity of cooked flavor decreases then acceptability decreases as stale flavors increase (Dunkley and Stevenson, 1987).

There are various procedures by which the sensory quality of food products can be measured. The methods were broadly classified into 4 categories and described by Sidel et al. (1981) as affective, discrimination, descriptive and quality tests.

- Affective tests are used to measure subjective attitudes such as product acceptance and preference by selecting, ranking or scoring samples. Examples include the paired-preference and the 9-point hedonic scale. Respondents are usually consumers selected on the basis of their current or potential use of the product. The size of panel is 25- 50 for laboratory studies or 75-200 for field studies.
- Discrimination tests are those used to determine whether samples are detectably different from one another. Examples include sequential, paired difference, duo-trio and triangle tests. Discrimination tests are laboratory tests using panels of 12-20 qualified subjects with replications or 24-40 without replication.
- Descriptive tests are designed to describe sensory properties of products and measure the perceived intensity of those properties. Examples include flavor profiles, texture profiles and quantitative descriptive analysis. Panels consist of 6-12 subjects screened for sensory acuity and trained to perform the descriptive task.
- Quality tests are those which provide a score or grade to summarize a product's proximity to a standard which may be an

Quality tests take on different forms depending on the product. In the dairy industry, for instance, subjects may use a scale consisting of numbers anchored by different word descriptors or a procedure that includes a checklist of deficiencies and assignment of a final grade depending on the number and type of deficiencies in the product. Some tests are a combination of 2 or more types and the results of one test may be used as the basis for another.

3. Microbial quality of UHT products

Just as milk is a rich source of nutrients for mammalian life, it is also a very favorable medium for growth of both spoilage and pathogenic microorganisms. Sources of contaminants for milk include the environment (water, soil, vegetation, air and animal bedding materials), the animal body, and milk handling equipment (milking machines, tanks and other vessels) (Suhren, 1989). Table 5 shows the microbial quality of good quality farm milk produced under sanitary conditions.

Table 6. Sources and levels of contamination of farm milk at different stages of handling.

Source of contamination	Number of bacteria (c.f.u/ml)
As it comes from the udder	500-1, 000
Milking equipment	1, 000- 10, 000
Refrigeration tank	5, 000-20, 000

Champagne and Goulet (1985).

Such milk, produced under sanitary conditions, has less than 10% of its microflora as psychrotrophs, while in milk produced under unsanitary conditions, more than 75% of its microflora can be psychrotrophs (Cousin, 1982). Cooling raw milk generally slows down bacterial growth but allows the predominance of psychrotrophic bacteria.

Effects of extended refrigeration of raw milk on the microbial quality of processed milk.

Extended refrigerated storage of raw milk can occur on dairy farms, where in many parts of the USA, collection occurs on an alternate day basis (Cousin, 1982); during transportation to the dairy plant; at the dairy plant before processing; and for processed products, in the supermarkets before purchase. A decrease in the number of dairy processing plants (Huff et al., 1993) may require milk to be transported for long distances before it reaches the processing plant. At the plant, the milk may be stored for 3-4 days before processing, particularly if the milk gets to the plant on Friday, for plants that operate on a 5-day week schedule. After processing, pasteurized milk is coded for 10-14 last-day-of-sale, but is expected to remain acceptable for an extra 5 days. Hence, the total age of conventionally pasteurized milk before consumption may be up to 21 days (Cousin, 1982).

Keeping raw milk under refrigeration for long periods of time prior to processing allows psychrotrophs to grow and produce heat resistant enzymes that may reduce the quality of the processed milk.

Psychrotrophs commonly growing in milk were broadly classified into 3 groups (Champagne et al., 1994):

1. The gram negative rods are the majority encountered in raw milk comprising at least 50% *Pseudomonas* spp. They increase in numbers during cold storage and most of them produce proteases associated with flavor and quality defects in milk.
2. The gram positive genera *Arthrobacter*, *Bacillus* and *Clostridium* are more common in processed than raw milk. *Bacillus* and *Clostridium* are heat resistant spore formers (HRSF) which may survive pasteurization and cause spoilage in pasteurized milk and milk products with low levels of post-processing contamination. Their spores are activated at the high processing temperatures so they cause spoilage in ESL products.
3. Pathogenic psychrotrophs include *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus*.

Methods of inhibiting and controlling psychrotrophs in milk were reviewed by Cousin (1982). They include proper cleaning and sanitization of dairy equipment, use of high and low temperature treatments, targeted destruction and physical removal of bacterial spores.

Spoilage of milk and dairy products can arise from growth of psychrotrophic bacteria prior to pasteurization, activity of thermal resistant psychrotrophic enzymes, growth of thermal resistant psychrotrophs and growth of post-pasteurization contaminants. The most common source of spoilage bacteria in processed milk is post-pasteurization contamination (Champagne et al., 1994). The UP process sufficiently destroys most spoilage microorganisms (Blake et al., 1995) and their presence in processed products implies post-processing contamination. The most common source of post-processing

contamination is at the filling stage (Moseley, 1980; Schröder, 1984) resulting from inadequately washed and sanitized equipment, as well as airborne contamination.

Role of thermoduric enzymes in milk spoilage.

Most psychrotrophs would normally not be a serious problem in processed milk and dairy products because they are eliminated by pasteurization. However, their extracellular enzymes, especially lipases and proteases are, in most cases, heat resistant and are not inactivated by pasteurization (Champagne et al., 1994). The major effects of psychrotrophs in pasteurized and UHT milk are flavor related. Bitterness results from release of peptides from proteolysis. The peptides also play a role in browning reactions during heat processing.

In an effort to maintain high microbial standards for their products, industries define acceptable levels of different types of microorganisms in raw farm milk coming into their plants. They also set targets for acceptable levels of different microorganisms in their products. Both of these industry target levels are usually more stringent than state legal requirements. The guidelines shown in table 7, for example were developed in 1994 by some large industries in NYS.

Table 7. Microbial standards for acceptable levels of different types of microorganisms in raw farm milk and pasteurized fluid milk.

Test	Raw milk	Pasteurized milk
	(c.f.u. /ml)	
SPC	< 300, 000 (300, 000)	< 33 (20, 000)
Coliform count	< 100	<1 (<10)
LPC / HRSF	< 300	< 10
PIC	< 20, 000	NA
RPC	< 1, 000	<1

Source: Circular 958, NYS Department of Agriculture and markets, April 1994.

PIC is preliminary incubation Count, RPC is rapid psychrotrophic count, Legal requirements where known are shown in brackets, NA means no known standard.

Changes in milk proteins in UHT products

Milk proteins were generally noted as the constituents that undergo the greatest change during UHT processing and storage. Changes mainly include alterations in flavor, gelation, sediment formation, loss of nutritional value and browning (Dunkley and Stevenson, 1987). On average, milk contains 3.2% protein of which 80% are casein and 20% are whey proteins (lacto-albumin and lactoglobulin). Whey proteins are the most heat labile of the milk

constituents (Jelen and Rattray, 1995).

Milk casein is present in the form of calcium phosphocaseinate. Casein can be precipitated by acidification at its average isoelectric point of pH 4.6 and consists of five families of casein, αs1, αs2, β, κ and λ. These caseins are present in milk either as polymers in spherical micelles or as soluble monomers (Riel, 1985).

The principle effect of UHT processing on casein was identified by Burton (1983) as changes in distributions of the sizes of the micelles accompanied by increases in numbers of very small particles and in the amount of soluble casein. An increase in the 12% TCA soluble fraction (amino acid and peptides) was also observed following UHT treatment (Samel et al., 1971). A decrease in the amount of soluble casein and an increase in the 12% TCA soluble fraction was observed when UHT milk was stored at temperatures of 4 to 37°C. The increase in soluble protein was attributed to the release of glycopeptides from casein. Generally, proteins undergo changes both during UHT processing and post-processing storage, some of which may affect the physical, chemical and sensory properties of the products.

RESEARCH OBJECTIVES

The overall objective for this project was to determine the microbial, sensory and chemical characteristics of 2% UP fluid milk and how these characteristics change during post-processing storage. The specific objectives were to:

1. Assess the quality of the raw skim and whole milk used in the manufacture of the 2% UP and determine if it bears a relationship to the quality of the finished product.

2. Assess the initial microbiological quality of the 2% UP milk and monitor subsequent changes with time.
3. Determine the sensory quality of the 2% UP milk and monitor quality changes with time.
4. Measure the initial level of proteolysis in the 2% UP milk and monitor changes with time.
5. Measure the level of lipolysis in the 2% UP milk and assess changes with time.
6. Determine the effect of UP treatment on Vitamins A and D.
7. Determine the levels of Vitamins A and D in the 2% UP and monitor the changes during storage.

MATERIALS AND METHODS

Experimental design

The experiment was set up to have two treatments. These are two UP processing plants in NY State from which samples were obtained. From each factory, 4 batches of samples (replications) were obtained at approximate three month intervals. A batch consisted of five sets of samples. Each sample group was comprised of 15 half pint paper cartons of milk picked off the production line at five randomly chosen time points. Raw milk samples (whole and skim milk used in the formulation of the 2% UP milk, as well as the raw 2% milk with the added Vitamins A and D) were also collected. One of each of the five 2% UP samples was analyzed during the first, fourth, seventh and tenth weeks of post processing storage at 7 °C. Raw milk samples were only analyzed during the first week.

Sample collection

The 5 samples were picked from the production line at 5 randomly selected times points. They all came off the same filler. The 15 packets collected at a given time point constituted a sample set. The samples were placed into insulated boxes and /or coolers with cooling packs and transported to Cornell. On arrival, they were immediately placed into a refrigerator maintained at 7+/-1° C (45 °C). This sampling procedure was repeated for both factories and for all batches.

Microbiological analysis

Packages containing the milk sample were gently tilted 25 times to mix, the package spouts were cleaned by wiping with ethanol before opening, and appropriate sample aliquots were drawn from the package using sterile disposable plastic pipettes. Appropriate dilutions were made in previously prepared phosphate buffered dilution blanks (Potassium phosphate and magnesium sulfate from Fisher Scientific; Fair Lawn, NJ). Samples were then plated.

Microbial plate counts included the Standard Plate Count (SPC) to assess over-all microbial quality, the Psychrotrophic Plate Count (PPC) to determine prevalence of psychrotrophs, the Laboratory Pasteurization Count (LPC) to determine presence of heat resistant species, the Coliform Plate Count (CPC) to detect indicators of fecal contamination and the gram negative count using Crystal Violet Tetrazolium Agar (CVTA).

Raw milk was analyzed for SPC on plate count agar, (PCA; Difco Inc., Detroit, MI) at dilution levels 10^{-1}, 10^{-2} and 10^{-3} followed by incubation at 32 +/- 2°C for 24 +/- 2 hours. PPC on plate count agar at dilution levels 10^{-1}, 10^{-2} and 10^{-3} followed by incubation at 7 +/- 2 °C for 10 days. Coliform plate counts were done using Violet Red Bile Agar (VRBA; (Difco Inc., Detroit, MI) at dilutions 10^{0}, 10^{-1} and 10^{-2} followed by incubation at 32°C for 24 +/- 2 hours. Gram negative count using CVTA at dilutions 10^{0}, 10^{-1} and 10^{-2} followed by incubation at 21 +/- 2 °C for 24 +/- 2 hours. LP counts were done using PCA at dilutions 10^{0}, 10^{-1} and 10^{-2} followed with incubation at 32+/- 2 °C for 24 +/-2 hours.

The 2% UP milk samples were examined for SPC and PPC at dilutions 10^{0} and 10^{-1}. A test was done for heat resistant spore-formers. A 2.2 ml aliquot of each sample was placed in a glass tube with a lid, a heating block was heated to 85 °C, the samples were placed in the block and heated for 15 minutes. The tubes were then removed and immediately cooled in an ice-water bath. This was followed by plating on PCA at dilutions 10^{0} and 10^{-1} and incubation at 32 °C for 48 +/- 2 hours. When plates with the 2% UP milk did not show growth within the 48 hours incubation times, they were incubated another 48 hours to allow slow growing colonies to grow. All plates were counted using a dark field Quebec colony counter (American Optical Instruments, Buffalo, NY) and the counts recorded as c.f.u /ml choosing plates with between 25 and 250 c.f.u./ml.

SENSORY ANALYSIS

Sample preparation for sensory analysis

Two packs of each of the 5 samples were removed from the refrigerator taking care to protect the milk from exposure to light by switching off lights and drawing blinds. The samples were assigned random numbers and 50 plastic cups with tightly fitting lids were labeled with the same numbers.

Each of the milk packs was mixed by holding it in one hand and slowly tilting it 25 times through ~180 ° angle. About 80-100 ml of the milk was poured into each of the cups and the lids were put in place. Samples were further protected from light by placing the cups with milk in cardboard boxes with lids before delivering them to panelists.

Sensory analysis was carried out by 10 trained panelists consisting of faculty, staff and students in the Department of Food Science, Cornell University. Panelists were selected based on previous training and experience on panels evaluating dairy products. A combination of descriptive and quality tests (Sidel et al., 1981) was used to evaluate the sensory quality of the 2% UP milk samples.

A quantitative descriptive analysis of the samples was done based on absence or presence of 19 flavor defects listed on the score sheet and the intensity of the defects (see appendix 1 for sample score sheet). Defects present were assigned numbers to indicate their intensity: 1 for slight, 2 for definite, 3 for pronounced and 4 for very pronounced defect. This was followed by a quality test in which a final score in the range of 1 to 10 was assigned to the sample depending on presence or absence of any of 19 flavor defects and their intensity (see appendix 2 for guideline).

A sample which was judged as good received an overall score of between 8 and 10, a fair sample was given either 6 or 7 and, a poor sample, less than 6. Panelists were trained to have a clear knowledge of the taste of excellent quality milk which they were to keep in mind while evaluating the samples.

CHEMICAL TESTS

Modified copper soap solvent extraction method for measuring free fatty acids in milk.

Variation II of a modification (Shipe et al., 1980) of the original copper soap method developed by Koops and Klomp (1977) was used.

In this method, free fatty acids in the sample are converted to copper salts which are extracted and reacted with a color reagent. The copper-color complex is then measured colorimetrically. The method was selected for use in this research because it is highly sensitive and rapid.

Unless otherwise specified, all reagents were certified A. C. S. Grade from Fisher Scientific Co. (Pittsburgh, PA). A copper reagent was prepared by mixing 5 ml of triethanolamine and 10 ml of 1M aqueous Cu $(NO_3)_2.3H_2O$ diluted to 100 ml with a saturated sodium chloride solution. The pH was adjusted to 8.3 with 1N NaOH and the mixture was stored in an opaque bottle at room temperature. Under these conditions, the mixture is stable for 5 months (Shipe et al., 1980). The color reagent used was a 0.5% sodium diethyl dithiocarbamate solution in n-butanol. The solvent was a mixture of chloroform-heptane-methanol (CHM) in the ratio 49:49:2: vol:vol:vol. The solubilizing agent for the color reagent was Ethylene Diamine Tetra Acetate (EDTA), di-sodium salt, 8% wt/vol. in distilled water.

A 0.5 ml aliquot of milk was added to 0.1 ml 0.7N HCl in a 16 x 25 mm test tube. The mixture was shaken on a vortex test tube mixer (GENE™, Scientific Industries Inc., Bohemia, NY) at speed 4. Copper reagent (2 ml) was added, followed by mixing. 6 ml of the CHM solvent was then added and the sample shaken for 30 minutes in a rotary Eberbach model shaker (Eberbach Corporation, Ann Arbor, MI) at 240 rpm then centrifuged for 10 minutes at 1940 x g in an IEC clinical centrifuge (International Equipment Co.). 3.5 ml of the solvent layer was then transferred to an acid washed tube containing 0.1 ml of the color reagent followed by mixing on a test tube vortex mixer. The color

was measured within 1 hour at 440 nm using a Beckman D4-640 spectrophotometer (Fullerton, CA).

Absorbencies were converted to Acid Degree Values (ADV). ADV is the ml of 1N base required to neutralize the acids in 100g of fat (Std. methods). The regression equation for the relation ship between the absorbency at 440 nm (y) and the meq FFA/liter of milk (x) is (Shipe et al., 1980):

$y = 0.849 x - 0.053$

$x = (y + 0.053) / 0.849$

In this work, the ADVs were calculated using a VP-planner computer program and a VP-planner soft-ware package as follows;

Raw milk ADV = {[(A700 - A blank + 0.053)/0.8491 - 0.002}/ 0.398

Homogenized milk ADV =

{[(A700 - A blank + 0.053)/0.849}] x 2.816}+0.311

Determination of vitamin A by High Performance Liquid Chromatography (HPLC)

A modification by Rosenberry et al. (1994) of the methods of Thompson and Maxwell (1977) and Thompson et al. (1980) was used. The method involves several steps.

1. Preparation of the retinol palmitate standard

The Vitamin A (retinol palmitate) in the milk was determined based on a retinol palmitate standard prepared by dissolving 0.0291g of retinyl palmitate (Sigma Chemical company, St. Louis. MO) in isopropanol. This weight was previously determined as the expected retinol palmitate content of the sample aliquot used in the analysis (2 ml

of milk). A working solution of retinol palmitate was made by mixing 250µl of the stock solution for every 100 ml of isopropanol.

The actual concentration of the working solution was then determined spectrophotometrically using a 1:10 dilution of the working solution in isopropanol. The absorbency was then measured at 325 nm wavelength and an extinction coefficient of 960. The absorbency was used to calculate the actual concentrations of the working solutions as follows;

Retinol palmitate (g/200 ml) = {(Absorbance x 100)/960} x 100

where 960 is the extinction coefficient for trans-retinol palmitate (E 1%, 1 cm) at 325 nm.

2. Extraction of vitamins

Extraction of the vitamins was necessary before quantification because of the close association between the fat-soluble vitamins and the milk lipids. A room temperature milk sample was thoroughly mixed and a 2 ml aliquot placed in a 16 x 125 ml centrifuge test tube. Laboratory grade (LG) water (2 ml) was placed in 2 tubes (controls) and another 3 recovery tubes (to check the recovery of the standards through the extraction). Ethanol (5 ml) was added to each of the tubes followed by mixing on a vortex mixer for 30 seconds. The tubes were then allowed to stand for 5 minutes. Hexane (5 ml) was then added to each of the tubes, except the recovery tubes, to which 5 ml of a serial dilution of the working solution was added (125, 250 and 500 µl/100 ml of hexane).

The tubes were vortexed again for 30 seconds followed by standing for 2 minutes. The mixing and standing was repeated twice. LG water (3 ml) was then added to each tube, the tubes were stoppered

and inverted to mix, then centrifuged for 10 minutes at 1,000 x g.

3. HPLC quantitation

An aliquot of about 0.5 ml of the hexane layer sample was placed in an actinic glass vial with a lid and analyzed. A 600E multi-solvent delivery HPLC system with a detector, auto-sampler and a Millennium 2010 software data system (Waters Corporation, Milford, MA) were used. The detection was done at 264 nm using a Vydac 201 TP 54 Carbon 18, 5 ul, 4.6 x 250 mm column with 100 µl injector.

Quantitative analysis of vitamin D using a modification of the High Performance Liquid Chromatography (HPLC) method

Vitamin D is used to refer to both D2 (ergocalciferol) and D3 (cholecalciferol), the latter being the form most commonly used to fortify milk. In this method, D2 was used as an internal standard to quantify D3. The quantitation involves several steps and an internal standard is needed to provide analytical confidence. The analysis is complicated by the low natural concentrations of vitamin D in foods (100 and 1000 IU /100g for most foods) and by the close association between vitamin D with vitamins A, E, sterol and other fat-soluble compounds (Kobayashi et al., 1986). Vitamin D2 was chosen to be the internal standard because it is virtually identical to D3 in physiochemical properties (recovery, extinction coefficient and wavelength for maximum absorption) and is not resolved from D3 during silica clean up (Indyk and Woollard, 1985). The method has a 5% analytical error (Rosenberry et al., unpublished).

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1. Preparation of the internal standard (ergo-calciferol, vitamin D2)

A stock solution of Vitamin D2 was prepared by weighing 16 to 20 mg of D2 (Sigma Chemical company, St. Louis. MO) into 100 ml amber colored volumetric flask and dissolving in room temperature methanol (Fisher Scientific, Fair Lawn, NJ). Exact concentrations of all stock solutions were determined by measuring absorbencies at 264 nm wavelength and 960 extinction coefficient. The stock solution was labeled and kept under refrigeration.

The daily working standard was prepared by allowing the stock solution to come to room temperature then transferring exactly 1.0 ml into a 10 ml volumetric flask. Using room temperature methanol, the contents of the flask were brought to volume.

2. Saponification

Milk (15 ml) was pipetted into 50 ml amber colored glass flasks with stoppers. 15 ml of 1% ethanolic pyrogallol (Fisher Scientific, Pittsburgh, PA) w/v and 100 µl of internal standard vitamin D2 solution added. The flasks were cooled on an ice bath to below 10 °C to prevent the destructive build up of the heat of solution and de-aerated with nitrogen gas to prevent oxidative destruction of vitamins. While still under the stream of nitrogen and on the ice bath, 6 gm of KOH pellets (Fisher Scientific, Pittsburgh, PA.) were added. The flask was immediately stoppered and swirled continuously on the ice until the KOH was dissolved. A positive pressure was kept on the stopper to keep it on (a pressure build up results from the heat of solution). The sample was mixed for an additional 4 minutes at room temperature then allowed to

stand for 18 hours in a covered shaker-water bath at 22 °C.

3. Solvent extraction

The saponified contents of the Erlenmeyer flask were transferred under a hood to a 125 ml amber colored glass separatory funnel. The flask was rinsed with 15 ml LG water followed by 5 ml ethanol, then 45 ml hexane, each of which were added to the separatory funnel. The funnel was then stoppered and shaken vigorously for 1 minute. The stopper was loosened and the funnel left to stand for at least 4 minutes to allow the phases to separate. The stop cock of the separatory funnel was then opened and the aqueous layer transferred back into its original Erlenmeyer flask. The hexane layer was then carefully transferred into an amber colored 250 ml separatory funnel. Using hexane only, the extraction of the aqueous layer was repeated two more times transferring each hexane layer into the 250 ml separatory funnel bringing the final volume to ~135 ml.

To back wash, 50 ml of 5% KOH solution was added to the pooled hexane layers. The stopper was tightly placed on and the flask shaken in a horizontal direction for 1 minute. The flask was then allowed to stand for 4 minutes after which the aqueous layer was removed and discarded. The hexane layer was then washed with 100 ml water followed by 50 ml and finally 100 ml 55% ethanol discarding the lower layers each time. The hexane layer was transferred into a 250 ml round bottom boiling flask and the hexane was evaporated to dryness over water at 40 °C using a Rotavapor-R model (Rinco Instruments, Switzerland). Care was taken to protect the samples from light at all stages.

To facilitate complete removal of all the water, 5 ml of ethanol was added to the round bottom flask after the hexane had been evaporated. The flask was swirled gently to incorporate the water droplets into the ethanol then the ethanol was evaporated to dryness. To the contents of the round bottom flask, 5 ml hexane was immediately added, the flask was swirled gently to wash the sides. The flask contents were then transferred to a 50 ml conical glass stoppered test tube wrapped in aluminum foil. The flask was washed two more times with 3 and 1 ml of hexane which were also added to the test tube.

D. Solid phase extraction (Silica clean-up phase)

Under a hood and using a stream of nitrogen and a water bath at about 35 to 40 °C, the hexane fraction was evaporated to about 0.5 ml (500 µl) then vortexed for 30 seconds. A solid phase silica extraction column was conditioned by introducing 5 ml hexane through the column under positive pressure generated by a syringe placed over the column. The column was placed into a clean 15 x 150 mm test tube. The vitamin D2/ D3 concentrate was then introduced into the column and allowed to be adsorbed by gravity feed. The tube containing the sample was then rinsed with about 0.5 ml hexane and vortexed for 30 seconds.

The contents were then added to the silica column. When the hexane wash had gone through the column, the column was transferred to a clean 15 x 150 mm glass test tube. To remove interfering compounds, the column was washed with a mobile phase of 1 ml of hexane / chloroform (22: 78, v/v). A yellow band eluted off with the

hexane /chloroform wash leaving a pale yellow band of vitamin D2/D3 together with vitamin A and like compounds adsorbed to the column.

The vitamin D containing fraction was stripped from the column by eluting with ~ 1 ml of methanol into a clean conical-bottom test tube. The residual hexane /chloroform was removed by evaporating using a stream of nitrogen. The sample was evaporated to approximately 0.2 ml. The volume of the sample was immediately adjusted to approximately 0.7 ml.

5. HPLC quantitation

The sample was placed in an actinic glass vial with a lid and analyzed in a similar manner to the vitamin A sample.

Determination of proteolysis as measured by the Tyrosine Value (TV).

The procedure developed by Juffs (1973) was used. TV is defined as the amount of acid-soluble amino acids and peptides in 1 ml of milk. It is expressed in terms of the equivalent color yield of a 1 mg/ml tyrosine solution.

The milk samples were thoroughly mixed and 10 ml aliquots placed into plastic 50 ml tubes with caps. 30% TCA (5 ml) was added with an Oxford pipette, the lids were placed on and the samples mixed on a vortex mixer (GENE ™. Scientific Industries, Bohemia, NY) for 20 seconds at speed 3. After 15 minutes the samples were filtered into 50 ml plastic tubes using Whatman # 1 (11 cm) filter papers (Fisher Scientific, Pittsburgh, PA.). Filtrate (1 ml) was placed in a 16 x 125 ml disposable glass test tube. Juffs' reagent (1 ml of 1% $CuSO_4.5\ H_2O$: 1

ml 2% Na_2 $C_4H_4O_6.2H_2O$: 100 ml 2% Na_2CO_3) was prepared and 5 ml of this reagent was added to the sample. The mixture was vortexed and left to stand for 10 minutes. Color reagent (0.5 ml) composed of a 1:1 Folin and Cio calteu's reagent (Fisher Scientific, Pittsburgh, PA): water was added, immediately followed by vortexing for 20 seconds at speed 3 on a vortex mixer (GENE ™, Scientific Industries, Bohemia, NY).

The color reagent was allowed to react with the mixture for 30 minutes, after which the mixture was filtered into 16 x 125 ml disposable glass test tubes with Whatman #1 (9 cm) filter paper (Fisher Scientific, Pittsburgh, PA). The absorbency of the filtrate was read on a Spectrophotometer (Beckman D4-640, Fullerton, CA) using 1 ml quartz cuvettes (Fisher Scientific, Pittsburgh, PA) at 700 nm wavelength. The spectrophotometer was set to zero with distilled water followed by the sample blank. The samples were then read.

A solution of 1 mg/ml of tyrosine (Fisher Scientific, Pittsburgh, PA.) was prepared, a serial dilution of the tyrosine solution was made and used to construct a standard curve. Using the standard curve developed, a regression equation relating absorbency at 700 nm and the TVs was established and applied to the mean absorbency (A-700) of the samples to determine their soluble protein content.

Mg /ml soluble protein = regression constant + slope of the regression line (absorbency at 700 nm).

Tyrosine concentration = - 0.071 + 1.46 (A-700)

Soluble protein concentration = - 0.071 + 1.46 (A-700).

Statistical analysis

A two way ANOVA was performed at $\alpha = 0.05$ using Minitab Version 10 (Minitab Inc., State College, PA) to determine if results varied significantly between weeks within a sample batch. A repeated measures split plot ANOVA was then done at $\alpha = 0.1$ using JMP Statistical Analytical Systems (SAS Institute, Inc., Cary, NC) computer software to determine if samples varied significantly between batches within the same factory and also to determine if significant differences existed between factories. The 0.1 level of significance was used because 4 duplicates (batches) would not give reliable results at the 0.05 level of significance.

RESULTS AND DISCUSSION

Microbiology

Tables 8 and 9 show results of the different microbial tests done on the raw milk samples (whole, skim and 2%). The whole and skim milk are samples from milk used as raw material for the 2% UP milk. The raw 2% milk is fortified with vitamin A and D before processing. Batches 2, 3, and 4 are 3 of the 4 replications from each of the factories.

Table 8. Bacterial counts (c.f.u./ml) for raw whole (W), skim (S) and 2% raw milk from factory H.

Test	Whole milk			Skim milk			2% raw milk		
SPC	68,000	+/-	37,486	34,900	+/-	50,316	77,750	+/-	92,277
GNC	36,667	+/-	23,099	7,354	+/-	9,426	93,000	+/-	94,752
PPC	37,333	+/-	24,363	24,793	+/-	40,049	23,250	+/-	23,688
LPC	4,040	+/-	5,488	4,167	+/-	6,786	6,050	+/-	8,415
CC	1,867	+/-	818	368	+/-	319	300	+/-	141

CC = coliform count, GNC = gram negative count, LPC = laboratory, pasteurized count, PPC = psychrotrophic plate count, SPC = standard plate count.

Note: Mean counts for batches and the standard deviations about the means are shown in tables 8 and 9.

Table 9. Bacterial counts (c.f.u./ml) for raw whole, skim and 2% milk from factory F.

Test	Whole milk			Skim milk			2% raw milk		
SPC	42,500	+/-	6,364	6,500	+/-	2,121	49,500	+/-	68,589
GNC	9,750	+/-	7,425	76	+/-	105	42,350	+/-	53,245
PPC	16,500	+/-	16,263	70	+/-	100	24,850	+/-	28,496
LPC	652	+/-	775	30	+/-	5	43	+/-	4
CC	76	+/-	106	1	+/-	5	84	+/-	105

Note: Mean counts for batches and the standard deviations about the means are shown in tables 8 and 9.

General observations in microbial counts.

The results of microbiological analyses done on raw milk samples varied both between factories and between batches within the same factory. In all cases, the total plate counts were less than the legal requirement of < 300, 000 cfu/ml. The PPC, CC, HRSF, and GNC were high in some cases. The high PPC are probably due to the increasingly long pre-processing refrigeration periods currently common in the dairy industry (Cousin, 1982).

Variation of bacterial types with milk types

Generally, skim milk had the lowest counts for all types of microorganism. The low counts are probably because of the pre-processing heat treatment that skim milk received during the skimming

process that the other milk types did not get.

Variation in microbial counts between batches and factories

Possible reasons for the difference in counts between batches and factories are the varying numbers of days the milk is held at the factory prior to processing (Cousin, 1982) and varying microbial quality of incoming farm milk.

Relation between raw milk and UP microbial quality

Regardless of the counts in the raw milk samples, the 2% UP milk samples were found to have no bacteria as evaluated by the standard plate count (SPC) and psychrotrophic plate count (PPC) and heat resistant spore formers (HRSF) when plated during the first, fourth, seventh and tenth weeks post processing. The results indicate that UP processing, 140 °C (284 °F) for 4 seconds and 145 °C (293°F) for 2 seconds adequately destroyed the microorganisms found in the skim and whole milk used as raw material for the 2% UP. These findings agree with results reported by Blake et al. (1995) indicating absence of psychrotrophs, mesophiles or thermophiles in UP samples processed at 134° C or higher for 4 seconds. These products are microbiologically stable.

Sensory quality

Figures 2 and 3 give the trends in sensory scores for 2% UP during weeks 1, 4, 7 and 10. The total number of samples analyzed for each factory was 800.

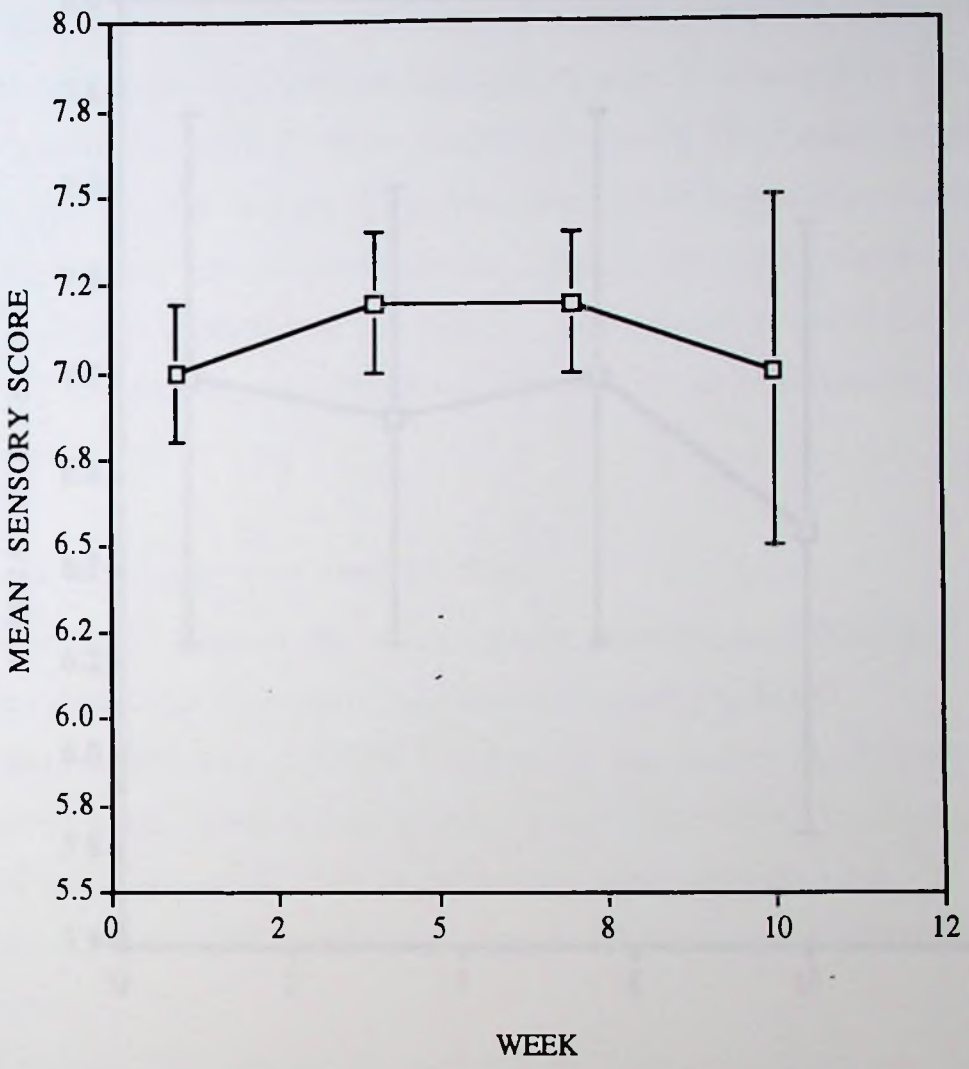

Figure 2. Changes in the acceptability of 2% UP milk from factory H stored at 7°C.

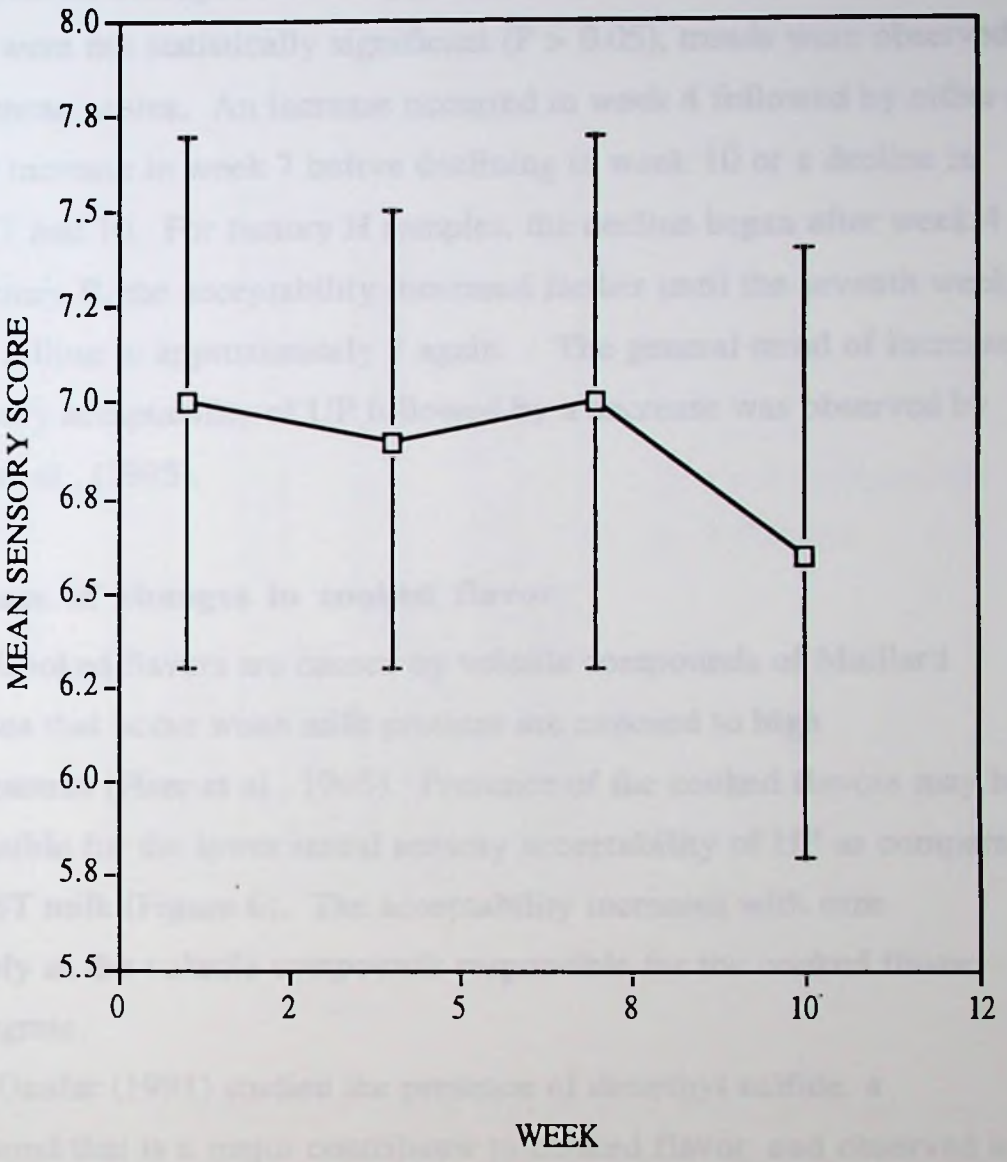

Figure 3. Changes in the acceptability of 2% UP milk from factory F stored at 7°C.

Overall acceptability

For both plants, samples were judged to be "fair" during the first week with an average score of approximately 7. Although change in scores were not statistically significant ($P > 0.05$), trends were observed in the mean scores. An increase occurred in week 4 followed by either a further increase in week 7 before declining in week 10 or a decline in weeks 7 and 10. For factory H samples, the decline began after week 4. For factory B, the acceptability increased further until the seventh week before falling to approximately 7 again. The general trend of increase in sensory acceptability of UP followed by a decrease was observed by Blake et al., (1995).

Causes of changes in cooked flavor

Cooked flavors are caused by volatile compounds of Maillard reactions that occur when milk proteins are exposed to high temperatures (Heer et al., 1995). Presence of the cooked flavors may be responsible for the lower initial sensory acceptability of UP as compared to HTST milk (Figure 6). The acceptability increases with time probably as the volatile compounds responsible for the cooked flavor disintegrate.

Gaafar (1991) studied the presence of dimethyl sulfide, a compound that is a major contributor to cooked flavor, and observed it to rapidly decrease during storage, and completely disappear following 2 months of storage at 4, 20 and, 35°C.

Trends in lack of freshness

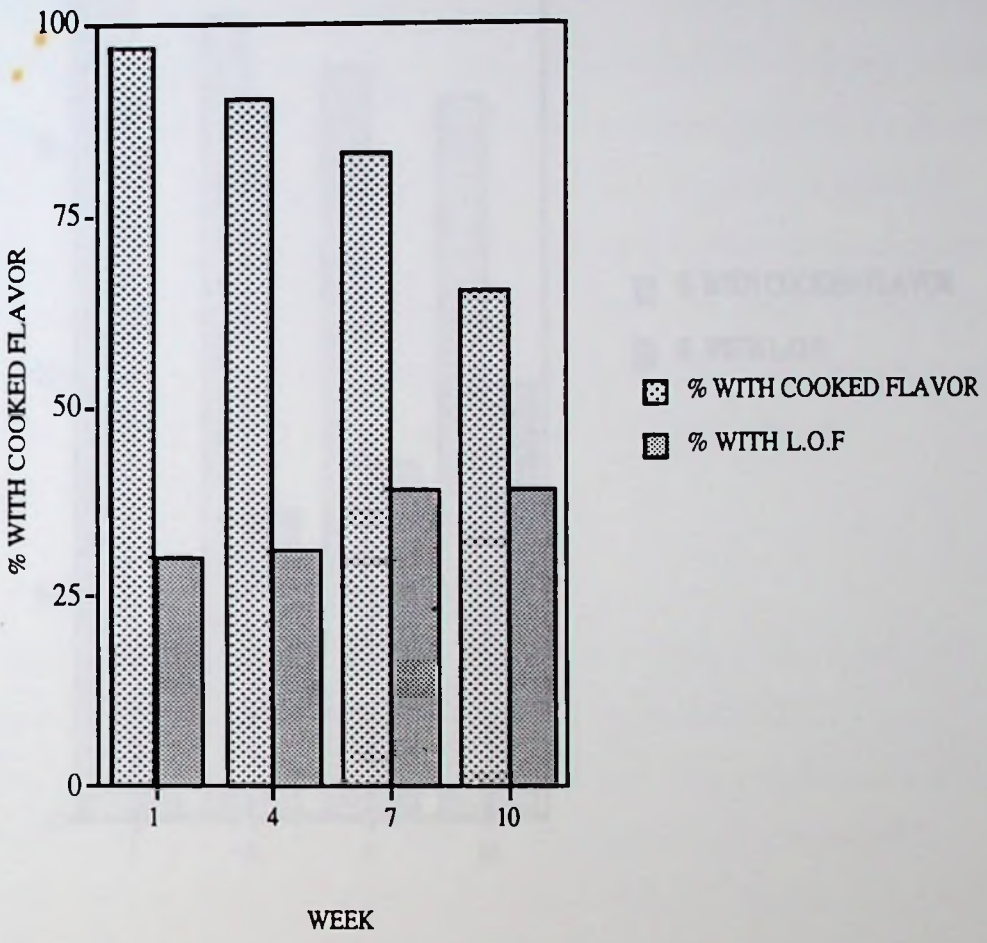

Figure 4. Trends in cooked flavor and lack of freshness (L.O.F) observed in 2% UP milk samples from factory F stored at 7°C.

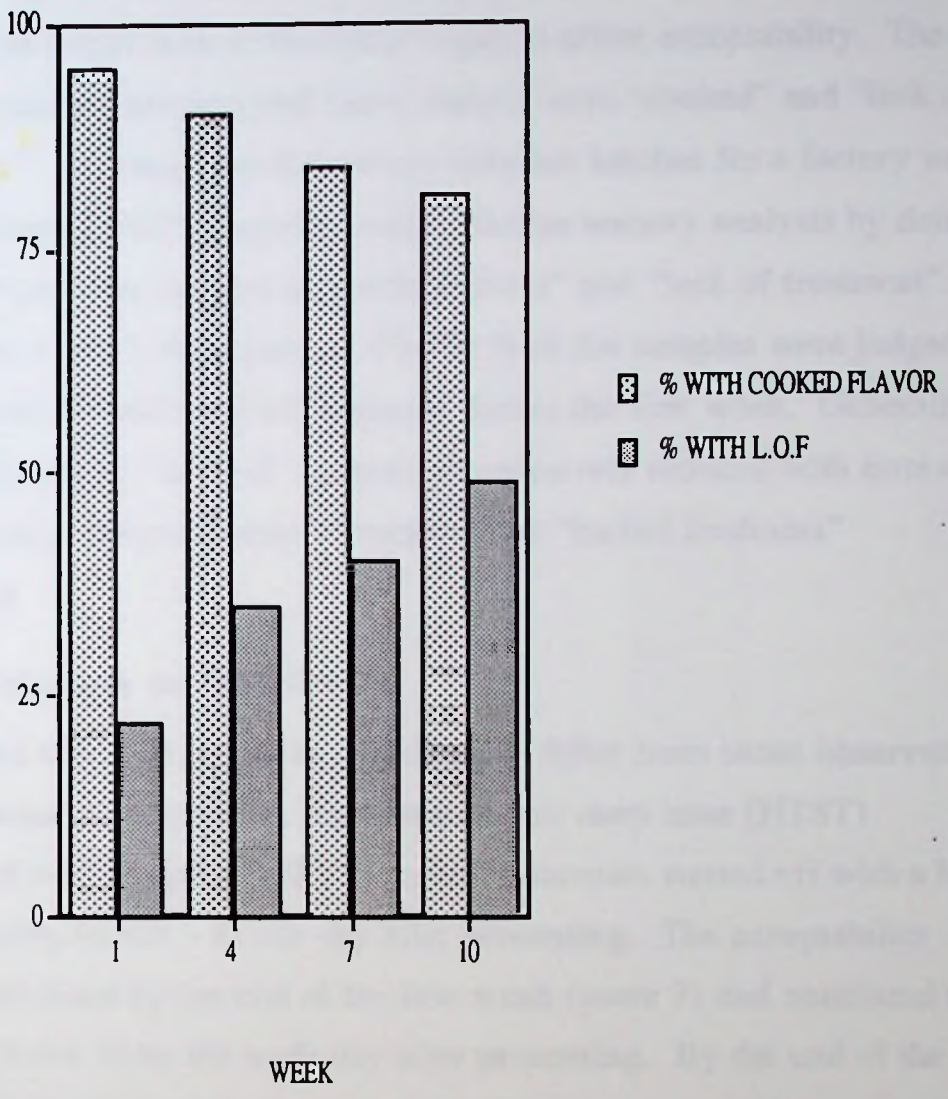

Figure 5. Trends in cooked flavor and lack of freshness observed in 2% UP milk samples from factory H, stored at 7°C.

While cooked flavor decreased with time, other flavor defects collectively described as "lack of freshness" increased (Figures 4 and 5). The milk attained maximum acceptability when the cooked flavors were lowest and before lack of freshness began to affect acceptability. The most commonly encountered flavor defects were "cooked" and "lack of freshness". Although the differences between batches for a factory were insignificant ($P>0.05$) between weeks, further sensory analysis by defect revealed trends in changes in "cooked flavor" and "lack of freshness". In figures 4 and 5, for example, 90-100 % of the samples were judged cooked and 30- 40% lacked freshness during the first week. Generally, the percentage of "cooked" samples progressively reduced with time of storage while the percentage of samples that "lacked freshness" increased.

Flavor changes in HTST milk

The trends observed in 2% UP milk differ from those observed in conventionally pasteurized, high temperature short time (HTST) processed milk samples. HTST processed samples started off with a high acceptability (score ~ 8) one day after processing. The acceptability rapidly declined by the end of the first week (score 7) and continued to decline (score 6) by the tenth day after processing. By the end of the second week, the milk had deteriorated into the unacceptable range (score 4) with visible signs of spoilage such as coagulation. At this point, the samples were frequently rejected by panelists.

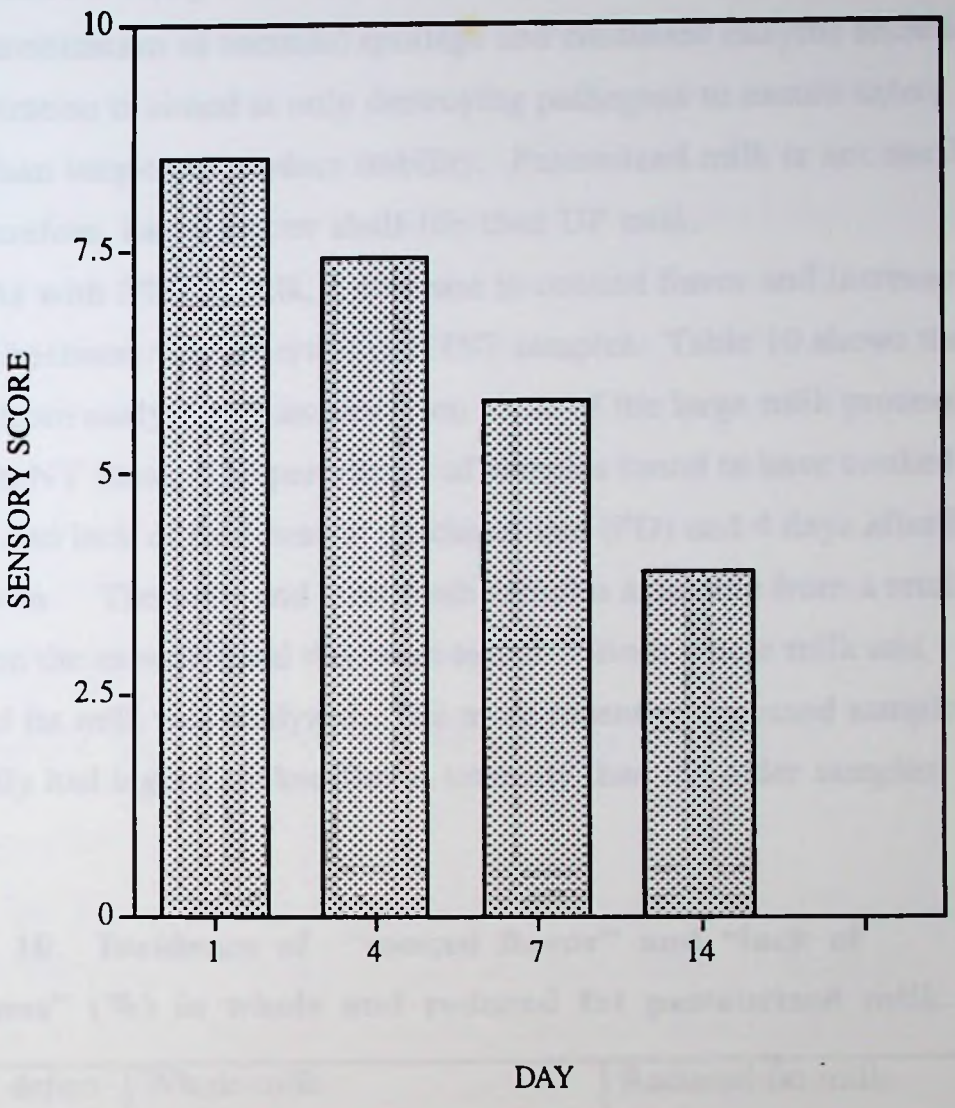

Figure 6. Typical trends in the acceptability of conventionally pasteurized, (HTST) milk from some large processing plants in NY State.

(Bandler et al., 1985).

The higher loss of sensory acceptability in HTST as compared to 2% UP milk is likely to be related to the presence of microorganisms and a combination of bacterial spoilage and continued enzyme secretion. Pasteurization is aimed at only destroying pathogens to ensure safety rather than long-term product stability. Pasteurized milk is not sterile and, therefore, has a shorter shelf-life than UP milk.

As with 2% UP milk, a decrease in cooked flavor and increase in lack of freshness was observed in HTST samples. Table 10 shows the results from analysis of samples from some of the large milk processing plants in NY State. The percentage of samples found to have cooked flavors and lack of freshness at purchase date (PD) and 4 days after PD are shown. The most and least fresh samples available from a retail outlet on the experimental day were tested. Both whole milk and reduced fat milk was analyzed. The more recently processed samples generally had higher cooked flavor intensity than the older samples.

Table 10. Incidence of "cooked flavor" and "lack of freshness" (%) in whole and reduced fat pasteurized milk.

Flavor defect	Whole milk				Reduced fat milk			
	Most fresh		Least fresh		Most fresh		Least fresh	
	PD	PD+4	PD	PD+4	PD	PD+4	PD	PD+4
Cooked	25	4	17	0	4	0	6	11
L. O. F.	33	63	39	70	25	46	11	61

Source: Bandler et al., 1995.

Unlike 2% UP, in HTST milk, lack of freshness was more pronounced than cooked flavor. Whereas cooked flavor is more pronounced in UP milk, L.O.F. and other defects that are related to microbial spoilage are more common in HTST milk. The changes in L. O.F. in HTST milk occur in a much shorter time (4 days after purchase day) leading to rejection of samples by the end of the second week. With UP samples under similar storage conditions (refrigeration), development of "lack of freshness" is slow and samples remain acceptable after 10 weeks. The difference in rates of increase in LOF point to its relation to microbial activity which is higher in HTST milk than 2% UP milk. UP treatment stabilizes the sensory quality of milk for a longer period of storage than conventional pasteurization.

Proteolysis as measured by the tyrosine value (TV)

Background

The TVs of freshly drawn milk vary from 0.31 to 0.92 mg/ml (310 to 920 µg/ml). The differences are attributable to variations between cows: age, stage of lactation, diets and breed (Juffs, 1972). In his studies, Juffs analyzed 192 milk samples from individual cows. 60% of the samples had TVs ranging from 0.36 to 0.50 mg/ml (360 to 500 µg/ml). This was also the range for TVs for bulked herd production.

Based on a number of studies (Juffs, 1975), 0.55 mg/ml (550 µg/ml) is considered the limit for natural variation in most bulked milks. Samples with TVs beyond this level may be considered to have undergone some proteolysis, the TV increasing with increasing levels of

proteolysis. The relationship between TV and sensory quality is a very general one but sensory rating tends to decrease with increasing TV (Juffs, 1975). TV for HTST milk at 1 week ~ 500 µg/ml (Bandler et al., 1985).

TVs of raw milk samples

All raw milk samples had undergone some proteolysis (TVs higher than 550 µg/ml). There is no definite relationship between type of milk, and level of proteolysis. Skim milk, which had the lowest SPC did not have correspondingly lower TVs.

Table 11. TVs for raw whole, raw skim, raw 2% and 2% UP milk from factories H and F.

Milk type	Factory H			Factory F		
	TV (ug/ml)					
Raw whole	540	+/-	225	619	+/-	171
Raw skim	662	+/-	31	778	+/-	399
Raw 2%	701	+/-	112	855	+/-	346
2% UP	247	+/-	214	213	+/-	165

TVs of raw milk as compared to TVs of 2% UP milk

TVs for the raw milk are generally higher than those for 2% UP and conventionally pasteurized milks (Table 11). This is probably because of higher numbers of bacteria in the raw milks. They continue to produce enzymes while bacterial destruction in 2% UP halts enzyme production and probably destroys naturally existing milk enzymes thus slowing down proteolysis.

In processed 2% UP milk samples, the proteolytic bacteria are destroyed. Heat labile bacterial and milk enzymes are probably destroyed as well. Proteolysis, however, still occurs during post-processing storage. Since no bacterial growth occurs in these products, the protein breakdown must be caused by thermoduric proteolytic enzymes that survive the heat processing. The slight increase in TV with time during storage did not cause bitterness (known to result from hydrolysis of proteins into peptides) judging from sensory analysis results.

The levels observed in the 2% UP milk studied (< 500 μg/ml) were not high enough to affect organoleptic quality. 160 samples from each factory were analyzed.

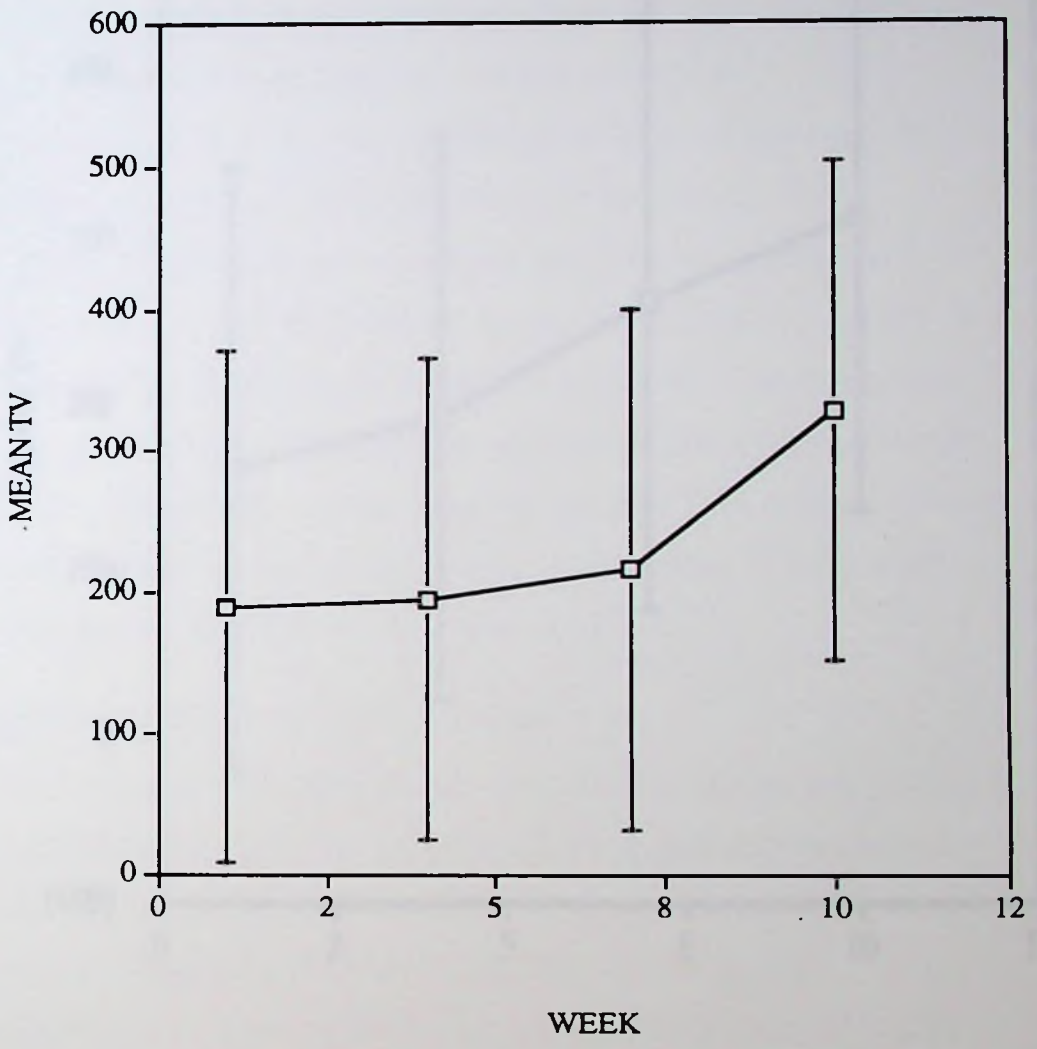

Figure 7. TV for 2% UP milk samples from factory H, stored at 7°C.

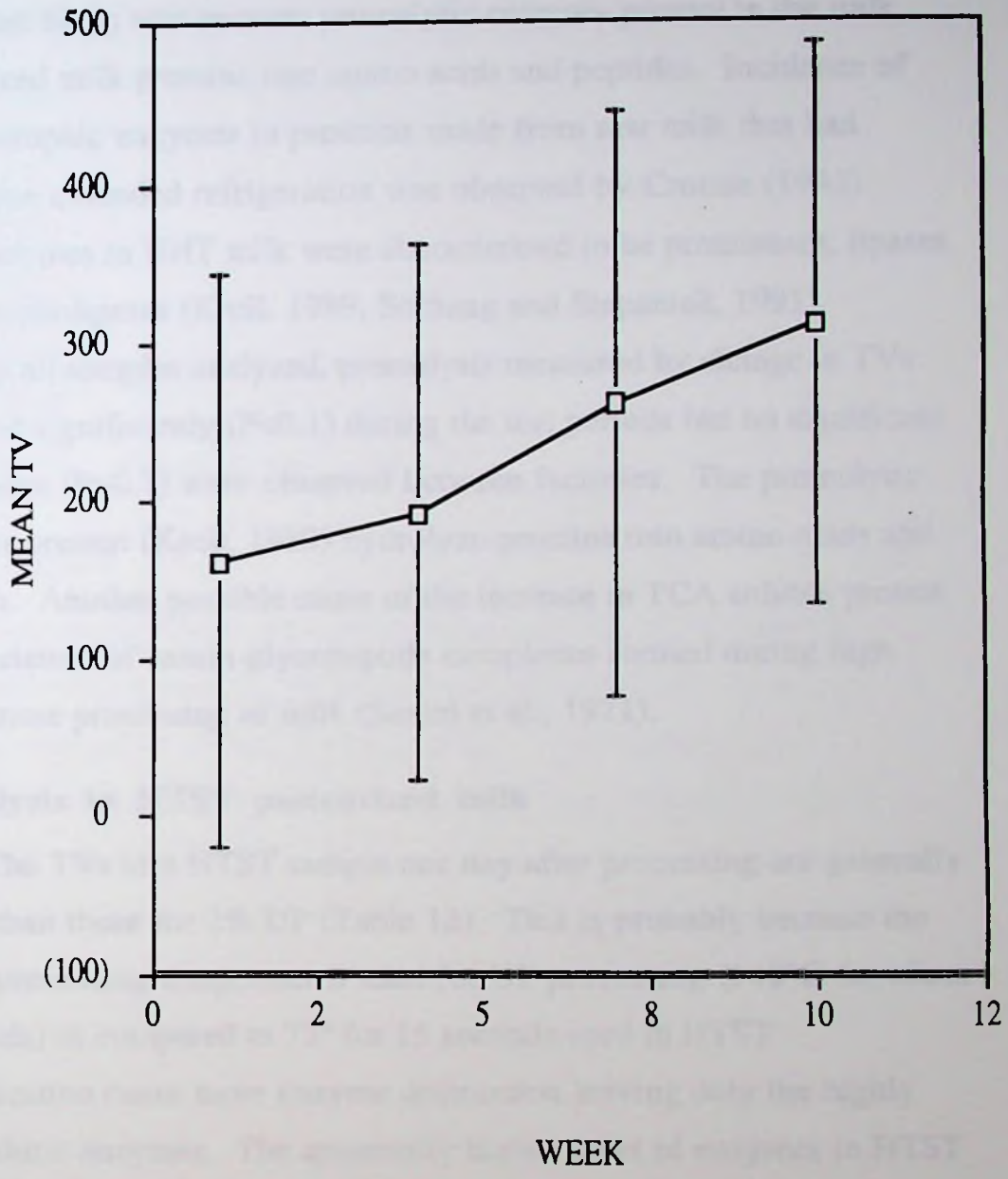

Figure 8. TV for 2% UP milk samples from factory F, stored at 7°C

Table 12. Trends in TV in HTST pasteurized milk during refrigerated storage.

Day After Processing	Tyrosine Value (TV) (µg/ml)
1	340
4	420
7	490
10	780

(Bandler et al., 1985).

Lipolysis as measured by ADV

Background

Normal raw milks are known to have ADVs of 0.25 to 0.4 (meq/liter) with rancid flavor detection occurring at ADVs of 1.2 meq/liter and above (Bradley et al., 1992). While conventional pasteurized milks should have ADVs of less than 1 meq/liter, acceptable UHT milk was found to have ADV values as high as 1.5 to 3 meq/liter (Duncan and Christen, 1991). UP milk, whose processing conditions approximate those of UHT could have ADV as high those reported in UHT with no rancid flavor detection.

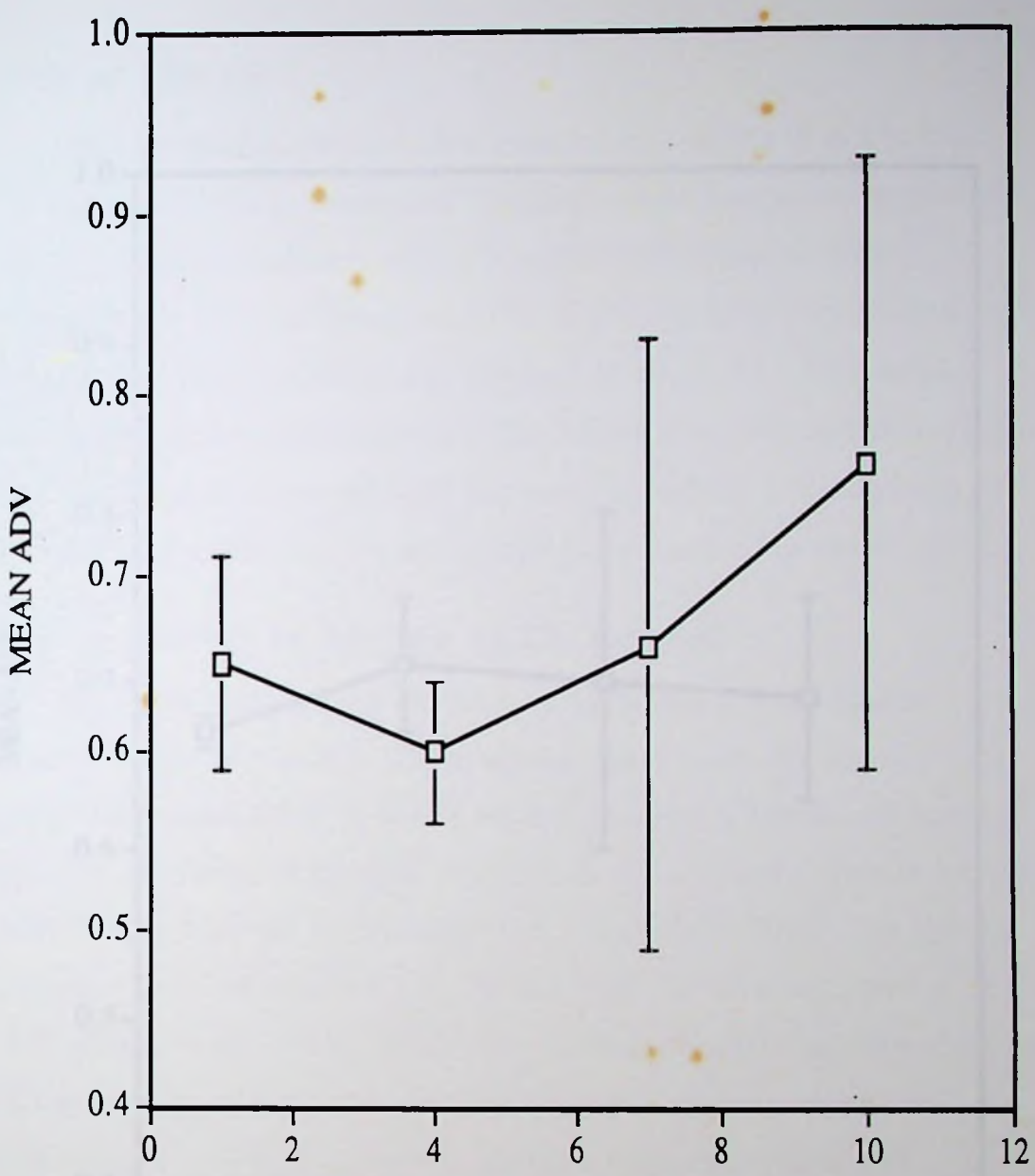

Figure 9. Changes in ADV of 2% UP milk from factory H stored at 7° C

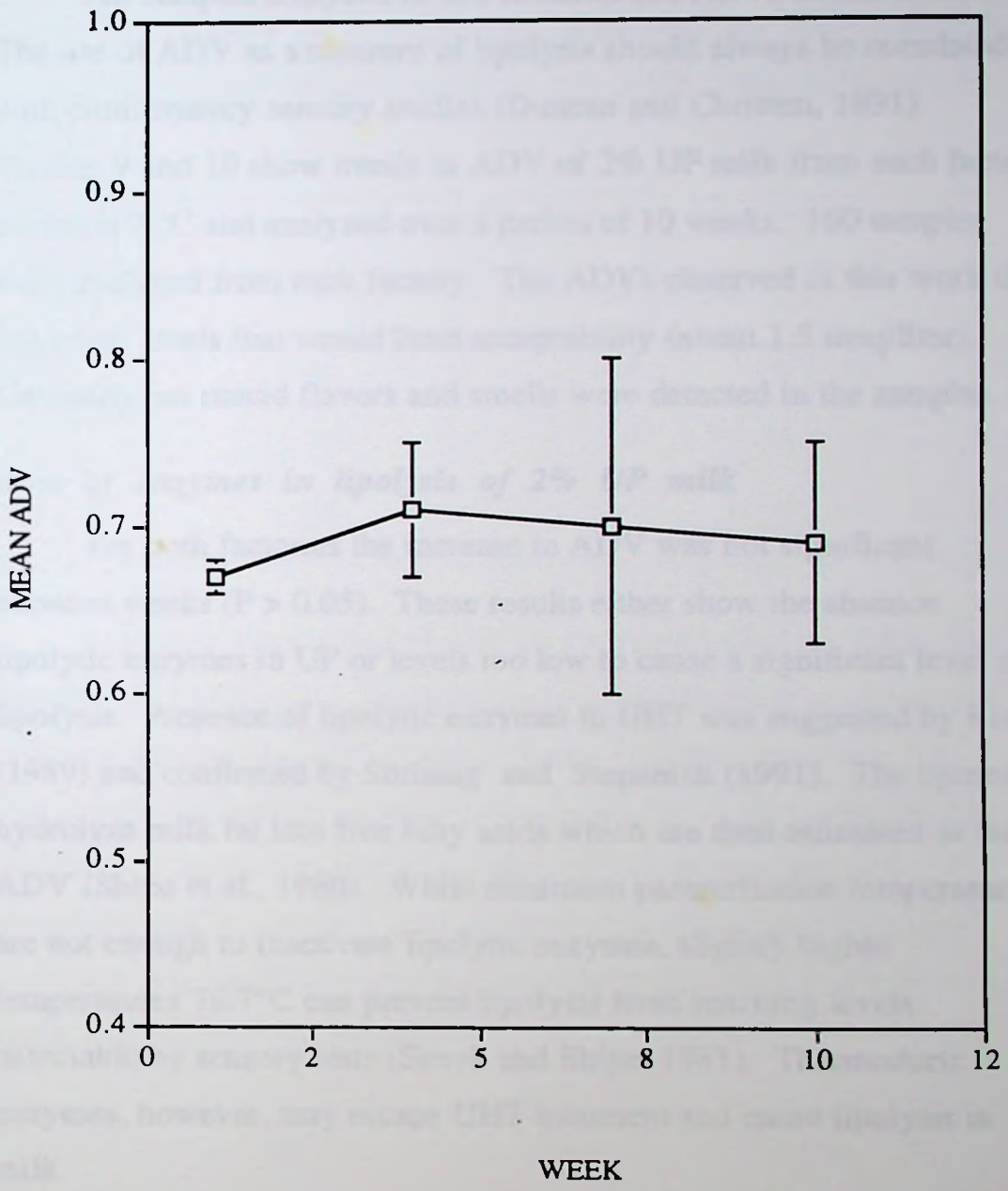

Figure 10. Changes in ADV of 2% UP milk from factory F stored at 7° C.

ADVs of 2% UP

All samples analyzed in this research had ADVs of 0.2 to 0.8. The use of ADV as a measure of lipolysis should always be correlated with confirmatory sensory studies (Duncan and Christen, 1991). Figures 9 and 10 show trends in ADV of 2% UP milk from each factory stored at 7 °C and analyzed over a period of 10 weeks. 160 samples were analyzed from each factory. The ADVs observed in this work did not reach levels that would limit acceptability (about 1.5 meq/liter). Generally, no rancid flavors and smells were detected in the samples.

Role of enzymes in lipolysis of 2% UP milk

For both factories the increase in ADV was not significant between weeks ($P > 0.05$). These results either show the absence lipolytic enzymes in UP or levels too low to cause a significant level of lipolysis. Presence of lipolytic enzymes in UHT was suggested by Kroll (1989) and confirmed by Sörhaug and Stepaniak (1991). The lipases hydrolyze milk fat into free fatty acids which are then estimated as the ADV (Shipe et al., 1980). While minimum pasteurization temperatures are not enough to inactivate lipolytic enzymes, slightly higher temperatures 76.7°C can prevent lipolysis from reaching levels detectable by sensory tests (Senyk and Shipe, 1981). Thermoduric enzymes, however, may escape UHT treatment and cause lipolysis in milk.

Lipolysis in raw milk samples

Table 13. Levels of lipolysis, as measured by ADV, observed in raw skim, raw whole and raw 2% milk from factories H and F

Milk type	Factory H			Factory F		
	ADV (meq/liter)					
Whole	1.60	+/-	0.9	1.98	+/-	0.1
2%	1.18	+/-	0.6	1.55	+/-	0.6
Skim	0.64	+/-	0.6	0.53	+/-	0.3

ADVs are generally higher in unprocessed milk (see Table 13) than 2% UP (<1 meq/l). This is because of the presence of enzyme secreting microorganisms in the raw milk. Lipolytic enzymes accumulate and hydrolyze lipids giving high ADVs interpreted as higher lipolysis. Lipolysis is lower in skim milk which received pre-processing heat treatment, has lower bacterial counts and probably lower levels of lipolytic enzymes. Skim milk also has a lower fat content which is substrate for lipolysis.

Lipolysis in HTST pasteurized milk

The level of lipolysis in HTST pasteurized milk one day after processing (table 13) is much higher than that of 2% UP during week 10 Again, the presence of microorganisms and their lipolytic enzymes in the raw and HTST milk is the likely cause.

Table 14. ADVs of HTST pasteurized milk during post processing refrigerated storage.

Day	ADV (meq/liter)
1	1.2
7	1.4
10	1.8
14	2.4

(Bandler et al., 1985).

Vitamin D

The changes in vitamin D3 before and after processing varied significantly between factories (Table 15) being higher for factory F than H. The decrease in vitamin D after processing can be attributed to heat losses during processing. The milk is fortified with vitamin D before UP processing.

Table 15. Levels of Vitamin D3 in the 2% milk before and after processing and the processing loss expressed as a percentage of the original Vitamin D3

	Factory H			Factory F		
Sample Type	Vitamin D (IU/Qt.)					
Raw-2%	408	+/-	13	511	+/-	6
2% UP	395	+/-	10	420	+/-	26
Mean Processing loss	13	+/-	4	92	+/-	32
% Processing loss	3	+/-	1	18	+/-	6

The role of processing conditions

The differences between factories are probably due to differences in processing and handling conditions (280 °C for 4 seconds and 285°C for 2 seconds for factories F and H respectively). In spite of the losses, the resulting vitamin D levels still met the federal requirement of 400 IU/Qt. (Renken and Warthesen, 1993) given a 5% analytical error of the HPLC method (Rosenberry et al, 1994). This work shows reasonable adherence to the federal vitamin D level requirement. The results contrast with the findings of Tanner et al. (1988) that vitamin D levels were not always in agreement with label claims. The results of this work may be an indication of better adherence to these standards by industry.

Post-processing trends in vitamin D3

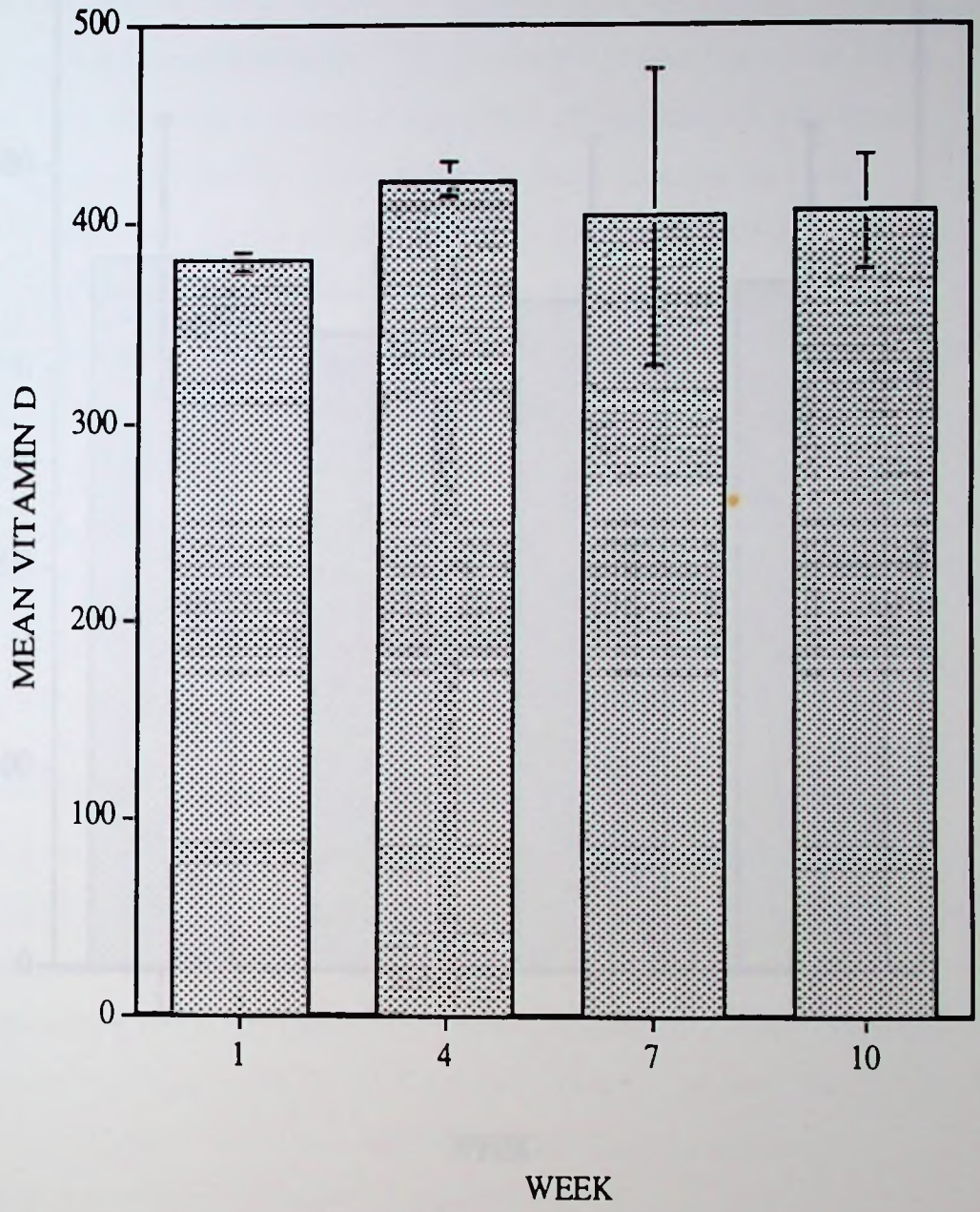

Figure 11. Vitamin D3 in 2% UP milk from factory H stored at 7°C.

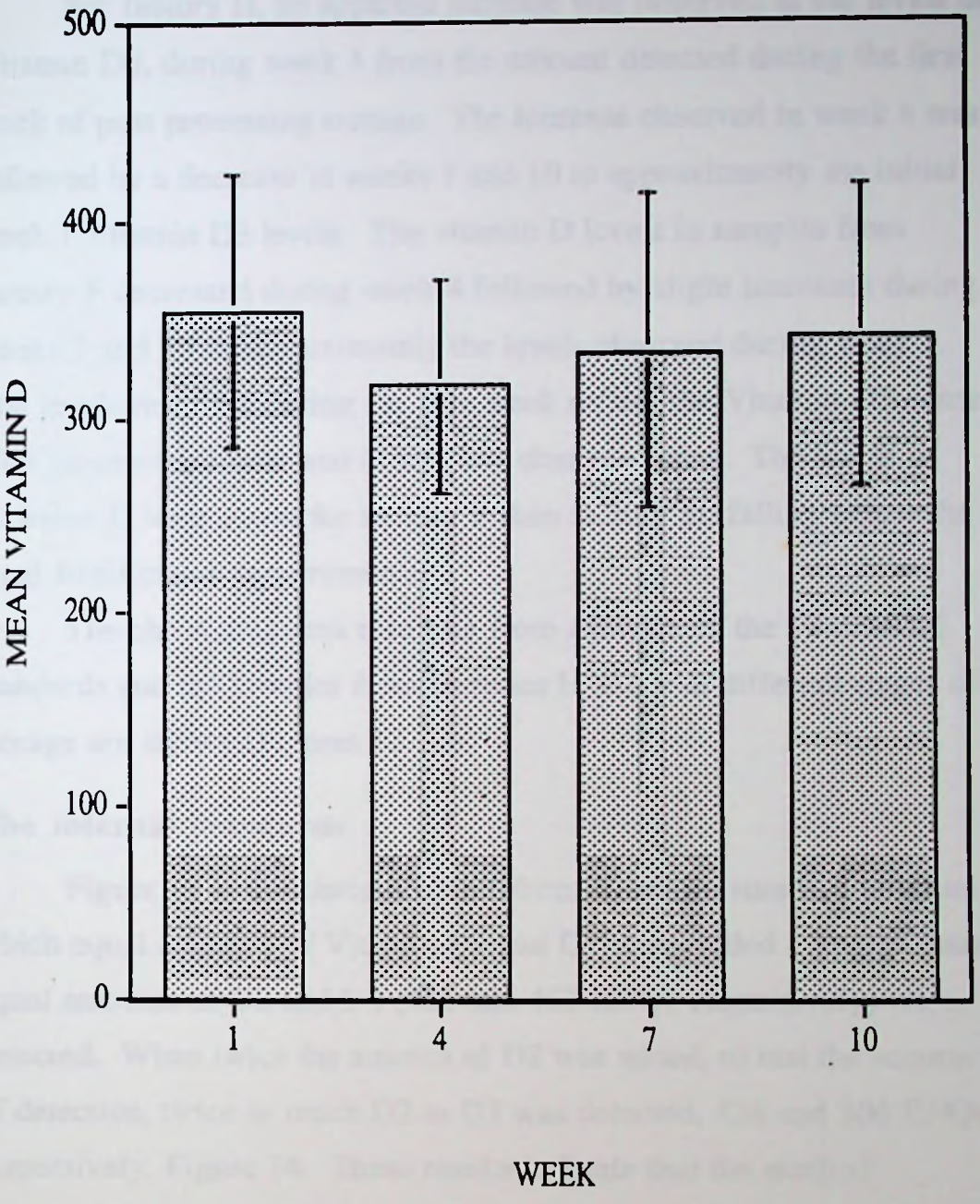

Figure 12. Vitamin D3 in 2% UP milk from factory F stored at 7°C

For factory H, an apparent increase was observed in the levels of Vitamin D3, during week 4 from the amount detected during the first week of post processing storage. The increase observed in week 4 was followed by a decrease in weeks 7 and 10 to approximately the initial week 1 Vitamin D3 levels. The vitamin D levels in samples from factory F decreased during week 4 followed by slight increases during weeks 7 and 10 to approximately the levels observed during week 1. The levels recorded during the first week reflect the Vitamin D3 content after processing losses and before any changes occur. The levels of vitamins D were lower for factory F than factory H, falling below the legal fortification requirements.

The chromatograms resulting from analysis of the vitamin D2 standards and the samples from factories H and F at different stages of storage are shown (Figures 13-22).

The internal standards

Figure 13 is the chromatogram from a sample standard solution to which equal amounts of Vitamin D2 and D3 were added. Approximately equal amounts of D2 and D3 (456 and 462 IU/Qt. respectively) were detected. When twice the amount of D2 was added, to test the accuracy of detection, twice as much D2 as D3 was detected, 426 and 206 IU/Qt. respectively, Figure 14. These results indicate that the method accurately detects both vitamin D2 and D3.

For all vitamin D chromatograms:

PDA is Photo diode array, vitamin D2 is the internal standard added before analysis, vitamin D3 is the native plus the vitamin D added during fortification.

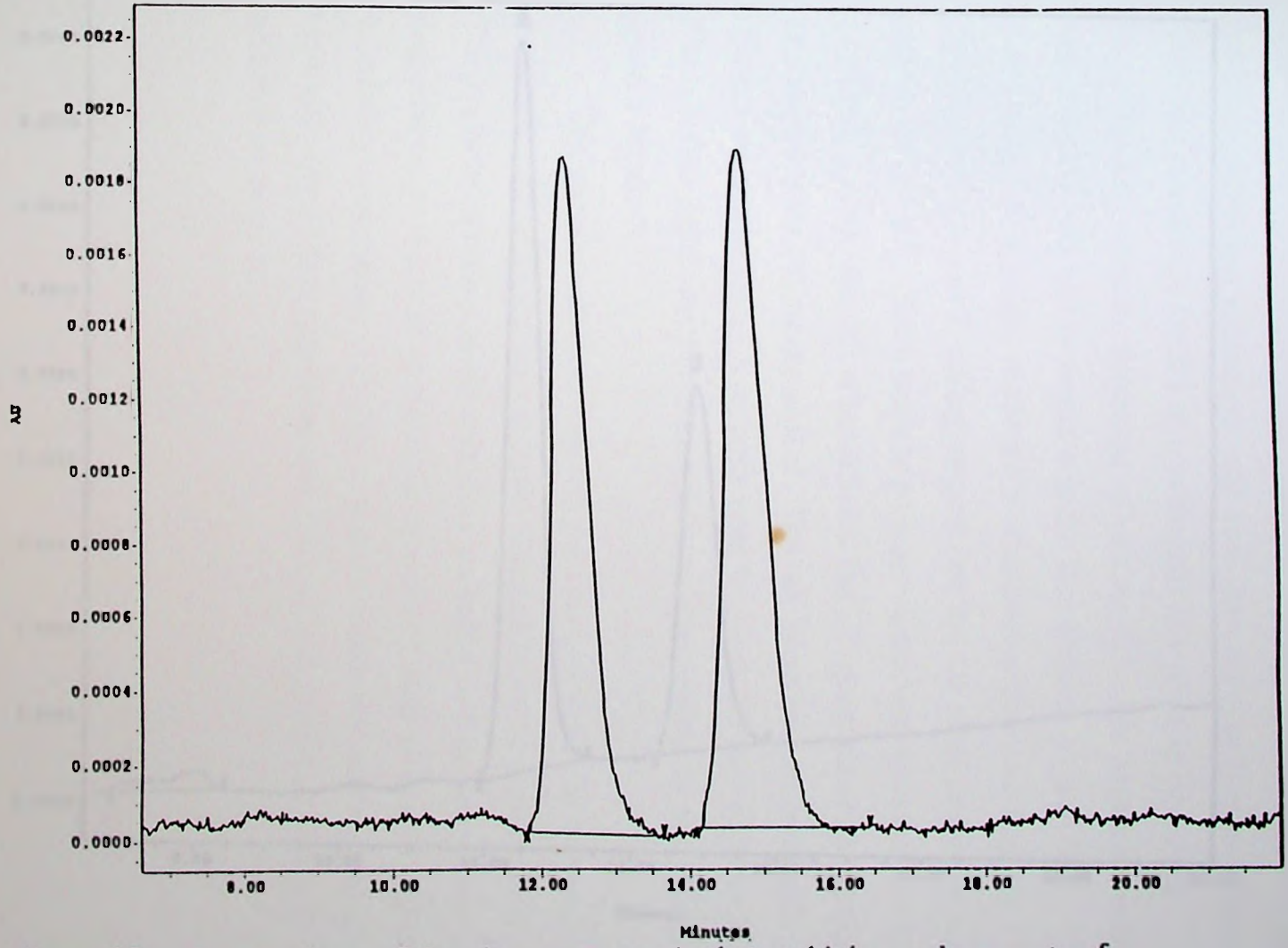

Figure 13. PDA spectrum of the vitamin D standards to which equal amounts of vitamins D2 (left) and D3 (right) were added.

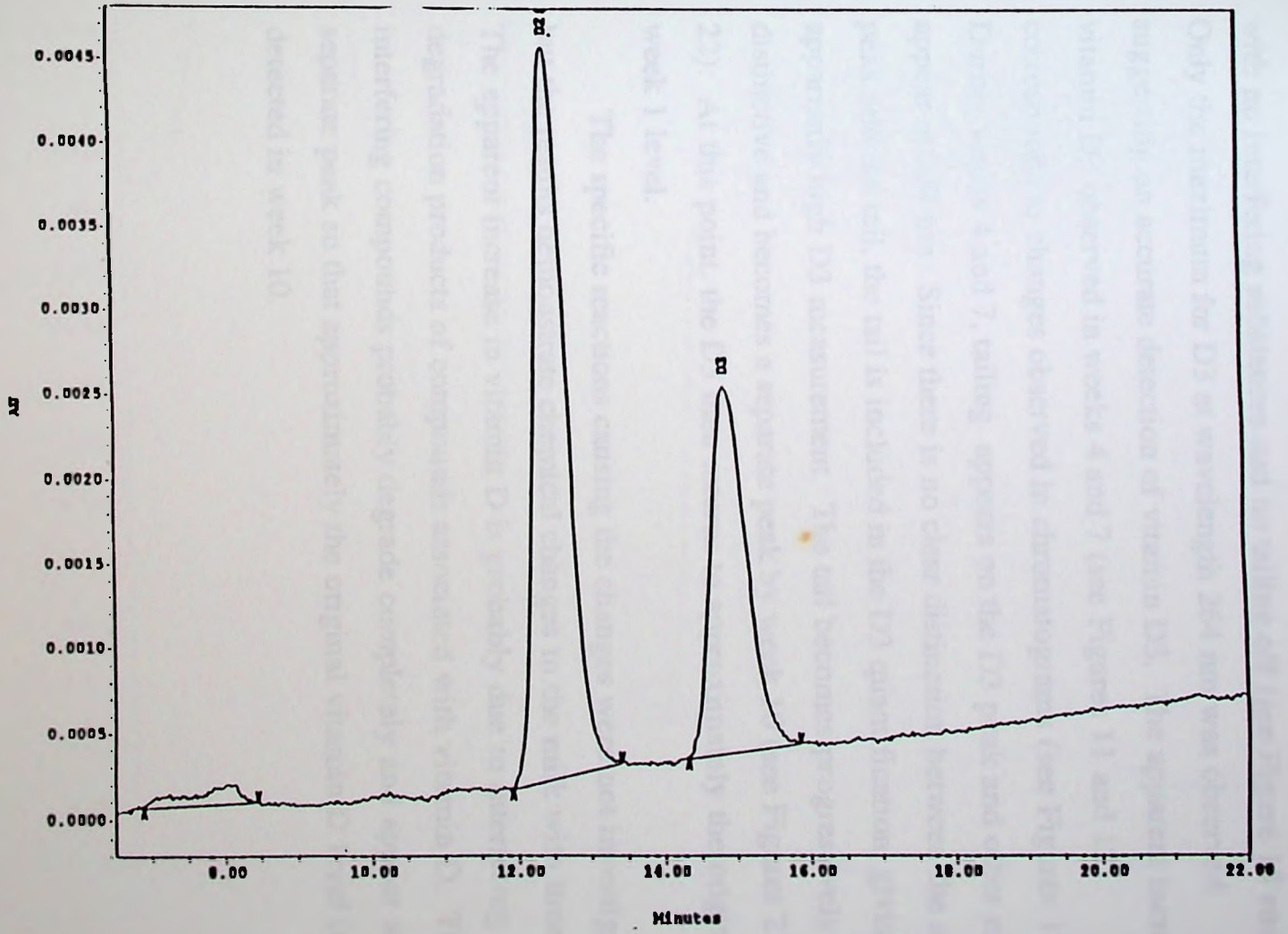

Figure 14. PDA spectrum of the vitamin D standards with twice as much D2 (left) as D3 (right).

The D3 is quantified as the area under the chromatogram curve. The chromatogram from week 1 analysis shows the expected D3 peak with no interfering substances and no tailing off (see Figure 15 and 16). Only the maximum for D3 at wavelength 264 nm was observed, suggesting an accurate detection of vitamin D3. The apparent increase in vitamin D3 observed in weeks 4 and 7 (see Figures 11 and 12) corresponds to changes observed in chromatograms (see Figures 17-20). During weeks 4 and 7, tailing appears on the D3 peak and other maxima appear at 264 nm. Since there is no clear distinction between the actual peak and the tail, the tail is included in the D3 quantification, giving an apparently high D3 measurement. The tail becomes progressively distinctive and becomes a separate peak by week 10 (see Figures 21 and 22). At this point, the D3 then returns to approximately the original week 1 level.

The specific reactions causing the changes were not investigated but the results demonstrate chemical changes in the milk with time. The apparent increase in vitamin D is probably due to interfering degradation products of compounds associated with vitamin D. The interfering compounds probably degrade completely and appear as a separate peak so that approximately the original vitamin D level is detected in week 10.

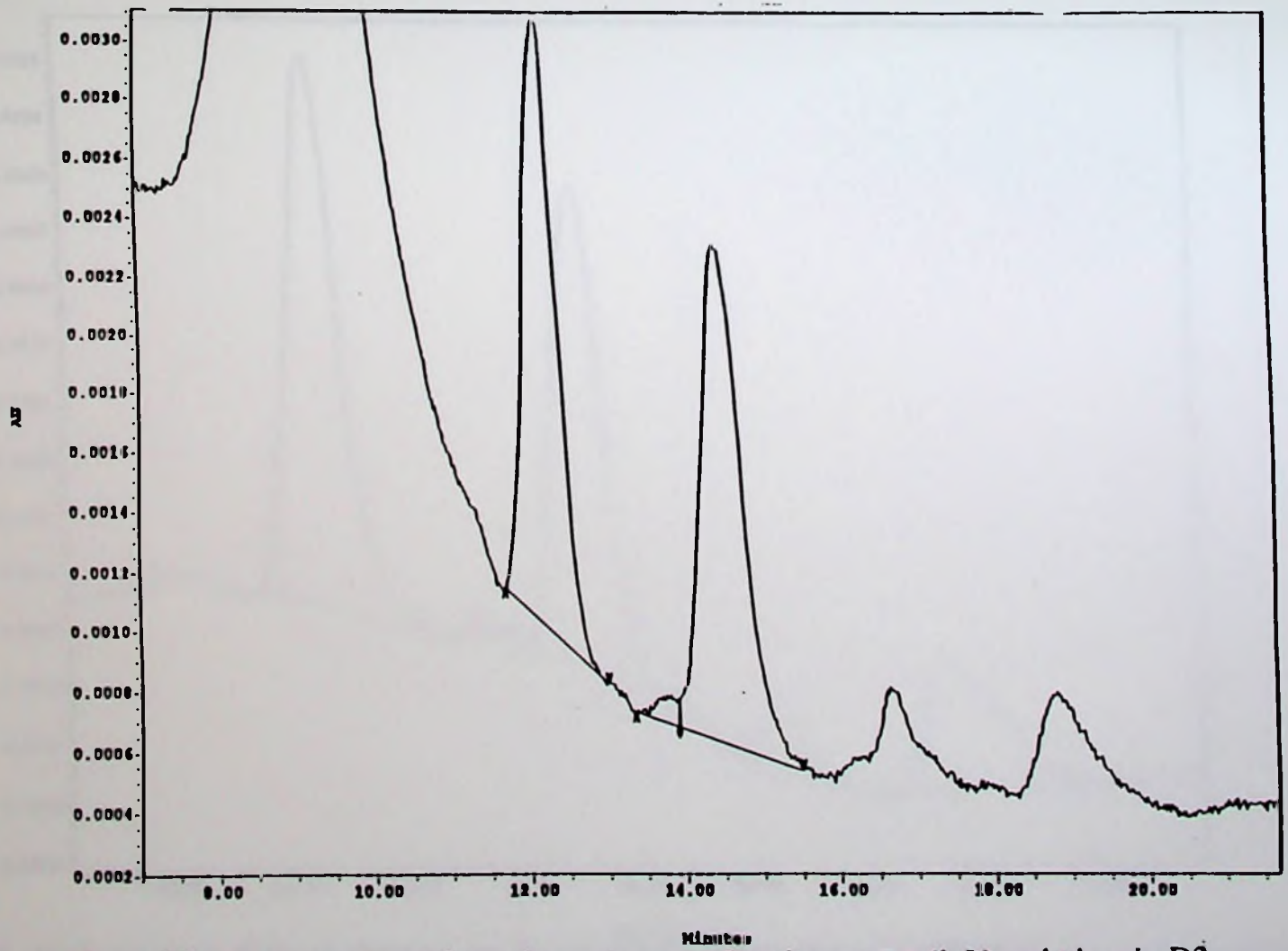

Figure 15. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory H during the first week of storage at 7 °C.

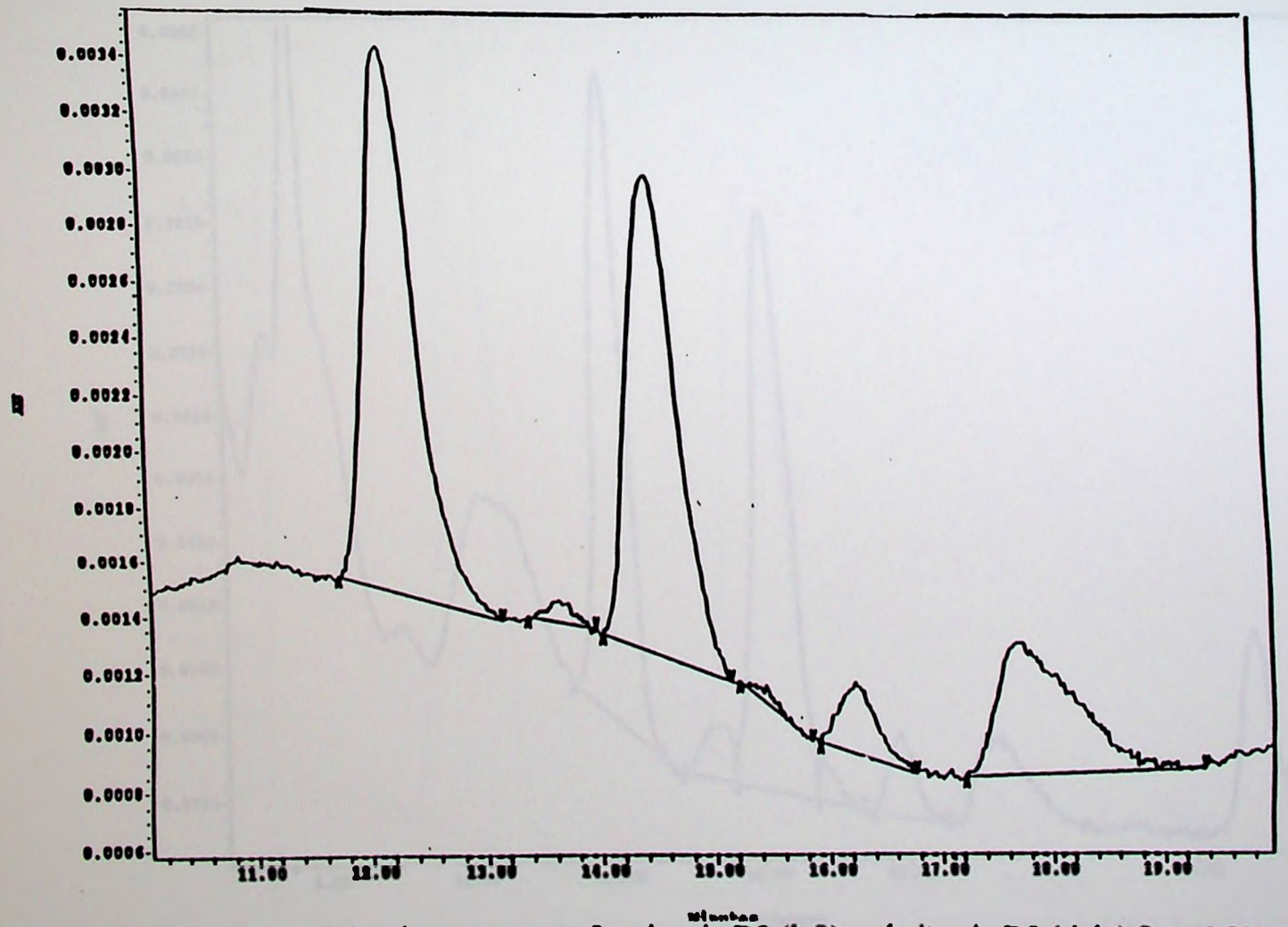

Figure 16. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory F during the first week of storage at 7 °C.

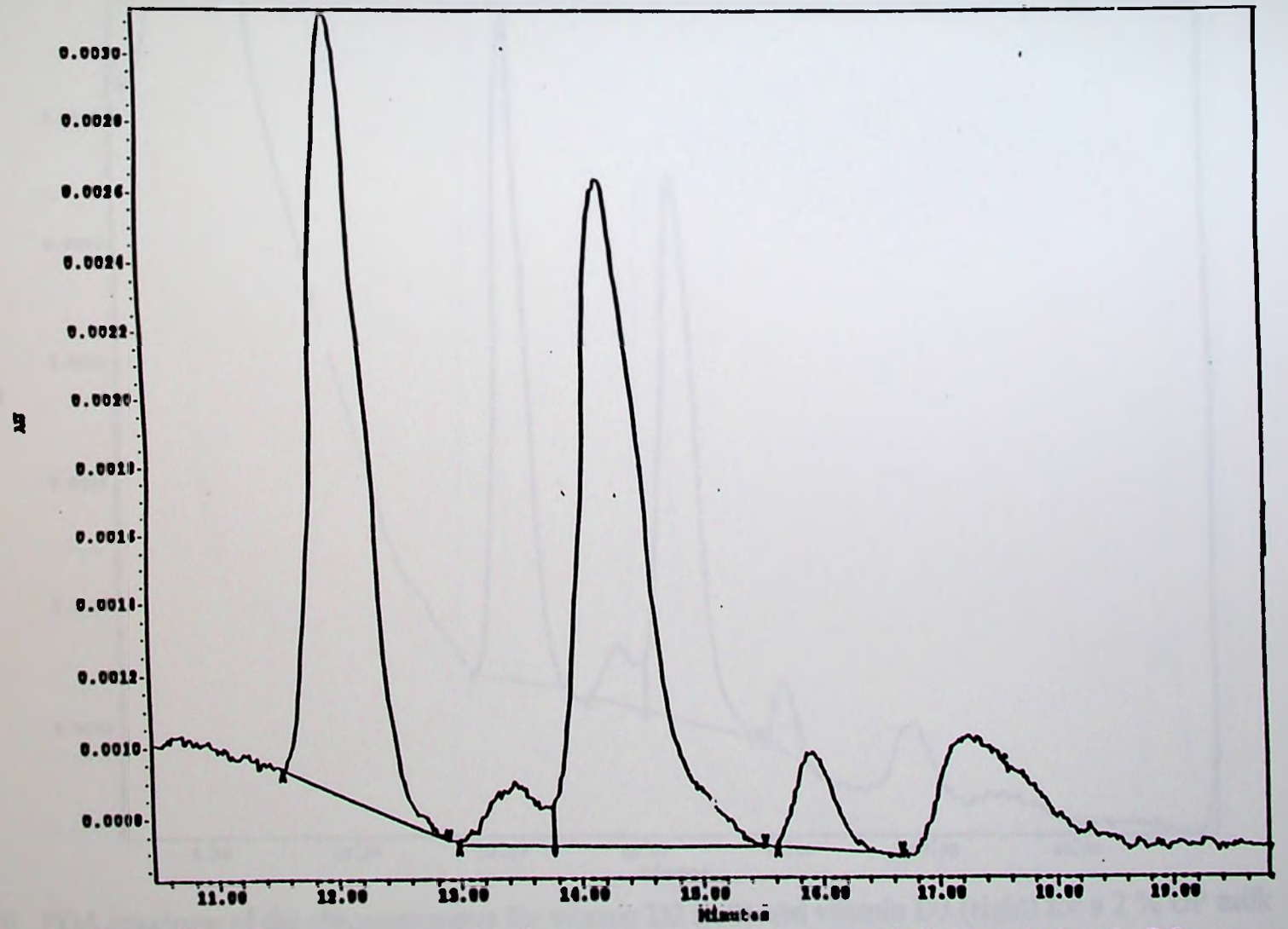

Figure 18. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory F during the fourth week of storage at 7 °C.

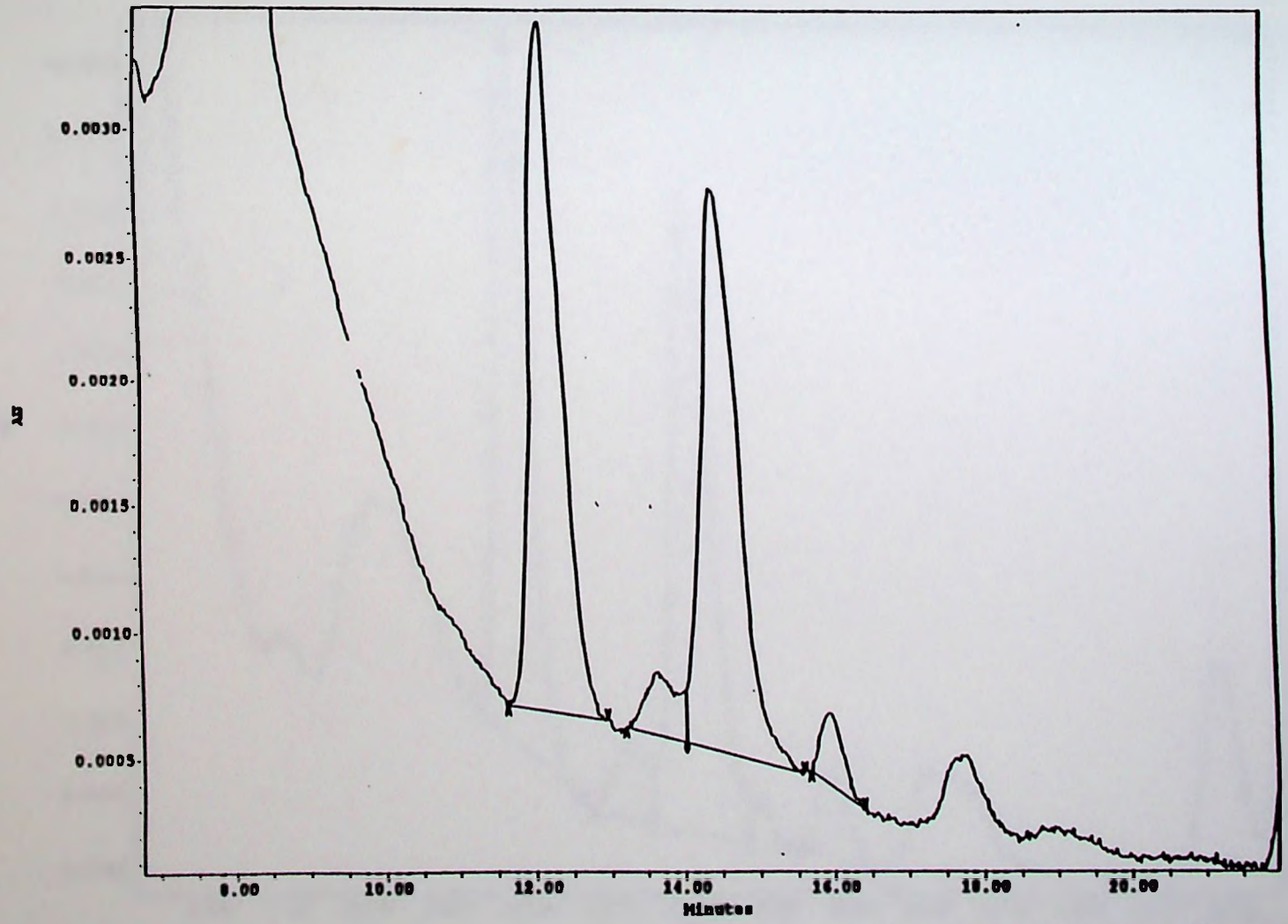

Figure 19. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory H during the seventh week of storage at 7 °C.

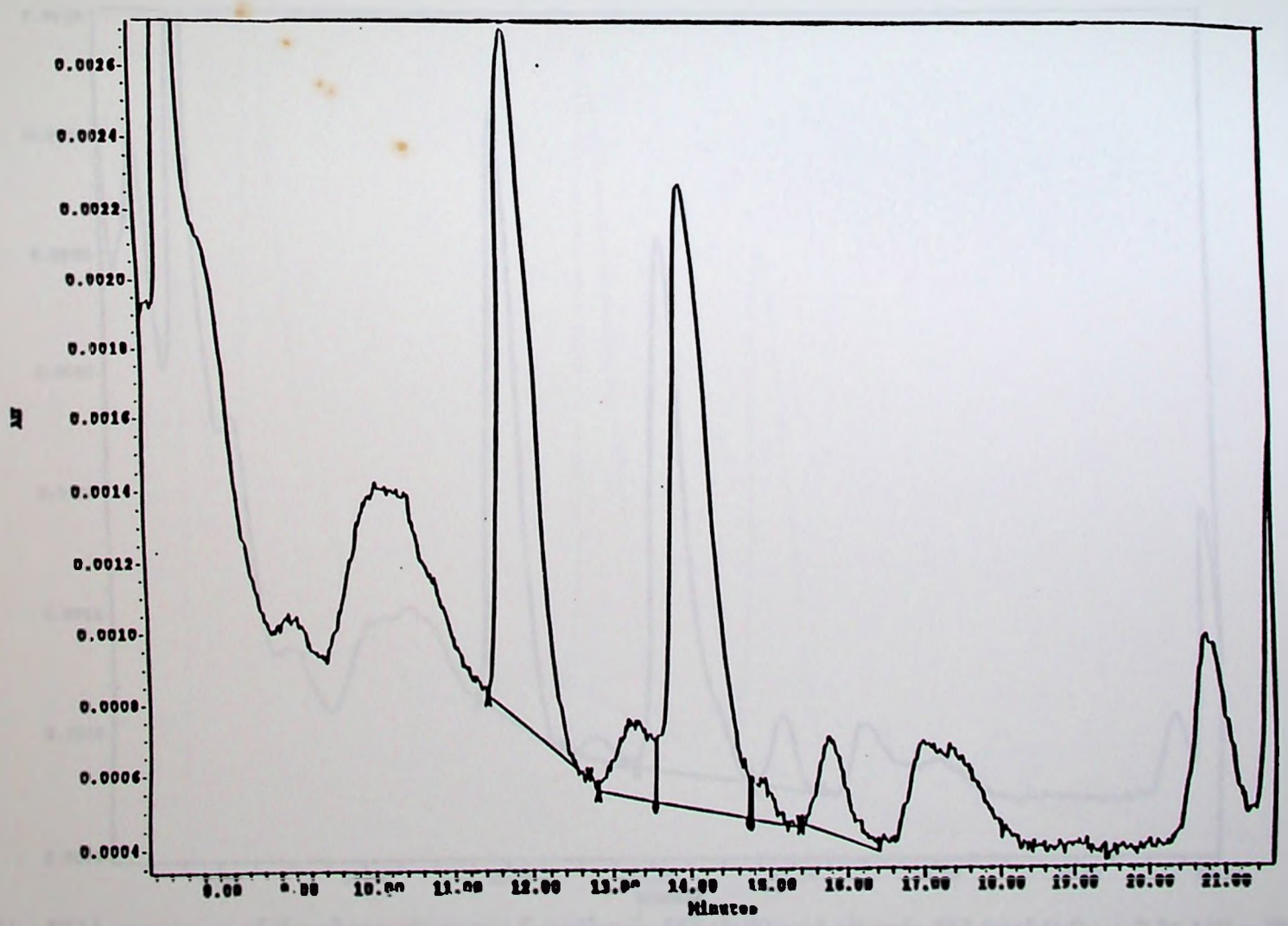

Figure 20. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory F during the seventh week of storage at 7 °C.

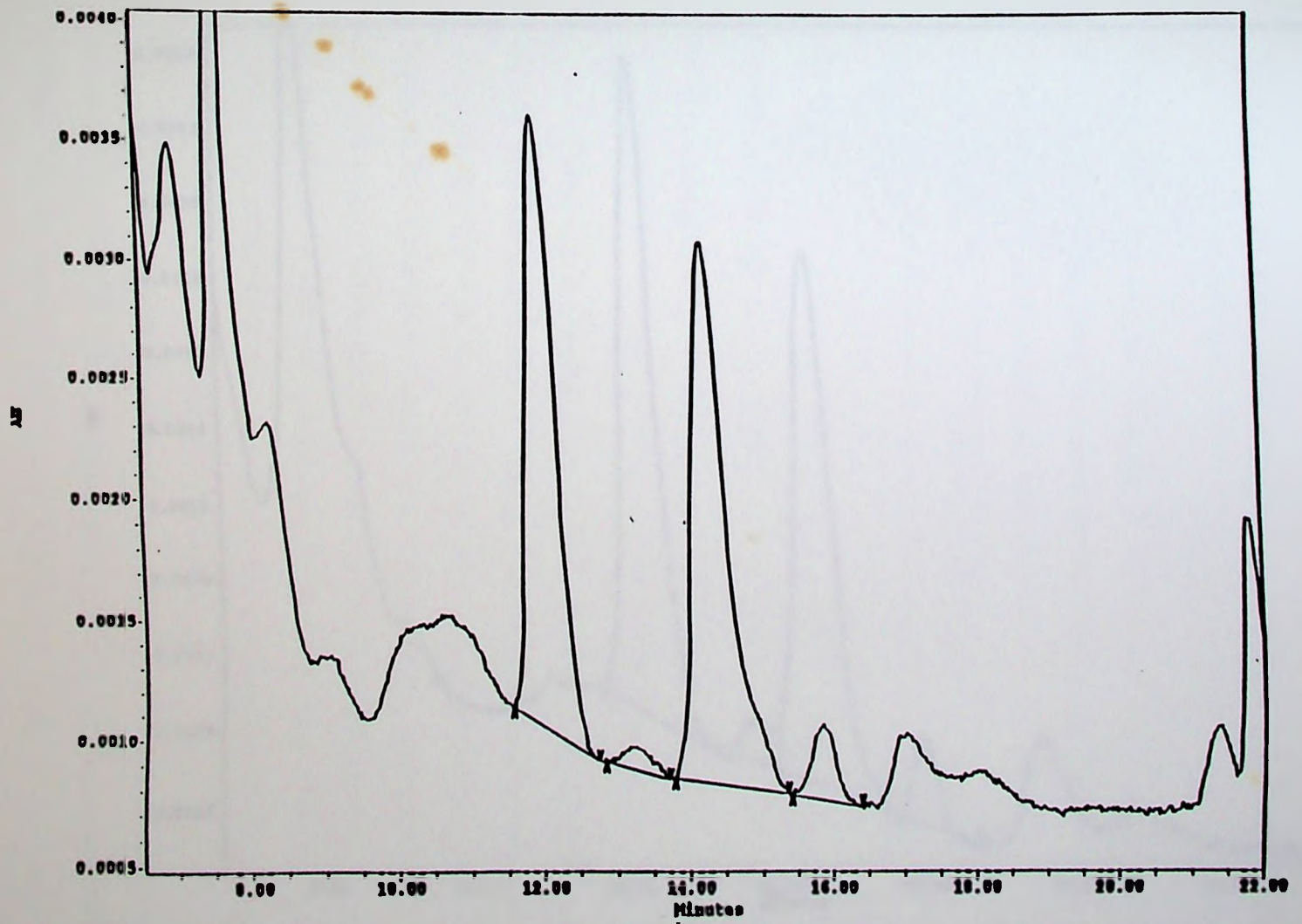

Figure 21. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory H during the tenth of storage at 7 °C.

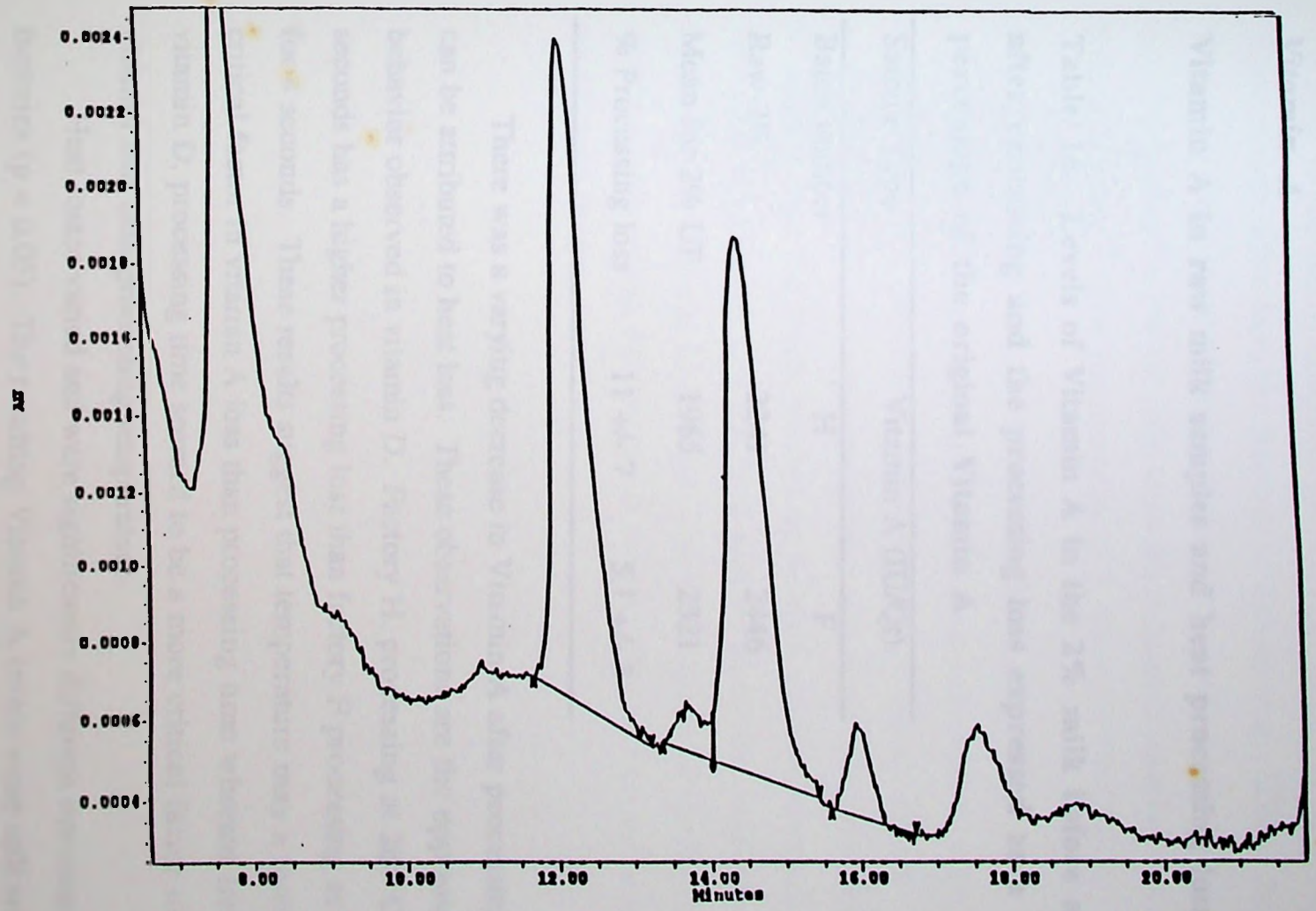

Figure 22. PDA spectrum of the chromatograms for vitamin D2 (left) and vitamin D3 (right) for a 2 % UP milk sample from factory F during the tenth week of storage at 7 °C.

Vitamin A

Vitamin A in raw milk samples and heat processing losses

Table 16. Levels of Vitamin A in the 2% milk before and after processing and the processing loss expressed as a percentage of the original Vitamin A

Sample Type	Vitamin A (IU/Qt).	
Batch number	H	F
Raw-2%	2241	2446
Mean for 2% UP	1965	2321
% Processing loss	11 +/- 7	5.1 +/- 3

There was a varying decrease in Vitamin A after processing which can be attributed to heat loss. These observations are the opposite of the behavior observed in vitamin D. Factory H, processing at 285°C for 2 seconds has a higher processing loss than factory F processing at 280 °C for 4 seconds. These results suggest that temperature may a more critical factor in vitamin A loss than processing time whereas for vitamin D, processing time seemed to be a more critical factor to vitamin loss than processing temperature.

Heat losses varied and were significantly different between factories ($p < 0.05$). The resulting Vitamin A levels were still within the federal requirement of 2,000-3,000 IU/Qt. (Fellman et al., 1990) given a

5% error of the HPLC analytical method (Rosenberry et al, 1994). These results differ from the findings of Tanner et al., (1988) that vitamin A levels were not always in agreement with label claims. This may imply that the 2% UP milk from the industries sampled met the federal vitamin A level requirements of 2, 000 to 3, 000 IU/Qt.

Post processing changes in vitamin A in 2% UP

The samples examined meet and maintain the Federal requirement levels (2, 000 to 3, 000 IU/Qt) all through the 10 weeks of post processing storage. An apparent increase was observed in the levels of Vitamin A from the fourth through the seventh week of storage, followed by a decrease in week 10 to approximately the level detected in the first week (Figures 23 and 24). The levels recorded during the first week probably reflect the Vitamin A content after processing losses, before any changes occur.

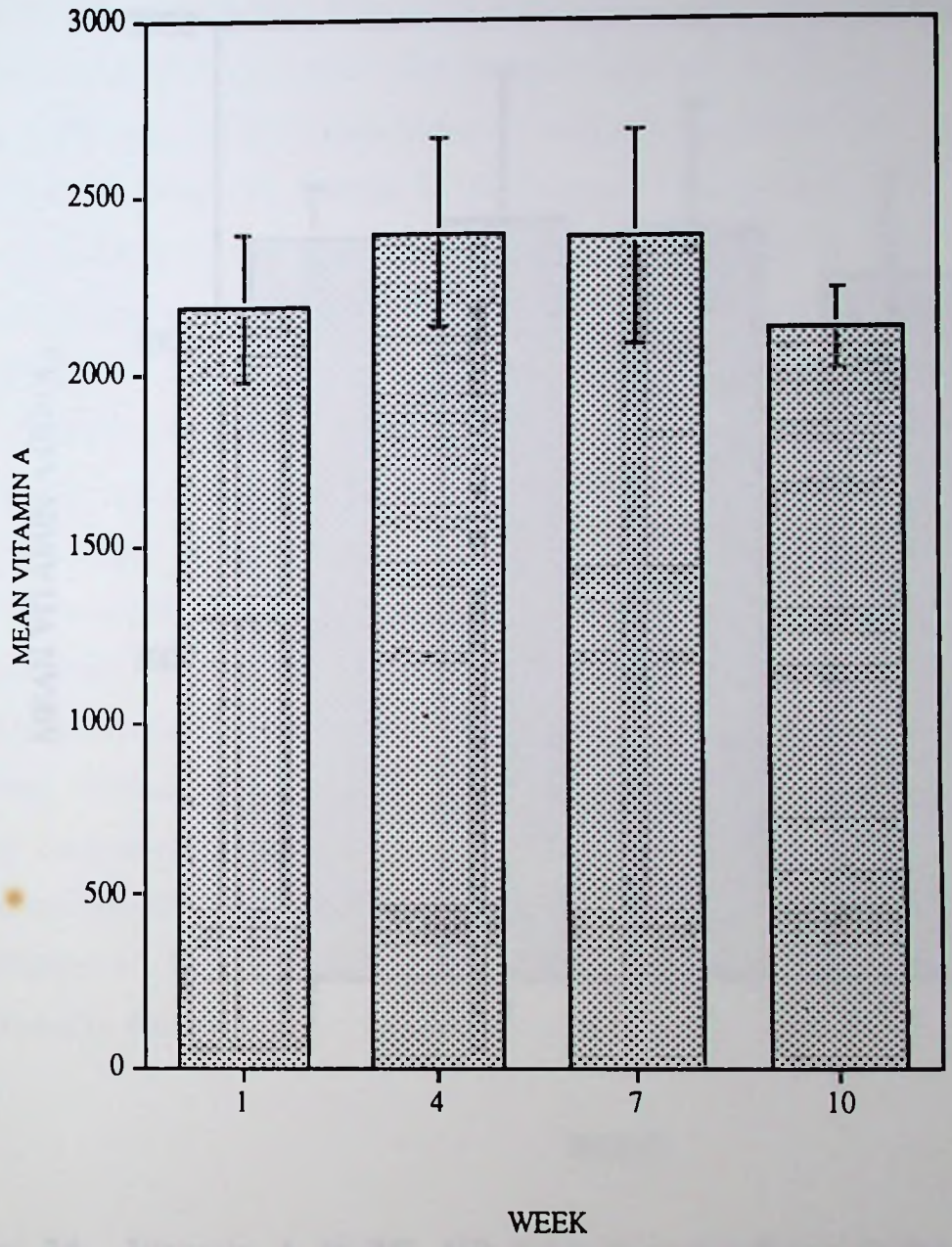

Figure 23. Vitamin A in 2% UP milk from factory H stored at 7°C

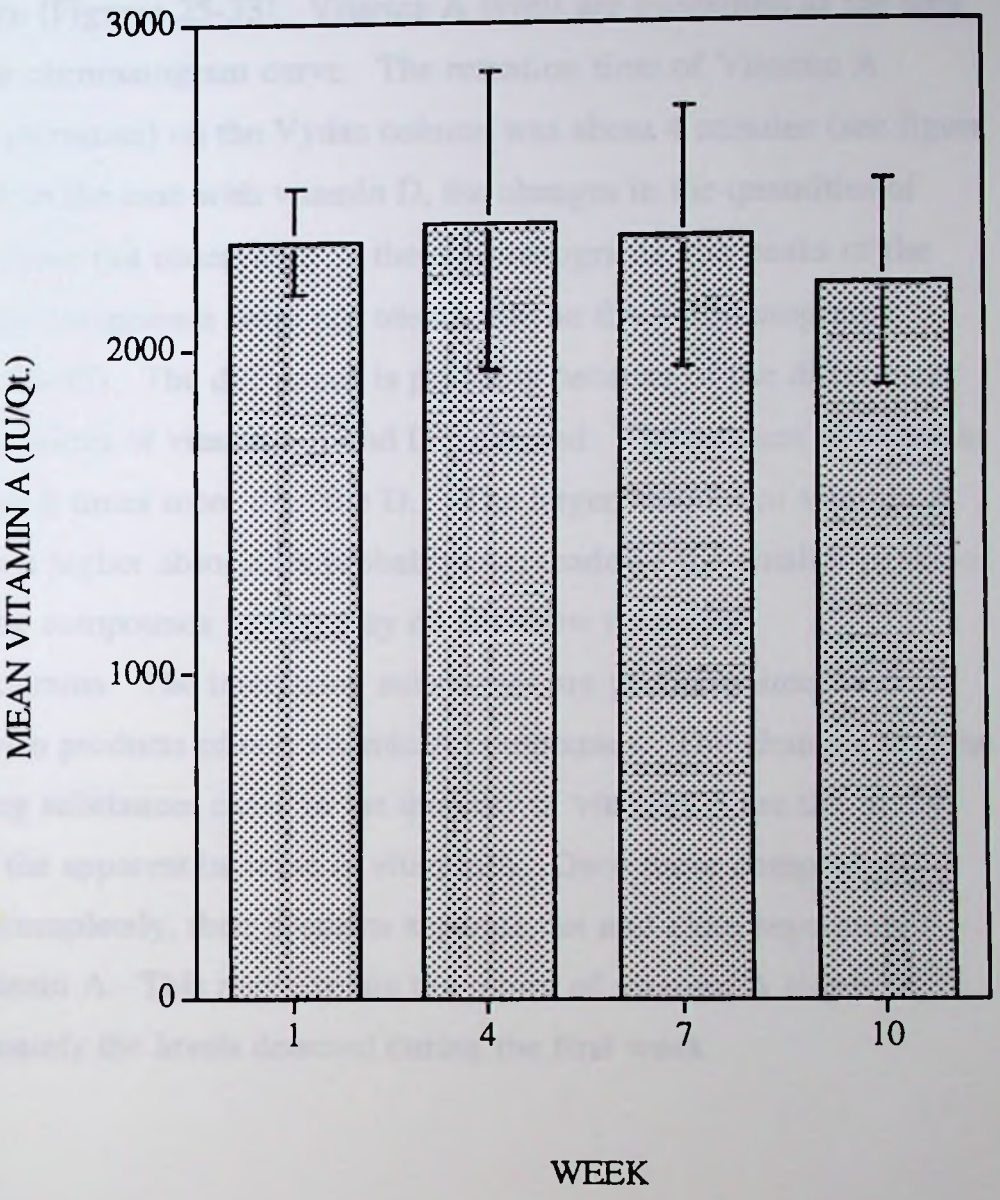

Figure 24. Vitamin A in 2% UP milk samples from factory F stored at 7°C.

The chromatograms from the analyses of vitamin A standards and the samples from factories H and F during the different stages of storage are shown (Figures 25-33). Vitamin A levels are quantified as the area under the chromatogram curve. The retention time of Vitamin A (Retinyl palmitate) on the Vydac column was about 4 minutes (see figure 25). Unlike the case with vitamin D, the changes in the quantities of vitamin A are not observable on the chromatograms, the peaks of the interfering compounds were not observable on the chromatograms (Figures 26-33). The difference is probably because of the differences in the quantities of vitamins A and D measured. The amount of vitamin A is about 8 times more than the D. The larger amount of vitamin A results in a higher absorption probably overshadows the smaller peaks of interfering compounds so that they do not show up on the chromatograms. The interfering substances are probably intermediate break down products of non vitamin A compounds. The changes that the interfering substances cause in the quantity of vitamin A are the likely cause of the apparent increase in vitamin A. Once these compounds degrade completely, their products separate out and elute separately from vitamin A. This may explain the return of vitamin A levels to approximately the levels detected during the first week.

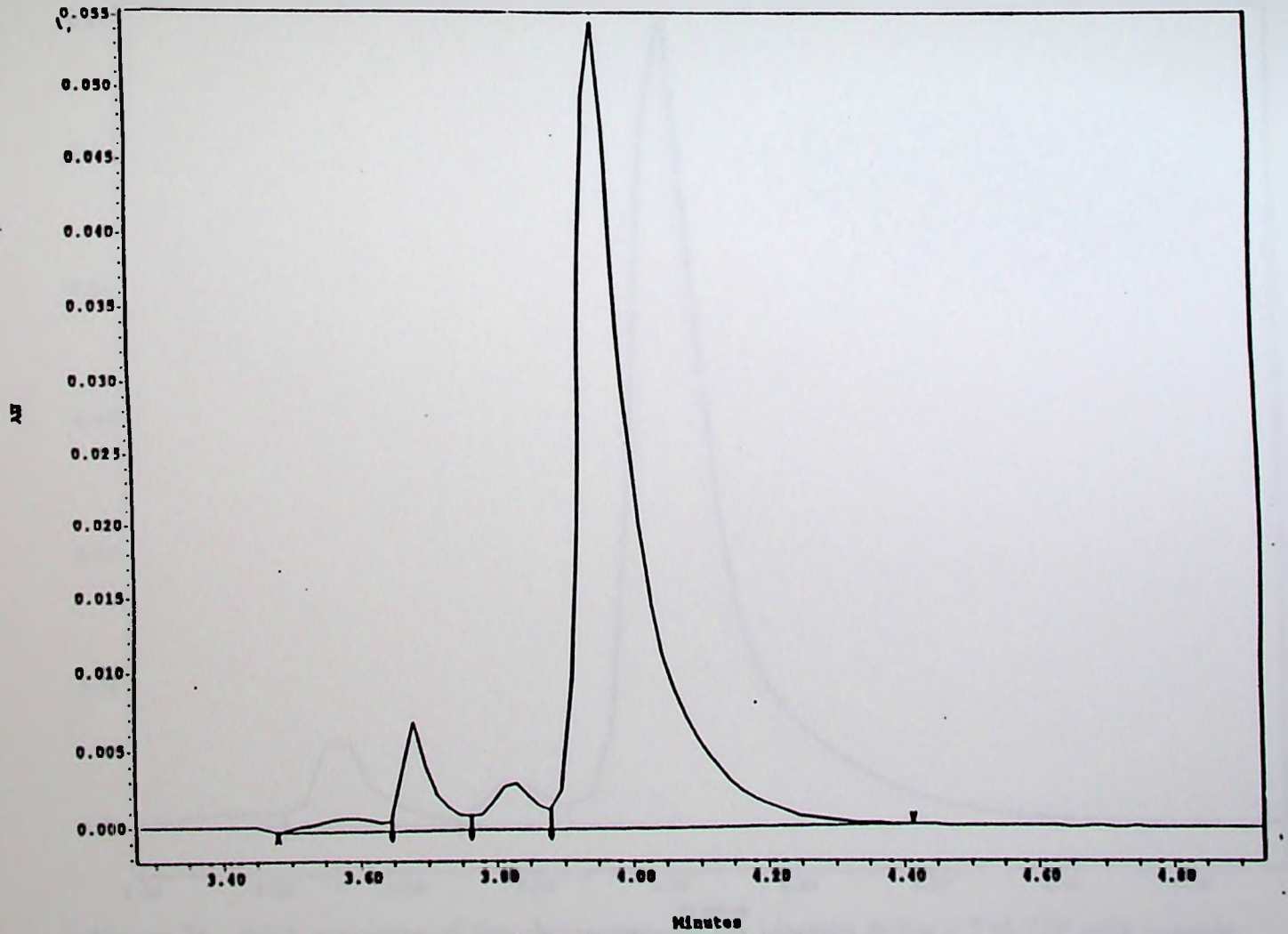

Figure 25. PDA spectrum of thevitamin A (Retinal palmitate) standard.

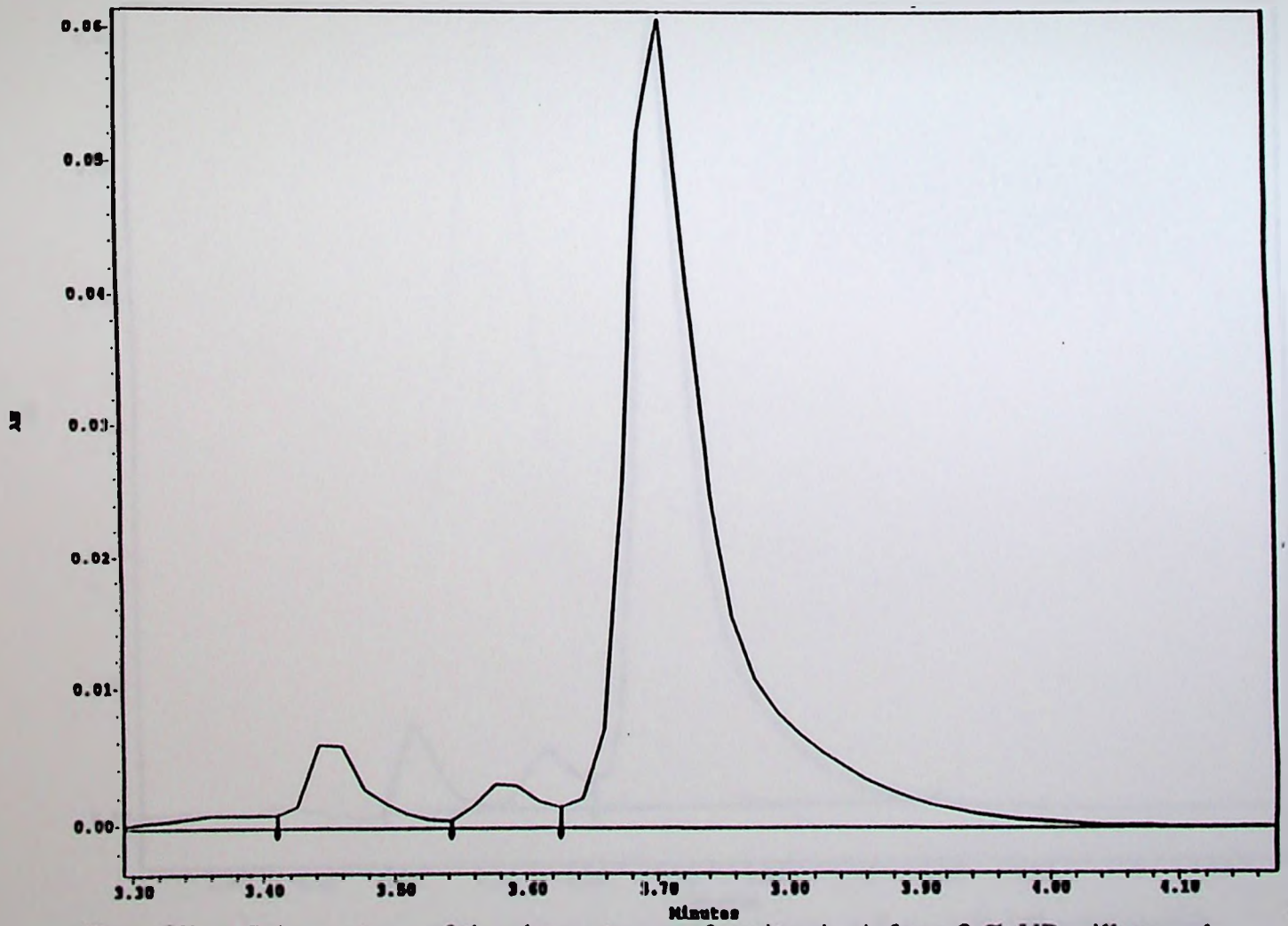

Figure 26. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory H during the first week of storage at 7 °C.

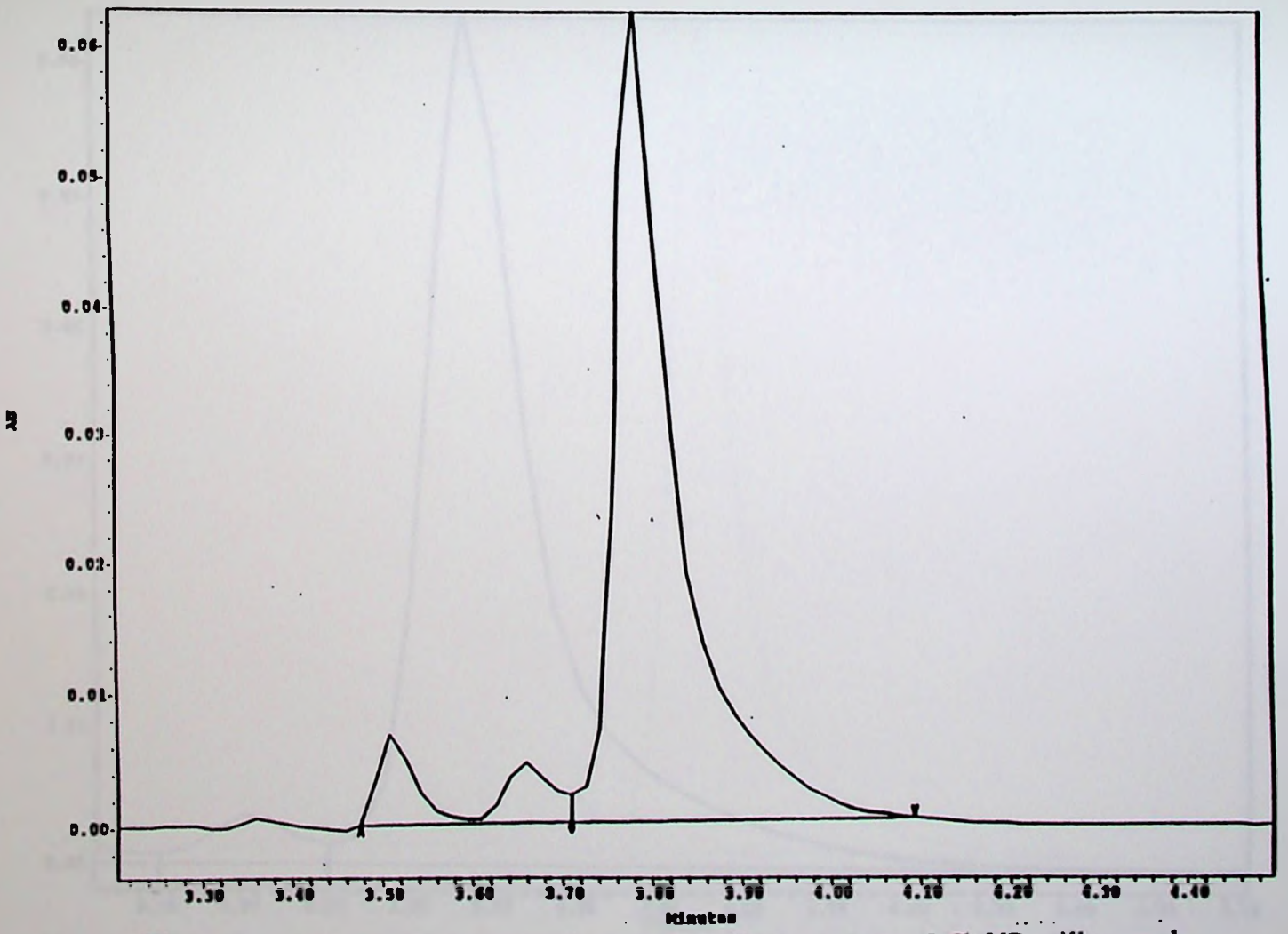

Figure 27. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory F during the first week of storage at 7 °C.

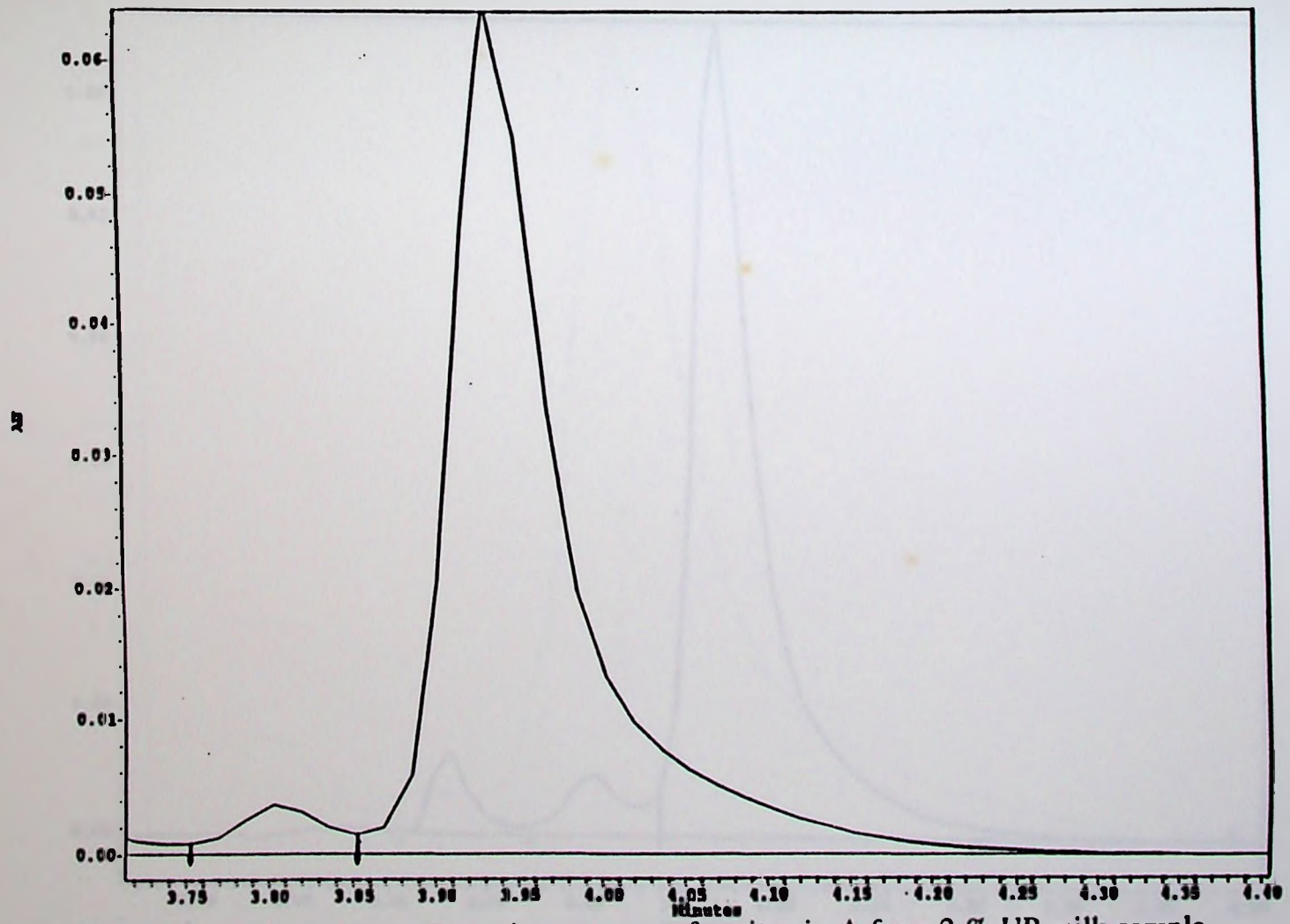

Figure 28. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory H during the fourth week of storage at 7 °C.

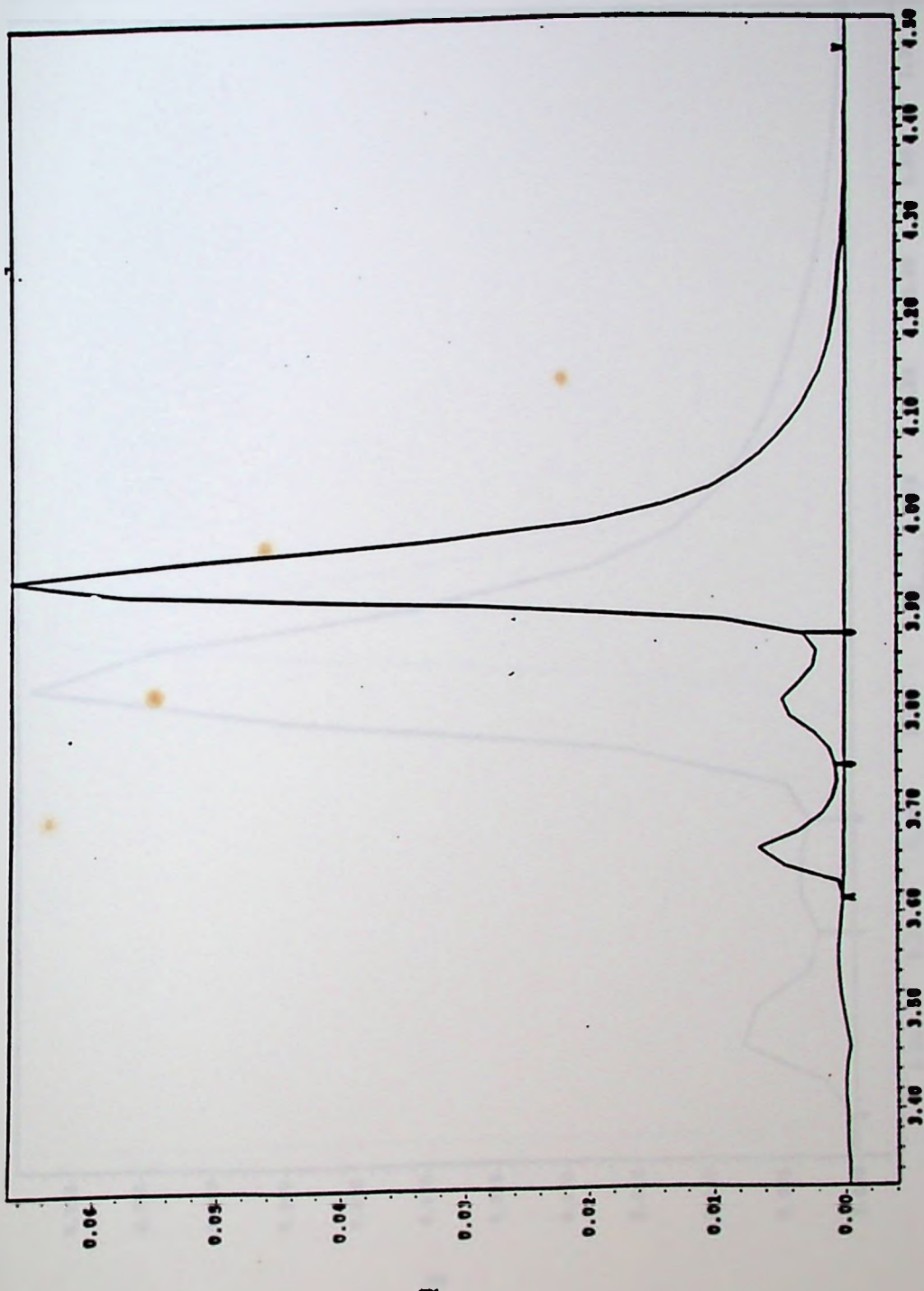

Figure 29. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory F during the fourth week of storage at 7 °C.

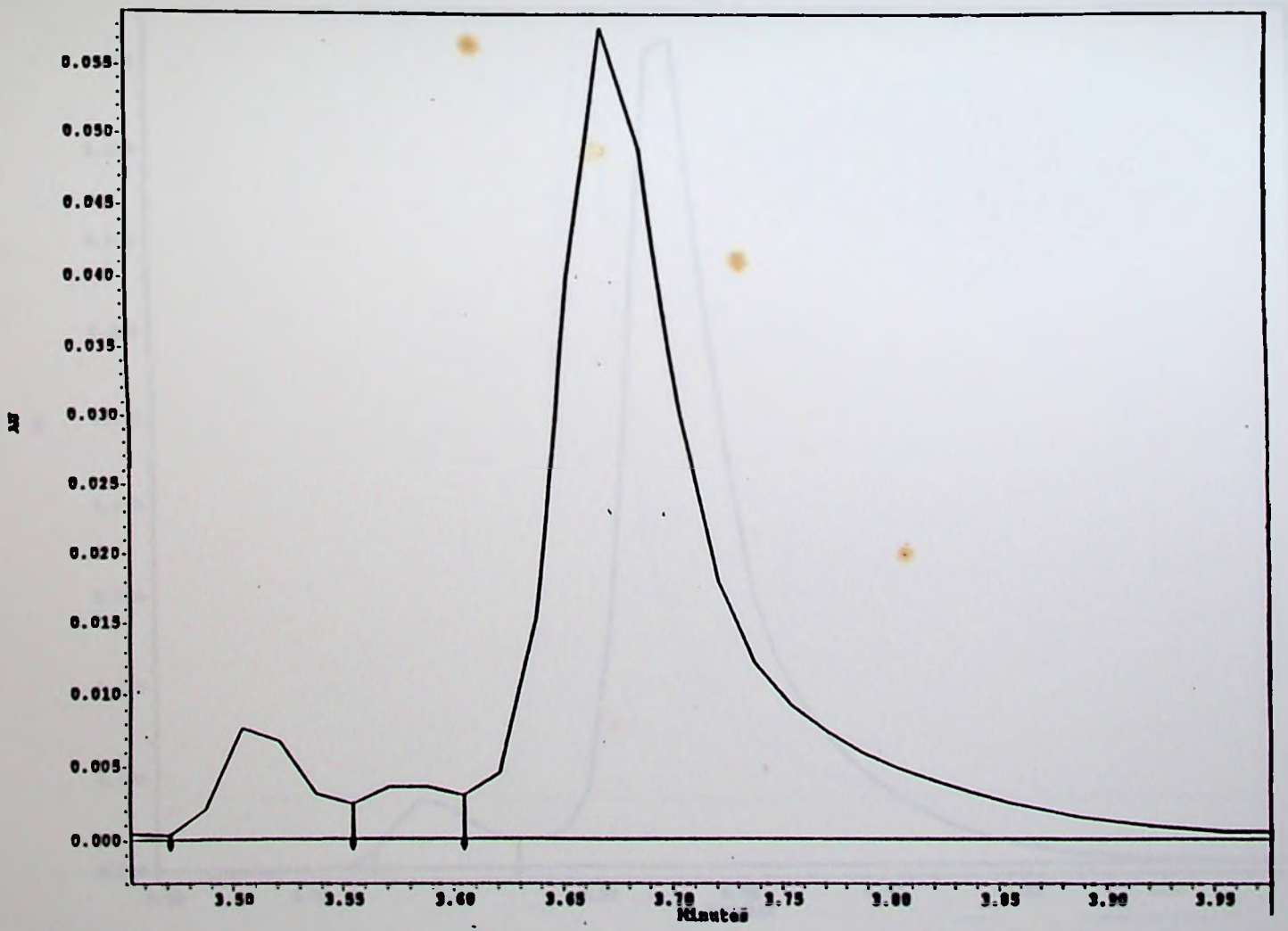

Figure 30. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory H during the seventh week of storage at 7 °C.

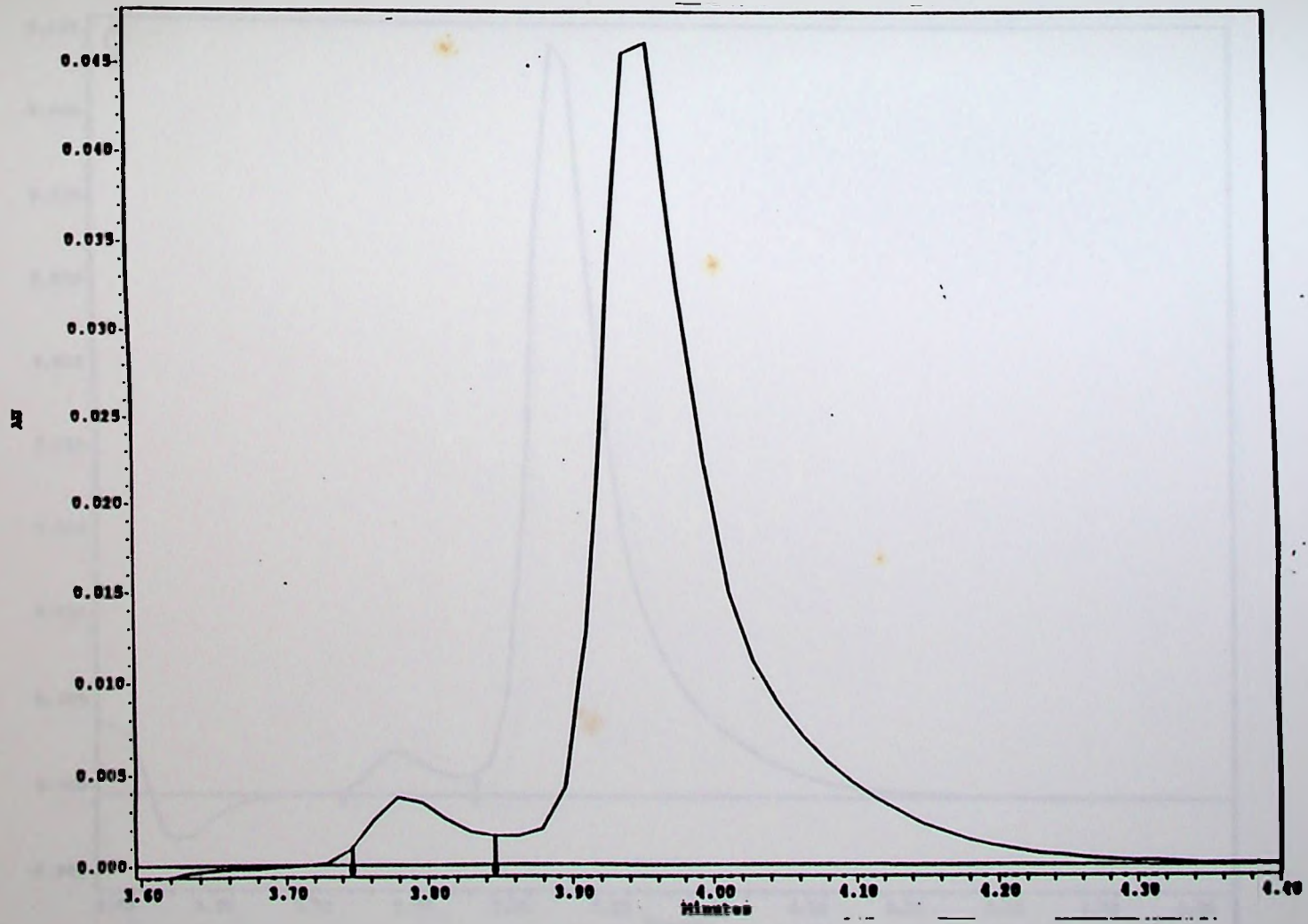

Figure 31. PDA spectrum of the chromatograms for vitamin A for a 2 % UP milk sample from factory F during the seventh week of storage at 7 °C.

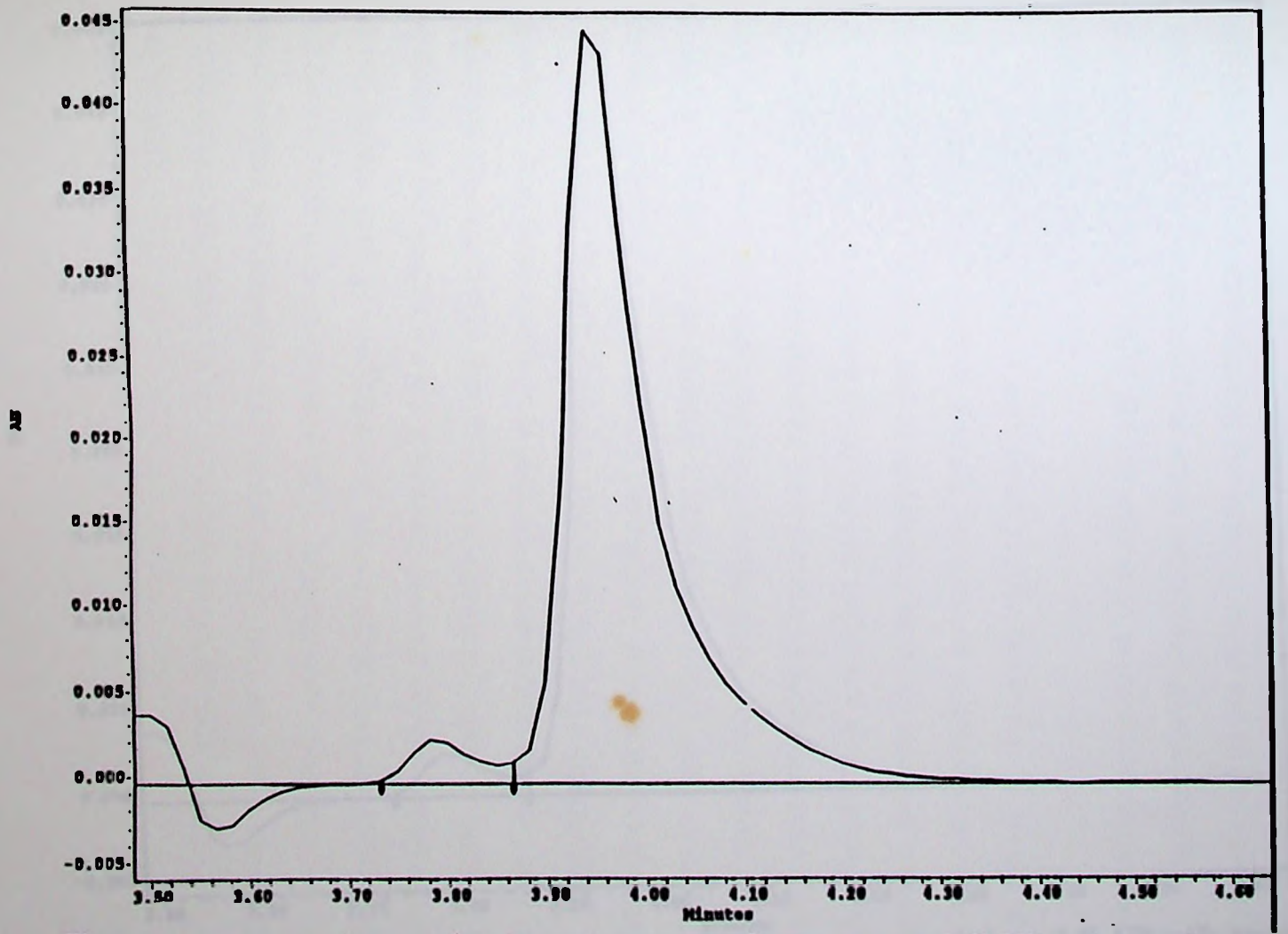

Figure 32. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory H during the tenth week of storage at 7 °C.

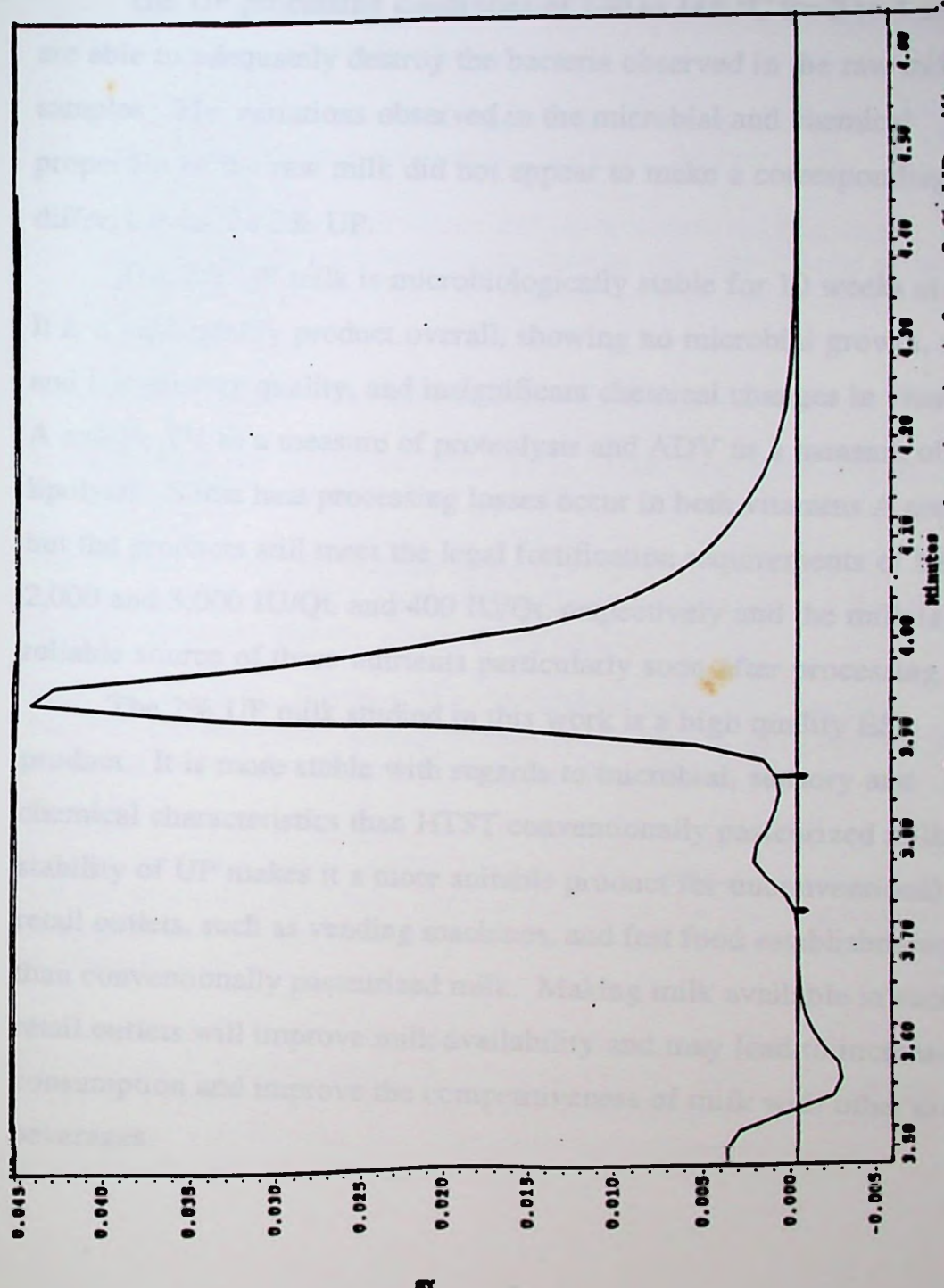

Figure 33. PDA spectrum of the chromatogram for vitamin A for a 2 % UP milk sample from factory F during the tenth week of storage at 7 °C.

SUMMARY AND CONCLUSIONS:

The UP processing conditions of 140 to 145 °C for 2 to 4 seconds are able to adequately destroy the bacteria observed in the raw milk samples. The variations observed in the microbial and chemical properties of the raw milk did not appear to make a corresponding difference in the 2% UP.

The 2% UP milk is microbiologically stable for 10 weeks at 7°C. It is a high quality product overall, showing no microbial growth, stable and fair sensory quality, and insignificant chemical changes in vitamins A and D, TV as a measure of proteolysis and ADV as a measure of lipolysis. Some heat processing losses occur in both vitamins A and D but the products still meet the legal fortification requirements of between 2,000 and 3,000 IU/Qt. and 400 IU/Qt. respectively and the milk is a reliable source of these nutrients particularly soon after processing.

The 2% UP milk studied in this work is a high quality ESL product. It is more stable with regards to microbial, sensory and chemical characteristics than HTST conventionally pasteurized milk. The stability of UP makes it a more suitable product for unconventional retail outlets, such as vending machines, and fast food establishments, than conventionally pasteurized milk. Making milk available in such retail outlets will improve milk availability and may lead to increased consumption and improve the competitiveness of milk with other cold beverages.

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