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ULTRA-HIGH TEMPERATURE PROCESSING OF MILK

UTAH STATE UNIVERSITY

MARCH 2 -3, 1994




WESTERN CENTER

WEDNESDAY, MARCH 2, 1994

TIME	TOPIC/SPEAKER
8:00 AM	Continental breakfast and registration
8:45 AM	Welcome and introductions ROD BROWN, Utah State University
8:50 AM	SESSION 1: CHANGES INDUCED IN MILK BY UHT PROCESSING Chairman: Harry Harwalkar
8:55 AM	History of UHT milk HARRY HARWALKAR, Agriculture Canada
9:00 AM	Lipase activity in UHT treated dairy products BART WEIMER, USU
9:30 AM	Reactions of lactose in UHT milk M. van BOEKEL, Wageningen University
10:10 AM	MILK BREAK: GOSSNER'S FOODS
10:30 AM	Denaturation and complexing of milk proteins MOHAN REDDY, USU
11:10 AM	Changes in casein micelle structure DONALD MCMAHON, USU
12:00 PM	LUNCH
1:30 PM	SESSION 2: SELECTING A UHT PROCESSING SYSTEM Chairman: Paul Savello
1:35 PM	UHT processing: From research to production PAUL SAVELLO, USU
1:40 PM	Heat transfer aspects of UHT processing RAUL NUNES, Richardson-Vicks, Mexico
2:20 PM	Spiral tubular heat exchanger for UHT processing: Benefits and advantages WILFRED HERMANS, STORK
3:00 PM	BREAK
3:20 PM	Applications of direct steam UHT heating PER OKE PERRSOR, Tetra Pak
4:00 PM	Regulatory control points in UHT processing STEVE GRALL, Cream Products
4:40 PM	Scraped surface UHT heat exchange systems DERRYL WERNIMONT, Cherry-Burrell
5:20 PM	END SESSION
6:30 PM	DINNER

THURSDAY, MARCH 3, 1994

TIME	TOPIC/SPEAKER
8:00 AM	SESSION 3: CHANGES DURING STORAGE OF UHT MILK Chairman: Greig Zadow
8:05 AM	Bringing consensus to UHT milk research GREIG ZADOW, J.G. Zadow & Associates, Australia
8:10 AM	Effect of proteolytic enzymes on the stability of UHT treated milk SUZANNE NIELSEN, Purdue University
8:50 AM	Flavor changes during UHT heating of milk FLOYD BODYFELT, Oregon State University
9:20 AM	Using recombined milk for UHT processing GREIG ZADOW, J.G. Zadow & Associates, Australia
9:55 AM	MILK BREAK: HERSHEY
10:15 AM	Flavor changes during storage of aseptically packaged dairy products ART HANSEN, North Carolina State University
10:55 AM	Changes in protein structure during storage DONALD MCMAHON, USU
11:25 AM	Stability of concentrated milks HARRY HARWALKAR, Agriculture Canada
12:00 PM	LUNCH
1:30 PM	SESSION 4: DEVELOPING NEW UHT MILK PRODUCTS Chairman: Donald McMahon
1:35 PM	Maximizing UHT technology DONALD MCMAHON, USU
1:40 PM	UHT lactose hydrolyzed milk GREIG ZADOW, J.G. Zadow & Associates, Australia
2:10 PM	UHT products from around the world LYNN ANDERSON, Tetra Pak
2:50 PM	STORK BREAK
3:20 PM	Concentrating milk: New UHT product concept PAUL SAVELLO, USU
4:00 PM	Product development: From concept to production REED ERNSTROM, Heart to Heart Foods
4:40 PM	Closing remarks DONALD MCMAHON, USU
5:00 PM	END SYMPOSIUM



Heat Stable Enzymes in Dairy Products



Bart Weimer, Ph.D.
Utah State University
Department of Nutrition & Food Sciences



Sources of Heat Stable Enzymes

- ❖ **Milk itself**
 - Plasmin
- ❖ **Bacterial metabolism**
 - *Pseudomonas*
 - *Flavobacterium*
 - thermophilic psychrotrophs

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Pseudomonads

- ❖ Commonly found in raw milk (contaminant)
 - *P. fluorescens* dominates
- ❖ Requires oxygen to grow
- ❖ Grow at low temperature (psychrotroph)
- ❖ Cells are heat sensitive (pasteurization)
- ❖ No acid produced during growth
- ❖ Post-processing contamination common

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Flavobacteria

- ❖ Commonly found in raw milk (contaminant)
- ❖ Pigmented (orange to yellow)
- ❖ psychrotroph
- ❖ slow growth

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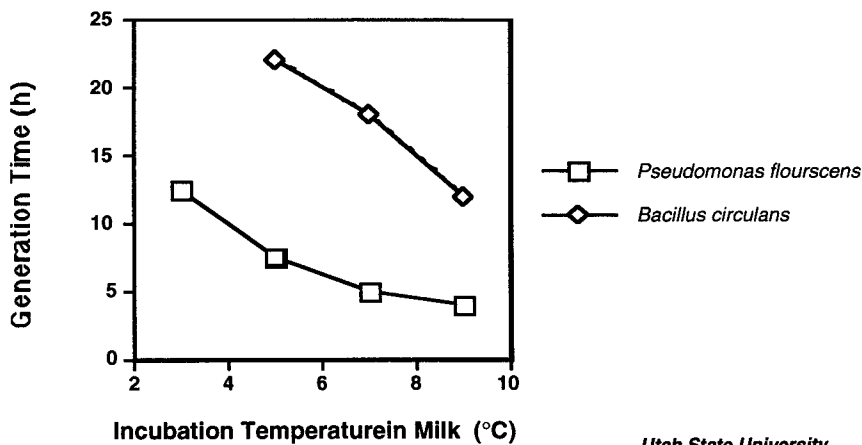
Thermophilic Psychrotrophs

- ❖ Spores survive high heat, grow well at low temperature
- ❖ Diverse group - *Bacillus* is main concern
 - *B. circulans*
 - *B. coagulans*
 - *B. cereus*
 - *B. subtilis*
- ❖ Spores are of concern, not active cells

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Effect of Temperature on Growth



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Metabolites of Bacteria

- ❖ Most metabolites are heat labile
- ❖ Requires 10^5 - 10^7 CFU/ml to detect influence
- ❖ Enzymes
 - Proteinases - degrade protein , zinc & calcium important
 - Lipases - degrade lipid (fat)
 - Glycosidases - degrade carbohydrate (sugar)
- ❖ Acid - bitty cream defect

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Heat Stable Proteinases

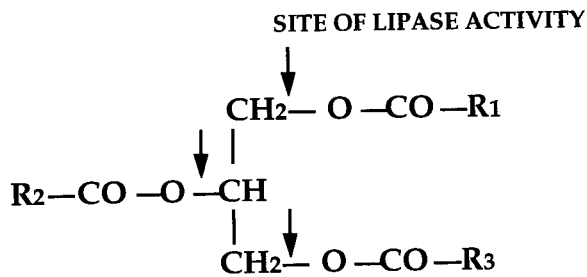
- ❖ Pseudomonas primarily
- ❖ Metals stabilize enzymes
 - zinc maintains active site
 - calcium increases heat stability
- ❖ Less heat resistance at pasteurization than UHT
- ❖ complete inactivation is 9 min at 120°C (4,000 times more heat stable than *B. stearothermophilus* spores)
- ❖ Strain dependant (usually more than 1 enzyme)
- ❖ Secreted during the growth phase (maximum at 0°C , pH 7.0)
- ❖ Active at 4°C

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Lipase Activity

- ❖ **LIPASES:** A group of enzymes responsible for cleavage of fatty acids from triacylglycerides.



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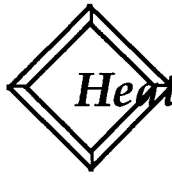


Industrial Concerns

- ❖ **EXTENDED SHELF LIFE MILK (UHT):**
Cumulative off flavors due to the release of short chain fatty acids from milkfat
- ❖ **CAPPUCCINO STYLE MILK:**
Poor frothing of milk due to increased free fatty acid concentration

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Heat Stable Pseudomonas Lipase

- ❖ Produced during the exponential phase
- ❖ Inhibited by metals
- ❖ β -lactoglobulin, κ -casein protect activity during heating
- ❖ Active at 10°C
- ❖ Yield varying flavor changes
 - short (4:0-6:0) = rancid, goaty
 - medium (10:0-12:0) = unclean, soapy
 - long (>14:0) = no flavor change
- ❖ Decreases steam frothing
- ❖ More than one lipase in UHT milk
- ❖ heat stable phospholipases also known - bitty cream
- ❖ Lipase and protease interact to cause defects

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Detection Methods

- ❖ Time/Activity = Detection of psychrotroph enzymes require 10^5 - 10^7 CFU/ml; extended incubation allows detection of 10^3 - 10^4 CFU/ml
- ❖ Current assays are not sensitive enough
- ❖ Measure FFA's released over time
 - directly measures total free fatty acids released
 - incubation period, solvent extraction, labor intensive
- ❖ ELISA-based detection
 - very sensitive to antigen enzyme, very fast
 - expensive, doesn't correlate with lipase activity in milk
- ❖ Spectrophotometric methods
 - sensitive, inexpensive
 - requires clarification step

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Colorimetry - Color wheel

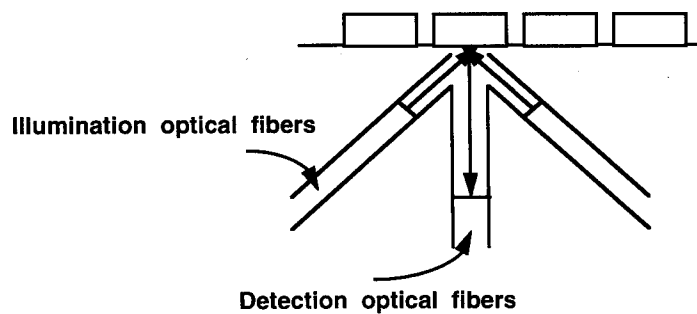
- ❖ Color spectrum is divided into 3D color space
 - L^* measures white to black
 - a^* measures green to red
 - b^* measures blue to yellow

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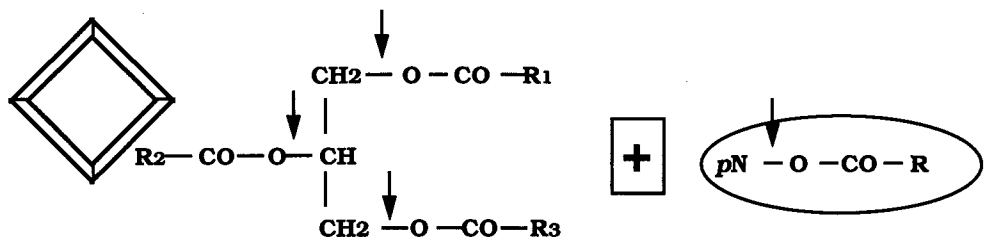
Detection System

- ❖ Colorimetric detection of color change
- ❖ Advantage: Reflectance rather than transmittance measured .



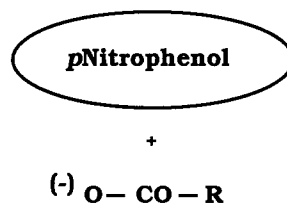
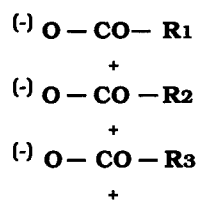
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Triglycerides

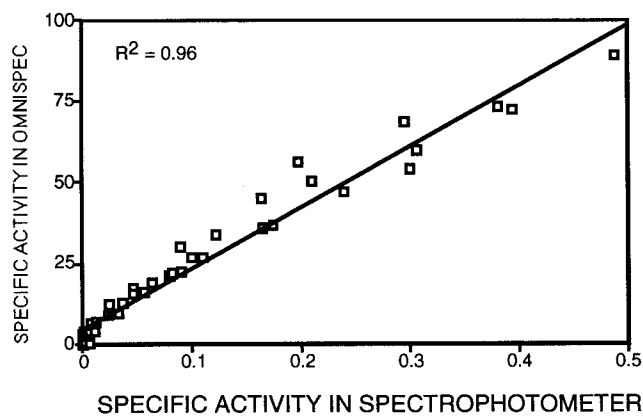
Chromogenic Substrate



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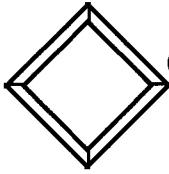


Colorimetry vs. Spectrophotometry

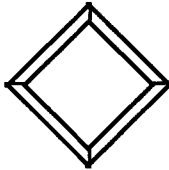
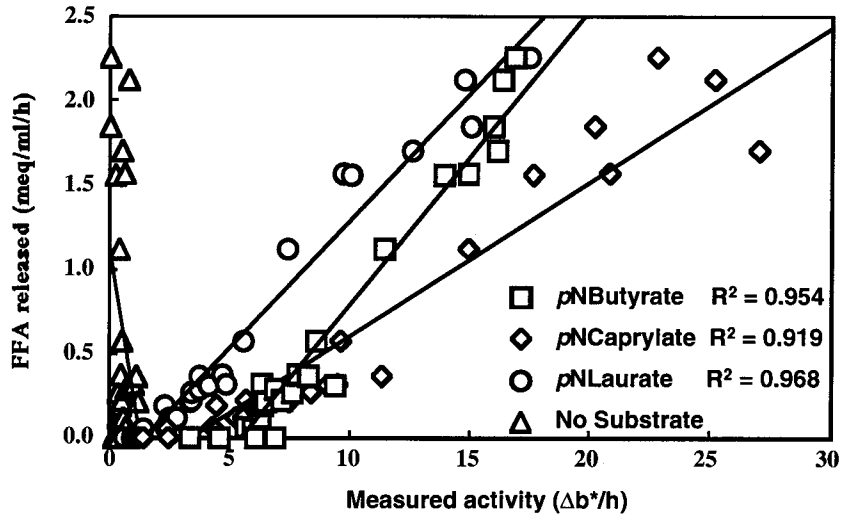


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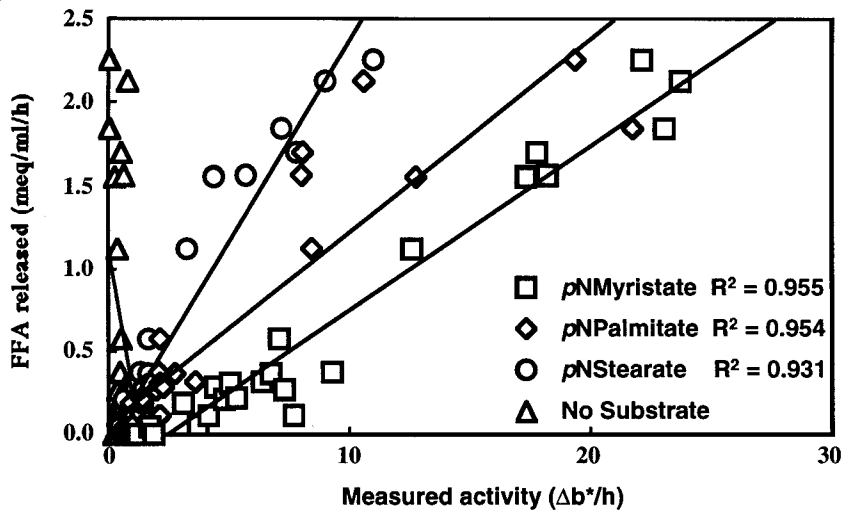




Correlation of Chromogen to FFA's

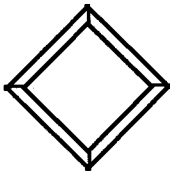
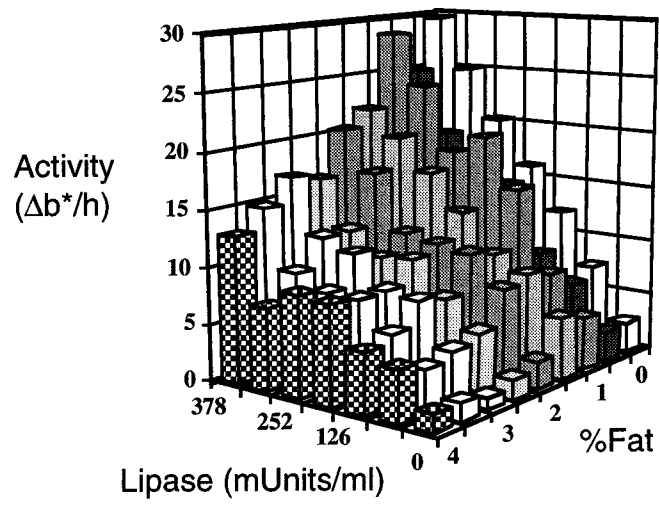


Correlation of Chromogen to FFA's





Effect of Fat on Lipase in Milk



REACTIONS OF LACTOSE IN UHT MILK

M.A. Van Boekel

Department of Food Science

Wageningen Agricultural University

The Netherlands

Summary

An overview is given of heat-induced reactions of lactose. There are two main reactions, namely the isomerization/degradation reaction and the Maillard reaction. Next, it is considered which of these reactions are important during UHT treatment. The main reaction products in UHT heated milk appear to be lactulose and galactose (due to isomerization/degradation) and the early Maillard reaction product lactulosyllysine (bound to protein). Hydroxymethylfurfural was only a minor reaction product and is not a major reaction product of the Maillard reaction in milk. A kinetic model is proposed that appeared to describe the experimental results well; it was found that the isomerization reaction was more temperature dependent than the Maillard reaction, while the degradation reaction was hardly temperature dependent. It is also discussed that direct UHT treatment causes less heat damage (as measured from lactose reaction products) than indirect UHT, probably due to the dilution during direct UHT treatment. Furthermore, it is reported that a higher fat content protects the milk from heat damage, perhaps due to a decrease in heat transfer with an increase in fat content.

Reactions of Lactose in UHT Milk

M.A. Van Boekel
Wageningen Agricultural University
The Netherlands

In this lecture I will present you some results about our research on heat-induced reactions of lactose. Most of this research was not concentrated on the UHT process, but we are currently studying this in more detail. What I present today is a combination of our view what happens with lactose during heating in general and some preliminary results plus literature data on UHT heating.

Why is it interesting to study lactose in the first place? That is because reaction products of lactose, among other things, determine the quality of heated milk; well known is, for instance, the Maillard reaction, leading to browning and off-flavours. Also, some reaction products (lactulose, HMF, furosine) have been proposed as indicators for the heat load given to milk. Our goal in this research project was to identify the main reaction products and paths of lactose and the kinetics of the formation of such products.

Slide 2 shows the two main reactions of lactose and the main reaction products we have identified in (severely) heated milk. First, I would like to discuss the isomerization reaction. Lactose isomerizes via the base-catalyzed Lobry de Bruin-Alberda van Ekenstein transformation. Slide 3 shows the reaction mechanism for this transformation, resulting in a mixture of lactose, lactulose and epilactose; epilactose, however, is only formed in small quantities. The crucial thing is now that lactulose is more unstable than lactose and is to some extent decomposed into C5/C1 and C6 compounds with the simultaneous release of galactose (slide 4). Slide 5 summarizes the isomerization/decomposition. Two C5 compounds have been identified, namely 2-deoxyribose and 3-deoxypentulose, and these compounds were found to be quite unstable in heated milk. The C1 compound is formic acid. The C6 compound is unknown and probably very unstable; it is not glucose. The second reaction is the well known Maillard reaction (slide 6), in which lactose reacts with lysine-residues to form (via some transformations) the Amadori product lactulosyllysine (bound to protein); the Amadori product can be decomposed again, as a result of which galactose is released along with formation of formic acid and intermediate Maillard products (IMP) and reformation of lysine-residues. IMP's can react further with lysine-residues to form advanced Maillard

products (AMP). To be complete, galactose and lactulose can also react with lysine-residues (slide 6).

What I have shown you so far, is an overview of the main reactions of lactose, as established by us in severely heated milk. The question is now whether or not this complex reaction network is also applicable to UHT milk. Before answering that question I like to show you some results for UHT milk, partly found by ourselves and partly from literature. Slide 7 shows the main reaction products for UHT heating at 120 °C, slide 8 for 150°C. It was found that lactulose, galactose and the Amadori product were the main reaction products; there was a tendency that lactulosyllysine was broken down again at the longer heating times; we are currently studying this in more detail. It appeared that the isomerization reaction was quantitatively of more importance than the Maillard reaction (because galactose originates also from the isomerization reaction). Slide 9 shows the formation of hydroxymethylfurfural (HMF) at 140 °C; it is noteworthy that HMF is formed in much smaller amounts than the other reaction products. This is especially remarkable for total HMF because this total HMF is supposed to be a marker for the Amadori product, which is however formed in much higher concentrations (slide 7 and 8). This leads me to conclude that formation of HMF is not at all of importance in heated milk, contrary to common belief.

Having shown you what the main reaction products are, I will now present a simplified kinetic model for reactions of lactose in UHT milk (slide 10). The reaction rate constants k_4 and k_5 are introduced to allow for the breakdown of the Amadori product lactulosyllysine at prolonged heating times, but these reactions are not of any significance in normal UHT milk. We have used this kinetic model to derive values for the three important reaction rate constants, k_1 , k_2 and k_3 . It is important to realise that reactions occur simultaneously, in other words, it is not correct to only study the formation of lactulose, for instance, because lactulose is partly broken down again into galactose. How to do this in the correct way is shown in slide 11. I present you one result in slide 12, which shows that the model adequately fits the experimental data. This was also the case for the other temperatures (120, 130 and 150 °C). From the numerical values of the reaction rate constants at different temperatures, we calculated the temperature dependence using the absolute rate theory of Eyring (slide 13). It is seen that the formation of lactulose is more temperature dependent than the early Maillard reaction, while the formation of galactose is only slightly temperature dependent. However, because galactose formation is dependent on lactulose formation, the

actual values formed are still temperature dependent! If one only studies the formation of galactose without taking into account that it is actually formed from lactulose, one obtains a completely wrong idea about the temperature dependence. This shows the power of kinetic modelling of the complete reaction network.

Having shown this, I would like to point out two more practical aspects related to reactions of lactose in UHT milk. The first is that there is a difference in direct and indirect UHT heating even if one compares these two processes based on the same equivalent heating times and temperatures. This is summarized in slide 14. It appears that the direct UHT treatment causes less heat damage even though the heat treatment is the same. According to German researchers (Klostermeyer and Reuter and coworkers) this is due to dilution of the milk due to steam injection. We plan to study this more in detail, using our kinetic model. The second practical point is that the fat content of a milk product has a remarkable effect on heat damage: the higher the fat content, the less heat damage (slide 15). The reason for this is not clear, but we assume that it may have something to do with heat transfer which may be less with higher fat content, for instance because of a turbulence depressing effect of fat.

This brings me then to the conclusions summarized in slides 16 and 17.

REACTIONS OF LACTOSE IN UHT MILK

M.A. Van Boekel

Department of Food Science
Wageningen Agricultural University
The Netherlands

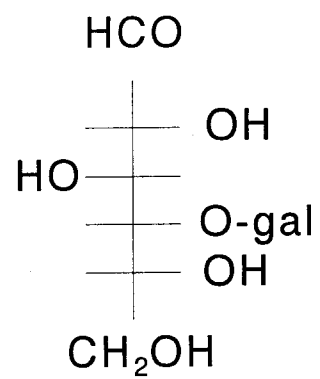
REACTION PATHS OF LACTOSE

Two main reactions in heated milk:

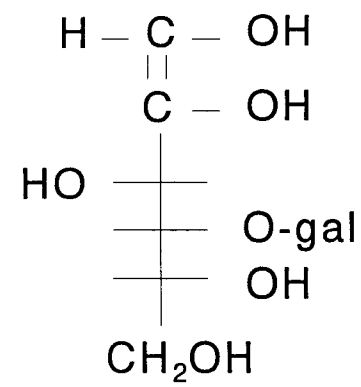
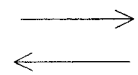
- Maillard reaction
- Isomerization reactions

Identification of reactants:

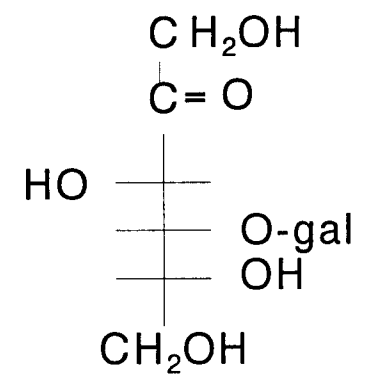
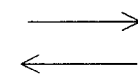
- ✓ lactose
- ✓ lactulose (epilactose)
- ✓ galactose (tagatose)
- ✓ formic acid
- ✓ lysine-R
- ✓ lactulosyllysine-R
- ✓ (HMF, furfural, furfuryl alcohol)
- ✓ (advanced Maillard products)



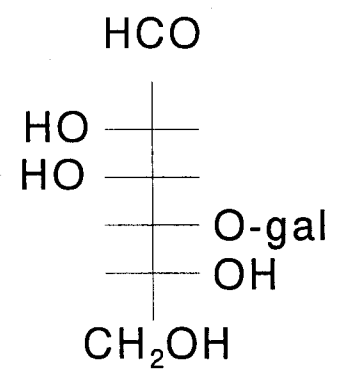
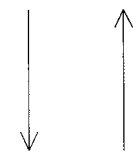
LACTOSE



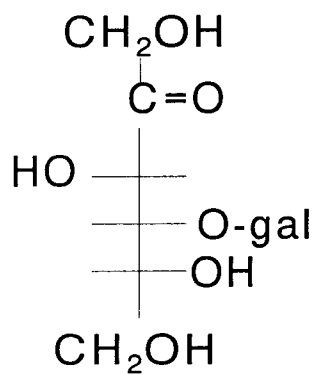
1,2-ENEDIOL



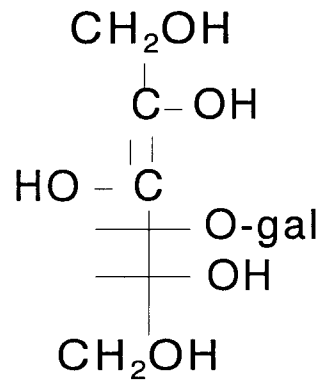
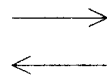
LACTULOSE



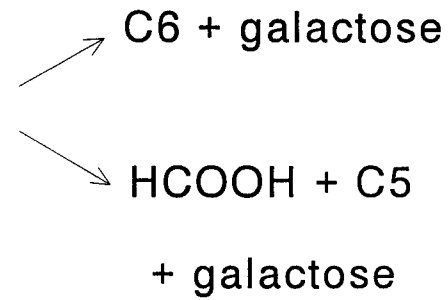
EPILACTOSE



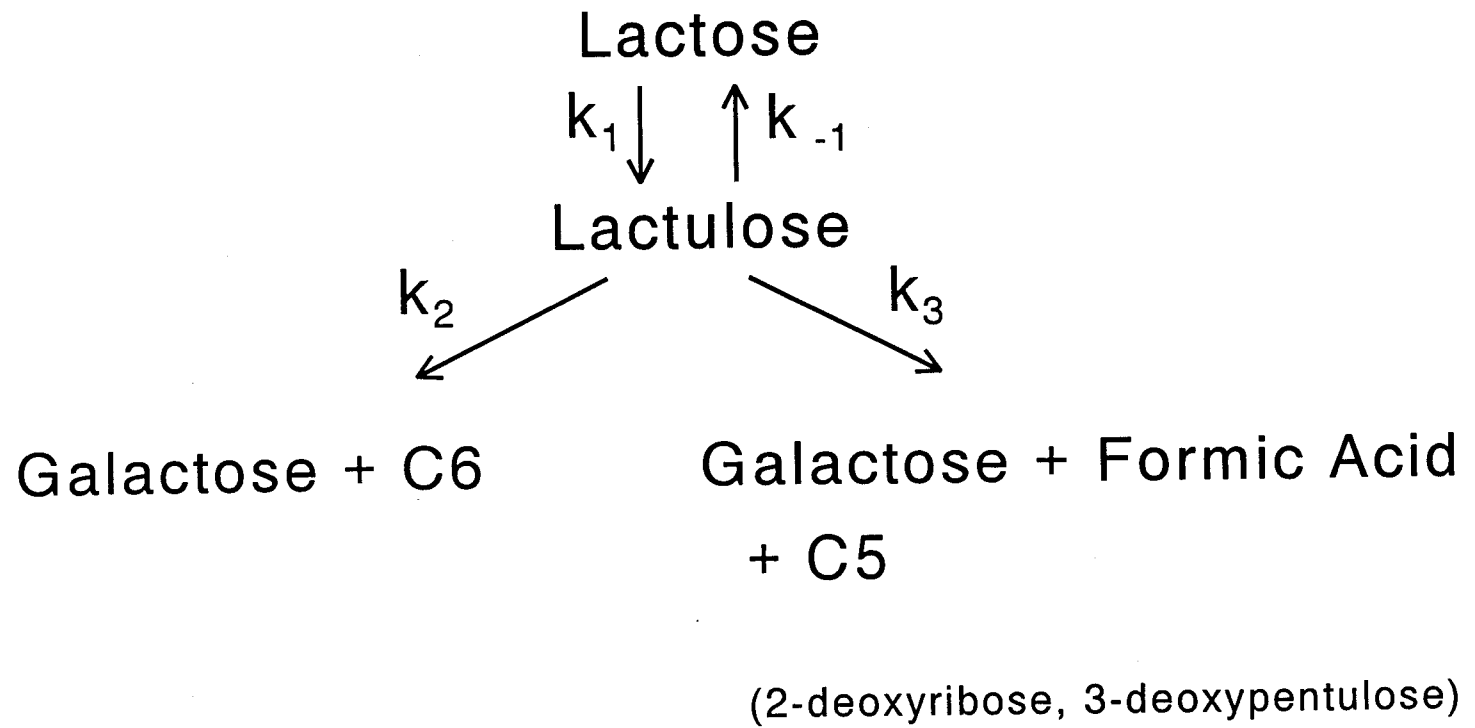
LACTULOSE

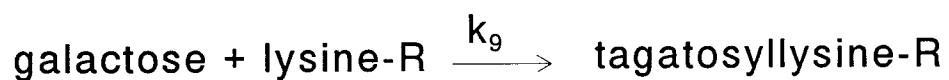
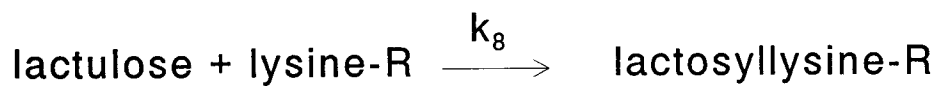
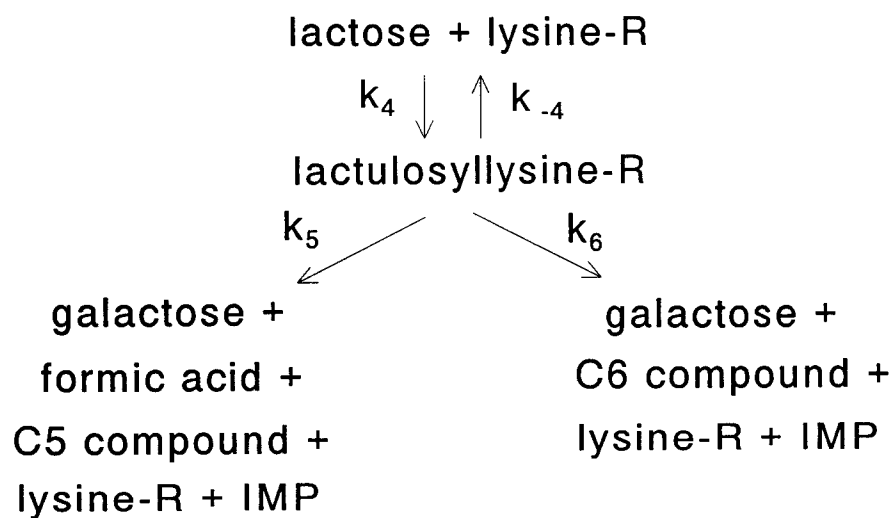


2,3-ENEDIOL

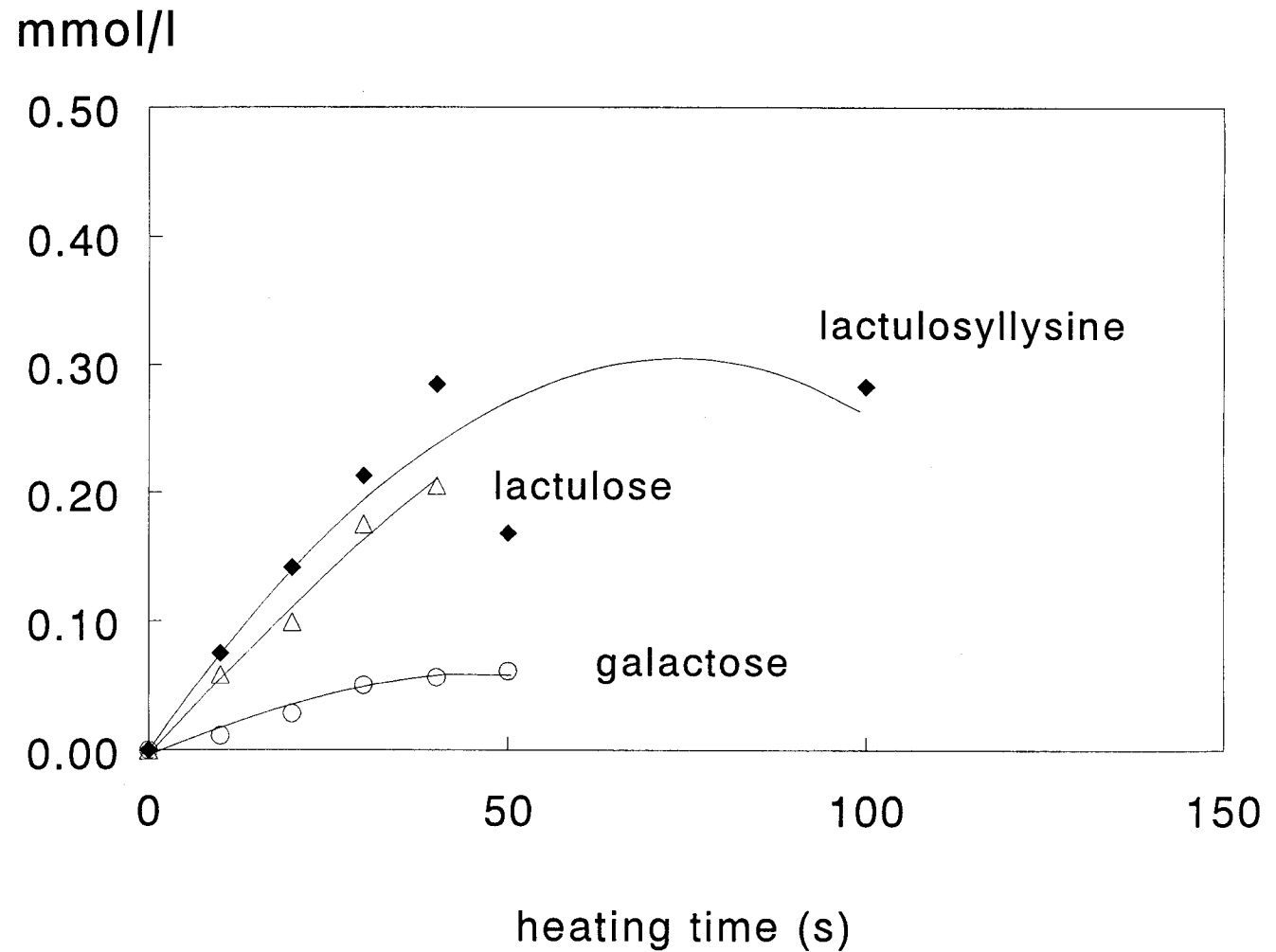


DECOMPOSITION

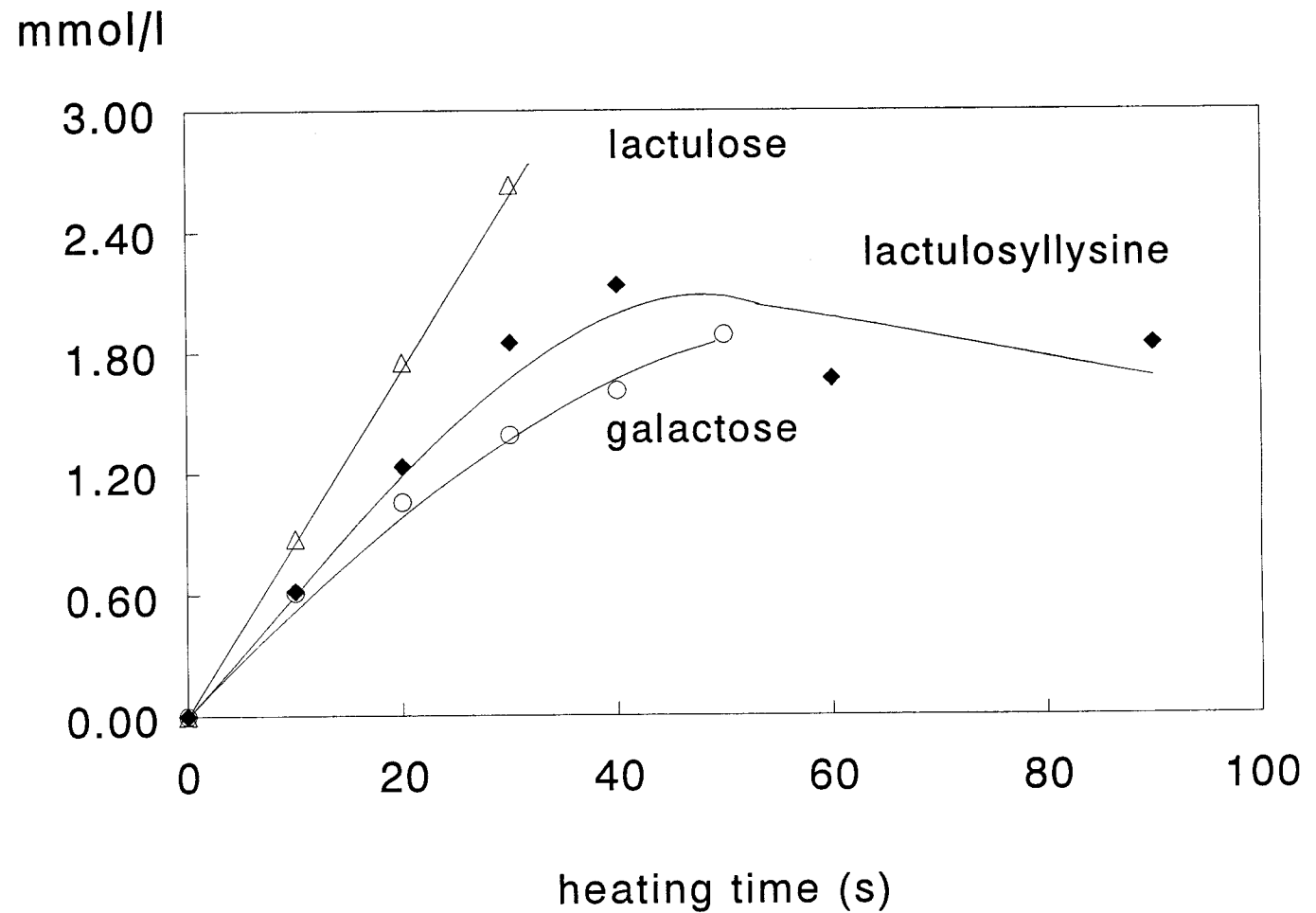




Indirect UHT heating 120 °C

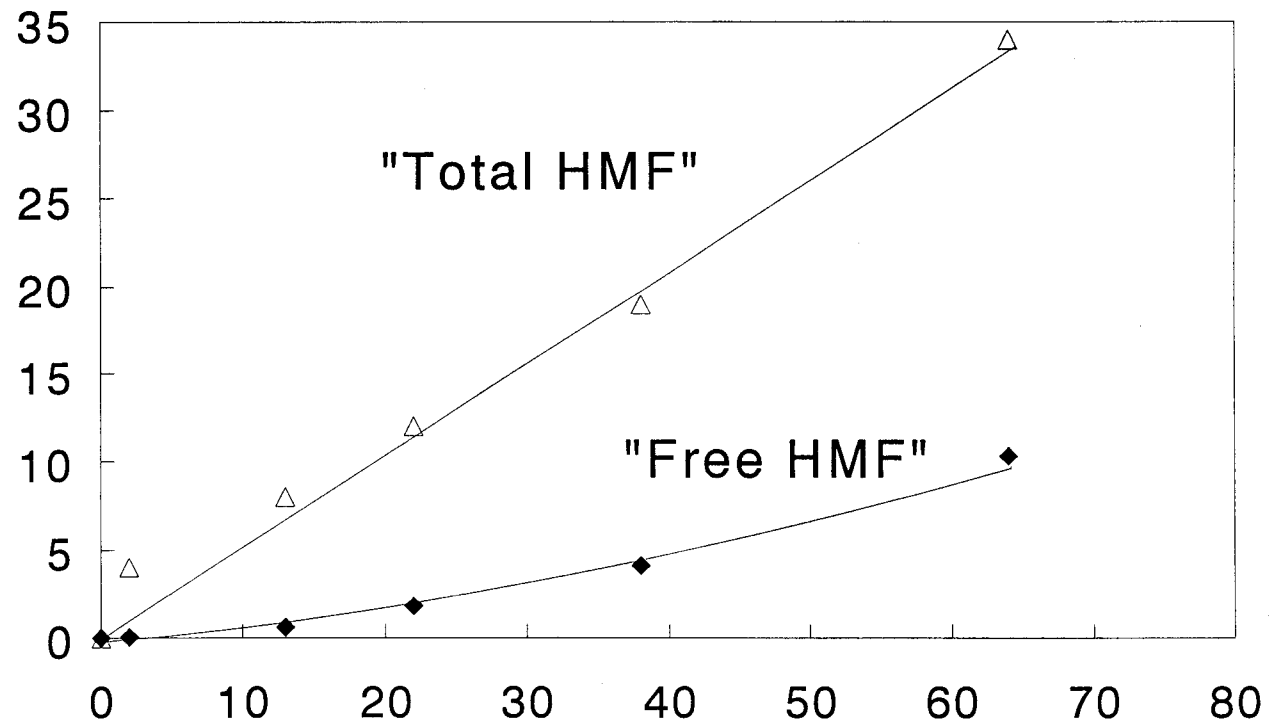


Indirect UHT heating 150 °C



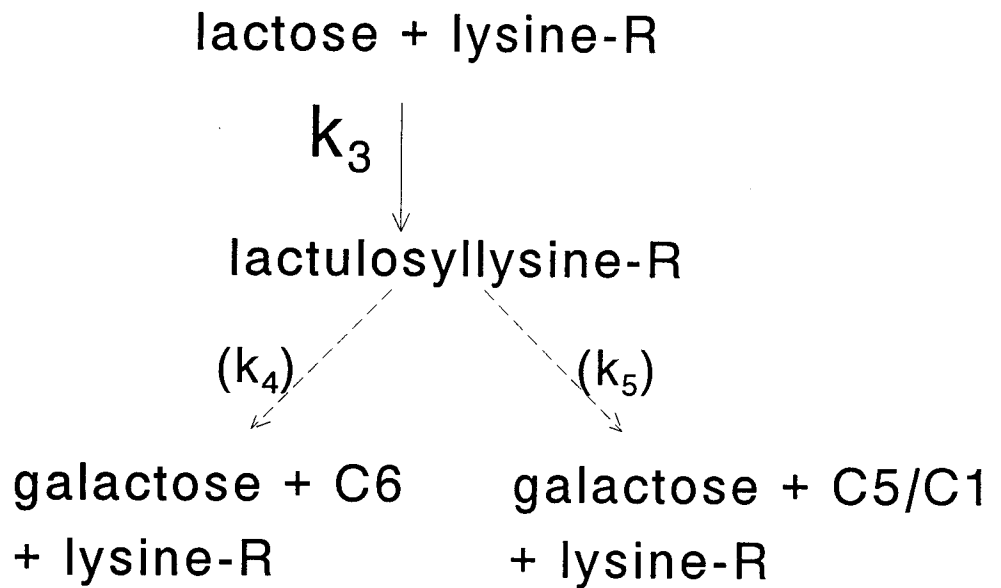
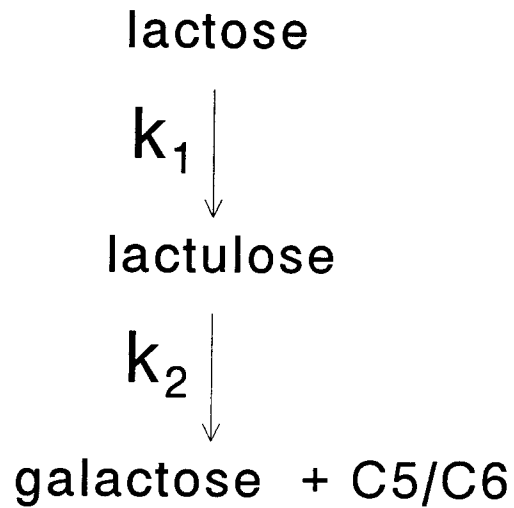
Indirect UHT, 140 °C

$\mu\text{mol/l}$



heating time (s)

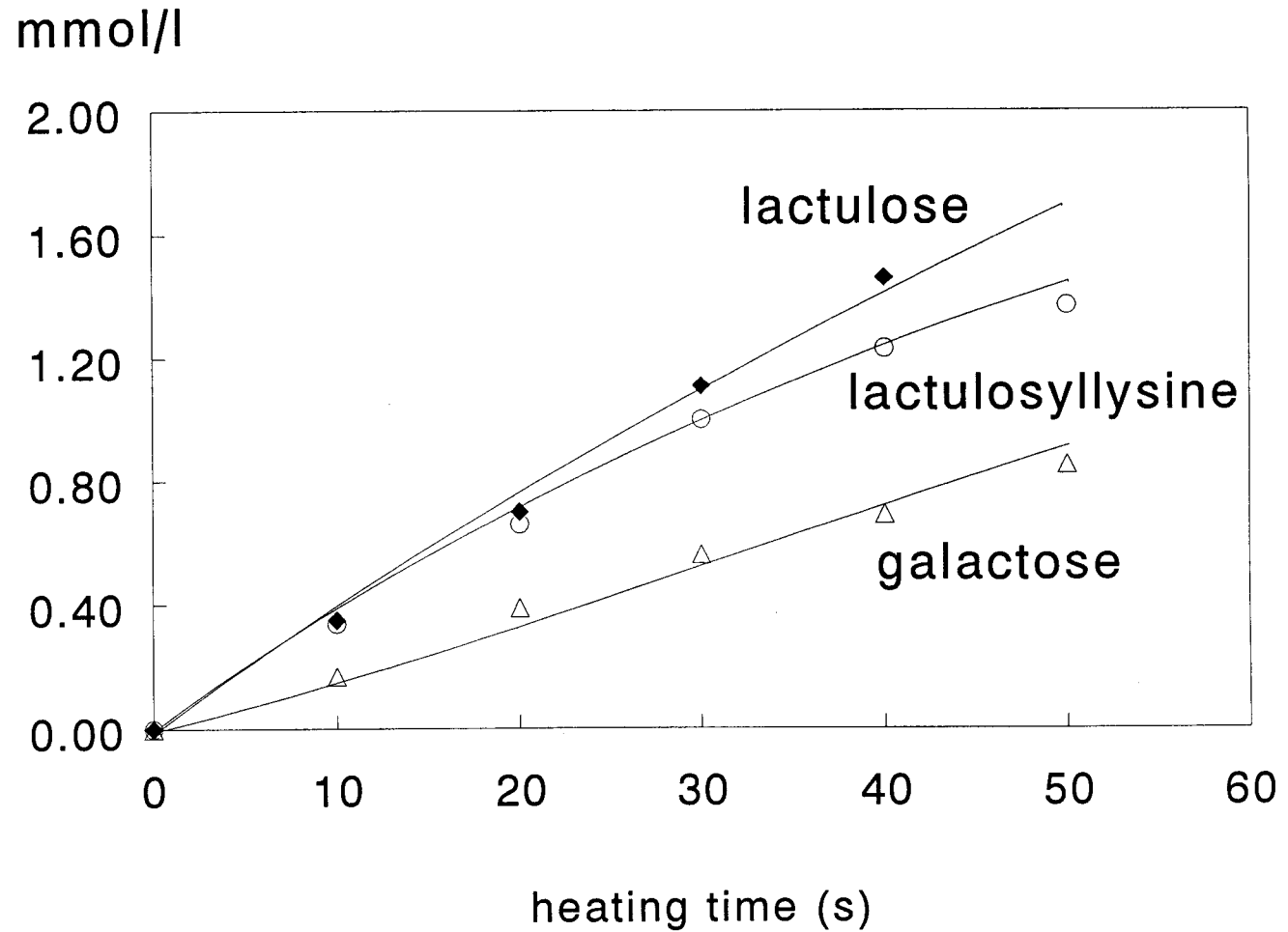
KINETIC MODEL FOR UHT HEATING



KINETIC MODELLING

- derivation of coupled ordinary differential equations (ODE's) based on the kinetic model
- solving of the ODE's by numerical integration
- fitting of the model to the data by a statistical procedure

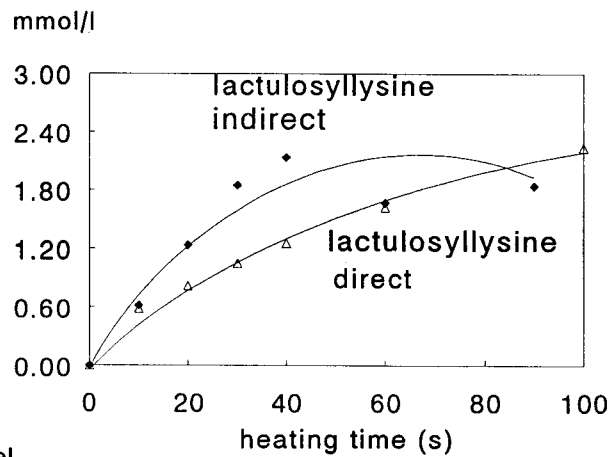
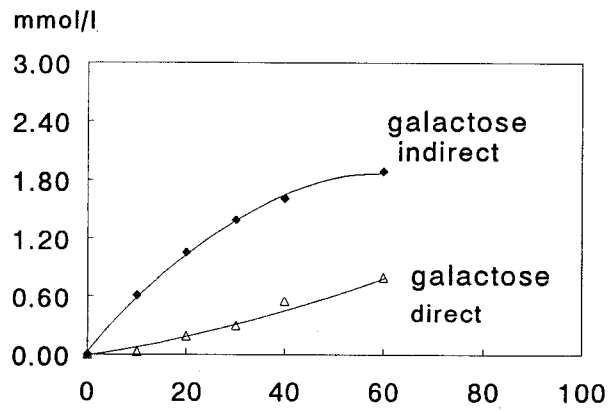
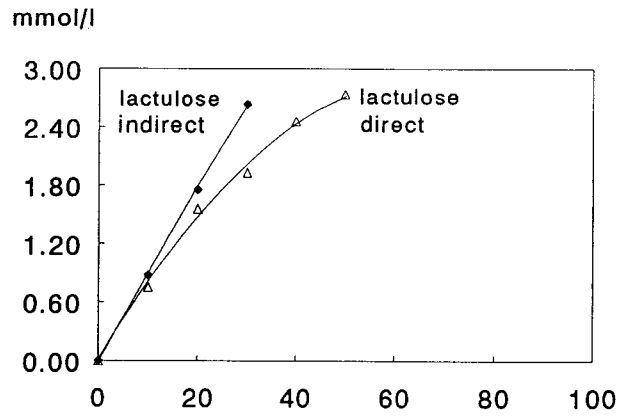
Fit of the model, indirect UHT 140 °C



Temperature dependence

Rate constant	$\Delta H^\#$ kJ/mol	$\Delta S^\#$ J/mol/K	Q_{10} (140°C)
k_1	129	2	2.5
k_2	22	-225	1.2
k_3	94	-112	1.9

Comparison direct and indirect UHT, 150°C



Effect of fat content on lactulose

Fat %	UHT treatment	Lactulose (mg/l) product	Lactulose (mg/l) plasma
3.5	direct, 4s 151°C	157	163
23.1	direct, 4s 151°C	90	117
3.5	indirect, 1s 139°C	400	415
31.0	indirect, 1s 139°C	200	290

CONCLUSIONS (contd)

- Difference between direct and indirect UHT: dilution effect?
- Increased fat content reduces heat load: difference in heat transfer?

CONCLUSIONS

- Kinetic model describes results well
- More loss of lactose due to isomerization than to Maillard
- HMF formation not important in heated milk

Changes in Casein Structure During UHT Processing of Milk

Donald J. McMahon
Director, Western Center for Dairy Protein Research & Technology
Department of Nutrition & Food Sciences, Utah State University

Introduction

The biological function of bovine casein micelles is to provide efficient nutrition to the young calf. It does not require inherently a high degree of ordered structure but, rather, an effective mechanism for secretion of a highly concentrated solution of protein, calcium, and phosphate. During the 30 years research has been extensive to determine the composition and structure of casein micelles and to identify forces that maintain their integrity.

Proteins in Milk

The proteins in milk can be divided into two groups, as shown below, the caseins (which are insoluble in milk at 20°C when pH is lowered to 4.6), and the whey proteins which are so named because they are found in cheese whey.

Protein	Approximate percent of total milk protein
<u>Caseins</u>	(80%)
α_{s1} -casein	34%
α_{s2} -casein	8%
β -casein	25%
κ -casein	9%
β -casein peptides (γ -caseins)	4%
<u>Whey Proteins</u>	(20%)
β -lactoglobulin	9%
α -lactalbumin	4%
β -casein peptides (proteose-peptones)	4%
bovine serum albumin	1%
immunoglobulins	2%

Caseins

The caseins interact with each other (and calcium phosphate) to form large spherical complexes called casein micelles. They range in size from about 0.030 to 0.300 μm with their average size being about 0.160 μm . This makes them about $1/20$ the size of fat globules. The casein micelles are not large enough to separate from milk under the force of gravity but they do contribute to light scattering and the white color of milk. On a dry basis, the micelles consist of about 92% casein and 8% colloidal calcium phosphate. They are, however, highly hydrated and hold about 4 grams of water for each gram of protein. The average ratio of α_{s1} -, α_{s2} -, β -, and κ -caseins in the micelles is 3:1:3:1 with κ -casein tending to be on the outside of the micelles. It is the characteristics of the casein micelles that determine the behavior of milk and milk products during processing (pasteurization, sterilization, concentration, freezing) and so an understanding of the structure of casein micelles is very important. There are about 10^{18} (or a trillion trillion) casein micelles in a glass of milk.

Whey Proteins

Whey proteins do not associate into large colloidal particles like the caseins and are in solution in the milk serum. Unlike the caseins, the whey proteins (especially β -lactoglobulin and the immunoglobulins) are denatured quite rapidly at temperatures above 70°C. When they are denatured, whey proteins become less soluble and more sensitive to calcium. Also, denatured β -lactoglobulin complexes with κ -casein in the

casein micelles and thus retards their coagulation by rennet both by slowing the enzyme hydrolysis of κ -casein and interfering with aggregation of the casein micelles. They also hold more water so that when milk is heated above pasteurization temperatures not only does it take longer to coagulate but it is more difficult to remove moisture from the curd.

Casein Micelle Model

The most widely accepted theory on the structure of casein micelles is that they are formed from submicelles of approximately 0.015 to 0.020 μm diameter. These submicelles are made up by a few α_{s1} -, α_{s2} -, β -, and κ -caseins grouping together. It is thought that the submicelles have the nonpolar portion of each protein chain oriented inward, while the charged acidic residues of the calcium-sensitive caseins and the hydrophilic carbohydrate portion of κ -casein are located on the submicelle surface. If the κ -casein is located primarily in one area of the submicelle there would be phosphate-rich and phosphate-depleted areas on the submicelle surfaces as well as hydrophilic and hydrophobic areas.

The phosphate groups of the α_s -caseins and β -caseins are potential calcium binding sites for forming ionic bonds with colloidal calcium phosphate.

The grouping of these submicelles causes the micelle to have a raspberry-like appearance. Submicelles with low κ -casein content are buried in the interior of the micelle while submicelles with a high percentage of κ -casein are unable to aggregate together and are found on the micelle surface. Because of the highly negatively macropeptide portion of κ -casein that protrudes from the micelle surface (giving it a "hairy" appearance) the casein micelles carry a net negative electrostatic charge. This is known as their ζ (zeta)-potential and in fresh milk has a value of negative 15–20 mV.

The casein micelles are very porous and the colloidal calcium phosphate is interspersed between the submicelles. The components of the micelles are in equilibria with the serum

phase of milk and cooling milk causes some β -casein and colloidal calcium phosphate to be lost from the micelles. This is reversible when milk is pasteurized. Colloidal calcium phosphate is also solubilized when milk is acidified.

Using Electron Microscopy to Study Casein Micelle Structure

An understanding of the structure of casein micelles can be obtained by examining them using an electron microscope. Both scanning electron microscopy (SEM) which looks at the surface of particles and transmission electron microscopy (TEM) which gives a composite view through a sample have been used. At Utah State University we have been refining both of these methods in the study of casein micelles.

In TEM the samples are usually impregnated with heavy metals (osmium and uranium) then embedded in a resin and cut into very thin slices ($\sim 0.070 \mu\text{m}$). Casein micelles are then observed as dark (electron dense) spheres of slightly irregular shape. However, without additional preparation it is not possible to distinguish between proteins. Electron spectroscopic imaging can be used with TEM to show the presence of calcium in the micelles.

The use of SEM has typically been limited to magnifications of 20,000 times which is insufficient to provide information on casein micelles. With a newly developed procedure, we have now been able to increase that magnification to 150,000 times and still obtain high resolution. This requires additional heavy metal impregnation of the samples to increase their conductivity and use of a very thin (0.002 μm) coating of iridium on the sample surface.

When conducting electron microscopy work it is important to realize what are its limitations. Of most importance, care must always be taken to avoid the production of artifacts (changes in structure caused by the way the samples were prepared) or if they cannot be avoided to be aware of their presence and account for them. Preparation of

milk for electron microscopy requires special techniques if the artifact-free microstructure of milk is to be observed

Agar Embedding of Milk

To look at milk by TEM it is first necessary to convert it into a solid (liquids cannot be studied because the TEM operates under a high vacuum and all traces of water in the sample must first be removed). The liquid milk has to be in a form which allows it to be fixed, dehydrated and embedded as if it were a solid structure. Various methods have been employed to produce micrographs from milk samples: freeze drying, freeze etching, freeze fracturing, ultracentrifugation, mixing with agar sol, microencapsulation, and replica techniques

Mixing with agar sol has often been used because of its simplicity but presents the problem of the visibility of agar fibres within the sample under the electron microscope. By using microencapsulation it is possible to avoid artifacts caused by concentrating, diluting or contaminating the milk sample and thus disrupting the initial particle distribution.

Disadvantages of using agar capsules are the difficulty of sealing the capsules and the dexterity required to make the capsules. We have developed a "microcube" encapsulation method which is simpler, more versatile, reliable and reproducible than the other microencapsulation methods used to prepare samples of milk. A significant reduction in defective capsules and blocks was realized by using the microcube method.

Denaturation of Whey Proteins

When milk is heated, whey proteins are irreversibly denatured and interact with the casein micelles. The major portion of the denatured protein is β -lactoglobulin. Although α -lactalbumin is denatured at a lower temperature it is considered more heat-resistant because this denaturation is reversible.

We conducted an experiment to determine how much whey protein denaturation occurred when milk was processed through a UHT

system (Alfa Laval Sterilab™). Milk and skim milk were ultrafiltered to 3 \times concentration and then heated to 72°C (residence time through the plate heat exchanger was 58 s) with 8 s holding time and then either held at 72°C or heated to 89, 106, 123, or 140°C in the second plate heat exchanger (residence time 97 s) with a holding time of 4 s. The milk was cooled to 60°C in a third plate heat exchanger (residence time 36 s) and passed through a homogenizer using two-stage homogenization with 14 MPa first stage and 3.5 MPa second stage pressures at a constant milk flow rate of 100 L/h. The milk was then cooled to 30°C and packaged.

Whey protein denaturation increased with heating temperature and was higher in concentrated milks than in the corresponding unconcentrated milks. There was no difference in the patterns of whey protein denaturation.

Ultra-high temperature heating caused the casein micelles to increase in size with additional protein material adhering to the casein micelles, especially in the 3 \times skim milk heated to 140°C. This diffuse layer of material around casein micelles was not observed in 3 \times whole milk. It was thought that this denatured protein in whole milk was adsorbed onto the fat-water interfaces during homogenization and was therefore not observed on the micelles.

The extent of whey protein denaturation measured for whole milk heated to temperatures <140°C was greater than that for skim milk. This suggested that some whey proteins adhered and unfolded at the newly formed fat-water interfaces when the fat globules were homogenized rather than being denatured during the heating process

We observed that when milk was heated, some casein micelles aggregated, while the incidence of submicellar casein (or micelle fragments) was increased. The shape of casein micelles was also altered depending on the intensity of heat treatment, with some formation of so called "spikes" or "hairs" occurring.

Homogenization decreased the fat droplet size as observed and many casein micelles

became adhered to the surfaces of the fat droplets. This is because of the turbulence and cavitation that occurs during homogenization. In contrast, fat globules in nonhomogenized milk existed independently of the casein micelles.

Changing the pH of Milk

Pasteurized skim milk was ultrafiltered to 3X and then pH-adjusted to pH 6.38 by adding 1.0 N HCl, or pH 6.85 or pH 7.32 by adding 1.0 N NaOH with the control milk having a pH of 6.68. They were then UHT preheated to at 76°C followed by heating to 140°C by direct steam injection, flash cooled to 76°C and then further cooled to 24°C and packaged.

The casein micelles were generally quite round but had a rough surface. They were usually well separated and displayed a wide size distribution. There were some differences in casein micelle microstructure observed in milk samples before or after UHT processing. In some micrographs of the pH 7.32 milk it appeared as though some protein had dissociated from the micelles. This was more apparent after UHT processing.

In the UHT-processed pH 6.38 samples a continuous network was present in which individual micelles could no longer be distinguished. At this pH the retentate precipitated in the flash evaporator during UHT processing. The UHT processing had little or no effect on room temperature viscosity of the other retentates.

The UHT processing decreased the pH of the pH 6.68 samples ($P = .038$). A decrease in pH of the pH 6.85 and pH 7.32 retentate samples was observed, but it was not statistically significant at the $P = .05$ level perhaps because of a smaller sample size ($n=3$). The mean pH drop was almost as great for samples adjusted to pH 6.85 and nearly 3 times as great for samples adjusted to pH 7.32 as compared to the pH 6.68 samples.

Using Immunogold Labeling to Identify the Proteins in Micrographs

Immunogold Labeling

Colloidal gold particles are formed by chemical reduction of an aqueous solution of tetrachloroauric acid. The gold particles are then converted to gold probes by reaction with a protein (such as Protein A) having the property of binding immunoglobulins. The gold complexes are used as components in indirect two-step immunolabeling. The first step involves the interaction of a specific (primary) immunoglobulin with the antigen under investigation. In the second step, the molecules of protein A surrounding the gold particle or the molecules of immunoglobulin-gold complex, interact with the Fc fragment of the primary immunoglobulin. The presence of the gold particle thus allows the indirect localization of the antigenic site. Electron microscopy can then be used to elucidate the position of the antigen under investigation.

Milk Proteins

We employed immunolocalization to elucidate the positions of β -lactoglobulin, α -lactalbumin, α_{S1} -casein, β -casein, and κ -casein in milk at various stages of treatment. These treatments comprised fresh whole milk, skim milk, pasteurized milk, ultrafiltered milk, and direct and indirect sterilized (110, 120, 130, and 140°C) milk.

Heating of milk through pasteurization and UHT sterilization affects the distribution and alters the conformational state of some milk proteins. This was more pronounced with β -lactoglobulin where interaction with whey and micellar casein protein was observed as a function of processing temperature. α -Lactalbumin and κ -casein show a weaker response. α_{S1} -Casein and β -casein showed heavy specific labeling concentrated on the micelles, but no effect of heating on protein distribution was evident with these proteins. α_{S2} -Casein did not respond to these immunolocalization procedures.

β -Lactoglobulin forms complexes with α -lactalbumin, α_{S2} -casein, β -casein, and κ -casein. The presence of α -lactalbumin reduces the direct interaction of β -lactoglobulin and κ -casein but has no effect on the denaturation process of β -lactoglobulin. Complexes formed between κ -casein and β -casein, and between κ -casein and α_{S1} -casein may interfere with κ -casein and β -lactoglobulin complex formation.

A more open micellar structure was observed in some samples due to the lack of osmium tetroxide staining in these preparations. Fixation with osmium tetroxide significantly reduces antigenicity of protein but imparts heavy metal staining to the samples. This staining confers a compact appearance to the micelles which may be artifactitious. We believe the open structure of the micelle is more representative of its actual structure. The outline of micelles in many samples appeared rough as though having short tendrillar appendages.

α -Lactalbumin

The samples showed sparse labeling for α -lactalbumin in the order: whole = skim < pasteurized = UF < UHT 110°C < UHT 120°C < UHT 130°C < UHT 140°C. The labeling was mainly within the intermicellar matrix. The heat treatment of the milk at pasteurization and UHT sterilization caused complexing of α -lactalbumin which resulted in a higher retention of this protein during pre-labeling preparation of samples for TEM. There appears to be a direct correlation between the amount of heating and the amount of complexing of α -lactalbumin within the samples. The labels were frequently observed as doublets and triplets suggesting self aggregation of the α -lactalbumin, with higher processing temperatures leading to more aggregated material.

β -lactoglobulin

The labeling for β -lactoglobulin was more intense than that for α -lactalbumin, but the trend in labeling density was similar. There was little difference in the labeling density

between the UHT samples from 110-140°C or from direct to indirect. However, there was a definite trend in the labeling pattern. From whole milk through to the UHT (110°C) samples (Figures 7.2a to 7.2c), the labeling was concentrated within the intermicellar matrix and often appeared as doublets, triplets and higher order linear multiples.

From UHT (120°C) to UHT (140°C) the labeling density shifted mainly to the surface of the micelles as well as some intermicellar material. This suggests that β -lactoglobulin was exhibiting greater complexing with higher heat treatment and that more complexing with the micellar casein occurred at higher UHT treatment. Initially the complexing of β -lactoglobulin may be with itself, α -lactalbumin, and/or serum caseins and upon higher-temperature-treatment complexing with micellar κ -casein and/or α_{S2} -casein occurs.

α_{S1} -Casein

All samples showed heavy labeling for α_{S1} -casein especially on the micelles. There appeared to be no change in labeling through heat treatment for this protein and there was labeling throughout the micelles showing an even distribution of α_{S1} -casein. The UHT (140°C) sample had less labeling density near the surface of large micelles, which after UHT-induced complexing would be predominantly β -lactoglobulin complexed with κ -casein.

α_{S2} -Casein

From ELISA testing, the antibodies were observed to be active against α_{S2} -casein (with some cross reactivity with α_{S1} -casein) but no labeling was observed with our sectioned samples. labeled for α_{S2} -casein. This suggests that the serum α_{S2} -casein may have been completely leached from the samples during pre-labeling TEM preparation or that the epitope of both micellar and serum α_{S2} -casein was inaccessible to the primary antibody, through conformational or steric restriction. This occurs if the antibody is raised to a different form of the protein than the form found in the intact material.

β -Casein

Labeling for β -casein was similar to that for α_{s1} -casein. All samples showed heavy labeling concentrated on the micelles as well as some intermicellar labeling of the non-UHT samples. The absence of label from certain areas of the micelles, indicates that β -casein is not uniformly distributed within micelles. The indirect UHT (140°C) sample appeared to be labeled more specifically on the micelles than the other samples. This suggests that the higher heat treatment caused serum β -casein to migrate to the micellar surface and participate in complex formation with β -lactoglobulin and κ -casein.

κ -Casein

All samples showed sparse labeling concentrated mainly in the intermicellar matrix

for κ -casein. This suggests that only the serum κ -casein was being labeled. The micellar κ -casein through conformational or steric hindrance was inaccessible to the primary antibody. No labeling of κ -casein was observed within the micelle interior supporting other work which suggest that κ -casein is located predominantly on the micelle surface. Being located on the micelle surface, the probability of κ -casein molecules being oriented correctly to bind with the antibody is small, since labels only attach to proteins on the sectioned surface. These results suggest that the antibody for κ -casein may have been raised against parts of the κ -casein molecule now hidden as a result of aggregation within the micelle surface. The antibody may have been raised against the para- κ -casein moiety of κ -casein.

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"IN-FLOW ASEPTIC PROCESSING OF LIQUIDS AND PARTICULATES"

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Introduction

Foodproducts like vegetables, meat, fruits and milk are an excellent medium for bacterial growth and hence subject to microbiological deterioration if nothing is done to prevent this.

Heat preservation has the aim to stop microbiological activity and includes the destruction of bacteria as well as microbiological spores, however with the least possible decrease of the quality of the food-product itself, in order to obtain a shelfstable product that can be stored for a long time under non-refrigerated conditions.

Such processes are performed at temperatures well above 100 °C, even up till 150 °C, and indicated as heat preservation processes.

In principle there are two methods to perform a heat-preservation process (Fig. 1):

- In-Container processing, in which case the product is first filled into a container (can, glass jar, bottle or plastic package) which than is hermetically closed before it is submitted to the process.
- In-Flow Aseptic processing in which case heat-exchangers are used to heat up the product to sterilizing temperature and subsequently cool down, after which it is filled under aseptic conditions into a pre-sterilized container.

The traditional idea of preservation in the food and dairy industry, i.e. providing seasonal produce throughout the year, is gradually changing into a more refined objective: "Integrated Shelf Stability", thus balancing shelf stableness with quality and convenience.

With the present availability in most of the developed countries, i.e. fresh food produce of all kind almost the whole year around, a market has been developed for which "long shelve-life" has become a lesser issue than the need for quality and convenience.

Quality in this respect stands for "as were it just prepared from fresh ingredients".

Convenience stands, apart from "Ready to Serve", also for shelf stability though not for periods of years but merely for sufficient length of time to bridge the shopping frequency or the "special offer" waves, quite often boiling down to periods of weeks or months.

Specifically the equipment for "In-Flow" processing, designed for rapid heating and cooling of pumpable products, and hence well suited for controlling t-T profiles, is increasingly designed to meet these latest demands. Quality and safety by aseptics are key-items in this respect.

Principle lay-out of In-Flow processing equipment

"In-Flow" processing equipment consists of heat exchangers and allows product in direct contact with such equipment. Hence all surfaces and ducts need to be cleaned and sterilized before it may come into contact with the product. Therefore three basic operating stages are recognized with "In-Flow" equipment:

- pre-sterilization of the equipment
- processing
- cleaning

The principle lay-out of "In-Flow" processing equipment consists of (Fig. 2):

- product infeed system, including a feed pump and often a metering system for detergents
- positive displacement pump for exact metering of the product flow
- arrangement of heat-exchanger sections to heat up, hold and cool down the product
- control system to set and monitor the process during each of the operating stages.

The equipment is designed as such that pre-sterilization and cleaning can be done "in-place" by so called S.I.P. and C.I.P. procedures. Automation with "In-Flow" equipment including the dialogue with the connected aseptic fillers has been developed nowadays to such extent that human supervision requires minimal effort and that the operator may concentrate on for instance the fillers or the total line.

Dependent on the type of product to be processed "In-Flow" equipment comes in many modifications mainly concerning:

- type of pump: in all cases a positive displacement pump
- type of heat exchangers: plates, tubes, SSHE's
- arrangement of the heat exchanging sections

As for the heat exchangers the concentric tubular heat exchangers, especially the coiled ones, are offering great advantages in terms of:

- single pass uninterrupted product channel without dead corners and without gaskets
- very small mixing zones when switching between S.I.P., production and C.I.P., saving product as well as detergents
- high pressure resistant
- no damage of particulates

Criteria for thermal processing

"In-Flow" processing by means of specifically designed heat exchanger arrangements provides fast heating up and cooling down of pumpable products.

So called "Primary products" like milk and fruit-juice, which are also

consumed in the raw or fresh state, need to be processed in such a manner that taste and flavour remain as near as possible to the untreated product. Other products, however, that need specific cooking flavour. For this reason processing values have been introduced in order to judge the result of any thermal treatment.

Processing values are expressing the changes in food caused by thermal treatment. They are based on first-order reaction kinetics and expressed in following equation:

$$F = \int_0^t 10^{\frac{T - T_{ref}}{z}} \cdot dt$$

- F - processing value, dimensionless, expressing the number of decimal reductions during a process
- T - product temperature at time interval dt
- T_{ref} - reference temperature at which one decimal reduction is obtained in one time-unit
- z - slope of the inactivation line in the "Thermal Inactivation Diagram" and expressed as the number of degrees C to traverse one log cycle (see fig. 3)
- dt - time-interval

A selection of frequently used processing values is given in the next table:

Processing Value	Symbol	T _{ref}	z	Time-unit
Lethality	F ₀	121.11	10.0	minutes
	F ₀	138.89	10.0	seconds
Microbiol. Inactivation	B*	145.44	10.4	seconds
Thiamine destruction	C*	181.61	31.4	seconds
Bact. Protease inactivation	E	140.00	32.0	seconds
Lactulose formation (mg/l)	L	111.00	25.8	seconds
Cooking value	C ₀	100.00	33.0	minutes
Pasteurization effect	P	72.00	8.0	seconds

Processing values are derived from product's t-T profile during the thermal process. They can be plotted in the so called "Thermal Inactivation Diagram" (Fig. 3) in which also the areas for typical thermal treatment are indicated such as:

- "In-Container" sterilization area
- "In-Flow" (UHT) area for milk and milk products
- Pasteurization area for milk

Often processes have to satisfy specific processing values:

- Commercial sterility requires a F_0 between 6 and 10
- UHT-treatment of milk may not exceed the formation of more than 350 mg lactulose/litre and should preferably yield a C^* -value of less than 1
- Pasteurization of milk should have a minimum P-value of 15 during the holding time (which equals 15 seconds at 72 °C)

With this respect processing values are an excellent tool to compare thermal processes as well as to evaluate the design of "In-Flow" equipment.

"In-Flow" processing of homogeneous liquids

Homogeneous liquids are "In-Flow" processed in UHT-equipment. Amongst these products are milk and milk products (cream, custard, liquid baby food), juices, soy bean products, tea and coffee drinks, liquid infant food and pharmaceutical products.

A great deal of these products, i.e. milk and fruit-juice are so called primary products with little additional value, for which processing is done in large throughputs up to 20.000 l/hr, in order to be economical. Given the smaller capacity of currently available aseptic filling machines these UHT-installations often feed two, and sometimes even up to four of those fillers. As a consequence either an aseptic buffering tank between processing equipment and fillers is required to overcome temporarily stops of one or more fillers, or the processing equipment itself is designed to automatically adapt its throughput to the number of fillers in operation. This latter option is often favoured since not only it saves the investment and complexity of the total production line, but also excludes additional risk for re-infection of product.

Variable throughput extends the product residence time in the installation whilst at less than nominal capacity. In order to keep the processing values within the required limits modern UHT-installations enable to automatically adapt the active heat exchanging surfaces in relation to changes in capacity.

A first adaption is obtained by dividing the main-heating section into several sub-sections, each of those curtaineable for steam-supply dependent on the actual product throughput. Product coming from the upstream part of the regenerative section remains at that temperature in the non-active part of the main heating section and is heated up only in the remaining active part.

With increasing percentage of heat-recovery in regenerative sections (85 - 88%) the product temperature at the end of the upstream part has become in the order of 120 - 125 °C which is too high to allow unnecessary dwell time. Therefore, as a second step, the upper part of the regenerative section is provided with an extra channel for supplementary cooling water. At throughput less than maximum, a small flow of cooling water not only decreases the product temperature in the upstream part of the regenerative section but also increases the cooling

rate in the downstream part. In concert with de-activated parts of the main heating section these two measurements are providing an excellent tool to shape the product t-T profile smoothly at any variation in throughput.

To meet regulatory agency's standards (F.D.A.), a third modification (Fig. 4) is introduced by including a water circuit between upstream and downstream part of the upper regenerative section. By this the already processed product can never contact non-processed product since the pressure in this water circuit is always less than product pressure. Furthermore, by introducing a supplementary heat exchanger in this water-circuit, it creates the possibility to adapt the product heating and cooling curves in this section in relation to the actual throughput (Fig. 5).

The latest modification in process control provides constant product quality regardless variations in throughput of the UHT-installation. This is obtained by:

- measuring product temperatures throughout the circuit
- measuring temperature differentials between product and heating media
- incorporating the actual production throughput.

The recordings are continuously processed in a Real Time Computer System which then controls the UHT-installation no longer on sterilizing temperature but on processing value.

Throughput control via aseptic buffering tank

In standard modification the UHT-installation is feeding one or more aseptic filling machines each one requiring its specific product pressure at its entry. In order to fulfil these pressure requirements a slight overflow (3-5 %) of product is by-passing the fillers via a pressure release valve and returned to the infeed of the UHT-installation. Some regulations however do not allow a repeated heat-treatment of the same product in which case the overflow needs to be directed to another destination which can be problematic sometimes. For this reason a small aseptic buffering tank has been designed including a level control which regulates the throughput of the UHT-installation. The volume of this controlled level tank is just enough to take the total product content of the UHT-installation in case all fillers need to be stopped in an emergency situation.

"In-Flow" processing of liquids containing particulates

When particulates are included in the liquid the lay-out of the product channel with "In-Flow" equipment needs to be free from obstructions so that particulates cannot jam. As for the heat exchangers the uninterrupted concentric double tube coils have proven to be excellent in this respect (Fig. 6).

Valves, being notorious obstructions for particulates, are selected (for instance ball-valves) or specially designed such as the Stork disc-valve

with tangential infeed.

The positive displacement pump is specially selected for the type of product: mohnopump, lobe pump or other.

Particulate containing product can be distinguished in two types:

- sauce type with low to medium viscosity of the carrier and a high percentage (30 - 55 % wt) of particulates. Examples are spaghetti sauces, meal-type soups, diced tomato and diced fruit.
- cream type product with a low percentage (10 - 15 % wt) of relatively large particulates. Examples are cream style soups with whole mushrooms, asparagus tips or meat chunks.

The flow characteristics of the "sauce type" product inside tubes are as such that the densely packed particulates can hardly separate, nor overtake each other. The product is pushed through the tubes as kind of a plug with only the carrier liquid moving radially, too.

For this type of product a conventional holding tube is mostly applied, holding liquid and particulates equally for the same period of time at processing temperature. Also, since particulates do not easily separate, the product can be stored in an aseptic buffering tank including a gentle mixing device, and from there filled in an one-stage filling operation.

The "cream-type" product, because of its low concentration on large particulates, requires more care in handling the particulates. The flow characteristics inside tubular heat exchangers are designed as such that, given the viscosity of the carrier, sufficient velocity is maintained in order to prevent the particulates to precipitate.

Furthermore the carrier may contain delicate components like aroma's or starches, that may not stand the required holding times for sufficient heat penetration to the centre of particulates. For this reason the "Selective Holding Section" has been designed, providing a short holding time for the liquid, whereas particulates are mechanically held for any desired period of time (Fig. 7). A typical example of such "SHS" is the Stork Rota-Hold consisting of a number of rotating forkblades inside a cylindrical vessel and provided with one static forkblade between product's infeed and discharge port to prevent particulates taking a short cut. Rotating and static forkblades are sliding closely along each other thus cleaning the spacing continuously.

The great importance of the selective holding section is that holding time for particulates can be mechanically set at measurable temperatures of the meanwhile surrounding flow of liquid, which makes heat penetration of particulates calculable.

Products with low viscosity of the carrier liquid and with also a low concentration of particulates often need to be filled in a two-stage operation: one stage for the particulates the other stage for the liquid. For this purpose the aseptic buffering tank can be provided with a particulate settling compartment where particulates are assembled in high concentration. From this compartment the required volume of particulates can be measured by a dosage system and transferred to the filling nozzle. From another location in this tank the additional liquid can be dosed to a separate filling nozzle.

"In-Flow" processing of granular products

Particulates without any carrying liquid, so called granular products, can be continuously processed in the Stork Bokfard system (Fig. 8).

Examples of such products are rice, cereals, soy beans, spices, cocoa nibs and powder, as well as chunks of vegetable and meat.

The Stork Bokfard system consists of at least one horizontally positioned pressure vessel with a continuous infeed valve on the one end at the top and a discharge valve on the other end at the bottom. Inside this vessel several transportation means can be applied, dependent on the type of granular product:

- paddles
- rotating drum with or without a helical screw on the inside
- shaking bed

Product is continuously fed to the system via the rotary infeed valve. Inside the first vessel the product is heated up by saturated steam of up to 125 °C. After its residence time in this vessel product is released by the discharge valve.

Since product has taken up moist by its direct contact with steam, it needs to be dried, as well as cooled down. For those products that can take the sudden pressure drop upon leaving the steam vessel, cooling and drying takes place on a shaking bed conditioner. This is done for rice, cereals and spices.

For products that cannot take the sudden pressure drop after the heating stage the first cooling step needs to be done under pressure as well. Dependent on the product and its further destination there are a few options for pressurized cooling:

- a second pressurized vessel for cooling by means of air or inert gas, eventually combined with flashing at a somewhat lower pressure than in the heating vessel. Final cooling and drying can take place on a shaking bed conditioner.
- cooling by means of separately sterilized and cooled liquid, either by spray-cooling in a second pressurized Bokfard vessel, or by mixing the chunks with liquid in a tubular cooling section.

This latter application can be typically designed for aseptic processing.

Some examples of continuous Bokfard processes are:

- pre-cooking of rice, in which case the rice is heated for about 20-30 minutes at 110 - 120 °C by which the moisture content may increase to up to about 40 %, and then subsequently cooled and dried to a final moisture content of 10 - 12 %
- sterilization of spices either granular (pepper beads) powder (curry mixtures) or leafy type (oregano), in which case the product is heated for a very short period (15-90 sec.) at a temperature between 105 - 120°C and subsequently cooled and dried in about 5 minutes. Decimal reductions in the order of 3-4 are obtained in this process which ensures total inactivation of pathogenic organisms (coli, salmonella).

Summary

"In-Flow" aseptic processing of pumpable food products, including products with particulates, is increasingly applied. Primary products, such as milk and fruit juices, are processed in high throughputs and, by keeping control over the processing values, yielding a quality nearing the one of the fresh product.

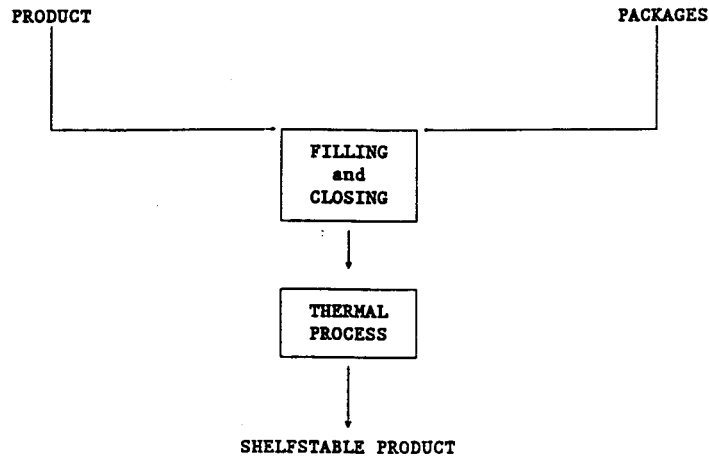
For value-added products "In-Flow" processing offers excellent possibilities to quickly vary formulations so as to satisfy the increasing requirements for variation in the market.

Also granular products without any carrying liquid, such as spices, cereals and rice can be processed "In-Flow", either for cooking purposes or for sterilization.

.....

February 1994
Nieuw-Vennep, The Netherlands

"IN CONTAINER" PROCESSING



"IN FLOW" PROCESSING

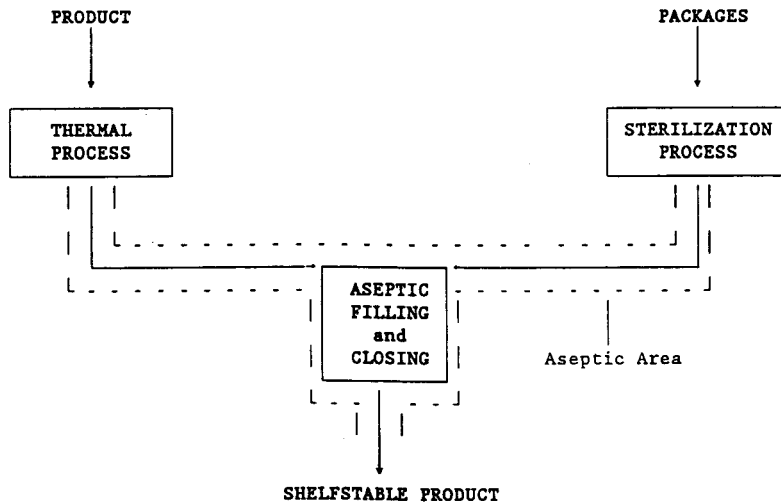


Fig. 1.: Basic functional diagram of "In-Container" respectively "In-Flow" processing

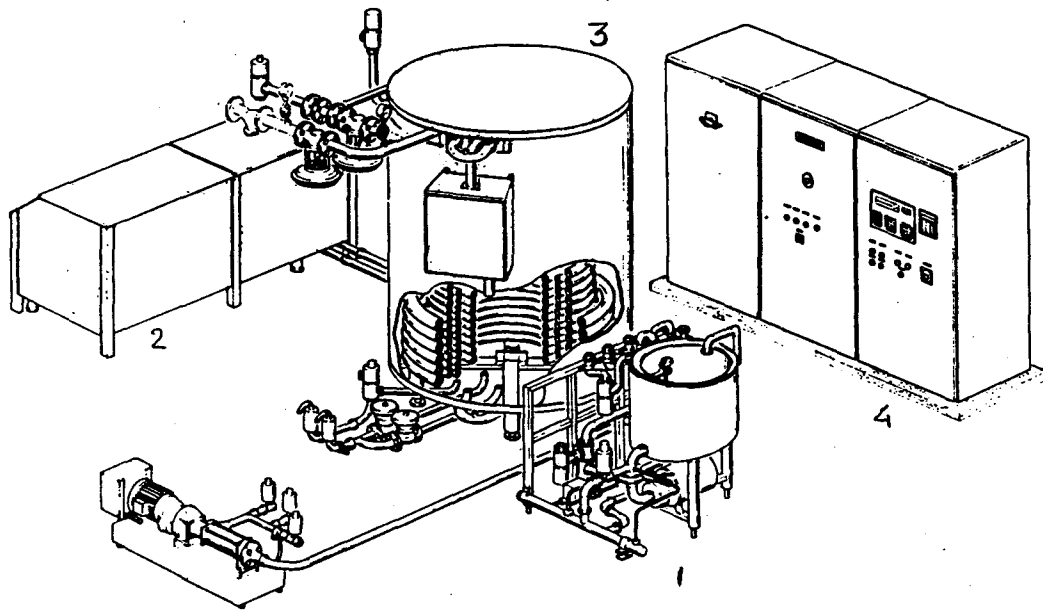


Fig. 2.: Typical arrangement of "In-Flow" UHT processing equipment

- 1.: Product Infeed System
- 2.: Positive Displacement Pump
- 3.: Tubular Heat-exchanging Sections
- 4.: Process Control System

Fig. 3.:
Thermal
Inactivation Diagram

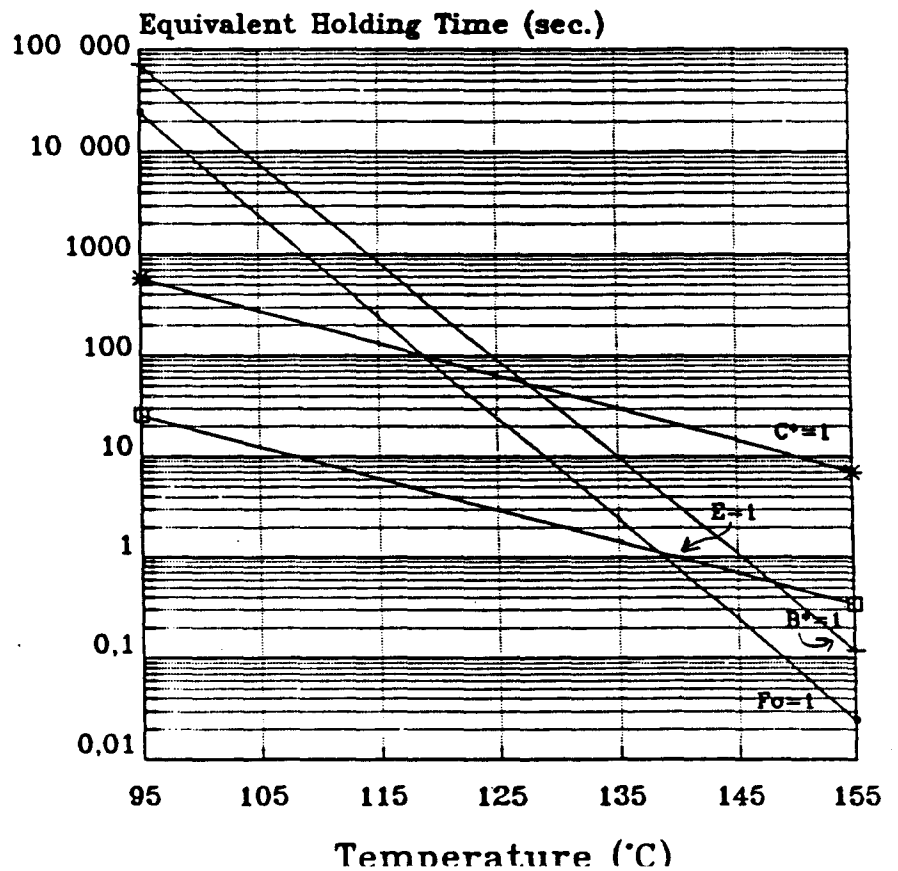


Fig. 4.:
Flowdiagram of a
UHT-installation
with variable
throughput

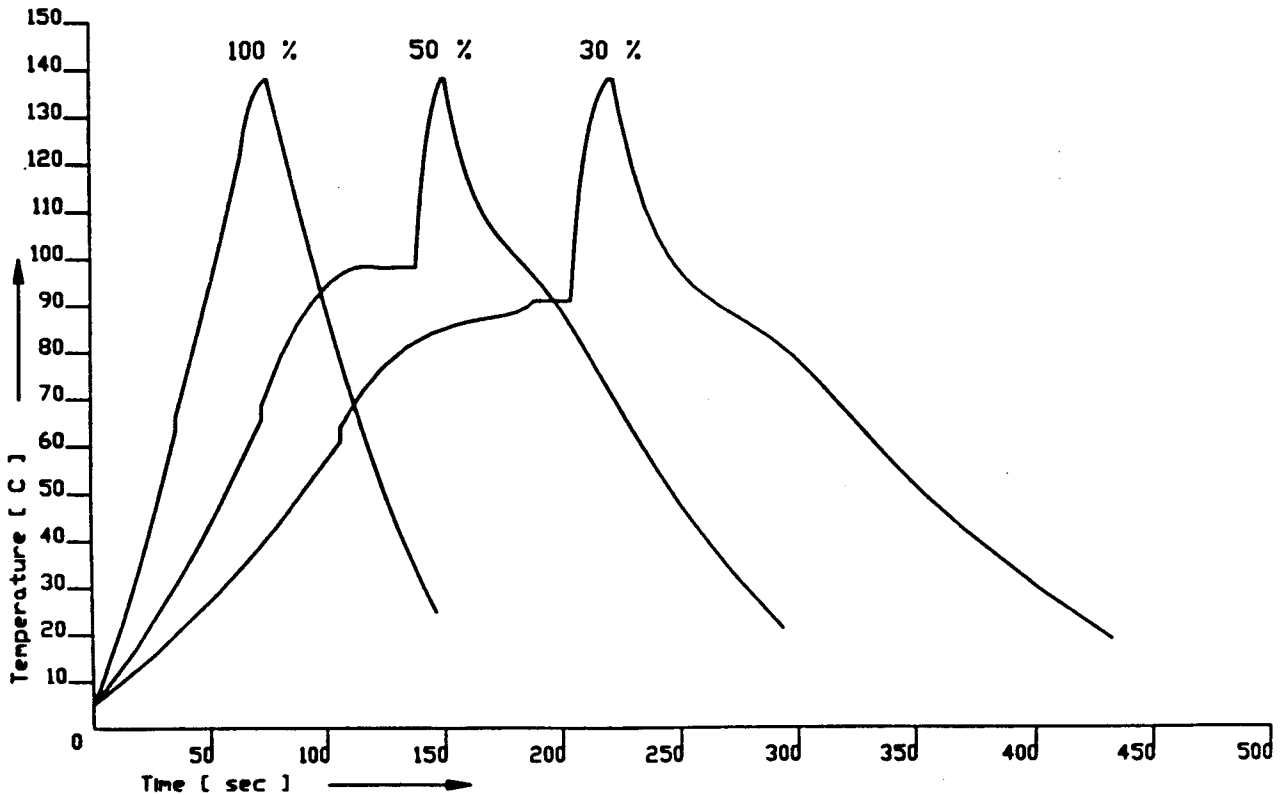
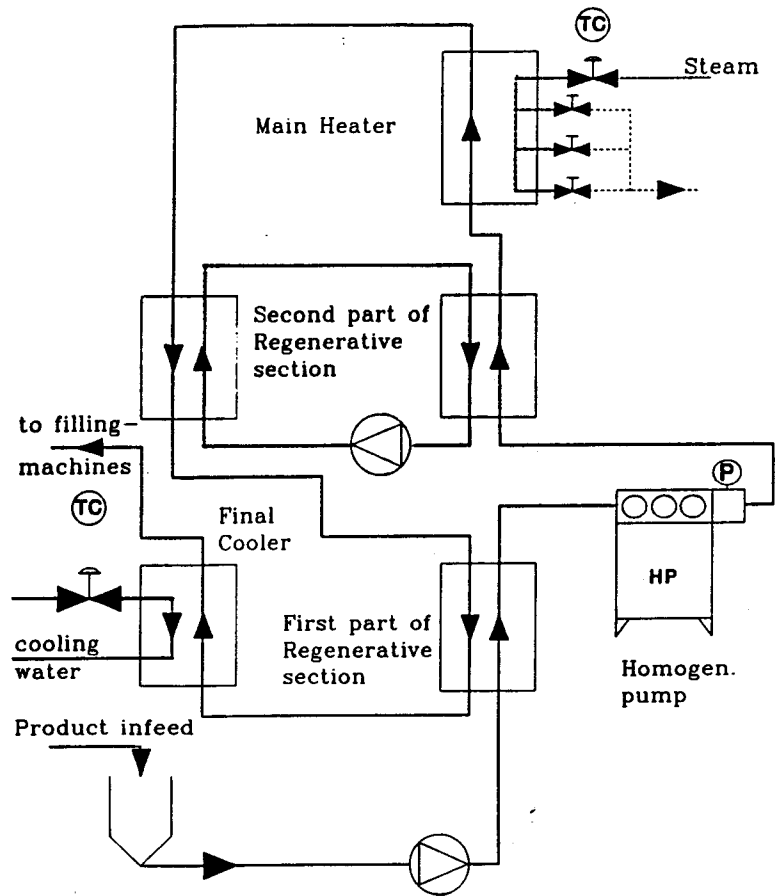


Fig. 5.: "t-T" profiles in a UHT-installation with variable throughput

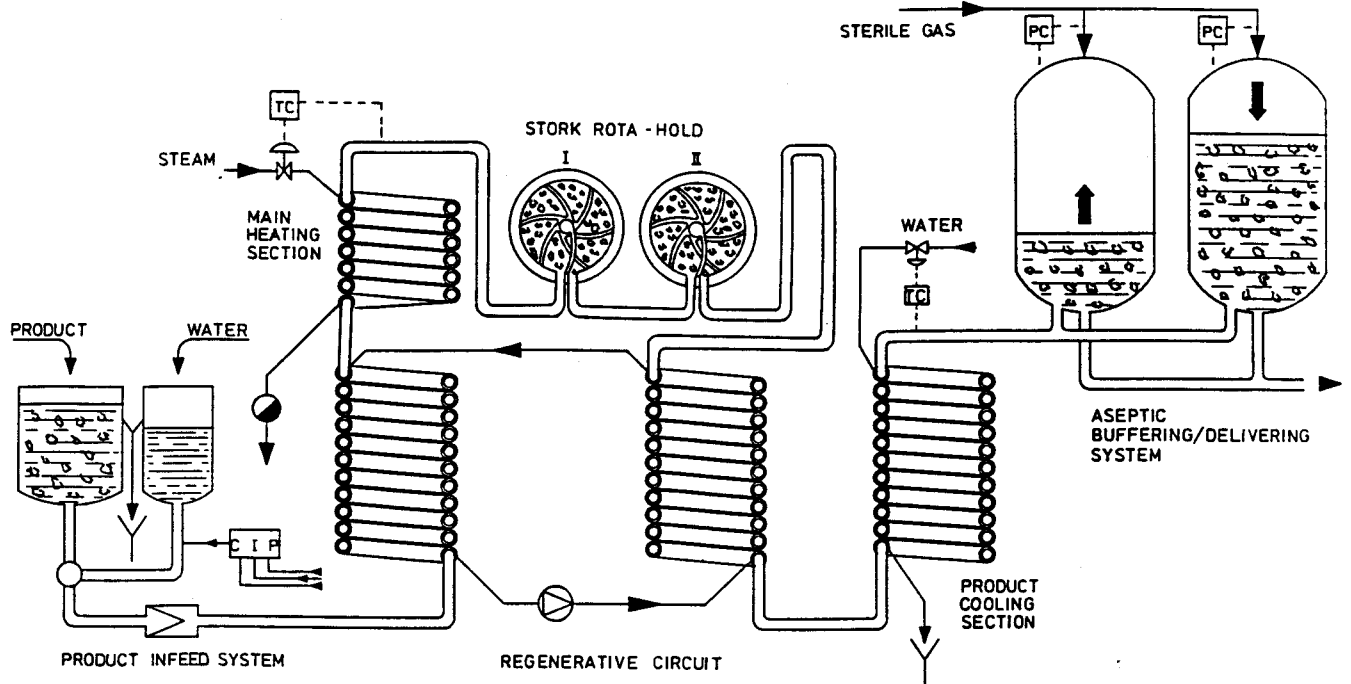


Fig. 6.: Flowdiagram of the STORK STERIPART System including two STORK ROTA-HOLD for "In-Flow" processing of liquids containing particulates

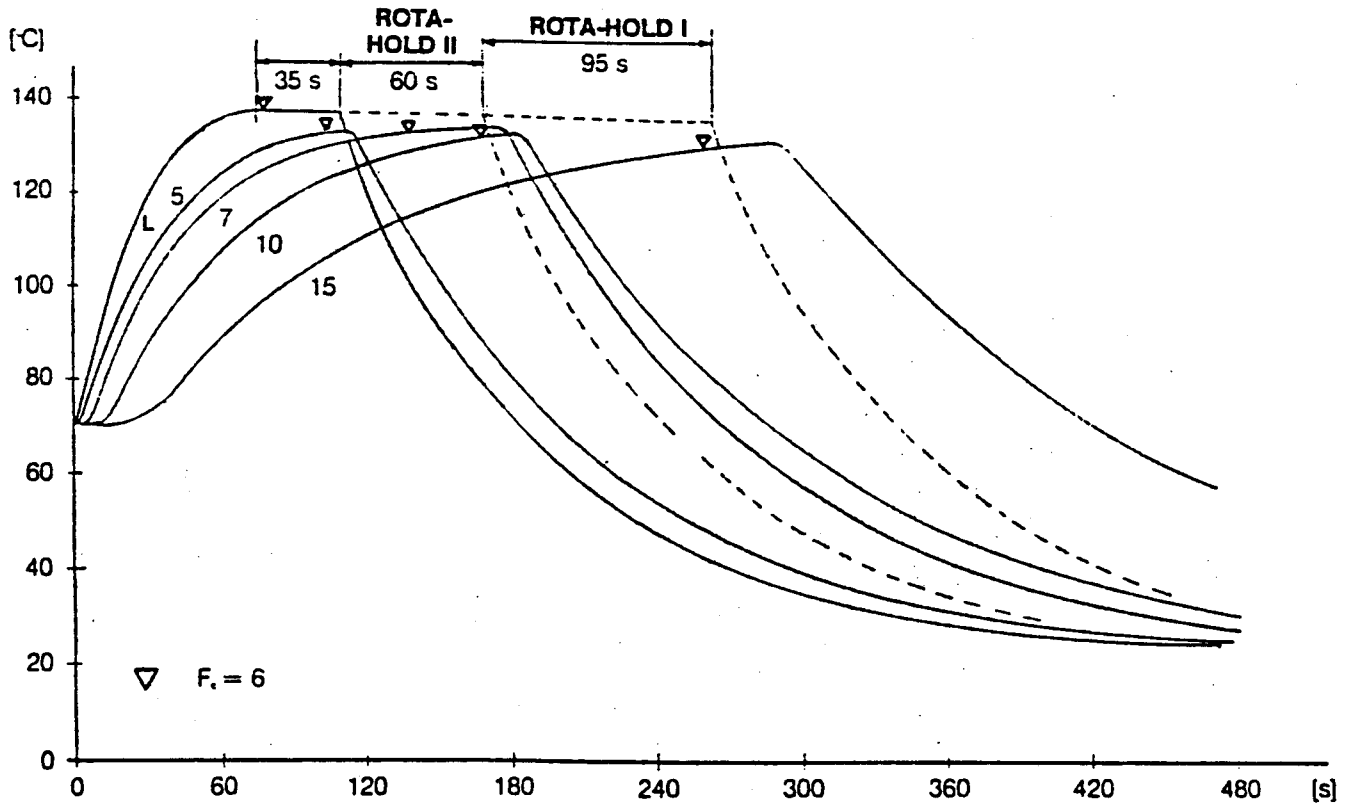


Fig. 7.: "t-T" profiles of liquid and differently sized particulates in a Stork SteriPart with two Stork Rota-Holds

STORK®

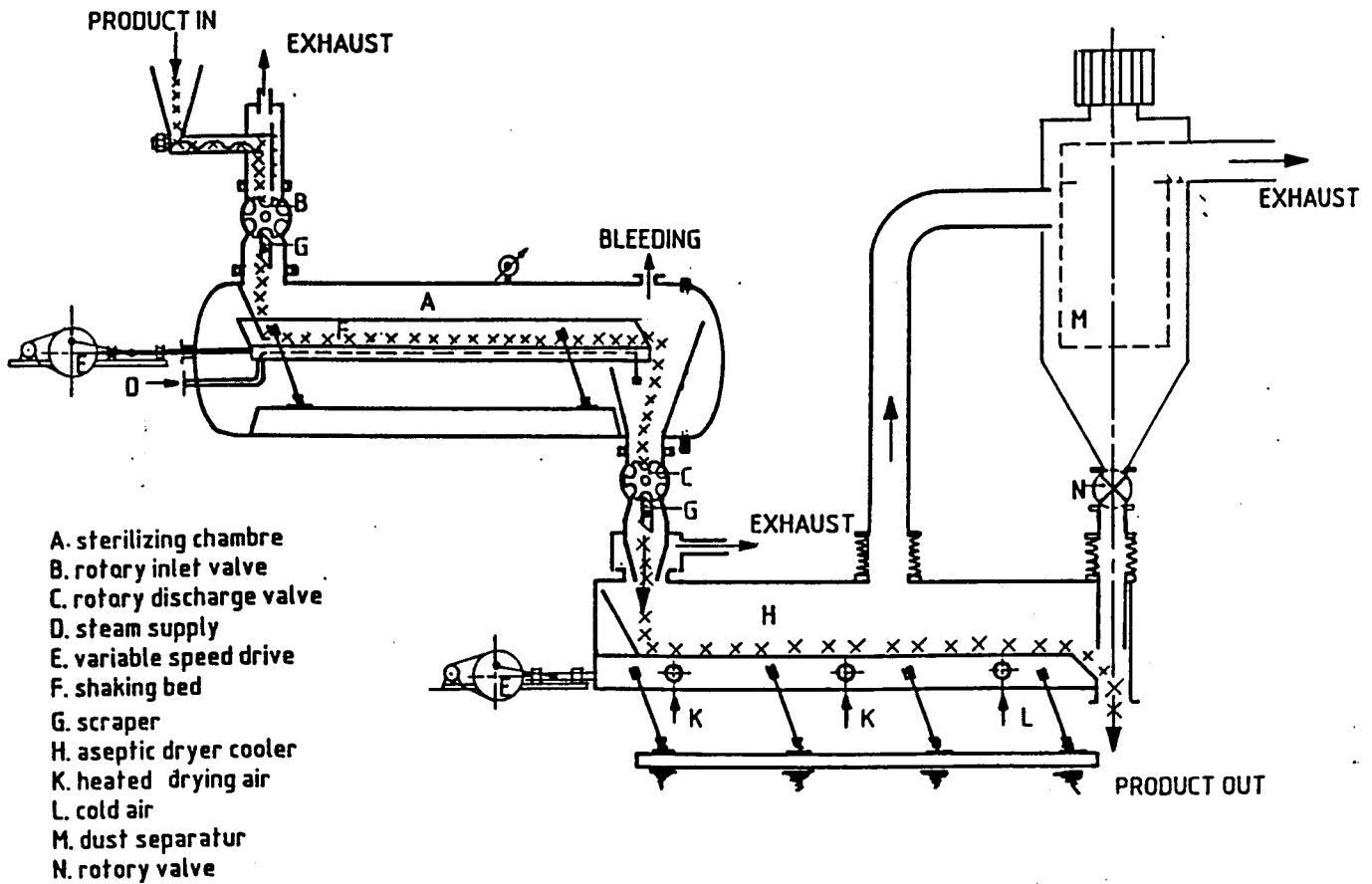


Fig. 8.: STORK BOKFARD System for the continuous "In-Flow" sterilization of spices



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ABSTRACT

Various regulatory agencies impact milk production and processing. They have a successful history of assuring the prominent position of milk as wholesome and nutritious. As some segments of the milk processing industry seek to extend shelf life for milk and milk products, regulation and assurance likewise work together to oversee that these products meet and exceed the quality expectations presently held for milk and milk products.

Meeting the higher temperatures required for extending shelf life requires many changes from that of common pasteurizing systems. There are many technical considerations for achieving ultra pasteurized products. Beyond these more technical and financial considerations must be given for achieving commercially sterile products. The regulatory control points similarly escalate as processing moves from pasteurization to ultra pasteurization and then to aseptic/UHT.



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REGULATORY HISTORY

No one wants to see a bad product, but it happens. At best we hope to catch bad product at the earliest possible time in its life cycle from raw material to the final consumer. Milk's reputation as wholesome and pure was not always the case.

Milk and milk products were staple foods for most people throughout history. Distribution was from Ol' Bessy to the table. Excess was made into cheese and butter right there in the kitchen. As people moved into cities they carried their tastes for milk and milk products with them. These people wanted milk for their children and themselves. To get milk to these people required more time than from Ol' Bessy to the table. It also required consolidating the milk from many cows. The milk production and distribution industry was made up of many Mrs. O'Leary's, one cow operations, who thankfully didn't all kick over lanterns and start Great Chicago Fires. Most milk industry pioneers knew that sanitation and healthy animals produced the best milk, but the demand for milk meant that the best wasn't required to make a buck. Some milk operations were pretty bad. They would milk any cow that could stand and even water down this milk. Milk borne diseases were prevalent. Infected milk caused outbreaks of Brucellosis/undulant fever, scarlet fever, typhoid fever, diphtheria, tuberculosis, and salmonellosis. Milk borne food diseases largely took their toll on children and the elderly. The industry would not regulate itself. Regulation and quality control had to come from outside of the industry.

For the Public Health

England established a public health service with the passage of the Public Health Act of 1848. Seeing the need and using Britain's public health act as an example, many United States cities and states founded public health departments. Most states and localities established public health services in the last half of the 19th century.

The United States Public Health Service (PHS) formally began in 1912 being renamed from the Marine Hospital Service. The Marine Hospital Service had conducted immigrant inspections, quarantine, and health related services for people in uniform since 1798. The PHS began as a uniformed branch of the military, but it now operates as a division of the Department of Health and Human Services. Many agencies make up the Public Health Service. Of these the Food and Drug Administration (FDA) has the most impact on milk processing and product identification.

Many cities and states had already established public health departments and instituted many ordinances before the United States established the PHS. These local public health departments made great public health advances by introducing the concept that microbes were the cause of disease. They implemented vaccination programs, introduced sanitation practices in the community, and established ordinances and regulations designed to prevent outbreaks of contagious disease.

The United States Public Health Service really established itself as it worked to keep the food supply safe for the allied armies during World War I. The safety of condensed milk and the benefits of pasteurization became wide knowledge during this time. The campaigns of Emile Berliner for pasteurized milk were widely heard. Berliner, the inventor of the telephone receiver and gramophone, devoted his later years to child health and nutrition, particularly to the promotion of milk pasteurization.

Each locality's public health department established ordinances, equipment standards, and practices designed to prevent unsafe milk products; however some problems still surfaced. Different equipment design requirements for each locality gave equipment manufacturers problems. Local sanitarians were



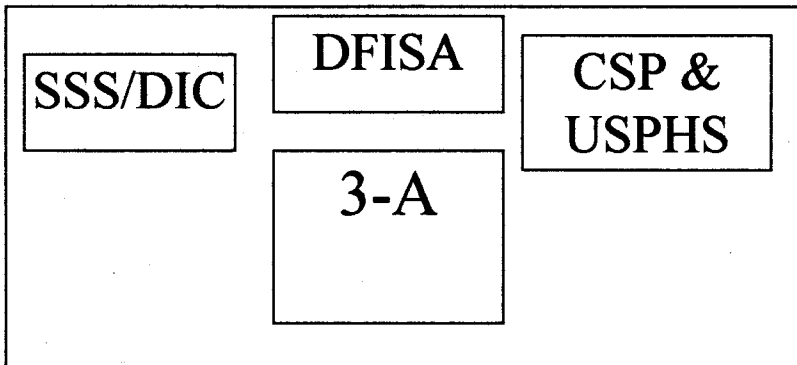
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subject to conflicting interests and interpretations, so enforcement was not evenly based on a set of agreed upon criteria. A common, uniform, and equitable criteria was needed. These criteria had to be forged by a consensus that represented all the interests in the dairy industry for the good of the reputation of the dairy products.

The Three Associations

Two trade associations wrestled with the lack of a consensus on what were good designs in dairy and milk plant equipment. These were the International Association of Milk Dealers (now the Milk Industry Foundation, MIF), the Dairy and Ice Cream Machinery Supply Association (now the Dairy and Food Industries Supply Association, DFISA). These associations together worked during the 1920's with many cities, states, and federal sanitarians to develop understandings in equipment design and plant practices. The International Association of Dairy and Milk Plant Inspectors (now the International Association of Milk, Food, and Environmental Sanitarians, IAMFES) in the late 1920's set up a committee on Dairy and Plant Equipment. In the 1930's, the work of the two trade associations and the professional association became known as 3-A Sanitary Standards. In 1944, the USPHS agreed to collaborate in the development of standards which helped to shape the 3-A organization as it is today.

Today the group still has the three basic legs. The Dairy Industry Council (DIC), referred to as the users group, the Dairy and Food Industries Supply Association (DFISA), referred to as the technical or task committees, and the IAMFES Committee on Sanitary Procedures (CSP) in conjunction with the USPHS.



The development of standard criteria and procedures continues today in the 3A organization. The standards continue to move forward with advances in sanitary science.

This UHT Symposium is part of the ongoing effort to further the ability to safely distribute milk and milk

products over longer distances and times. The understanding of the equipment and practices for ultra high temperature processing of milk has advanced significantly in the past 10 years. The development of ultra high temperature processing equipment and its method of operation is taking a development cycle similar to the introduction and acceptance of pasteurization at the turn of the century and of Clean-in-Place in the 1950's and 60's. Advancements in Ultra high temperature processing are aided by the international nature of the Dairy and Food Industry.

Prevention and Prosecution

UHT processing is being developed during a time of major shifting in the Dairy and Food Industry. Consolidation of the industry has been significant, both on the product as well as the equipment manufacturing side. The industry consolidation is merging the Dairy, Food, and Pharmaceutical sectors.

Since the historical milk industry was one of many small milk producers and processors, the focus of the public health professionals was to design equipment and manufacturing practices that prevented unsafe, adulterated, or fraudulent claims concerning milk and milk products. If the equipment was designed and



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operated according to 3A standards and the product handled according to the city, state, and federal ordinances, the product would be safe, wholesome, and meet minimum standards of identity. The Pasteurized Milk Ordinance (PMO) is the milk sanitation standard for the nation. Local inspectors make periodic audits of the systems and the procedures to assure that the equipment and procedures are performing to the PMO. These inspectors are trained and coordinated by the USPHS/FDA's Milk Safety Branch. Any person who violates provisions in the PMO are guilty of a misdemeanor punishable by fines within that state. All of these measures have worked well to prevent milk borne illnesses. The reputation milk holds is testament to this effort.

The USPHS and its FDA agency has authorities beyond that of milk and milk products. Many of the products overseen by the FDA must be processed and packaged in ways that make and keep them commercially sterile. The USPHS/FDA has long been associated with retort processing. Retort processing in the canning industry brings the product to the temperature and time required to render the product commercially sterile. The raw product, processing and packaging equipment, and procedures must all be understood and kept in perfect operation to produce a commercially sterile product. To prove that the system will perform, the product as well as the process and packaging system are validated by challenging the total system with known bacteria. When this is done and record keeping procedures and specific operator training are in place, the scheduled process is written. The Code of Federal Regulations (CFR) contain the regulations and procedures necessary to file and operate under a scheduled process.

The USPHS/FDA uses a slightly different procedure in the pharmaceutical industry. This industry uses many varied techniques to provide for safe product distribution and use. The product specific unique techniques of manufacture make standard methods of product handling and standard equipment design very difficult. In the pharmaceutical industry the process is validated according to the process filing known as a Drug Master File.

Management is ultimately liable for failure to comply with the requirements as written down in the Process Filing or Drug Master Filing. This allows for a wide variety of equipment and procedures, but puts the public health and safety issues on the company's management. Management is subject to federal prosecution if the filed process is not followed.

The PMO, the CFR, and the USDA

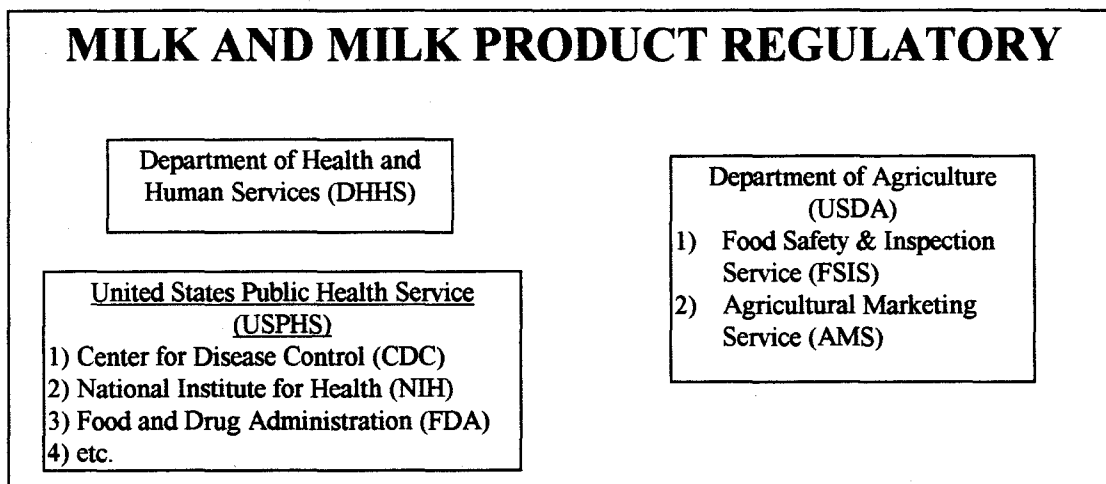
The USDA and the DHHS

All the regulatory agencies and the standards and recommendations look to one goal, to uphold the highest quality for milk and milk products. The best plans and best equipment need to be kept in top condition for nothing is foolproof. "*Foolproof systems do not take into account the ingenuity of fools*"
Gene Brown in Danbury CT News-Times



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The following is a chart of the Government Departments that oversee milk and milk products.



Most of the government departments have their hand in the regulatory process, but not as directly. OSHA prescribes how certain practices must be done to insure worker safety. EPA has some regulations as to how products are handled. The Department of Commerce sets up trade and import/export rules.

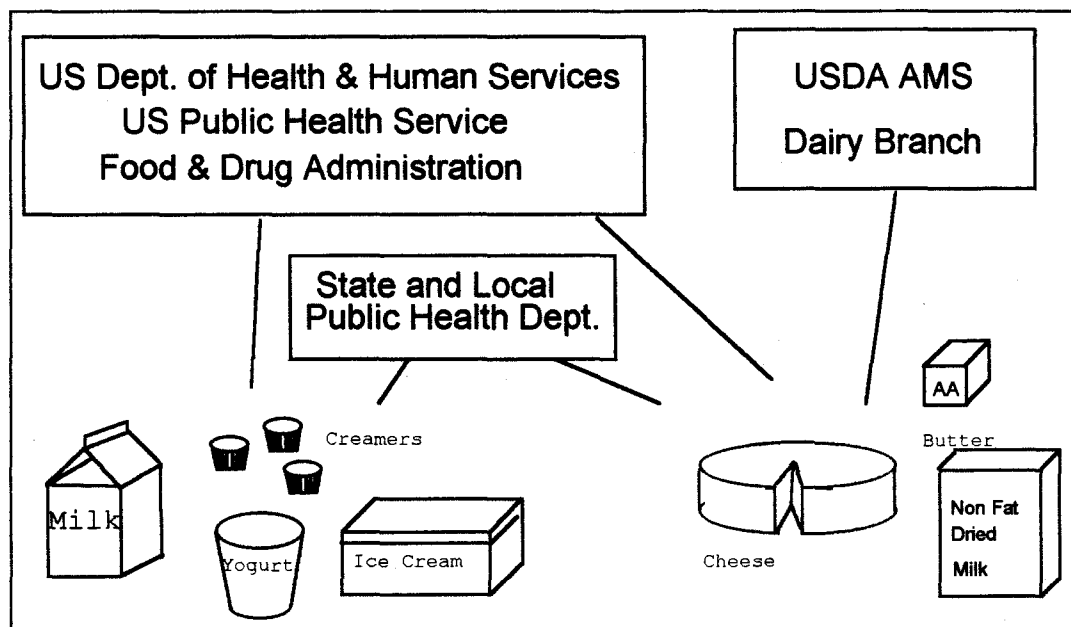


Figure 1 Influences of Various Government Oversight Departments.

The USDA is responsible to work with dairy farmers, milk processors, and consumer representatives to write milk marketing orders for specific regions. These orders determine the regional prices to be paid farmers for grade A raw milk. Such prices must be at least as high as the federally supported price. The orders also establish classes of milk, formulas for pricing, and a system for assuring proper testing and accounting procedures.



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The federal government, through the USDA, seeks to assure a stable supply of milk and to maintain orderly marketing procedures. It buys milk--in the form of butter, cheese, and nonfat dried milk--that has not been sold commercially, in effect establishing minimum prices.

To buy cheese, butter, and non fat dry milk, the USDA must grade these products. Milk product grading is a service offered to manufacturers on a voluntary basis by the Agricultural and Marketing Service(AMS) of the Department of Agriculture. Grading is operated on a self-supporting basis and is funded from fees paid by the users. Although this is voluntary, most plants cannot refuse. The product must be graded in order for a plant to sell product to the USDA or to out of state markets. The USDA purposes of grading are to establish and maintain uniform trading standards and to aid in the determination of milk pricing.

In order to establish the grade of the product, USDA inspectors must review and inspect the plant, its procedures, and its equipment. The USDA impact on a producer is because of these inspections and the approval of equipment and procedures. USDA people serve on the groups writing standards and reviewing equipment for which no standard has been written. Because of the marketing aspect of the USDA's AMS, the suggestions and recommendations of USDA inspectors carry the significance of regulation.

The DHHS has the public health regulatory role. Within it, specific regulations deal with standards of identity, procedures, and guidelines that deal with safeguarding public health. The Food and Drug Administration (FDA) oversees milk and milk products through the Pasteurized Milk Ordinance (PMO) as well as the wider food industry within the Code of Federal Regulation (CFR).

DHHS/FDA/The Pasteurized Milk Ordinance (PMO)

In simplest terms, the Pasteurized Milk Ordinance works to prevent unsafe, or adulterated, milk or milk products from getting to the consumer. Every ordinance in the PMO and effort by the local, state, and federal public health inspector works to assure safe and wholesome milk and milk products. The PMO has been through many revisions. The first version was written in 1924. Many revision have been issued since. The PMO is a consensus formed from the current knowledge and experiences of the whole of the nations dairy industry.

The PMO is the basic standard used in the voluntary Cooperative State PHS program for Certification of Interstate Milk Shippers. The National Conference on Interstate Milk Shipments (NCIMS) recommends changes and modifications to the PMO. The FDA has formal Memorandum of Understanding on how these recommendations will be included in the PMO. An appendix in the PMO addresses the 3A Standards that are involved when and where the PMO refers to equipment and practices. Relevant sections of the CFR are included in the appendix as well provisions that make the PMO State Law.

The official term of UHT and the treatment of milk and milk products at temperatures like 280°F(138°C) are different. UHT as defined in the PMO is officially the label needed to indicate that the milk or milk product has been aseptically processed and packaged. Stress this point because it is confusing. Consumers as well as many industry people do not understand meaning of aseptic, UHT, or ultra-pasteurized.



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Ultra-Pasteurization

By using 3A approved equipment in properly designed systems, and following proper practices, a milk plant can be locally inspected and begin processing and distributing milk and milk products that are treated using ultra high temperature processors. The product can be labeled as ultra-pasteurized, but it must be distributed under refrigeration. The PMO provides for the design and testing. Because the public health must be safeguarded and the goal is to prevent unsafe product, the PMO directives for ultra-pasteurized production are stringent when it comes to temperature and time. The temperature can actually be higher for ultra pasteurization than for aseptic processing. The design of the heat treatment processor and the flow diversion device must adhere to the Higher Heat Shorter Time (HHST) pasteurizer as specified in the PMO. Ultra pasteurized product requires no changes in the filling equipment or in how packaged product is handled from that needed for an HTST. The same records kept for pasteurization are needed.

If the product has been pasteurized prior to an ultra pasteurization treatment, the public health has been satisfied. Additional Safety thermal limit interlocks and PMO approved flow diversion devices are not required on the Ultra pasteurizing system. Failure to meet the 280°F(138°C) for 2 seconds at the hold tube exit would constitute fraud if it were packaged as ultra-pasteurized. This is a matter addressed in the CFR under standards of identity and labeling. The product though, would not endanger public health so is not addressed in the PMO.

The local, state, and federal public health officials have approved cases where the ultra high temperature heating system is the only heat treatment system. The main consideration is that the PMO approved flow diversion device must be after the final cooler to provide for unrestricted free draining and all product contact surfaces must be heated in excess of the pasteurization temperature for the required minimum time. Systems that meet Higher Heat Shorter Time(HHST) conditions like 191°F(88°C) for 2 seconds and then continue heating and holding times to conditions of commercial sterility have been allowed. Chart recorders in the range of 280°F are not part of the approved PMO pasteurization equipment. The use of HTST approved 2 pen plus event recorders have been used with other recorders charting the temperatures above 191°F(88°C).

Technical Considerations from HTST to HHST and Ultra High Temperature

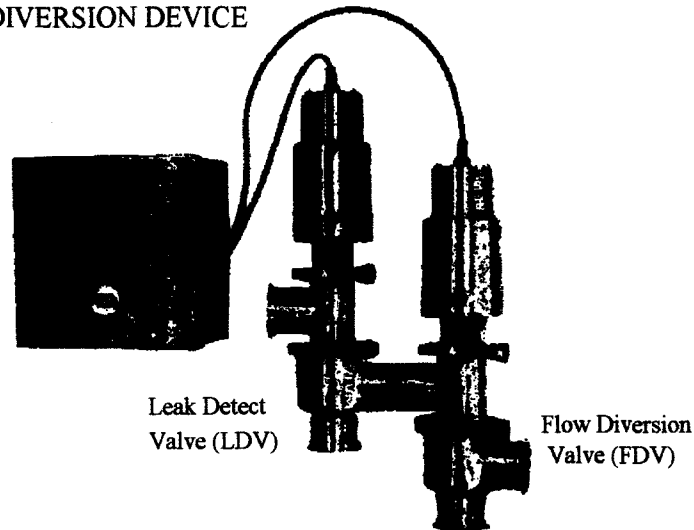
The Flow Diversion Device (FDD) is the main item that distinguishes HTST's, HHST's, and UHT's. FDD's are the means used to prevent under processed product from being distributed to consumers. The 3-A practices for High Temperature Short Time (HTST) systems and the Pasteurized Milk Ordinance (PMO) describe and show flow diversion devices (FDD).



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HTST Flow Diversion

FLOW DIVERSION DEVICE



Used by permission Courtesy of Alloy Products Company, Waukesha, Wisconsin

Figure 2 Photo of Flow Diversion Device

The vertical position of these two valves located at the end of the holder tube with drain lines running to the constant level tank is standard for an HTST.

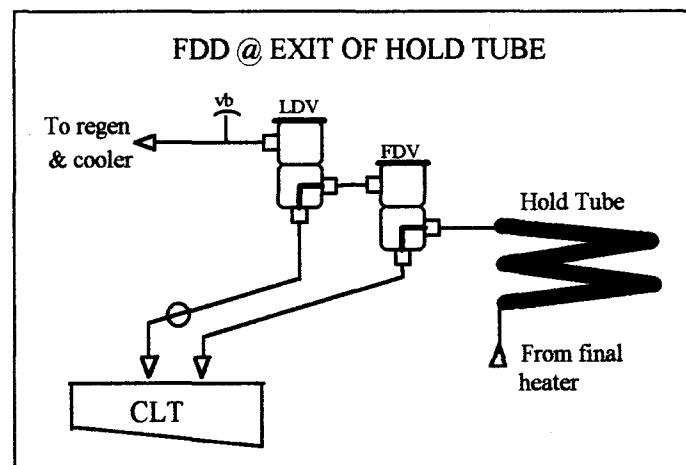


Figure 3 Schematic of FDD at Exit of Hold Tube

The operational logic and the physical layout of this flow diversion device work well to prevent under-processed product from being packaged when looking at HTST of milk and milk products.

A High Temperature Short Time (HTST) system effectively operates with the ability to cycle in and out of forward flow. Forward Flow means that product is moving past the point of diversion. This was essential with older control systems especially at start up, when the HTST is trying to reach equilibrium. The cycle from forward flow to diverted flow and back can be done on product without a significant change in



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product quality. In an HTST, the FDD is at pasteurization temperature. Since product is diverted at the pasteurization temperature, the valves and the valve seats are seeing pasteurization temperatures. This means that the under processed product clinging to the valve seats and surfaces of the drain lines will be thoroughly pasteurized. Product in these areas will not become growth media for bacteria of public health significance.

HHST Flow Diversion

The temperature required at the holder tube exit for aseptic and ultra-pasteurized products is well above the atmospheric boiling temperature. A potentially dangerous expansion of steam could occur if the flow diversion device were located at the exit of the hold tube and product temperature there was near or above the atmospheric boiling temperature of 100°C (212°F). The flow diversion device located at the exit of the hold tube would divert product that would flash. Flashing is the nearly instantaneous expansion to a gas when a liquid is released from the pressure that keeps it from boiling. Because of this dangerous possibility, allowances are made for an alternative location of the flow diversion device. From the Pasteurized Milk Ordinance 1989 revision SECTION 7, ITEM 16p(B).2.b.(7),

In the case of higher-heat, shorter-time (HHST) pasteurizing systems utilizing the temperatures of 89°C (191°F) and above and holding times of 1 second or less, the flow diversion device may be located downstream from the regenerator and/or cooler section: *Provided*, that when the flow diversion device is located downstream from the regenerator and/or cooler section, the flow diversion device shall be automatically prevented from assuming the forward flow position until all product contact surfaces between the holding tube and flow diversion device have been held at or above the required pasteurization temperature for at least the required pasteurization temperature continuously and simultaneously for at least the required pasteurization time as defined in Definition S of this Ordinance.

The flow diversion device must be located downstream from the cooler section whenever the temperature at the hold tube exit is near or above the atmospheric boiling temperature. Temperatures are usually above the atmospheric boiling temperature when designing processes for aseptic processing.

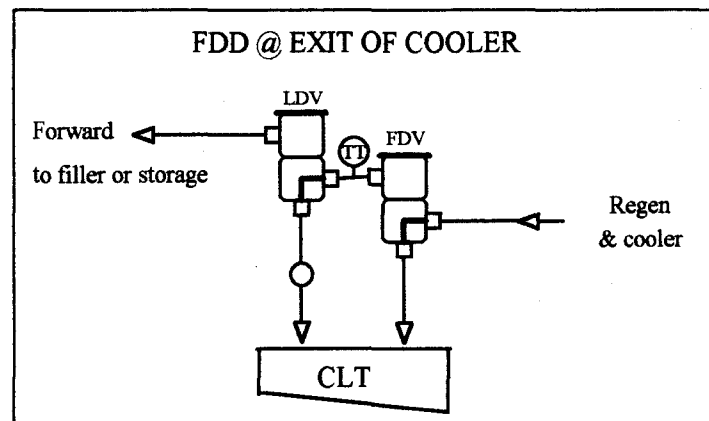


Figure 4 Schematic of FDD at Exit of Final Cooler

Several technical considerations must be dealt when using the FDD arrangement of Figure 3.



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Has a More Time Consuming and Complicated Operation

A system built with the flow diversion device downstream of the regenerator and/or cooler, does not operate as simply. Product is diverted by the operational logic at the occurrence of an under processed product. The regenerator and/or cooler section will then be filled with under processed product. The logic requires that all product contact surfaces meet pasteurization temperatures and holding times before forward flow can occur. The temperature transmitter between the two valves of the FDD signals that pasteurization temperature of 89°C (191°F) has been met. This requires logic and systems that disable regeneration and cooling so that all the product contact surfaces can be heated to the pasteurization temperatures and then cooled down to the filling temperature. This can be time consuming and if done with product, wasteful.

Most systems allow diversion of product only once when using post cooler flow diversion. In most cases, these systems must then be cleaned, or least purged, and then started on water before switching to product. The loss in production time when this occurs has to be figured into the cost of production.

Requires a Pressurized System to Reach Temperature

The temperatures in the system could never exceed the atmospheric boiling temperature at the valve discharge unless the system has some means of building pressure. To achieve sterilization temperatures some means to hold pressure is required so that the temperature in the holder tube and downstream product contact surfaces can reach the desired temperature. If a pressurized recirculation system is not used during sterilization, a cooler will be needed to remove the flash heat before recycling back to the constant level tank.

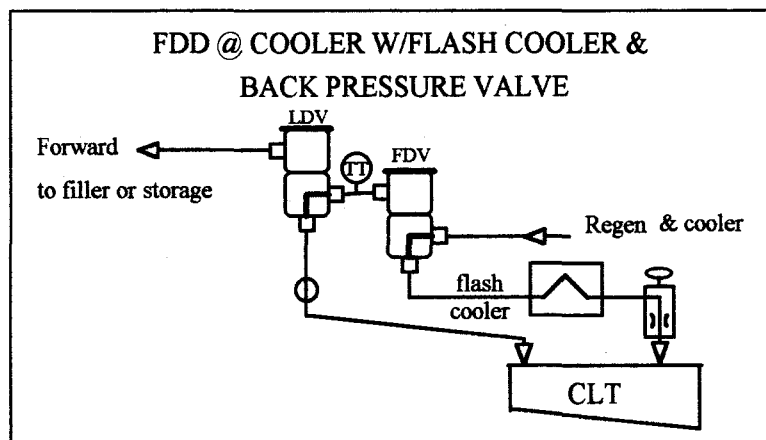


Figure 5 Schematic of FDD at Exit of Final Cooler w/Flash Cooler & Back Pressure Valve

Allows Bacterial growth at the FDD

The single valve seat and stem seal pose sites for bacterial entrance between sterile and unsterile product. Bacteria are present in the air, water, or product on the unsterile sides of the flow diversion valve seats and stems. These valve seats and stems are generally at cold or ambient temperatures well below pasteurization or sterilization temperatures. This means that the bacteria are in viable environment. Product and water clinging to the surfaces of the valve seats and stems are culture growth media. Eductor type suction forces can draw in these bacteria if the valve seat and stem seals are worn or improperly assembled or the actuator forces are weak, also the actuation of the valve moving the stem can introduce



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bacteria into the product stream. Even without the valve parts moving, bacterial cultures can grow against pressure and cross the valve seats and stems into the product stream.

The PMO provisions try to ensure that product does not sit on the valve seats or surfaces of the drain lines from the flow diversion device. From the Pasteurized Milk Ordinance 1989 revision SECTION 7, ITEM 16p(B).2.b. 7 and 8, as well as the similar ordinances contained in SECTION 7, ITEM 16p(C).2.g. 8 and 9,

(8) The pipeline from the diversion port of the flow diversion device shall be self draining, and shall be free of restrictions or valves, unless such restrictions or valves are so designed that stoppage of the diversion line cannot occur

(9) When it is used, the pipeline from the leak detector port of the flow diversion device shall be self draining, and shall be free of restrictions or valves.

Figure 5 is a flow diversion flow diagram and arrangement that satisfies the PMO. The PMO authorities understand the problems and potential risk. The risk is loss of shelf life, but the product will be properly processed. Pathogenic bacteria will be destroyed. The advantage is in not having to file the process or to keep the records required by the CFR for aseptic processing.

Pasteurized Milk Ordinances Control Points

The PMO calls for several control points to guarantee product processing and to ensure that no adulteration can be done to the product.



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Table 1 PMO Regulatory Control Points

<p>Pasteurized Milk Ordinances</p>	<p>Process design and controls</p>
<p>That each particle of milk receive the proper holding time.</p>	<p>Hold tube length is measured or salt tested, then sealed against tampering. The hold tube must also rise at minimum of 2.1cm/meter(0.25"/foot)</p> <p>All flow promoting devices not used as timing devices such as vacuum tanks, separators, and pumps must be free to recirculate on themselves.</p> <p>Air breaking anti-siphon piping designs are located around constant level tank.</p> <p>Product to product regeneration requires vacuum breakers at high point leading into cooling regenerator section and the heating regeneration section have drain back holes.</p> <p>On steam injection systems, a differential pressure across injection chamber to ensure that steam is not a motive source. Also a differential pressure across the holder tube to ensure that the product does not boil. That the hold tube be longer to correspond to the increase volume of condensed steam.</p> <p>Timing pumps or Meter Based timing systems must be checked out every three months. Adjustment systems are sealed against tampering.</p> <p>Flow promoting devices shall be wired so as not to run unless the flow is diverted, satisfying pasteurization conditions or in a Clean in Place mode.</p>
<p>That each particle of milk receive the proper heat treatment.</p>	<p>The Safety Thermal Limit Controller (STLR) and the Flow Diversion Device (FDD) isolate pasteurized product from non-pasteurized product with several degrees of redundancy.</p> <ol style="list-style-type: none"> 1) Dual stem to overcome switching time and breakage in a seat seal. 2) Unrestricted drain back to balance tank or drain to prevent back pressure from forcing raw product forward. 3) Position indication on valves to prove ease and speed of diversion. 4) User adjustments shall not prevent proper operation. 5) That air or electrical failure automatically cause diversion. <p>Pasteurized product over pressure to regenerator to ensure that regenerator leakage is toward raw side product or intermediate water.</p> <p>Mercury in Glass thermometer or an equivalent temperature indicating device with accuracy of 1/10° F or C.</p> <p>A circular chart recorder adhering to specific graduations be located to record temperature at the exit of the holder tube.</p> <p>No portion of the holder tube can receive heat.</p>
<p>Prevention of product adulteration by added water. Steam injection systems</p>	<p>For steam injection systems ratio control by temperature of the product prior to steam injection and after vacuum flashing.</p> <p>If steam is added after the flow diversion device, a means to shut off the steam when product is diverted is needed.</p> <p>If direct condensers are used on the vacuum system, the water supply must stop if over a safe level.</p>



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DHHS/FDA/The Code of Federal Regulations (CFR 21 Food & Drug)

The Code of Federal Regulations (CFR) cover many aspects of federal legislation. The specific title concerning Food and Drugs is called Title 21. Title 21 has over a thousand parts. Parts that are pertinent to Milk and Milk products include the Standards of Identity, Thermally Processed Foods Packaged in Hermetically Sealed Containers, and Good Manufacturing Practices (GMP). Standards of identity for milk and milk products are defined in parts, 131 for Milk & Cream, 133 for Cheeses and Related Cheese Products, and 135 for Frozen Desserts. The thermally processed foods sections are under part 113, and the GMP is under part 110.

The Federal Food, Drug, and Cosmetic Act have pertinent references on adulterated and misbranded foods.

Aseptic/UHT

Ultra high temperature treatment of milk and milk products packaged for unrefrigerated distribution falls under the Code of Federal Regulations. These regulations are in CFR 21, 110-113. UHT is the PMO official labeling requirement indicating that the product is aseptically processed and packaged. Other labeling options have been allowed. This is due to the lack of consumer awareness and understanding of what UHT means. It is not apparent to most consumers that the UHT label or one saying Aseptic means No Chill Needed prior to opening.

A world of difference exists between producing an Ultra Pasteurized product and an Aseptic or UHT defined product. The processing systems are generally the same. Processing systems for both Ultra pasteurized and Aseptic/UHT must withstand the rigors of the higher temperature. They must run in accordance with all but two of the HHST provisions of the PMO. These provisions are not exceptions to the PMO, but are really extensions. The PMO allows these extensions under the Aseptic processing clause. From the Pasteurized Milk Ordinance 1989 revision SECTION 7, ITEM 16p(C). Aseptic Processing Systems, Administrative Procedures,

Aseptic Processing Systems... Provided that nothing shall be construed as barring any other aseptic processing system which have been recognized by the Food and Drug Administration to be equally effective and which is approved by the regulatory agency

These extensions are a significant step up from production of ultra pasteurized products. They constitute significant added costs to operating procedures, levels of talent, and mean generally lower production efficiency.

The first extension from the PMO regards what constitutes a flow diversion device and the introduction of a flow diversion system. The second requires the challenging of the system and preparation of a scheduled process. The scheduled process and current good manufacturing practice requires:

- 1) Experimentation and definition of the unique times and temperatures required to sterilize the product with the specific sterilizer.
- 2) Spelling out of the procedures needed to sterilize the processing, distribution, and packaging equipment.
- 3) Definition of Critical Control Points and operational reports and records indicating observation and adherence to the predefined standard.
- 4) Formal procedures, tests, and documentation that assure that the product remains commercially sterile throughout distribution.



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- 5) Formal means to handle any unforeseen problems.

Some other processes are defined along with the scheduled process.

Scheduled process -- the process selected by the processor as adequate under the conditions of manufacture for a given product to achieve commercial sterility. This process may be in excess of that necessary to ensure destruction of microorganisms of public health significance, and shall be at least equivalent to the process established by a competent processing authority to achieve commercial sterility.

Operating process -- the process selected by the processor that equals or exceeds the minimum requirements set forth in the scheduled process.

Minimum thermal process -- the period of time at a temperature scientifically determined to be adequate to ensure destruction of microorganisms of public health significance by application of heat to food, either before or after sealing in a hermetically sealed container.

The temperature and times dictated in the PMO would be similar for milk and milk products under the CFR 21: 113 scheduled process. The minimum thermal process could be that for HHST production of 191°F(89°C) for 2 seconds at average particle velocity hold time or any of the other time-temperature pasteurization profiles. The scheduled process would usually be at a temperature less than operating process. The operating process would typically be 280°F(138°C) for 2 seconds for an indirect heating system. The operating process is usually at a higher temperature for direct heat steam injection systems. The shorter total residence time in these systems usually requires higher temperatures to reach the same reduction in microbial loading.

Aseptic Flow Diversion

An Ultra-Pasteurized product pasteurizer and Aseptic UHT sterilizer look very similar. The major difference is in the control philosophy and in the Flow Diversion Device (FDD). Free drainability issues on the flow diversion devices are not relevant in an aseptic system. A contamination site is likely where a single valve seat or shaft seal separates an area that should contain sterile product from a contaminated area such as room air.

The following table outlines the needs of an aseptic flow diversion system.

TECHNICAL DESIGN NEEDS	
1)	Isolate sterile product from non-sterile product.
2)	Prevent bacterial contamination from valve seats and stems.
3)	Achieve and maintain sterility.

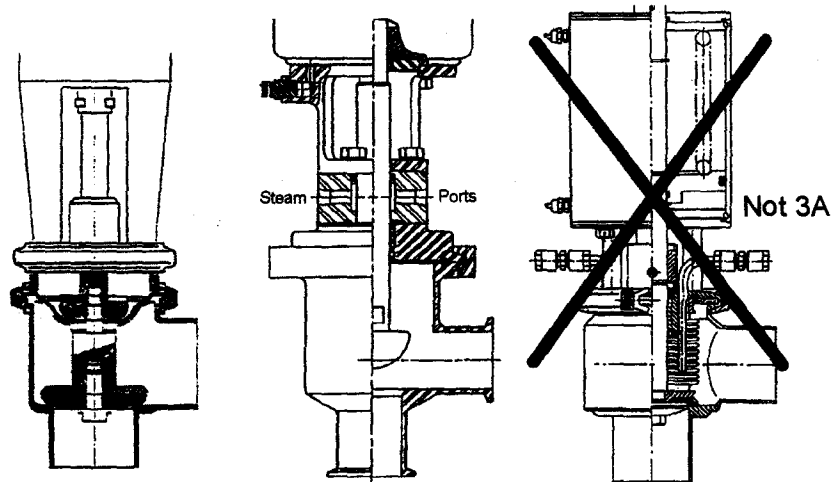
Table 2 Requirements of Aseptic Flow Diversion Valving

The typical flow diversion device of an HTST or HHST, is not readily adapted to being an aseptic flow diversion device. An aseptic flow diversion system requires many valves. These valves are designed to prevent bacterial contamination from the stem seal. Steam prevents bacterial contamination from the seat side of the valves by the arrangement of valves in the aseptic flow diversion system.



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Most of the sanitary valve supply companies have solutions to the stem seal problem. Steam tracing the external area of the stem seal is one method. Other means are diaphragms, and bellows encasements.



Used by Permission Courtesy of G&H Products Corp., Tri-Clover, and Kolttek.

Figure 6 Typical Stem Seal Isolation Techniques

Bellows encased valves do not have a 3A sanitary standard. The product contact parts of the valve may be able to meet cleanability guidelines, but the internal parts of the bellows pose a site for contamination. Fluid can condense in the bellows chamber under normal operation, and the potential for undiscovered failure of the bellows exists especially if the valve is hidden or used for vacuum processes. Undiscovered infection and contamination of the product could result. The FDA Milk Safety Branch authorities have concerns about bellows encased valves for these reasons, and so do not allow even provisional use of bellows type valves in plants subject to the PMO.

CFR Defined Flow Diversion System

From the Code of Federal Regulations, Title 21-- Food and Drugs, Chapter 1, Part 113.40 (g)(1)(h)(4-1-91 Edition)

Flow Diversion Systems. If a processor elects to install a flow-diversion system, it should be installed in the product piping located between the product cooler and the product filler or aseptic surge tank and should be designed to divert flow away from the filler or aseptic surge tank automatically. Controls and/or warning systems should be designed and installed with necessary sensors and actuators to operate whenever the sterilizing temperature in the holding tube or pressure differential in the product regenerator drops below specified limits. Flow diversion systems should be designed and operated in accordance with recommendations of an aseptic processing and packaging authority.

A flow diversion system should be designed according to recommendations of aseptic processing and packaging authorities. One such authority is the National Food Laboratories (NFL), a division of the National Food Processors Association (NFPA). Every plant has unique considerations of layout, flowrate, and production flexibility's. Since every valve is a potential site of product contamination each must be designed, piped, and operated in a manner that allows it to achieve and maintain sterility.



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Due to the confidential nature of NFL's work, recommendations are given only after flow diversion system design is done. Consulting firms and equipment supply companies usually layout and design flow diversion systems and the associated controls.

CFR 21:113g Aseptic Processing Control Points

The CFR adds more to the requirements than the PMO, but in a less structured manner. The CFR suggest following the recommendations of the process authority. More so it implies, do not endanger the public health by letting any questionable product out for distribution.

Since UHT in this review is still dealing with milk the PMO forms the foundation and all of the PMO must be followed unless by exception. Some additional regulatory controls are required.



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Table 3 CFR Regulatory Control Points.

Code of Federal Regulations 21 :113	Process Design and Controls
That each particle of milk receive the proper holding time.	In addition to the PMO. The CFR requires charting the temperature exiting the final heater. This ensures that no heat is gained and minimal heat is lost during holding.
That each particle of milk receive the proper heat treatment.	<p>The PMO requires tamper evident seals. The CFR allows a lock and/or a notice from management that says "ONLY AUTHORIZED PERSONS ARE PERMITTED TO MAKE ADJUSTMENTS"</p> <p>The CFR requires charting the sterilized regenerator pressure differential over the unsterilized regenerator.</p> <p>A Flow Diversion System is not required by the CFR since product may be diverted after packaging. So long as unsterile product does not get into distribution. Flow Diversion Systems are usually employed since diversion after packaging is extremely costly.</p>
That the complete aseptic processing and packaging system achieves sterility	<p>Every 15 minutes observations are recorded concerning vital temperatures and pressures and other phenomena around the complete aseptic processing and packaging system during sterilizing.</p> <p>That these observation be recorded by an individual that has been to a Better Process School and understands what constitutes sterility. This individual must have full authority to halt processing and/or packaging</p>
That the complete aseptic processing and packaging system maintains sterility	<p>Every 30 minutes observations are recorded concerning vital temperatures and pressures and other phenomena around the complete aseptic processing and packaging system during all times that any part of the system is said to sterile.</p> <p>That these observation be recorded by an individual that has been to a Better Process School and understands what constitutes sterility. This individual must have full authority to halt processing and/or packaging.</p>
That unsterile product does not get into distribution.	The final packaged product is held as unreleaseable inventory until analysis of its sterility is assured. To properly culture product may take up to a week, before the product can be cleared for distribution.
That consumption of product can be halted once product is in distribution.	Lot tracing and distribution mapping must be maintained so that product can be recalled. Procedures must be in place that assure quick response to a product recall.



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CONCLUSION

The history of healthy and nutritious food supplies proves the milk industry and regulations work. This was not always the case. Meeting the needs for safe and wholesome milk products is a continuing goal. The industry along with various agencies within all levels of government have a rich history of learning and applying and then standardizing the best and equitable production practices. All of the regulatory levels and agencies can become confusing. Working together to build a standard can be painstakingly slow. New technology introduction requires many years for acceptance. The outcome, though, is unchallenged. The United States has an enviable record of quality food supply. We trust our food supply.

The discussion of UHT begins with ultra high temperature processing and the changes such temperatures have on milk processing. Regulatory provisions allow for a product that is labeled as Ultra Pasteurized. This brings the benefit of extended shelf life to producers without the significant burden of meeting the requirements for aseptic processing and packaging. Design considerations are many when upgrading from an HTST pasteurization system to an HHST pasteurization system. An HHST that can achieve Ultra pasteurized temperatures and holding times, has a lower production efficiency, requires more equipment, and increases utility consumption over that of an HTST. The cost of production is higher.

In order to make an aseptic product, the CFR 21 requires several records be automatically taken, several manually noted, and that the system be challenged with inoculated products to what is required to meet commercial sterility for that product. The piping and filling system must also be able to achieve and maintain the products sterility. Beyond this, specially trained operators and quality assurance procedures must be in place. Assuring that the packaged product is commercially sterile is very costly. The testing costs are reasonable, but the warehousing and inventory costs of up to weeks of production are significant. What if the testing proves the product failed to achieve commercial sterility. The result can be devastating. Consider the loss of all that production, but also the costs of failing to satisfy customers who do not receive their orders.

When it comes to installing an Aseptic/UHT system none are simple extensions of HTST's. Installation of a system is the lesser part of the costs associated with Aseptic/UHT production.



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Effect of Proteolytic Enzymes on the Stability of UHT-Treated Milk

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I. Abstract

Age gelation and off-flavors are the major problems in UHT milk associated with proteolytic enzymes. These proteases may be native enzymes or those produced by psychrotrophic microorganisms. The problems from proteolysis arise because these enzymes are very heat-stable. The nature, location, and characteristics of heat-stable proteases produced by psychrotrophs are covered in this paper. The plasmin system and plasmin characteristics are described, as are the activation of plasminogen to plasmin, the proteolysis of casein by plasmin, its heat stability, and other factors that affect plasmin activity. The roles of native proteases and those produced by psychrotrophs in causing age gelation and off-flavors in UHT milk are described, along with related factors that influence these defects. Means to control protease activity are discussed, to prevent or minimize age gelation and off-flavors in UHT milk.

II. Proteolytic Activity in Bovine Milk

A. Proteases Produced by Psychrotrophs

a. Nature of the Problem

Bacterial proteases of most concern are those produced by psychrotrophic microorganisms that grow in milk held at refrigerated storage temperatures prior to processing (see reviews by Cousin, 1982; Fairbairn and Law, 1986). Trends toward a reduction in the frequency of milk collection result in milk being stored for longer periods before processing. This results in increased growth of psychrotrophic bacteria, many of which produce heat-stable protease that affect end-product quality. Psychrotrophic counts may reach 10^3 to 10^6 c.f.u./ml before processing. Proteases have been detected at these populations.

Pasteurization or UHT treatment of raw milk destroys the psychrotrophic microorganisms, except *Bacillus*, but the proteases produced by a number of species of psychrotrophic microorganisms are very heat-stable. Proteases of microbial origin have been shown in many studies to survive UHT treatment. These proteases in milk have been implicated as causing gelation and unclean and bitter off-flavors in UHT milk. Several authors have reviewed the importance of psychrotrophic enzymes on dairy products (Cousin, 1982; Fairbairn and Law, 1986).

b. Types and Characteristics

Microbial source proteolytic enzymes may be: 1) located within the microorganism cell (cell associated or intracellular), 2) near the cell wall (periplasmic), 3) cell wall associated, or 4)

excreted in the media (extracellular). By far the most information exists for the extracellular proteases produced by psychrotrophic microorganisms. *Pseudomonas* spp. secrete extracellular, alkaline metalloproteases that require divalent cations (Ca^{+2} , Zn^{+2}) for stability and activity, and possess remarkable heat stability. These proteases survive pasteurization, UHT processing, and low temperature inactivation (i.e. 55°C for 5 to 60 min., before UHT processing). The extracellular, heat-stable proteases produced by psychrotrophs are reported to have a broad temperature and pH range for activity (Mitchell and Ewings, 1985).

c. Proteolysis of Milk Proteins

Proteases from most psychrotrophs attack κ -casein, causing destabilization of the casein micelle and coagulation of the milk, similar to chymosin action (Fairbairn and Law, 1986). Therefore, considerable amounts of para- κ -casein in UHT milk, as visualized by electrophoretic techniques, can indicate a poor microbial quality of the raw milk. β -Casein is also hydrolyzed by proteases from psychrotrophs, with β -casein more rapidly hydrolyzed than α -casein. The hydrolysis of β -casein likely accounts for accumulation of bitter peptides. Degradation of whey proteins by proteases from psychrotrophs has been observed less frequently than the degradation of casein proteins.

B. Native Proteases

1. Overview

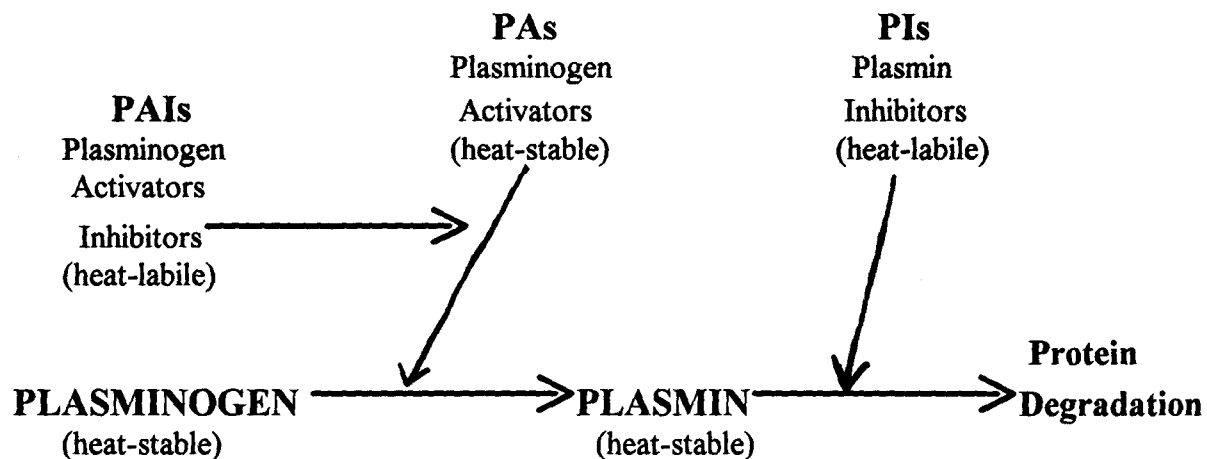
Grufferty and Fox (1988) have published an excellent review on native milk proteases, and in particular the alkaline protease plasmin. Normal bovine milk contains approximately 30 native enzymes, which are associated with either the serum phase, casein micelles, fat globule membrane, or microsomal particles. These enzymes originate from the 1) blood, or other organs via the blood, 2) secretory cell cytoplasm, or 3) fat globule membrane itself. Increased permeability of the mammary gland secretory cell membranes during late lactation and in mastitic infection results in increased levels and/or activities of most native enzymes in milk.

Before focusing in detail below on the major native milk protease, plasmin, other native proteases are noted. These other proteases explain the breakdown of casein not caused by plasmin in stored milk, and the proteolysis that occurs when plasmin activity is inhibited. The other proteases identified include 1) an acid proteinase, 2) thrombin (putative), 3) aminopeptidases, and 4) proteases from leucocytes. For reasons such as low pH optimum, high specificity, low heat stability, and low level and/or activity, these protease are not considered major contributors to problems caused by proteolysis in UHT milk. In contrast, plasmin has characteristics described in detail below that make it a major concern for problems caused by proteolysis.

2. Plasmin

a. Overview of System

The activity of plasmin, the major native protease in milk, is controlled in part by enzyme activators and inhibitors. This native enzyme/activator/inhibitor system has been illustrated as follows (Richardson, 1983):



Plasmin, the major protease naturally present in milk, exists primarily in its inactive form, plasminogen (Richardson, 1983). The ratio of plasminogen:plasmin in milk has been reported to be from 50:1 to 2:1. Plasmin and plasminogen in milk are apparently identical to those found in blood, and cross mammary membranes from blood into milk. Plasminogen in milk must be activated to plasmin by proteases (i.e., plasminogen activators, PAs) present in milk, before plasmin can degrade milk proteins. It has been suggested that protease inhibitors against PAs (i.e. plasminogen activator inhibitors, PAIs) and plasmin (i.e. plasmin inhibitors, PIs) also exist in bovine milk (Richardson, 1983), but none have been isolated and characterized. It appears that heat treatment of milk products may be destroying the natural balance between heat-stable proteases (i.e. plasmin, plasminogen, and PAs) and their heat-labile inhibitors (i.e. PIs and PAIs), which can lead to the proteolysis of heated milk products.

b. Characteristics

Plasmin is a serine proteinase, similar to the digestive enzyme trypsin in its activity and characteristics. Bovine milk plasmin is most active at about pH 7.5 (thus, the name "milk alkaline proteinase"), and at 37°C. It is reportedly stable over a broad pH range. Plasmin and plasminogen have been shown to be associated with casein micelles (Richardson, 1983; Politis et al., 1992). Other characteristics of plasmin are described below, including the proteolysis of casein by plasmin, its heat stability, and other factors that affect its activity, after first describing the activation of plasminogen to plasmin.

c. Activation of Plasminogen to Plasmin

Plasminogen activators (PAs) are present in many animal tissues and fluids, including mammary gland and milk. These PAs are serine proteinases, they are very specific, and they activate plasminogen to plasmin. Until recently, very little information was available concerning plasminogen activators in bovine milk. PAs were thought to be present, based on an increase in plasmin concentration and the concomitant decrease in plasminogen concentration during the storage of bovine milk and solutions of casein at 37°C. It was known that activator activity was associated with milk casein micelles, whereas inhibitors of PAs and/or plasmin were localized in milk serum. The PAs appeared to be heat stable, and increased in activity after pasteurization presumably due to heat inactivation of PA inhibitors (Richardson, 1983).

In 1991, Deharveng and Nielsen reported the partial purification and characterization of PAs from bovine milk. That work was continued by Lu and Nielsen (1993a, b, c), with improved assays to quantitate and visualize PA activity. Five bands with PA activity were detected by an electrophoresis technique, with molecular masses of approximately 93, 57, 42, 35, and 27 kilodaltons. Most, if not all, of the PAs in normal bovine milk seem to be urokinase-type PA, as opposed to another possible type referred to as tissue-type PA. Work with a partially purified bovine milk PA preparation suggested an optimum pH for activity of 8.0 to 8.5, stability in the pH range of 1 to 11, optimum activity at approximately 37°C, and measurable activity at 4°C. Study of kinetics of the heat-inactivation of milk PAs using a model system showed that the inactivation of PAs followed first-order kinetics in the temperature range of 60 to 140°C. The activation energy (E_a) for the inactivation reaction was 21.4 kcal/mol. The decimal reduction time (D-value) was 109 min. at 70°C, and 32 sec. at 140°C. This indicates that native PAs in bovine milk are not affected by pasteurization processes, and largely are not inactivated by UHT processing conditions used in the dairy industry.

It has been hypothesized that proteases produced by psychrotrophs might contribute to overall plasmin activity in milk by acting as plasminogen activators, to convert plasminogen to plasmin (Kohlmann et al., 1991b). The specificity determined for extracellular proteases produced by *P. fluorescens* strains (Mitchell et al., 1986) suggests they may be able to cleave the peptide bond needed to hydrolyze plasminogen to plasmin.

d. Proteolysis of Casein

The preferred substrates for bovine milk plasmin are α_{s2} - and β -caseins, which are hydrolyzed by plasmin at similar rates (Snoeren et al., 1979; Richardson, 1983). β -casein is hydrolyzed by plasmin to γ -caseins and certain protease peptones. Plasmin seems to have little or no activity on κ -casein. Plasmin can hydrolyze α_{s1} -casein, likely yielding λ -casein. Both β -lactoglobulin and α -lactalbumin are seemingly resistant to plasmin.

e. Heat Stability

The heat stability of isolated bovine milk plasmin is pH dependent. Pasteurization of milk at 72°C for 15 sec. has been reported to somewhat decrease plasmin content of milk, but plasmin

activity increases during storage of pasteurized milk (Richardson, 1983) The increase in plasminogen activation during storage of milk after pasteurization, which results in increased plasmin activity, has been attributed to inactivation of a plasminogen activator inhibitor (Richardson, 1983). To completely prevent proteolysis by plasmin during storage of the product, a heat treatment of 142°C for 18 sec. was reported for UHT milk, and a treatment of 120°C for 15 min was reported for milk in bottles (Driessen and van der Waals, 1978). Reported D values for plasmin are 35.7 (Driessen and van der Waals, 1978) and 12.4 min (Alichandis et al., 1986) at 72.5°C, and 7 sec (Driessen and van der Waals, 1978) and 10 sec (Alichandis et al., 1986) at 142.5°C. A study by Manji et al. (1986) suggested that plasminogen is more heat-stable than plasmin to UHT processing, and that the decrease in both activities is greater with indirect processed milk versus direct processed milk.

f. Other Factors That Affect Plasmin Activity in Milk

It has been suggested that the following factors other than heat treatment of milk affect plasmin content and/or activity (Grufferty and Fox, 1988; Bastian et al., 1991):

1. Breed of Cow - varied reports of differences and no differences.
2. Age of Cow - plasmin activity increases with increasing lactation number.
3. Lactation Stage - generally, higher concentration of plasmin and/or plasminogen in late lactation milk.
4. Mastitis - level of plasmin increases with mastitis infection.
5. Season - plasminogen activity increases during fall and winter.

Conclusions drawn about factors that affect plasmin, plasminogen, or plasminogen activator concentration and/or activity are dependent on how specific and quantitative are the assays for these proteases. Problems regarding substrates and conditions for these assays have been noted (Deharveng and Nielsen, 1991; Kohlmann et al., 1991b) and improved assays have been reported (Lu and Nielsen, 1993a). With improved assays, it should be possible to focus more attention on factors that affect plasminogen activators concentration and/or activity. These are key to control of the plasmin system, since they convert plasminogen to plasmin to cause the proteolysis of milk proteins.

III. Problems with UHT Milk Attributed to Proteolysis

A. Nature of the Proteolysis

Numerous investigations have reported protein breakdown during storage of both unconcentrated and concentrated UHT milk. There is a progressive decrease in the amount of casein nitrogen and a corresponding increase in non-casein and non-protein nitrogen during storage of UHT milk samples. However, there is considerable variation in the extent and rate of proteolysis (Corradini, 1975). Some researchers have found that the degradation of casein fractions correlates with residual protease activity in UHT milk, but that no correlation exists between onset of gelation and protease activity (Manji et al., 1986).

The techniques of polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography have been used to follow the breakdown of milk protein caused by plasmin and proteases produced by psychrotrophs. Use of electrophoretic techniques indicate a decrease in relative concentrations of α_{s1} -, α_{s2} -, β -, and κ -casein during storage of UHT milk, and an increase in γ -casein and para- κ -casein (Corradini, 1975). UHT concentrated milk gels sooner than unconcentrated milk, even though the rate and extent of protein breakdown is lower for concentrated milk (Harwalker, 1982). The time of gelation has been shown to differ markedly for UHT milk from different laboratories for which there was a similar degree of proteolysis (Harwalker, 1982). A higher degree of proteolysis during milk storage has been observed for UHT treatment by the direct method as compared to the indirect method.

B. Age Gelation

1. Nature of the Problem

The defect called "age gelation" limits the shelf life of UHT milk (Burton, 1988). Some (but not all) UHT milk forms a custard-like gel during storage. In the gelation process there is initial thinning, a long period with little viscosity change, then a sudden rise in viscosity. Changes in the casein micelle structure have been reported during this process, as has been the breakdown of casein proteins.

The causes of age gelation in UHT have not been clearly identified, but two theories have been proposed, both involving proteins and the casein micelle: 1) Enzymatic modification of casein to cause gelation, involving protease attack then rearrangement of the milk proteins, and 2) Physical-chemical effects that lead to nonenzymatic rearrangement of casein micelles and perhaps the whey protein, β -lactoglobulin. Gelation may likely involve a combination of these two theories (Kocak and Zadow, 1985).

2. Factors that Affect Age Gelation

Harwalker (1982) presented a comprehensive list of factors that affect gelation in UHT milk.

a. Processing Conditions

The onset of gelation in unconcentrated milk depends on both the time and temperature of processing. While UHT sterilized milk gels during storage, retort sterilized milk (115-120°C for 15-20 min) is resistant to gelation for long storage periods. Forewarming (e.g. 72°C for 30 sec) and increasing the sterilization temperature and holding time result in delayed gelation (Zadow and Chituta, 1975). Direct heating methods offer less protection from gelation than indirect methods (Harwalker, 1982; Manji et al., 1986).

In concentrated milk the delay in gelation as a result of forewarming is increased at higher temperature and a longer hold time. While higher temperature and longer times for HTST treatment of concentrated milk retards gelation, higher sterilization temperature with shorter hold time gives reduced protection from gelation. Homogenization and the sequence of operations affect the storage stability of unconcentrated and concentrated milks (Harwalker, 1982).

b. Milk Characteristics

The composition and microbial quality of UHT milk affect gel formation (Snoeren et al., 1979; Zadow and Chituta, 1975; Law et al., 1977). Increasing the total solids content of concentrated milk processed by HTST or UHT methods hastens gelation onset. Summer milk has been found to give a more stable product than winter milk. Early-lactation UHT milk is more susceptible to gelation than that from mid- or late-lactation. Mastitic milk is more susceptible to gelation than normal milk. Milk with a high pre-processing microbial count is more susceptible than milk with a low pre-processing count to gel formation.

c. Additives

Many studies on both unconcentrated and concentrated UHT milk have been undertaken with potential additives to delay or prevent gelation (Harwalker, 1982). Polyphosphates, manganous sulfate, lactose, sucrose, dextrose, sorbitol, and sulfhydryl blocking agents have all been shown to delay gelation. Sodium hydroxide and sodium carbonate have been shown to delay gelation in concentrated milk but not in unconcentrated milk. Addition of phosphate, citrate, and other anions that lower Ca ion activity hasten gelation, while addition of Ca ions delays gelation. Hydrogen peroxide and disulfide reducing agents have been shown to hasten gelation.

d. Storage Conditions

Generally for concentrated milk processed by HTST or UHT methods, the higher the storage temperature, the faster the gelation. For unconcentrated UHT milk, reports in the literature are inconsistent regarding the effect of storage temperature on gelation. Many researchers have observed improved resistance to gelation during storage at 35°C and above. Zadow and Chituta (1975) found milk to be most susceptible to gelation when stored at 25 to 35°C, and the storage life was much greater at 2°C or 40°C.

3. Role of Proteases from Psychrotrophs

As described above, milk with a high pre-processing microbial count is more susceptible to age gelation (Snoeren et al., 1979; Zadow and Chituta, 1975). Microorganisms that produce heat-stable enzymes cause the most serious gelation problems. Longer refrigeration prior to sterilization allows for increased growth of psychrotropic microorganisms, which can produce heat-stable enzymes.

Law et al. (1977) compared the time to gelation after UHT sterilization (140° for 3.5 sec) of uninoculated low count milk, and milk containing 8×10^5 c.f.u./ml, 8×10^6 c.f.u./ml, or 5×10^7 c.f.u./ml *Ps. fluorescens*. Gelation times for the UHT milk stored at 20°C were over 20 weeks, 8-10 weeks, and 10-14 days, respectively. Proteolysis was shown to be attributed to extracellular proteases.

Keogh and Pettingill (1984) reported a correlation between UHT milk gelation time and psychrotrophic intracellular proteolytic enzyme activity. Intracellular proteases may be a problem

in milk held before processing, especially if they are produced early in the growth cycle of the microorganism. This was shown to be the case for two *Pseudomonas* cultures grown in milk at 7 °C (Kohlmann et al., 1991a). Using a sensitive substrate for plasmin activity, intracellular proteases were shown to be present after only 20 hours of incubation, before detection of extracellular proteases. Intracellular protease activity was exceeded by extracellular protease activity as the incubation period was extended.

4. Role of Plasmin System

While proteases from psychrotrophs can play a role in age gelation of UHT milk, native proteases apparently are also important. Snoeren et al. (1979) showed that aseptically drawn UHT milk may coagulate after 3 months of storage. Corradini and Pecis (1979) also reported that an enzymic process can occur in UHT milk in the absence of psychrotrophic microorganisms, to influence the process of gel formation.

Kohlmann et al. (1988, 1991c) conducted two studies to help determine if gelation which may occur during storage of UHT milk can be attributed to proteolysis caused by native milk proteases. In one study, proteolysis and gelation were investigated in UHT milk following aseptic addition of combinations of plasmin, plasminogen, trypsin, trypsin inhibitor (Kunitz), and urokinase (plasminogen activator) (Kohlmann et al., 1988). Results suggested that plasminogen-derived activity promotes UHT milk gelation. A second study was conducted in which a low level of plasmin (0.5 mg/l) was injected into UHT milk containers and various indicators of gelation and proteolysis were monitored (Kohlmann et al., 1991c). Results obtained support the hypothesis that a relationship exists between the level of plasmin activity and the gelation of UHT milk. These studies showed that low levels of the native serine proteinase, plasmin/plasminogen, in UHT milk can cause proteolysis and lead to gelation of the product. The process can apparently not be speeded up greatly or made to occur with high levels of this protease. Results suggested that UHT processed milk contains heat stable PAs. These activators could convert plasminogen to plasmin, thus resulting in a gentle casein hydrolysis to give a gel.

Results from the studies by Kohlmann et al. (1988, 1991c) are consistent with current thought on the cause of age gelation in unconcentrated UHT milk. Some proteolysis is apparently necessary to partially hydrolyze the caseins before the modified caseins physically associate to form a gel structure (deKoning et al., 1985; Harwalker, 1982; Manji et al., 1986). However, the mechanism of gelation is proposed to be different for unconcentrated than for concentrated UHT milk. For unconcentrated UHT milk, deKoning et al. (1985) reported a good correlation between plasminogen or plasmin activity and time of gelation, but for concentrated UHT milk they observed gelation with no evidence of proteolysis.

C. Off-Flavors

1. Nature of the Problem

Off-flavors in UHT milk attributed to protease activity has been described as bitter and astringent. These off-flavors have been linked to the production of polypeptides generated when

native plasmin and other proteases that survive UHT treatment act on milk proteins (Harwalker et al., 1989). Harwalker et al. (1993) showed that astringent off-flavors are linked to the production of γ -caseins. These are specific C-terminal breakdown products of β -casein, generally produced by the action of plasmin. Harwalker et al. (1993) treated pasteurized skim milk with plasmin and with 10 proteases from psychrotrophic bacteria, then evaluated the products organoleptically for astringency and for proteolysis. Results led to the conclusion that astringent off-flavors in milk result from the action of proteases produced by *Ps. fluorescens* only if those enzymes have specificities similar to that of plasmin, to produce compounds similar to γ -casein. Kohlmann et al. (1991b) noted that an extracellular protease produced when a *Ps. fluorescens* strain was grown in milk at 7°C exhibited plasmin-like activity, as determined using a sensitive substrate specific for plasmin. Mitchell and Ewings (1985) found that proteases isolated from various *Ps. fluorescens* species had activity against various artificial substrates, some of which are plasmin substrates.

2. Role of Proteases from Psychrotrophs

Many of the heat-stable extracellular enzymes produced by psychrotrophs have been shown to hydrolyze proteins and cause off-flavors in UHT milk (Gillis et al., 1985). Significant proteolysis and bitter off-flavors in UHT milk are reportedly caused by proteases from *Ps. fluorescens*. Some researchers have reported that proteolytic activity in milk is not proportional to psychrotrophic populations, but others have reported that psychrotrophic counts in the raw milk were strongly correlated with the extent of proteolysis in stored UHT milk (Collins et al., 1993). Some inconsistencies in this area may be due to differences in methods to detect proteolysis, since some methods have been shown to be unreliable. However, large psychrotrophic populations are not needed for production of significant amounts of heat-stable proteases (Cousin, 1982).

The number of psychrotrophs required to produce off-flavors in milk varies between species (Cousin, 1982). It is determined by the growth rate at the temperature of storage, the length of the lag period, the proteolytic activity, and the heat resistance of the enzymes. Cousin (1982) summarized the many reports of numbers of psychrotrophs required to lead to off flavors in milk. Reported values differ greatly due to the difference in proteolytic activity and heat resistance of the proteases found. Generally, a psychrotroph count of at least 10^7 c.f.u./ml is reportedly needed before organoleptic changes are detected in milk.

IV. Control of Protease Activity

A. Proteases from Psychrotrophs

Fairbairn and Law (1986) and Champagne et al. (1994) reviewed the various methods that can be used to control psychrotrophs. Two important methods are: 1) low temperature, to slow the growth of psychrotrophs, and 2) high temperature, to cause denaturation of vital cell components. The growth curve for psychrotrophs when held at low temperatures is characterized by a long lag phase and a slower logarithmic phase. However, the effect of cold temperature or limiting growth is reduced when the initial contamination level of milk is high (Champagne et al., 1994). The shelf life of UHT milk was much shorter when processed from raw milk stored at 6°C than when processed from raw milk held at 2°C (Griffiths et al., 1988).

Numerous temperature and time combinations have been applied to eliminate psychrotrophs (Champagne et al., 1994). Pasteurization temperatures destroy most psychrotrophic bacteria. However, a thermization process, such as heating at 65°C for 10 sec, has been used before storage of the milk at a low temperature (Griffiths et al., 1986). Such a treatment reduces the total count and inhibits initial growth. The increase in storage life of raw milk obtained with a thermalization process is dependent on the temperature used and/or initial population of psychrotrophs (Griffiths et al., 1986).

The use of chemicals and other means to prevent psychrotroph-related problems in dairy products has been reviewed by Fairbairn and Law (1986) and Champagne et al. (1994). Chemicals can be used as sanitizing agents on dairy surfaces, to kill cells on equipment and prevent contamination of milk. Also, certain chemicals added to raw milk will prevent development of the psychrotrophic flora. However, the best way to control psychrotrophic microorganisms that can produce heat-stable proteases is to maintain good sanitary practices and effective control of production and processing conditions (Champagne et al., 1994).

B. Plasmin System

The heat stability of plasmin, plasminogen, and plasminogen activators make them difficult to inactivate by heat processing, to control proteolytic activity. Plasminogen activators are even more heat-stable than plasmin and plasminogen. Therefore, it has been suggested (Lu and Nielsen, 1993c) that the best chance for control of the plasmin system by heat treatment may come by heat denaturation of plasminogen, so it can no longer be activated to plasmin by plasminogen activators. However, even plasminogen is not easily denatured by UHT processing conditions.

Protease inhibitors are known to exist in normal bovine milk, but the only case of isolation and partial characterization is for a putative α_1 -antitrypsin (Weber and Nielsen, 1991). Native protease inhibitors of plasmin and plasminogen activators are thought to exist in bovine milk (Richardson, 1983), but they have yet to be isolated and characterized. While these protease inhibitors are seemingly heat-labile, control of the plasmin system might come from adding such inhibitors after heat processing.

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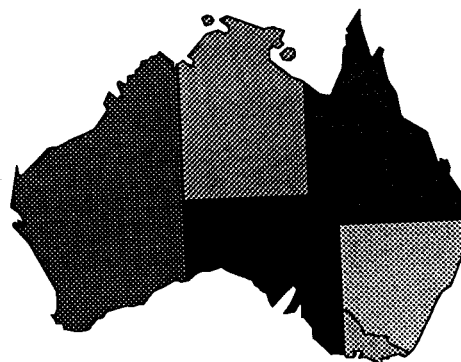
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USING RECOMBINED MILK FOR UHT PROCESSING

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1. Why recombine?

The development of a major recombining industry as an integral part of international dairy operations has been one of the great success stories of the past few decades. The market for recombined products has allowed the exporting dairy nations to develop alternative markets for their products, whilst at the same time the populations of the nations using this technology have gained from wide spread availability of nutritious dairy products at a reasonable price.

In essence, recombining involves the separation of milk into components which can at a later date be recombined into products very close to those made by traditional manufacturing methods. There are two obvious benefits:

- (i) the separate components may be stored for extended periods without the need for refrigeration, allow simple export and storage, and
- (ii) the total mass involved in export is reduced by a factor of more than 7 (in the case of milk) by the removal of water.

At this stage, it is important that a clear differentiation be made between the terms "recombination" and "reconstitution". This differentiation, originally suggested by Mr. G. Loftus Hills (1964) of the CSIRO Dairy Research Laboratory, is now generally accepted throughout the industry. In essence:

"*Recombination*" is defined as a process in which the milk is separated into a number of components, in particular skim milk powder and anhydrous milk fat (AMF), and these components are mixed with water and any other required stabilisers to form the end product. Recombining operations can thus result in the manufacture of a wide range of products of varying ratios of fat to milk solids-not-fat (SNF).

"*Reconstitution*" is defined as the process of addition of water only to whole milk powder to produce a product of similar fat to solids-not-fat ratio of milk itself.

Clearly, the main raw material involved in reconstitution is whole milk powder, and the raw materials involved in recombining are skim milk powder and AMF.

This paper will first discuss matters affecting the manufacture of recombined Ultra Heat Treated (UHT) milks (generally in plants in areas where there is limited local production of milk), and then discuss the formulation of other recombined UHT products (such as custards and other formulated products, for consumer consumption often in areas of high local production.)

1.2. Some background to the development of manufacture of UHT recombined products

Recombining technology was originally developed for the manufacture of recombined sweetened condensed milk and recombined evaporated milks. Much of the impetus for the development of this technology was the development of the European Economic Community, and the closure of much of the UK market to Australian and New Zealand Dairy products in the late 1960s. Many South East Asian countries, as a part of a policy to develop their own industries, invited the development of recombined

plants using joint ventures between local and overseas capital. More recently, many Middle East countries have also been involved in such activities, as have countries in South America and Africa. The logical development of the RSCM and REM markets has been the development of the market for recombined UHT products in these countries.

1.3. Market trends and potential

1.3.1 In areas of low local milk production

In such areas, recombining plants generally produce a range of white and sweetened white UHT milks. Many recombining plants are operating on this basis around the world. It is however very difficult to assess the exact number, and even harder to assess total production. In 1982, it was suggested by Gunnis (1982) that between 200 and 250 recombining plants were then in existence, utilising about 1 million tonnes of non-fat milk solids. Since then there has been considerable expansion in recombining operations, so that these figures will now be considerable underestimates.

The potential of recombining is clear when it is realised that 75% of the world's population live in countries which are deficient in dairy production. These countries have climatic conditions which do not in general support economic production of milk, and thus recombining is the only realistic option available for supply of dairy products in these areas.

As well as the obvious benefits to the populations of such countries, the trade benefits to the exporting countries are considerable. For example, Australia's share of world production of milk is comparatively small, (a little over 1%), but Australia holds 7% of the world export market (as measured in milk equivalents (see Figures 1 and 2, and Table 1).

Clearly therefore for a number of smaller countries the continuing health and development of international dairy trade, including the supply of raw materials for recombining operations, is vital to their survival. For example, New Zealand is the second largest supplier of manufactured dairy production in the world market, but produces only 1.5% of world milk production. Most of the New Zealand dairy products are intended for export.

The major market UHT in much of South East Asia is for a product which has added sugar - generally levels of about 3% are desirable. In South East Asia, the sales of sweetened UHT milk are about 50% of the UHT white milk market.

The overall market is expanding substantially, both in South East Asia, and close to the USA, both in Mexico, and in South America. A major producer in Mexico utilises about 200,000 tonnes of milk powder per annum, sourced mostly from the USA and Canada.

1.3.2 In areas of high local milk production

In such areas, UHT plants generally produce both white milks, and a range of formulated products. These latter products can in one sense be considered as recombined, as they often include significant amounts of materials such as starch, sugar and stabilisers. In many countries, such products attract a very high premium (compared to UHT milk itself) and the volume of production of such products is increasing rapidly. The continued development of these products and markets will improve the overall profitability of the world dairy industry.

2. Factors involved in selection of raw materials

2.1 Powder

In the manufacture of recombined sweetened condensed milk (RSCM) and recombined evaporated milk (REM), the selection of powder is very critical. In the case of RSCM, the stability of the product to changes on viscosity on storage is determined by the heat treatment of the powder. Similarly, the stability of REM to the sterilisation process is determined by the powder properties. In both cases, specialised manufacturing techniques have been developed over the past 30 years to ensure that the desired characteristics are built into the powder for its particular application.

Similarly, the selection of powder to be used in recombined UHT milk is critical to the satisfactory processing of the product and its storage characteristics. However the powder properties are not as critical as those required for the manufacture of satisfactory REM or RSCM. It is necessary however to select the powder with care, as incorrect selection will result in an unsatisfactory product.

Two major factors must be considered: stability to UHT processing, and the development of age gelation in the stored product.

2.1.1 Stability to UHT processing

As general background, milk powders for recombining are generally divided into three categories, high heat, medium heat and low heat. These terms refer to the heat treatment given to the milk prior to spray drying, and not to the stability of the recombined product to heat. Milk for low heat powder might for example be preheat treated at 72C for 15s, for medium heat powder at 73-75 for 1-3 min, and high heat powder at 80-85 for 30 min. The preheat treatment is generally determined by the Whey Protein Nitrogen Index of the powder (ADMI, 1971). Table 2 outlines the WPNI specifications for high, medium and low heat powders. Whilst WPNI values remain very useful, they have in many cases been supplemented by tests involving specific functional properties.

The preheat treatment used in powder manufacture does not appear to have any significant impact on the storage properties of the UHT processed product, either in terms of flavour or the onset of age gelation (there may be marginal flavour benefits through use of either medium or low heat powder, but these are not of major significance. Newstead (1990) has reported that low or medium heat powders are normally used for UHT recombined products, as high heat powders had a lower acceptance because they tended to impart more cooked flavour to the product.

The major concern is the stability of the reconstituted UHT milks to UHT processing itself. It has been known for many years (Zadow, 1971) that if milk of pH below about 6.65 is subjected to UHT processing, considerable sedimentation will develop in the product. At a pH below about 6.60, this results in the very rapid sedimentation of virtually all of the casein and denatured whey proteins in the milk. The sediment which forms is heavy, and settles in the product within a few minutes. The critical pH for most UHT operations is in the range 6.65 to 6.60, when some sediment will form, but perhaps not very severely. It will however lead to burn on in the holding section, with resultant processing problems, and can lead to consumer complaints regarding product sediment.

For this reason, there is a strict control on the pH of milk used in most UHT operations. The instability is believed to be calcium mediated - the higher the ionic calcium content of the product, the higher the pH at which instability occurs.

In a study comparing the stability of recombined milks concentrates and fresh skim milk concentrates to UHT processing, Zadow and Hardham (1978) have shown that the pH at which instability occurs is a little lower for recombined products than for fresh milk. It would be expected that similar

results would be obtained for single strength milks. The reason for the higher stability of milk powder based products was suggested to be that spray drying results in irreversible changes to the powder, so that on reconstitution, the product has a lower ionic calcium content than that present in the original product.

In studies comparing the stability of low medium and high heat powder based reconstituted UHT milks, Zadow and Hardham (1981) showed that the onset of severe sedimentation occurred about 0.8 of a pH unit higher in low heat powder based products. As suggested above, all samples showed increased stability compared to that expected for fresh milk based products.

Overall, therefore it may be concluded that recombining operations will result in an increased stability to pH induced sedimentation on UHT processing. Further, the use of medium or high heat powders will result in an even greater increase in stability compared to low heat powder products.

2.1.2 Control of age gelation

The control of age gelation is much more of a problem in UHT products based on milk powders. The cause of age gelation is well recognised to be the action of a protease in the UHT milk which survives UHT sterilisation. The action of this enzyme leads to the development of a gel in the product after a storage time as short in some cases as a few weeks, but is more common after a few months storage. The most rapid gelation occurs in samples stored at about 27C, temperatures often encountered in countries involved in the manufacture of recombined products.

The source of the enzyme is generally agreed to be psychrotrophic organisms present in the raw milk. These organisms are destroyed by the UHT treatment, but sufficient of the enzyme activity survives to cause gelation in the stored product. There is no commercial means of control of age gelation - the only means of reducing the problem is to use freshly drawn milk, with a minimum bacterial load, as the milk supply for the process. Every second day pick up of milk should be avoided for obvious reasons.

Milk powder to be used in the manufacture of UHT milk must be prepared with the above in mind. In particular, steps should be taken to ensure that the opportunity for the growth of psychrotrophic organisms is restricted. Thus:

- (i) the milk to be used for powder should be freshly drawn, and converted to powder as soon as possible after arrival at the factory.
- (ii) every second day pick up milk should not be used,
- (iii) The milk should have as low a bacterial load as possible
- (iv) Sjollema (1988) has suggested that powders with a pyruvate content of less than 9 mg/100g be used, as this may indicate a lower level of heat resistant enzymes in the powder.

Note that these precautions also will reduce the growth of organisms leading to a decrease in pH of the milk. This in turn will increase the stability of the end product to sedimentation on UHT processing.

In controlling the onset of age gelation, the type of pre-heat treatment employed in the manufacture of the milk powder is not critical.

2.1.3 Overall

Most powders used in UHT recombining powder operations are medium heat. Low heat powders are considered to have the possibility of an unnecessarily high bacterial load, and high heat powders may result in a somewhat poorer flavour.

2.2 Milk fat

Standard butteroil which meets usual manufacturing specification is adequate for use in recombined UHT milk. Normal care regarding prevention of oxidation is required to ensure that flavour defects are not introduced into the product. Provided that the fat is made from good quality milk, that care is exercised during manufacture and nitrogen flushing of containers is completed efficiently, the product will store well at ambient temperature. High storage temperatures accelerate the development of off flavours. It is also essential that a system for strict control of stock be introduced, to ensure that turnover is rapid. Once the fat has been melted, or if it is stored in opened drums, it should be used as rapidly as possible.

The milk fat used should conform to the IDF Standard 68A (1977). It should be noted that peroxide values and acidity values are not always good indicators of the stability of fat towards oxidative deterioration.

UHT filled milks are also commonly manufactured in many countries. In these products, the source of fat is generally a cheaper vegetable fat such as palm kernel or coconut oil. However in many cases, these fats are more unsaturated than milk fat, and can undergo much more rapid oxidative deterioration. In such cases, particular care must be taken not only during manufacture, but also in the packaging employed. Contact with oxygen must be kept to a minimum. Head spaces in the package (for example, in Combibloc systems and some TetraPak systems) can result in introduction of additional oxygen into the system and accelerate development of oxidative defects.

Lauric oils are very sensitive to lipolytic rancidity, and the shelf life of products based on these oils may be limited. Mono-unsaturated oils (such as canola) are more resistant to oxidative degradation and off flavour development from lipolytic reactions. It should also be noted that there is evidence that products based on harder fat fractions of milk fat showed less fat separation on storage than those based on the soft fat fraction (Mayhill and Newstead, 1992).

2.3 Water

Water is the single biggest component of UHT recombined products, but it is often the most overlooked. Colour flavour and odour may all affect the quality of the end product. Hard water can affect protein stability on heating. A system to ensure water quality is therefore an essential part of any UHT recombining operations.

2.5 Emulsifiers and Stabilisers

In general, stabilisers from specialist companies are preferred by most manufacturers. These components are added to the milk to ensure that the milk fat emulsion formed during the homogenisation process remains stable during processing and storage, as well as to improve the mouth feel of the product. A wide range of systems have been suggested over the years, but most plants now use proprietary mixtures provided by specialist companies. Generally a single product, comprising a mixture of stabiliser and emulsifier is provided to the UHT recombining manufacturer. These products are designed to increase the heat stability of the product, to improve fat dispersion and stability during processing and storage, and to improve the organoleptic qualities of the product, through development of a more desirable viscosity and a richer mouth feel. Some stabilisers also assist in the prevention of froth during mixing operations.

Emulsifiers commonly used in UHT recombining operations include mono and di-glycerides, and soya lecithin. Emulsifiers reduce fat separation by formation of a membrane at the fat/water interface. Hydrocolloids are often used as stabilisers in UHT products. These act by increasing the viscosity of the aqueous phase, increasing the viscosity of the product and improving mouth feel and reducing the rate of fat separation. Hydrocolloids commonly employed include carrageenans and alginates.

2.6 Flavour and sweeteners

As indicated earlier, sweetened products are very popular in much of the UHT market. It is common to introduce a low level of vanilla into such products for a further improvement in palatability. The vanilla used must be stable to UHT processing, and if steam injection with a vacuum flashdown is employed, the vanilla must be comparatively non-volatile. The same caveats apply to all UHT flavours.

Sucrose is the most commonly used sweetener in UHT recombined products, but other sweeteners may be employed, from saccharides such as glucose, fructose and glucose syrups, through to artificial sweeteners. In the case of saccharides, considerable care must be taken, as the higher level of reducing residues present in many of these products leads to increased browning of the product during processing and storage, because of increased Maillard reactions.

Any artificial sweeteners must of course be stable to UHT processing, and to extended storage.

3. Formulation of UHT recombined milks

The use of reconstitution is comparatively inflexible in terms of the ease of changing SNF/fat ratios. For this reason, recombining is the preferred methodology employed in UHT operations

The use of recombining for the manufacture of UHT milk allows for wide variations in the composition of the final product. The major factors are of course the level of solids-not-fat, and the level of fat present. A number of factors will influence the levels selected, the major ones being the economic factors (the relative cost of fat, and SNF) and those related to the acceptability of the product. In general, the lower the solids content of the product, the less the consumer acceptability. However, the reduction in acceptability due to reduced fat content can for example be overcome, to some extent by increasing the level of the (generally cheaper) solids-not-fat component.

A further key factor in many markets is the level of added sugar. A considerable portion of the milk drunk as a consumer product in many South East Asian countries is sweetened by the addition of between 1 and 4 % sugar. Thus UHT products destined for these markets should consider the level of sweetener.

As well as milk fat, skim milk powder and sugar, most UHT products also include an emulsifier and stabiliser.

In many recombined plants in South East Asia, the Middle East and Africa the local authorities require the inclusion of a certain volume of locally produced fresh milk to be included in the final formulation. This is done so as to encourage the development of a local dairy industry. The quality of such milk has sometimes been poor, and has posed major difficulties to the recombiner. However in general it has been found that as the industry develops, the quality of the milk improves substantially, and it can be included with little or no difficulty.

Typical Formulations might be:

Unsweetened Product

Anhydrous Milk Fat	3.6
SNF	9.3
Stabiliser	0.2
Water	86.9

This yields a product containing 3.5% protein and 13.1% total solids.

Sweetened product

Anhydrous Milk Fat	3.6
SNF	8.5
Sugar	3.5
Vanilla	0.1
Stabilizer	0.2
Water	84.1

This yields a product containing 3.2% protein and 15.7% total solids

As indicated above, the formations can vary very widely - SNF and butterfat contents and ratios can be selected to meet specific cost and product specifications.

4. Manufacture of recombined UHT milk

The following outlines typical methodology for the manufacture of UHT recombined products:

1. The required quantity of skim milk powder is blended into the water at 40-45C.
2. Other ingredients are added whilst agitating the mix. The butterfat must be added last, after liquefaction by preheating to 40-45C prior to use.
3. The butteroil should then be dispersed in the mix. This may be carried out by agitation, or by moderate homogenisation. Conditions will depend on the homogeniser, but might for example involve two stages of homogenisation (say 1500 plus 500 psi, (10 MPa plus 3 MPa) at 65C). In many plants, agitation is sufficient, as the recombined product is subjected to UHT processing and homogenisation with minimal storage. It is also generally wise to pasteurise the product (HTST, 73C for 35s) at this stage, if the product is to be stored for any period prior to UHT processing. The product should be cooled to 4C and UHT processed as soon as possible.
4. The mix is UHT processed for example at 138C for 3 sec, with homogenisation at say 3000 + 500 psi (20 Mpa + 3.5 Mpa)

The overall scheme is shown in Figure 3.

One key aspect of the process is to ensure that the product should not be stored for an excessive period after recombining, prior to UHT processing. Excessive storage can result in further growth of psychrotrophic organisms, leading to problems with age gelation storage of the UHT product.

It is generally agreed that downstream homogenisation (after sterilisation) is preferable to upstream homogenisation (before sterilisation) for recombined products. For whole milk, the benefits of downstream homogenisation, whilst real, are generally minor. For recombined products however, there are significant gains in terms of reduction of sediment formation in the product by use of down stream homogenisation. For this reason, in spite of the additional cost and complexity, many UHT plants processing recombined products utilise downstream homogenisation.

Burn on is a problem in all UHT operations, This occurs in general in the sterilisation section of the plant, and is the result of denaturation and precipitation of proteins occurring during the heating process, forming a deposit on the walls of the sterilisation section. Burn on is often a greater problem when processing recombined products than with fresh. Burn on can result in the reduction of the effective sterilisation time to which the product is subjected, and so can lead to increased levels of contamination in the product as well as increased sediment and poorer flavour. For these reasons, many UHT plants use

intermediate cleans to remove any sediment which has developed during processing. With products containing added sugar, the extent of burn on is generally substantially reduced, often to levels below that observed when processing fresh milk. The reason for this is not understood.

5. Specific Products

5.1 Flavoured milks

Stabilisers and emulsifiers are commonly employed in the manufacture of non-UHT processed flavoured milks, to prevent fat separation and/or sedimentation in the process. In general however the formulations used cannot be transferred to UHT operations satisfactorily. Coope (1983) has reported that milk reactive κ -carrageenan is frequently used in pasteurised formulations, at levels of about 0.04%. A UHT product based on this formulation however would tend to form soft gels because of the calcium reactivity of the carrageenan. Addition rates of only about 0.02% are possible in such products. By contrast, λ -carrageenan which does not gel in the presence of divalent ions is used at higher levels (>0.1%) to aid in the suspension of cocoa in chocolate milk

Egg nog products are particularly popular in Australia, and attract a high price premium. However they have posed major difficulties to the formulator. Carrageenan was not sufficient to prevent precipitation of the egg protein at UHT temperatures. Propylene glycol alginate was found to give satisfactory protection to the egg protein

5.2 Custards and Desserts

Much of the desirable properties of custards and desserts depends on the properties of the starch. The starch must be carefully selected so that it will not gel under UHT conditions (or whilst still in the plant or packaging machine), but which will thicken adequately in the pack. Some traditional starches gel immediately in the steriliser or even in the preheater of a UHT plant, and are thus unsatisfactory. Other commonly used starches are broken down at UHT temperatures, and result in a product which is too thin. The source of starch (potato, corn ...) and its chemical treatment (degree and type of cross linking) has a crucial effect on the performance of these products under UHT conditions. Generally cross linked starches (by esterifying or etherifying amylopectin) are employed - hydroxypropylated starches are the most suitable because of their thermal stability and shear resistance.

Vegetable gums such as carrageenan are also included in the formulations to improve mouthfeel and control syneresis.

5.3 Creams

Whilst not truly recombined products, additives are required to produce a satisfactory UHT cream. Homogenisation of cream destroys its ability to whip, and yet homogenisation of UHT cream is required to prevent fat separation on storage. Commonly, sodium alginate together with other vegetable gums such as guar or locust bean gum are used to achieve good whipping properties and reduce syneresis. Mono and di glycerides of fatty acids are also used as emulsifiers to assist in coating the new fat globule membrane created by homogenisation.

5.4 Low pH products

As discussed earlier, at a pH below about 6.60, milk proteins become unstable to UHT processing. However the use of poly-propyleneglycol alginate and carboxy methyl cellulose can assist in improving the stability of lower pH products, such as drinking yogurts, acidified milks and juice/milk drinks. High methyl pectins have also proved satisfactory over a restricted pH range.

5.5 Cocoa

Cocoa often poses particular problems to the UHT formulator. The pH of cocoa can vary widely depending on the grade and will influence the pH of the mix. Cocoa of pH about 7.2 is considered to be most desirable, rather than natural cocoa (pH 5.8) or highly alkalised cocoa (pH 8.0)

Shell content should also be as low as possible - it is a general indication of quality, and high levels of shell can cause wear on the homogeniser as well as being a source of bacterial contamination. All cocoa used in UHT operations should have a spore count of <500/g.

6. Control of product defects

6.1 Fat separation

Fat separation is perhaps the most common cause of consumer complaint. It is caused by the rise of fat globules, and is controlled in the main by the size of the globules, and the viscosity of the aqueous phase of the product. More often than not, it is the result of inadequate homogenisation because of poor homogeniser valve maintenance. Microscopically, very few globules should be greater than 1 μ in diameter.

6.2 Age gelation

This is caused by the action of heat resistant psychrotrophic enzymes in the UHT milk. There is no means of prevention, but it may be reduced by careful selection of raw materials of the best quality, and ensuring that products are processed as rapidly as possible

6.3 Flavour

This is influenced by the milk fat, the protein and the particular stabilisers as outlined above. It is also influenced enormously by storage temperature, and the type of packaging employed, particularly the presence or otherwise of oxygen in the system. Such oxygen may be present as dissolved oxygen in the product (where it has not undergone a vacuum flashdown, or has been filled at some stage into an aseptic holding tank prior to packing) or available as a head space in the pack. High oxygen availability will result in the rapid development of oxidative flavours in the product.

7. Shelf life.

Shelf life claims are always a trade off between the demands of the sales arms of the manufacturing organisations and the perceptions of the technical staff. In general, well formulated recombined products can have a longer shelf life claim than those acceptable for recombined white milks. Small storage induced changes in favour are more readily perceived in milk than in highly flavoured products. However I suggest that 6 months claim should be the maximum for any UHT product.

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Table 1
Major world milk producers and exporters

Country	% World milk production	% Export world milk trade	Ratio
Australia	1.3	7	5.4
Canada	1.7	3	1.8
Eastern Block	1.2	4	0.3
USA	13.8	12	0.85
New Zealand	1.5	15	10
EEC	21	50	2.4

Table 2.

Grades of Milk Powder by Whey Protein Nitrogen Index (WPNI)

Grade	WPNI Index (mg/g powder)
High	<1.5
Medium	1.5 - 6.0
Low	> 6.0

Figure 1

Exporters share of International market

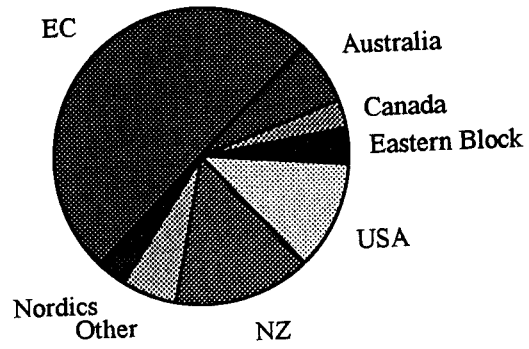


Figure 2

Milk Production

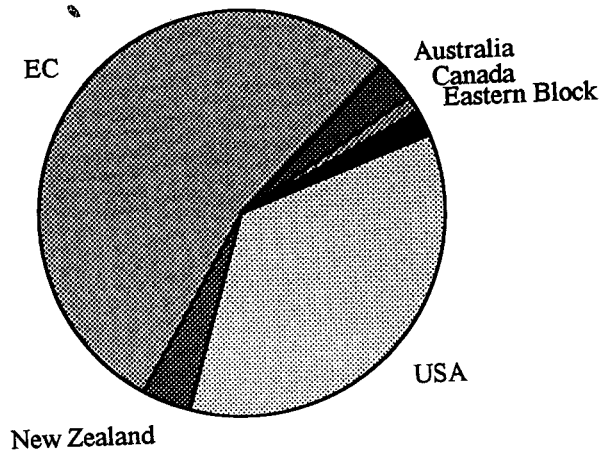
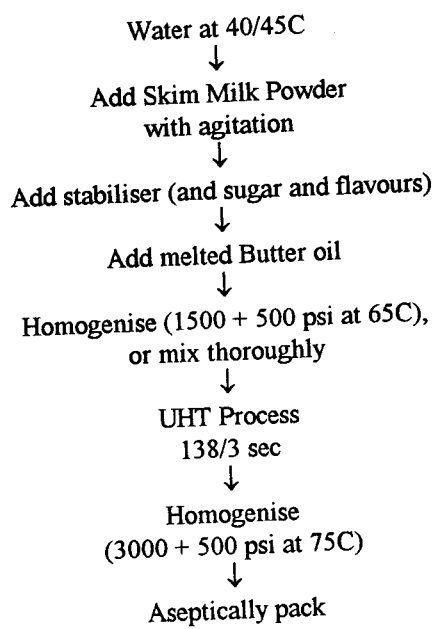


Figure 3

Manufacture of UHT Recombined UHT milk



Changes in Casein Micelle Structure During Storage of UHT Processed Milk

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Introduction

Age gelation of UHT-processed milk limits shelf-life and hinders commercial exploitation of UHT-processed concentrated milks. The mechanism of age gelation, however, remains unsolved. Gelation has been described as coagulation, sweet curd formation, thixotropic gel formation, age thickening, partial gelation, or lumpiness. It is usually preceded by a sharp rise in viscosity culminating in gel formation within 1 to 3 wk to a custard-like consistency. It is an irreversible condition.

Factors Affecting Age Gelation

Onset of age gelation is affected by heat treatment, homogenization and sequence of processing steps, milk solids content, composition of milk, quality of milk, and storage temperature. Processing concentrated milk at higher temperatures and longer holding times retards gelation. However, at equivalent sterilizing effectiveness, higher sterilization temperatures with shorter exposure times reduce the resistance to gelation. Gelation is also influenced by the UHT sterilizing method. Direct heating methods offer less protection against gelation during storage than does sterilization by indirect heating. Thus, more severe heat treatments probably make the micelles more resistant to the changes that promote aggregation during storage.

Opinions differ on the effect of storage temperature on the gelation of unconcentrated and concentrated UHT-sterilized milk. Some researchers report that lower temperatures accelerate gelation; others report that higher storage temperatures accelerate gelation.

Proposed Mechanisms for Age Gelation

Age gelation has been attributed to various changes in UHT milk during storage and to various conditions that alter the gelation time. In general, gelation occurs when casein micelles lose colloidal stability and form a three-dimensional gel network. The stability of casein micelles has been attributed to the presence of κ -casein, colloidal calcium phosphate, a high zeta-potential (-18 mV), and steric stabilization. Gelation is then preceded by changes at the surface of the casein micelles. These changes enhance interaction between micelles and can be categorized as changes that arise from proteinase activity or changes that arise from nonenzymic reactions.

Proteinase Hypothesis

Milk contains enzymes secreted by the mammary glands and those produced by bacterial growth. Some proteolytic enzymes that survive UHT treatment or reactivate during storage influence gelation of UHT milk during storage.

Nonenzymic Basis for Gelation.

The absence of a quantitative relationship between gelation time and proteolytic activity has prompted some researchers to attribute gelation to physicochemical processes, including: processes involving whey proteins, chemical modification of casein micelles by Maillard reactions, milk salts, modification of κ -casein during storage, sulfide-disulfide reactions, changes in casein micelle surface potential, or casein micelle dissociation. Our research implicates an important role of micelle dissociation.

Maillard Browning

Maillard-type reactions during storage could crosslink protein chains into very large complexes, because lactose, but not its degradation products, reacts with proteins. However, Maillard browning of UHT-concentrated milk is similar, regardless of rate of gelation. Both UHT casein micelle dispersions with lactose or sorbitol gel around the same time, as does UHT concentrated milk that had lactose replaced with sucrose.

When the extent of browning was measured using b^* values it was found that at 4 or 20°C the UHT concentrated milk did not appear brown to the naked eye. Browning increased gradually during storage at 35°C in samples containing lactose. No visual browning occurred at 35°C when the lactose had been replaced with sucrose.

All samples stored at 4 and 20°C gelled after 21 wk. Gelation was observed by the sudden rise in viscosity of the control sample and of those containing lactose or sucrose. None of the samples stored at 35°C gelled but, after 32 wk of storage, they showed slight sedimentation, which increased with storage.

Regardless of sugar composition, the electron micrographs of the gelled samples were all similar. All of the micrographs of the ungelled samples were similar.

Samples without lactose and samples with added sucrose that were stored at 4 and 20°C had gelled. These samples showed appendages on the surface of casein particles, yet they did not undergo Maillard browning during storage. Samples with added lactose stored at 35°C did not show hairy appendages protruding from the surface of casein particles; these samples underwent browning during storage. Thus, no correlation could be established between extent of browning and tendency of the samples to gel during storage.

Protein Changes During Storage

Proteolysis

The SDS-PAGE electrophoretograms of UHT concentrated milk samples after 22 wk of storage all showed evidence of proteolysis

occurring when compared to raw skim milk. New bands appeared in the gels of samples stored at 4, 20, and 35°C. In addition, a streaking pattern was also prevalent in samples stored at 35°C. The new bands could be differentiated into two types, bands that appeared in front of γ -caseins and between the κ -casein and β -lactoglobulin and bands that appeared above bovine serum albumin.

Both native and microbial proteinases have a maximum activity at 37°C. If proteolysis is considered to be the only cause of gelation, it should occur faster at 35°C than at 4 or 20°C. Proteolysis was observed in all samples, both gelled and ungelled. It cannot be ruled out as a cause of gelation, but it cannot be considered to be the only cause. After proteolysis, an aggregation process is required before the UHT-concentrated milk ultimately culminates in gelation during storage.

Gelation occurred in the 4 and 20°C samples but did not occur in the 35°C sample. Therefore, modification of protein during storage at 35°C must play a role in preventing aggregation.

Casein Micelle "Hairy" Appendages

Casein micelles in gelled samples are distorted and not spherical in shape and showed tendrillar or hairy appendages protruding from their surface. They appear connected by such appendages to form a continuous three-dimensional network.

In ungelled samples stored at 35°C, the casein particles are spherical and few tendrillar appendages protruded from their surfaces. Most of the particles are well separated although some were joined or in close proximity in the section viewed.

The absence of such appendages suggests that modification of milk proteins occurs during storage at 35°C that either prevents protein from being released from the micelle or prevents it adhering to the micelles.

These hairy appendages are most likely a linear aggregate of several polypeptide chains, because some tendrils are over 200 nm in length.

Protein Crosslinking

The electrophoretic pattern of ungelled samples stored at 35°C showed very little proteolysis. The bands identified as proteolytic products were very faint. These gels showed a streaking pattern throughout the lane with very intense bands near the top of the gel. Some material did not enter the stacking gel and some did not enter the resolving gel. In SDS-PAGE with β -mercaptoethanol, all forces involved in protein-protein interactions except covalent linkages (not including disulfide) are overcome. Protein molecules linked covalently will appear as a separate band if they have the same extent of crosslinking or a streak if a range of crosslinking exists with different number of protein molecules.

The streaking pattern in SDS-PAGE of stored UHT milks has been observed by other workers and had been attributed to Maillard browning. Our research has shown that while the Maillard reaction can induce reactions between proteins, the extent of crosslinking is the same whether lactose is present or not. Other reactions with activation energies equal to or less than Maillard browning can take place during storage at 35°C of UHT-processed concentrated milk

Heating of the proteins in milk may promote reactions involving serine phosphate, thiol, disulfide, lysine, and amide side chains and may also cleave peptide bonds. The side chains of amino acids in caseins are readily available for heat-induced or storage-induced reactions, and those of globular whey proteins are made available by the uncoiling and unfolding resulting from denaturation.

Using Immunogold Labeling to Identify the Proteins in Micrographs

Immunogold Labeling

Colloidal gold particles are formed by chemical reduction of an aqueous solution of tetrachloroauric acid. The gold particles are then converted to gold probes by reaction with a protein (such as Protein A) having the property of binding immunoglobulins. The

gold complexes are used as components in indirect two-step immunolabeling.

The first step involves the interaction of a specific (primary) immunoglobulin with the antigen under investigation. In the second step, the molecules of protein A surrounding the gold particle or the molecules of immunoglobulin-gold complex, interact with the Fc fragment of the primary immunoglobulin. The presence of the gold particle thus allows the indirect localization of the antigenic site. Electron microscopy can then be used to elucidate the position of the antigen under investigation.

A more open micellar structure was observed in some samples due to the lack of osmium tetroxide staining in these preparations. Fixation with osmium tetroxide significantly reduces antigenicity of protein but imparts heavy metal staining to the samples. This staining confers a compact appearance to the micelles which may be artifactitious. We believe the open appearance of the micelle is more representative of its actual structure.

Milk Proteins

We employed immunolocalization to elucidate the positions of β -lactoglobulin, α -lactalbumin, α _{S1}-casein, β -casein, and κ -casein in UHT concentrated milk during 12 months of storage.

When conducting electron microscopy experiments it must always be recognized that a long sample preparation occurs before examination in the microscope. The aim of such sample preparation is to fix the proteins so as to preserve the sample structure as much as possible. During the dehydration and resin embedding steps it is probable that soluble components including soluble serum proteins may be lost from the sample matrix. Consequently, in raw milk, there is little labeling of the whey proteins because they are in their native (uncomplexed) form and have a greater likelihood of being lost during sample preparation than complexed whey proteins which predominant in UHTmilk samples.

Heating of milk through pasteurization and UHT sterilization affects the distribution and

alters the conformational state of some milk proteins. This was more pronounced with β -lactoglobulin where interaction with whey and micellar casein protein was observed as a function of processing temperature. α -Lactalbumin and κ -casein show a weaker response. α_{S1} -Casein and β -casein showed heavy specific labeling concentrated on the micelles, but no effect of heating on protein distribution was evident with these proteins. α_{S2} -Casein did not respond to these immunolocalization procedures.

β -Lactoglobulin forms complexes with α -lactalbumin, α_{S2} -casein, β -casein, and κ -casein. The presence of α -lactalbumin reduces the direct interaction of β -lactoglobulin and κ -casein but has no effect on the denaturation process of β -lactoglobulin. Complexes formed between κ -casein and β -casein, and between κ -casein and α_{S1} -casein may interfere with κ -casein and β -lactoglobulin complex formation.

α -Lactalbumin

α -Lactalbumin labeling was most intense immediately after UHT-sterilization. Labeling at month 4, 8, and 12 was very sparse. Possibly this is result of complexed α -lactalbumin that formed during UHT heating breaking down with time, leading to leaching of the uncomplexed α -lactalbumin during pre-labeling TEM preparation. This is consistent with the reversibility of conformational changes in α -lactalbumin during heat processing. These aggregates would not be disulfide linked and, therefore, could revert to a non-aggregated state upon cooling.

β -lactoglobulin

Labeling for β -lactoglobulin was relatively heavy in all samples. At the beginning of storage the labeling was mainly associated with the surface of the micelles. At 4 months of storage the labeling was still associated with the micellar surfaces, but the intermicellar matrix was also well labeled. At 8, 10, and 12 months of storage the bulk of the labeling was on the intermicellar matrix with very little on

the micellar surfaces. This gradual transfer of labeling from the micelles to the intermicellar matrix suggested that the initial β -lactoglobulin— κ -casein complex moved into the intermicellar matrix on aging.

α_{S1} -Casein

All samples labeled heavily for α_{S1} -casein. At months 0 through 8 labeling was almost exclusively on the micelles, but at months 10 and 12 (at which time the milk concentrate had gelled) the micelles appeared less heavily labeled, and there was increased labeling in the intermicellar matrix. This suggested that gelation involved migration of some of the α_{S1} -casein from the casein micelles to the intermicellar spaces.

β -Casein

All samples labeled heavily for β -casein. β -Casein was not uniformly distributed within the micelles perhaps due to β -casein being proteolyzed by residual proteinases to yield β -casein fragments. At months 0 through 8 labeling was almost exclusively on the micelles, but at months 10 and 12 the micelles appeared less heavily labeled, and there was increased labeling in the intermicellar matrix. Thus some of the β -casein apparently migrated from the casein micelles to the intermicellar spaces.

κ -Casein

Labeling for κ -casein increased as the samples aged indicating that the site recognized by the antibodies became more available. The antibody for κ -casein may have been raised against para- κ -casein which, in native casein micelles, would be embedded within the micelles. There was sparse labeling for κ -casein within the intermicellar matrix at months 0-4. Labeling increased at months 6 and 8 within the matrix, mainly between adjacent micelles. The labeling appeared as linear patterns as well as clumps within the intermicellar matrix. There were few tendrillar appendages between micelles at months 0-8; these structures proliferated at months 10 and 12. The labeling at month 10 was very

dispersed, but involved linkages between micelles. This trend was accentuated at month 12 where there were linear clusters between adjacent micelles.

A Mechanism of Age Gelation

UHT sterilization of UF-concentrated milk denatures β -lactoglobulin, which forms complexes with micellar κ -casein. This interaction apparently destabilizes the κ -casein and predisposes it to dissociation from the micellar moiety.

The movement of κ -casein and β -lactoglobulin during storage seemed to be related to age gelation perhaps through interactions between these proteins leading to a complex. The tendrillar appendages observed in electron microscopy appear to be the remnants of the β -lactoglobulin- κ -casein complex, which are still physically associated with the surface of the coagulated micellar residues. As UHT ages, κ -casein leaves the micelles as the competitive binding of denatured β -lactoglobulin to κ -casein weakens its bond with the casein micelle.

As more β -lactoglobulin- κ -casein complexes are released into the serum phase of the UHT milk they tend to associate together. Eventually a time is reached in which there is sufficient material in the serum phase of milk to bring about gelation, entrapping what remains of the casein micelles within the gel.

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Maximizing UHT Technology

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In the design of new UHT products there are a number of parameters available for manipulation:

- Product Composition
- Pre-UHT Processing
- UHT Processing Conditions
- Post-UHT Processing
- Packaging
- Storage
- "Consumer" Processing

Each parameter has limitations, and may be affected by other parameters but taken together, they provide a myriad of combinations that can be used to develop unique food products.

Product Composition

Composition of the product is the basis from which all other parameters are built. It can be varied by separating (or adding) cream, concentrating the milk, using fresh or reconstituted milk, mixing milk with non-dairy fluids, adding stabilizers, emulsifiers, starches, and flavoring ingredients.

Fat content has long been manipulated to meet consumer demands. Apart from milks of different fat contents, there have already been UHT cream, "Half-and-Half" coffee creamers, specialty creams and other higher fat products developed. Solids content of milk can be increased by adding powders or concentrating milk by evaporation, reverse osmosis or ultrafiltration. The method chosen will depend not only on economics but which fraction of the milk solids you wish to increase. Evaporation and the membrane process of reverse osmosis only remove water. In contrast, ultrafiltration concentrates the protein and calcium phosphate but allows lactose and some salts to be removed with the water. You can also add whey powders, whey protein concentrates and isolates, or caseins to selectively increase the concentration of specific milk components.

The manufacture of UHT products does not have to be restricted to milk products and fruit juices. And in a sense, neither of those could be considered as novel because the technology, the products, and the markets for them are well established.

Pre-UHT Processing

The physical and chemical state of milk can also be altered by a pre-UHT treatment. Homogenization may be necessary to allow proper dispersion of added ingredients in a formulated product or to produce the appropriate fat droplet surface composition before any heat treatment is given to the product. A prolonged heat treatment may also be applied before UHT processing to increase whey protein denaturation. The milk can be acidified but only within the limits of product stability upon heating. If too much acid is added, the proteins will precipitate upon UHT heating. Enzyme treatment is another option available as a pre-UHT process and could be used, for example, to hydrolyze proteins.

UHT Processing

Within the limits of obtaining commercial sterilization of the product there are a number of variables that should be optimized for any UHT product. Doing so could be the difference between having a product that is stable and one that is not, or having a product that is marketable and one that is not.

The first choice to make is the type of heating system. Then it is necessary to determine the best preheating temperatures and times as well as the UHT temperature and holding time. It is not just a case of more heat is better, finding the most suitable temperature/time conditions along with the optimum formulation can take a lot of work.

Post-UHT Processing

After the product has been UHT processed it must be maintained in its sterile state until it is packaged. This does not, however, preclude

further processing provided it is done aseptically. An example of this is the injection of solutions into UHT processed milk using a sub-micron filter to remove bacteria. Such equipment is commercially available and allows for the addition of materials that are sensitive to heat, such as flavours, acids or enzymes.

Packaging

A lot of the recent innovations in UHT processing has come in the form of new packaging systems. During storage of UHT products there are a number of changes that can occur based on the product composition and the processing conditions. Most of these are detrimental to product quality although it could be conceived that given the appropriate product they may serve to improve some desirable product attribute.

"Consumer" Processing

The final opportunity to introduce a processing step into a UHT product is at the point of use by the consumer. These can be categorized into five groups:

- UHT products for immediate use without further modification such as with consumption of UHT milk, juices, or other drinks.
- UHT products for immediate use as part of a prepared food item. This would include such items as sauces and custards that could be poured over a food at the time of serving.
- UHT products used in cooking as an ingredient such as creams and sauces.
- UHT products used to manufacture a reconstituted product, most likely on a commercial basis with the reconstituted product being further processed before sale. This would include concentrated milks that could compete with milk powders in producing a reconstituted product more comparable to "fresh" milk.
- UHT products that require additional processing before use. An example of this

would be the production of a UHT milk concentrate that is shelf stable in a liquid form but then can be converted into a solid form after purchase.

Conducting the Product Development

So you see, there are a lot of opportunities possible for using UHT technologies in novel ways. What is needed now is for marketing people, food scientists, engineers and others to work together not only within their own company but with equipment suppliers and academic institutions so that these opportunities can be realized.

Very few companies have their own pilot UHT equipment so the best way to conduct your product development is with an institution such as Utah State University where the trials can be done for you. Not only is this cost effective but you need to test your product on a system comparable to that to be used for commercial production. It has been our experience that not only can we run far more permutations of formulations per day than is possible on a commercial packaging line but that they relate very well to the configurations used by those who have eventually processed and packaged the product. Samples can be obtained with as little as 5 gallons of milk. And it is possible to complete up to 10 or 12 runs per day depending on the number of samples to be collected.

Again, it must be stressed that before you travel too far along the UHT product development road you should decide the system will be used for commercial manufacture and then find a pilot scale unit to match it. Product development is easier when you can run 10 gallon batches rather than 1000 gallon batches. This is especially so when you may need to run dozens of samples to reach your product goal.

For more information on availability of UHT equipment for use in your product development contact either Paul Savello or Don McMahon, Department of Nutrition and Food Sciences, Utah State University, Logan, Utah 84322-8700. Telephone: (801) 797 2106 or Fax: (801) 797 2379.

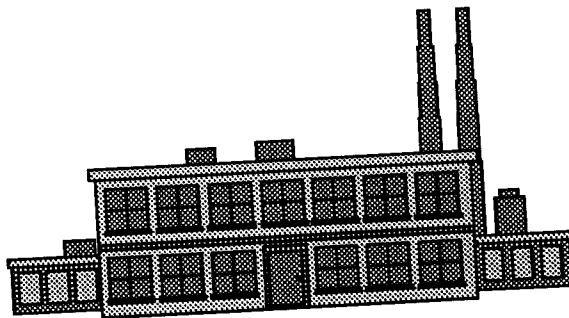
Lactose Hydrolyzed UHT products

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ABSTRACT

UHT lactose hydrolyzed milk is seen as having commercial potential, particularly in local markets where an educational campaign regarding the benefits of the product has been carried out. It is likely that these will develop as low-volume high added-value products serving niche markets.

1. Lactose Hydrolysis

Lactose hydrolysis offers the dairy processor an additional option for the manufacture of value-added fluid and dried dairy products. Lactose hydrolyzed products offer two market advantages over unhydrolyzed products:

(i) Lactose hydrolyzed products are sweeter than unhydrolyzed products. It is often forgotten that lactose, a di-saccharide similar to sucrose in structure, is a comparatively unsweet sugar. After all, milk contains about 5% lactose, but it is not a noticeably sweet product. On the other hand, a 5% solution of sucrose is very sweet indeed.

The process of lactose hydrolysis involves cleaving the di-saccharide, lactose, into its two component mono-saccharides, glucose and galactose. Each of these sugars is noticeably sweeter than sucrose, although galactose is still not a particularly sweet sugar. Thus, the outcome of hydrolysis is a noticeable overall increase in milk sweetness - complete hydrolysis of the lactose in milk results in an increase in sweetness equivalent to the addition of about 1.5% sucrose. Such an increase in sweetness can be beneficial, as formulated products containing hydrolyzed milks may require less addition of sugar to reach the desired level of sweetness. This can reduce costs and calorific content - one aspect helping profitability, the other assisting marketing. Examples of such applications include lactose hydrolyzed flavoured milks, ice cream and puddings. On the other hand, existing products may become more attractive to consumers when based on hydrolyzed milk. Examples include unflavoured

and flavoured yogurts, which are much less tart when based on hydrolyzed milk. Consumer trials have shown marked increases in consumer acceptance of such products.

(ii) A significant percentage of the population suffer from lactose malabsorption. Normally, digestion of lactose involves cleavage of the di-saccharide into glucose and galactose in the gut by the action of an enzyme, β -galactosidase. The resultant mono-saccharides are then absorbed and metabolized by the body. However, much of the population of the world lose the ability to secrete sufficient β -galactosidase to effectively digest their normal intake of milk, after the age of about 10 years. This decreased level of enzyme secretion results in a marked decrease in the ability of this segment of the population to properly digest lactose. Thus, when lactose malabsorbers ingest lactose-containing products, a significant amount of the lactose passes undigested into the lower intestine, where it is fermented, leading to production of gas, an increase in osmotic pressure and a flow of fluid into the bowel. This in turn leads to feelings of discomfort, bloating, and in severe cases stomach cramps and diarrhoea. Lactose intolerant populations include most of those of South East Asia, Japan, black Africans, black Americans, red Indians and Australian Aborigines. The nutritional benefits of many milk-based products are thus not readily available to most of these populations. Some of these populations are in areas where famine is a problem, and aid is commonly supplied. However, given the percentage of lactose malabsorbers in these populations, clearly the role of milk products in famine assistance and aid is limited.

The industrial process of lactose hydrolysis mimics precisely the natural digestive action taking place in the stomach on digestion of milk - in one sense, lactose hydrolyzed milk may be considered to be "pre-digested". Therefore, lactose hydrolyzed milks may be ingested and metabolized by lactose malabsorbers without difficulty, as the lactose has already been cleaved into glucose and galactose. It has often been suggested that there is therefore a considerable market for lactose hydrolyzed products in countries with high percentages of lactose malabsorbers in the population. In fact there has been a strong belief that lactose hydrolyzed products have an extensive and "natural" market in such areas. Thus, it has been often suggested that if only lactose hydrolyzed products could be developed at a reasonable price, the populations of these countries, who had been deprived of the benefits of milk for much of their lives, would fall over each other to buy and consume these products. There was an image of deprived populations, craving for milk, wishing for milk, but knowing that they were unable to drink it.

This concept has been clearly shown to be incorrect - it is a belief which should be thoroughly scotched. Studies undertaken in South East Asia, with the financial help of the Victorian Government, involving detailed discussions with a number of dairy marketers, manufacturers and suppliers in South East Asia have led to a key conclusion in this matter. It is now evident that lactose malabsorbing individuals who no longer drink milk will not readily be attracted back to the milk market through the provision of lactose hydrolyzed products. Having developed a diet satisfactory to their

needs, it will be a very difficult matter to persuade them to change their attitudes and start drinking milk again. Those members of the population that recognized that they were lactose malabsorbers have modified their diet to ensure that their lactose intake is controlled. They certainly have no great desire to start to consume milk - generally they are happy with their existing diet.

The only way to develop a market for lactose hydrolyzed products in such countries would therefore be to undertake a major educational and advertising campaign to try to get these people to change their diets and eating habits. Recognition of the fact that in general lactose intolerant populations were not going to be clamouring for lactose hydrolyzed products have currently put paid to attempts to develop markets for lactose hydrolyzed skim milk powders in South East Asia. Similar arguments mitigate against the development of significant markets for UHT lactose hydrolyzed milk in these areas.

This should not be taken as an indication that there is not a market for lactose hydrolyzed products in South East Asia - of course there is, but only for liquid products. The market already exists, at a low level - to develop it further will require a very significant commitment and considerable capital.

2. Some marketing considerations

For the manufacturer of UHT lactose hydrolyzed products, these considerations pose particular problems. In particular, at whom should the products be targeted? It would seem that there is not a significant market for lactose hydrolyzed UHT milks in South East Asia (with one or two exceptions which are State sponsored - for example the provision of lactose hydrolyzed UHT milk to Malaysian school children). UHT products in fact suffer from an additional drawback, as, if by some chance a market should develop in South East Asia for example, it would almost certainly prove to be more economic to supply lactose hydrolyzed milk powder for recombination of reconstitution in South East Asia than to supply the product (containing 87% water) from Australia.

Clearly therefore, the best market for local manufacturers will be niche local markets catering for the "health conscious" sector of the population, and local lactose malabsorbers (which is said to represent about 10% of the Australian population). It should be noted that some soy products (notably "So Good") promote their product in the basis that they contain "no lactose". It may be possible to develop a marketing approach to lactose hydrolyzed UHT products by utilizing the concern regarding lactose in the community aroused by such commercials.

The second option appears to be the development of lactose hydrolyzed products which utilize the increase sweetness of lactose hydrolyzed products. This option has not really been considered in Australia, or indeed throughout much of the rest of the world. Potential products include ice cream with a lower calorie content (through reduced sucrose content), lower calorie flavoured milks ("naturally

sweetened, no added sucrose!") and "Breakfast Delight" - a concentrated reduced fat milk, say 16% solids, 3% fat, lactose hydrolyzed. Such a product would be ideal for UHT processing. This should be rich in mouth feel sweet, and could be used on breakfast cereals without the need to add sucrose. Sadly, there is no sign of development of any such products of which I am aware.

3. Technological aspects

Lactose hydrolysis of milk can really only be effectively carried out by the use of the enzyme β -galactosidase. This enzyme may be readily extracted from a number of micro-organisms, and has been available commercially for some years. In principal, the procedure for hydrolysis of milk is straight forward. For pasteurized milk, an appropriate amount of β -galactosidase is added to the milk at an appropriate temperature, and the milk held for the required period until the hydrolysis is complete, prior to pasteurization and filling. Higher levels of lactase addition require shorter holding times for hydrolysis to reach the required level of completion. Similarly, the reaction proceeds more slowly under refrigeration temperatures than at blood heat. Pasteurization results in the denaturation (or destruction) of the enzyme.

This approach can be used for the production of UHT lactose hydrolyzed milk - the milk is hydrolyzed by the addition of the enzyme, and the hydrolyzed milk then subjected to UHT sterilization. It is not however the most desirable methodology. It suffers from the considerable disadvantage that it is the hydrolyzed product which is subjected to the UHT processing conditions of say 140°C. Unfortunately, the monosaccharides glucose and galactose are much more reactive than the original lactose, and the end product will have suffered considerably from browning reactions. These reactions lead to the development both of off flavours in the product, and also a noticeable of darkening of the product. In general, it is my belief that this approach should not be used for the manufacture of lactose hydrolyzed UHT milk - the product is of poor quality, and certainly will result in the product developing an unsatisfactory reputation in the community.

A much more preferable option has been developed by the TetraPak company (under the name TetraLac), and others. The procedure involves injection of sterile enzyme solution in very low quantities into the UHT sterilized milk prior to aseptic packaging. The process may use injection into an aseptic holding tank, or into the pack itself prior to sealing. In these circumstances, the hydrolysis takes place over a period of say 10 days after manufacture, in the sterile pack. Self evidently, the amount of enzyme required can be reduced very substantially compared to the process described earlier, as the reaction can take place over a much longer period - there is no concern regarding bacteriological growth in the product. Operational costs are thus considerably reduced. Further, the enzyme is added after heat processing, so that there is no excessive browning of the product occurring during sterilization.

Overall, this procedure offers considerable benefits. Its main drawback is that the "live" enzyme remains in the product, and is consumed by the consumer. This is of

no significance however from the nutritional or toxicological point of view - perhaps only from marketing.

A more major concern is the freedom of the enzyme preparation from protease contamination. Cheaper enzymes often contain significant levels of protease, which can lead to the development of bitterness in the product (and in some cases gelation) on extended storage. Operators using such systems should ensure the high quality of the enzyme used in the process.

4. Current Products

4.1 Human Foods

Some years ago, another Australian company decided to introduce a UHT lactose hydrolyzed milk to the Australian market. As I have indicated, there is really only one preferred way to do this - by the injection of sterile filtered enzyme into the pack prior to filling (as in the TetraLac system) or into the aseptic tank, prior to filling.

Being interested in such products, I purchased some from my local supermarket. To my concern the product was very noticeably brown in colour, and smelled badly cooked and caramelized. I considered, as a consumer, that the product was totally unacceptable. According to its "Use By" date, it still had some months of life. On inquiry from the manufacturer, I discovered that they had decided to carry out the hydrolysis stage prior to UHT treatment, for the sake of processing convenience. Thus, soluble enzyme was used to treat pasteurized milk, and after hydrolysis, the product was UHT processed. Of course, this meant that the hydrolyzed product was subjected to UHT processing, and the increased number of reducing residues in the product arising from the hydrolysis inevitably led to greatly increased browning during processing. The plant also used indirect UHT processing, which would also be likely to accentuate the degree of browning.

Needless to say the product did not meet with a high level of consumer acceptance and was withdrawn a short time later.

In another development, another Australian company has promoted a lactose hydrolyzed UHT milk under the umbrella name of a "range" of products. The range includes a high-calcium high-protein low-fat UHT skim milk, and lactose hydrolyzed full cream UHT milk. The packs for these products are almost identical, with the generic brand name covering a large part of the pack. Only by close examination can you tell if it is (a) lactose hydrolyzed *or* high calcium, and (b) whether it is low fat *or* full cream. This is most confusing for the potential consumer, and I am sure is retarding sales. I suggest that to develop the market, it is necessary to ensure that lactose hydrolyzed products are clearly marked and have a clear and separate niche in the market.

CONCENTRATING MILK: NEW UHT PRODUCT CONCEPTS

by

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Linking the two technologies of membrane concentration of milk and UHT processing of the concentrate can lead to innovative product concepts. Improvements in traditional dairy products can also be made using these technologies. This paper presents a summary of product and process research concepts that have been investigated at Utah State University using membrane concentration and thermal processing (including UHT) of milk.

RO and UF Concentration/UHT Processing.

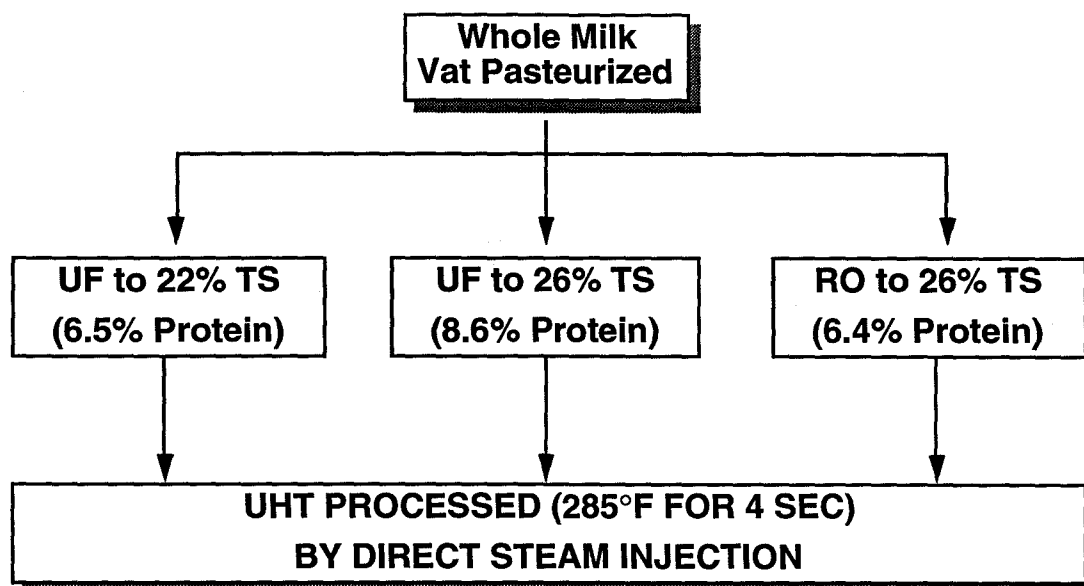
Reverse osmosis (RO) and ultrafiltration (UF) membranes have been used to concentrate milk components. A milk concentrate can be UHT-processed to provide a shelf stable concentrate. RO concentrates retain all the milk components because only water is removed by this membrane process technique; UF concentrates retain all the fat and protein, while having less of the lower molecular weight components (minerals, nonprotein nitrogen, free vitamins, lactose). The two membrane processes have been investigated to determine if the UHT-processed concentrates differ in their shelf stability, particularly in the time it takes (at room temperature storage) for the concentrate to exhibit the "gelation phenomenon."

RO is the most efficient way to remove water from milk. The data below indicate that RO concentration of milk uses significantly less energy than three other forms of concentration (evaporation, mechanical vapor recompression, and freeze concentration).

<u>Concentration Technique</u>	<u>Kwh Used per Metric Ton Water Removal</u>
Multi-stage evaporator	118
Multi-stage freeze concentration	65
MVR	12.7
RO	6.5

Experiments at USU concentrated whole milk to 2x level using RO (to 26% total solids and 6.4% protein). UF concentration (using 20,000 molecular weight cut-off membranes) of whole milk was performed to two different levels: 1) to 26% total solids

(with 8.6% protein), and 2) to 22% total solids (with 6.5% protein. The flow diagram below shows these concentration levels and basic analyses.



Analyses of the final concentrated/UHT products included: total protein, nonprotein nitrogen, total calcium, soluble calcium, ionic calcium, and viscosity (including time to gelation). These analyses were performed on a monthly basis until gelation occurred in the samples. The main statistically significant relationship was the soluble calcium-to-protein ratio and time (in months) to gelation ($R^2 = 0.84$). Other analyses did not correlate well with the time to gelation. UF samples gelled faster than RO samples. RO/UHT milk samples gelled between 6 and 12 months; UF/UHT milk samples gelled between 2 and 4 months.

Addition of appropriate salts can extend the shelf life (i.e. delay of gelation) of the concentrated/UHT milk samples. Phosphate and citrate salts have been investigated in the past to determine if their calcium-chelating abilities can further stabilize the milk proteins against gelation during long-term storage. Our research indicates that proper selection (and proper use level) of salts can help extend the shelf life of membrane concentrated/UHT milk.

Three salts were tested, each at six (6) levels. The salts used were: trisodium citrate, disodium phosphate, and sodium tripolyphosphate. The levels added to the milk concentrates prior to UHT processing were: 0, 1, 3, 5, 10, and 20 mM (as final concentration in the milk concentrate).

The citrate and disodium phosphate salts speeded up the gelation phenomenon when added at any levels greater than the control sample (with no added salts). The polyphosphate salt increased the shelf life of the milk concentrates when used at levels less than 5 mM; at the higher levels (10 and 20 mM), the concentrates gelled faster than the controls. These high levels of calcium chelating salts destabilized the milk protein system significantly fast so that "gelation" occurred within days after UHT processing.

RO milk samples with the lowest levels of tripolyphosphate (1 and 3 mM) extended the shelf life of the stored samples to 12 months. During this time, the samples remained fluid and did not indicate any significant increase in viscosity.

UF milk samples with the lowest levels of tripolyphosphate (1 and 3 mM) also extended the shelf life of the stored samples, but not to the extent of the RO samples with the same salt type and levels. The UF samples had a longer shelf life than the UF controls (no added salt).

Multiple Membrane Concentration/UHT Processing

Concentrating milk to higher than a 2x concentration level can be achieved by RO but the efficiency of the membranes drops after reaching the 2x level. Our laboratory has concentrated whole milk by RO to a 3x level (approximately 38% total solids), but greater pressure is required to remove water from the concentrate between the 2x and 3x levels. We have been achieving a 3x concentration level, however, using a "multiple membrane" sequence. This process uses both UF and RO to arrive at a 2.5x, 2.75x, and 3.0x concentration levels of whole milk.

The basic concept is to concentrate the protein and fat (large molecular weight components) using UF membranes. The UF permeate is then concentrated by RO. The two concentrate streams (UF and RO) can then be mixed to achieve the higher levels of concentration. In the final analysis, only water has been removed from the original whole milk, and (theoretically, at least) all the milk constituents are in the final concentrated milk product. The UHT process of these milk concentrates is performed using direct steam injection and aseptic homogenization.

The flow chart below shows how the basic multiple membrane concentration steps were performed.

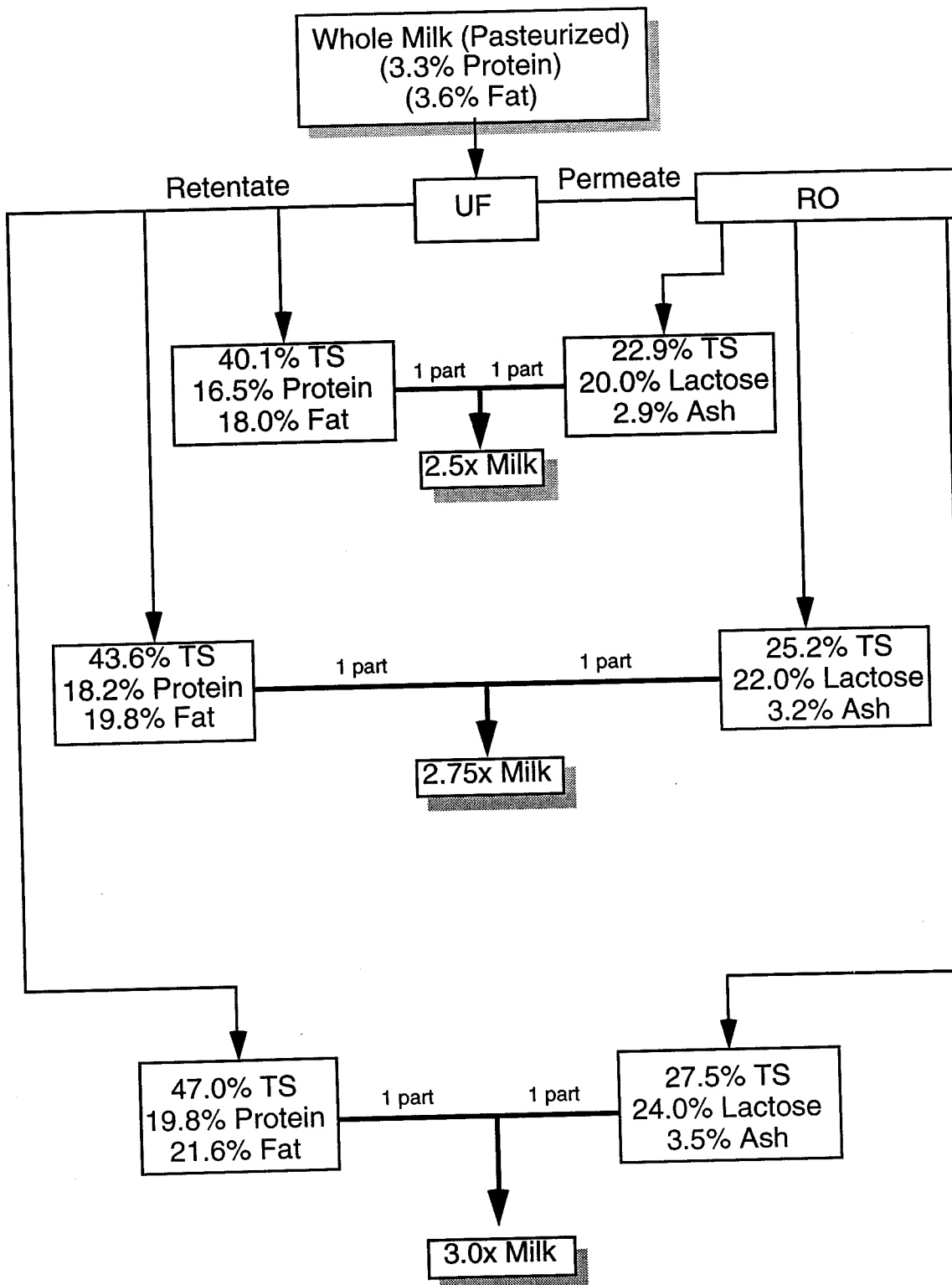


Figure 1. UF/RO Process Flow to Produce 2.5x, 2.75x, and 3.0x Milk Concentrate

Milk constituents were analyzed in all fluid streams and in final processed products over a six month period at room temperature storage. The fluid streams included: whole milk, UF retentate, UF permeate, RO retentate, RO permeate, and the blend of retentates to achieve the desired concentration factor.

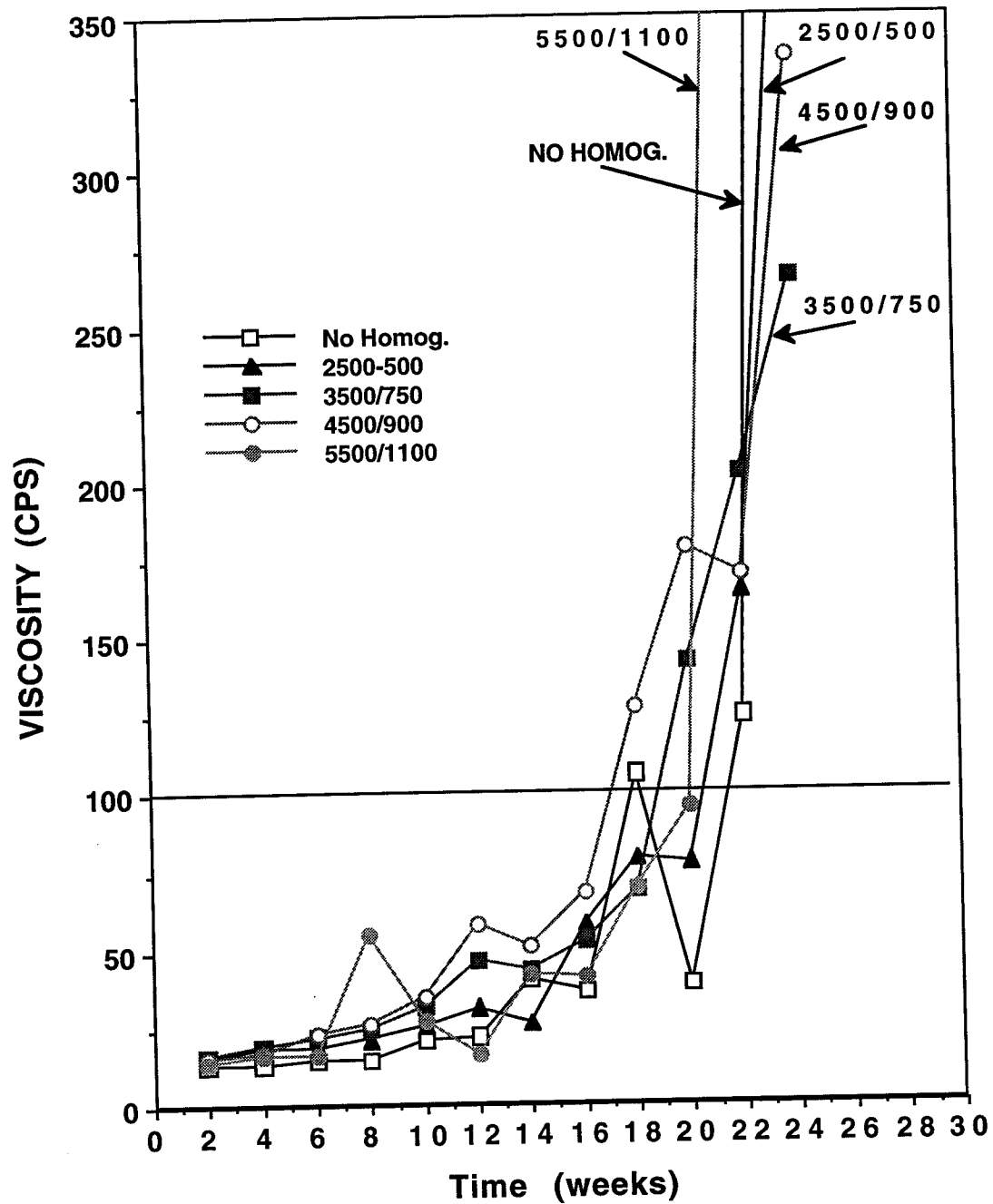
The analyses were performed every two weeks during that storage time. The physical properties and chemical analyses performed included: total solids, total nitrogen, nonprotein nitrogen (NPN), total ash, lactose, riboflavin, fat, and total calcium, viscosity, pH, sedimentation, and cream plugging.

The concentrated milks were UHT processed using direct steam injection (285°F for 4 sec). Following flash cooling, the concentrates were aseptically homogenized (two stage) at the following pressures: no pressure, 2000/500 psi, 3500/700 psi, 4500/900 psi, and 5500/1100 psi. All samples were stored at room temperature during the shelf studies.

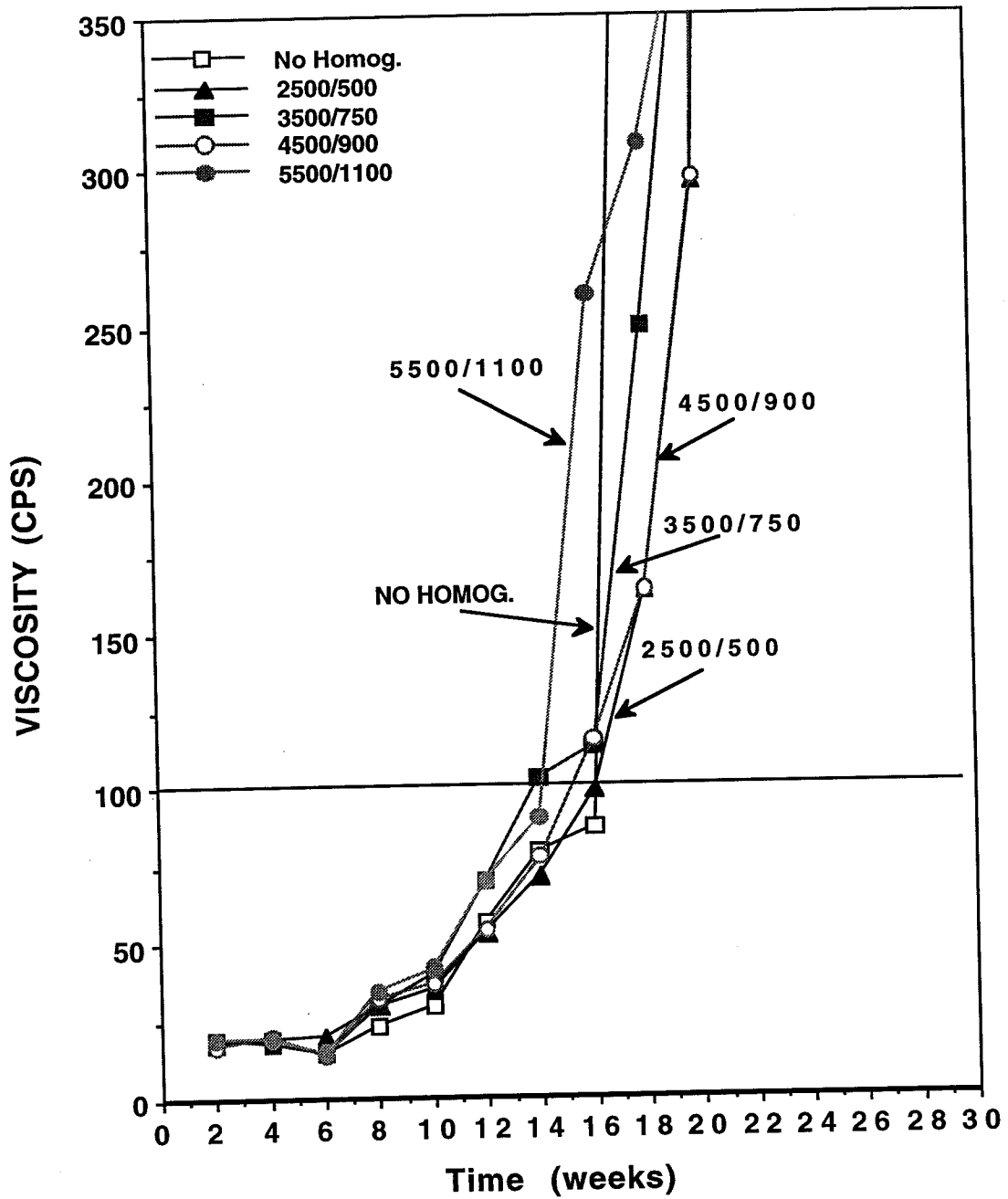
Data of all the runs and analyses are now being analyzed for statistical differences (significance). Preliminary analysis of the data indicates that although there seems to be minor loss of expected constituent return after the multiple membrane processing, the constituents should be accounted for within statistical error and measurement error.

The two graphs below show the gelation of 2.5x and 2.75x milk blends following multiple membrane concentration and UHT processing. These samples were stored at room temperature.

VISCOSITY OF 2.5x UF/RO CONCENTRATED WHOLE MILK STORED AT ROOM TEMPERATURE



VISCOSITY OF 2.75x UF/RO CONCENTRATED WHOLE MILK STORED AT ROOM TEMPERATURE

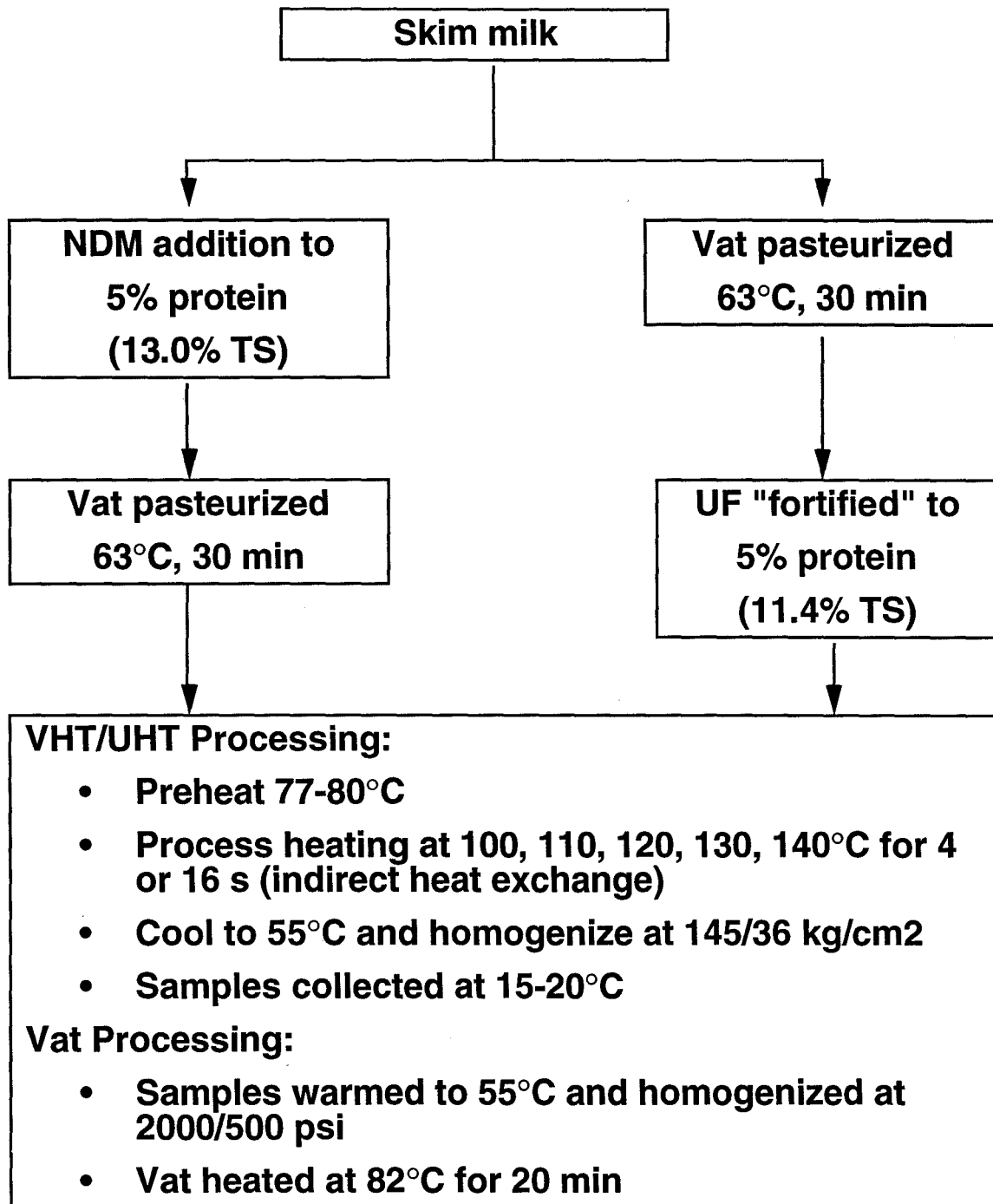


Homogenization pressure applied to the milk concentrates may have an impact on shelf life before the concentrates gel (or show increased viscosity). However, the differences among the pressures applied may not be statistically significant. The principal defect noted in the samples is increased sedimentation over storage time, particularly as the total solids increase in the different samples. We are not clear as to the reason for this sedimentation. None of the samples tested in this multiple membrane research were treated with a polyphosphate additive prior to UHT processing. Possibly, addition of a polyphosphate or a stabilizer could prevent the sedimentation defect.

The research to date shows that the multiple membrane concept works well in capturing the milk constituents. Energy consumption in this process has not been studied to determine if the multiple membrane process uses less energy than RO processing alone, particularly up to the 3.0x concentration level. This is one research area that should be performed, with a carefully designed set of experimental runs that can provide valid energy consumption data.

UF/NHT/UHT Processing of Yogurt Milk

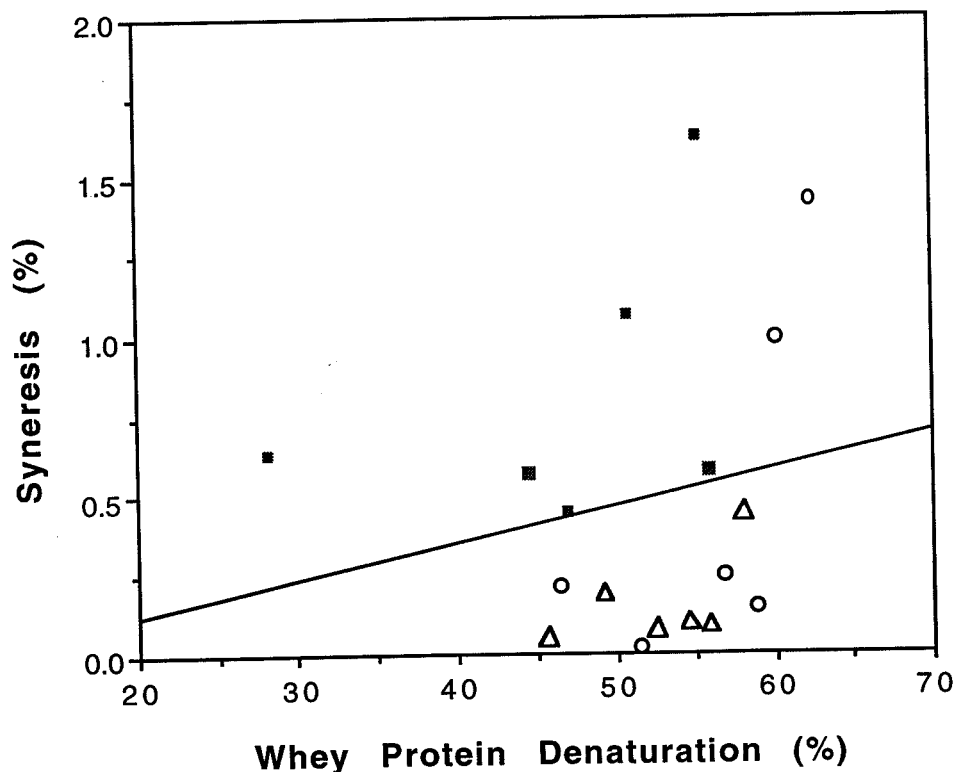
Our laboratory has researched the effects of different fortification methods and heating temperatures of yogurt milk on various physical properties of firm set yogurt. This research investigated the fortification of skim milk by either nonfat dry milk (NFDM) powder addition or by ultrafiltration. Both fortification methods raised the protein content of the yogurt milk to 5.0%. The fortified skim milks were heat treated by either vat heating (82°C for 20 min) or by heating in an Alfa-Laval Sterilab® UHT pilot plant. The fortified milks were heated to 100, 120, 130, or 140°C and held for either 4 or 16 sec followed by cooling to approximately 20°C. The heated yogurt milks were inoculated with commercial yogurt culture (1%) and incubated at 37°C. The samples were removed from the incubator when the pH reached 4.9 to 5.0. All samples were refrigerated at 4°C. The flow chart below shows the basic schema of fortification and heating methods.



The total solids levels of the two fortified nonfat yogurt milk were not the same because of the different ways in which solids were "added" to the milk. The UF fortified milk had a total solids level of 11.4%; the NFDM fortified milk had a total solids level of 13.0%.

No clear definitions or terms exist for the temperatures of 100, 110, 120, or 130°C when applied to thermal processing milk. We have created the term "Very High Temperature" (or VHT) for these temperatures. UHT is understood to mean temperatures in excess of 138°C.

The yogurt samples produced after treating skim milk by the two fortification methods and the different heat treatments were tested for physical properties after a 21-day storage at 4°C. The properties tested were: syneresis, water holding capacity, viscosity (stirred), and gel strength (penetrometer test). Many earlier research results tried to correlate whey protein denaturation (WPD) (resulting from heat treatment of the yogurt milk) to the extent of syneresis. The theory has been that increased WPD holds more water and yields a yogurt product with less syneresis. The data collected of all samples tested in this research indicate that no correlation exists between WPD and syneresis. The graph of these data is presented below.



The bar graphs that follow show the results of pooled data (of the 4 and 16 sec holding times and where $n = 16$ samples). There was no statistical significance between these two holding times on any of the physical properties measured. The four properties measured after 21 days of storage at 4°C were: syneresis, water-holding capacity, viscosity (stirred), and gel strength (penetrometer).

Syneresis of both fortification types had lowest values in the 110 to 130°C ("VHT" region). At higher heat treatments (140°C and vat heating), the syneresis level increased. Water-holding capacity did not change over the temperature range tested for the UF-fortified yogurt milks. There was statistical differences among the heat treatments of the NFDM-fortified samples. It is unclear what the different really mean because these water-holding capacity results do not correlate with syneresis results.

Viscosity and gel strength values were very similar. Of the VHT/UHT processed samples, the 140°C samples had the lowest viscosity and gel strength values. The highest viscosity and gel strength values re at 110°C . There was no statistical difference between the 110°C and vat heated samples of these two physical properties.

It appears that the VHT heating region (110 to 130°C) does have an effect on yogurt milk preparation and the resultant physical properties of the finished firm set yogurt. It is not established what the change(s) is (are) that can cause the physical properties noted in this research. It should be kept in mind that the samples tested in this investigation did not use any type of stabilizer as is commonly used in commercial practice. The yogurts tested in this research consisted of skim milk fortified to 5% protein by either NFDM addition or by ultrafiltration, and commercial yogurt culture. No sweetening agents nor stabilizer materials were used to confound the research results.

