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Effects of Stabilizers and pH Adjustments on Milk Proteins in UHT-Treated Citrus Juice/Skim Milk Blend Drink

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EFFECTS OF STABILIZERS AND pH ADJUSTMENTS ON MILK PROTEINS IN UHT–TREATED CITRUS JUICE/SKIM MILK BLEND DRINK

by

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ABSTRACT

EFFECTS OF STABILIZERS AND pH ADJUSTMENTS ON MILK PROTEINS IN UHT–TREATED CITRUS JUICE/SKIM MILK BLEND DRINK

by

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A UHT–processed skim milk (85%)/orange juice (15%) drink was developed. Product integrity and stability were maintained by two methods. Proper homogenization of the blend before UHT processing stabilized a drink formulation containing .25% carboxymethyl cellulose and .025% carrageenan. Adjusting the pH of the blend (pH 6.3 and 6.5) resulted in a different stabilization. After 28 days at room temperature, settling of milk solids was 5.2% of volume height in the prehomogenized sample and 86.9% of volume height in the same blend that had not been homogenized prior to UHT processing. After storage, the two treatments were analyzed to verify that there was no perceived textural difference between the pH adjusted and unadjusted blends. A consumer product acceptability evaluation resulted in a split population, and more panelists liked the product than disliked it.

(72 pages)

INTRODUCTION

In 1989 the U.S. consumed 25.3 gallons of fluid milk per capita compared to 51.1 gallons of soft drinks (40). The major dairy products available in the market today (cheese, butter, yogurt, and ice cream) were all invented before modern dairying (24), each one providing a specific market potential for fluid milk. In the last decade, the development of stable cream liquers has opened new doors for marketing milk. Increased consumption of fluid milk in the U.S. may be possible with the development of other milk drink products.

Milk drink beverages can be made shelf stable by ultra high temperature (UHT) processing (16). In developing a UHT–processed skim milk/citrus juice drink, processing conditions and blend pH must be considered to avoid protein precipitation. Changes due to salt, pH, or heat treatment may lead to irreversible changes in the milk colloid system (48).

The objectives of this study were to:

a. stabilize milk proteins against heat coagulation when 15% (w/w) orange juice is added to skim milk at different pH levels and UHT processed;
b. process the skim milk/orange juice drink under aseptic (UHT) conditions;
c. maintain a stable UHT–processed skim milk/orange juice drink blend over twenty-eight day storage at room temperature and 35°C storage with the use of stabilizers;
d. evaluate the effect of different processing conditions on product homogeneity through sensory testing; and
e. use taste panels to evaluate the consumer acceptance of the product.

LITERATURE REVIEW

Citrus Juice Milk Drinks

A complete mechanism for heat–induced destabilization of milk proteins is unknown. At most, only parts of the reactions are known and understood. Further understanding of the mechanisms involved would make possible the development of a variety of heated milk products. Currently there are several patents available for the manufacture of different citrus fruit–milk beverages (1, 7, 17, 25, 49); however, these patents do not work in the development of an UHT skim milk/orange juice drink. Futhermore, some of the patents do not use fluid milk but milk solids with milk being a minor ingredient.

Polysaccharides are used in the formulation of citrus juice milk drinks. Because polysaccharides interact with milk proteins and prevent acid heat coagulation (27), they can be used in citrus fruit milk drinks to stabilize the milk proteins. Milk proteins may be kept in solution at low pH with carboxymethyl cellulose (27). Under the appropriate conditions, polysaccharides will inhibit the precipitation of some water soluble proteins following thermal denaturation (27). κ–Casein and κ–carrageenan (and to a lesser extent ι– and λ–carrageenan) form a complex via electrostatic interaction between the negatively charged sulfate groups of carrageenan and a region of positively charged residues on κ–casein. κ– and ι– Carrageenan are capable of protecting α_S–casein from calcium precipitation at neutral pH (the mechanism is unknown) (27). Some polysaccharides increase skim milk viscosity (14), thus giving to a skim milk drink the mouthfeel of a thicker product. A network of polysaccharides could also help maintain thermally denatured water soluble proteins in solution. When manufacturer makes pasteurized fruit juice milk/ drinks, the stability of the sample is both pH and stabilizer type dependent (28).

The Milk Colloid

The stabilizing factors in milk are sufficient to maintain the casein-calcium phosphate phase in suspension after certain heat treatments. On prolonged heating, coagulation is observed. Heat stability is defined "as the time taken for coagulation to occur under standard conditions, usually 140°C for milks of normal concentration or 120°C for concentrated milks" (24, p. 3619). To measure heat stability objectively, nitrogen depletion curves are used. A nitrogen depletion curve is the "the amount of protein remaining in the supernateant on gentle centrifugation decreasing sharply at the coagulation time" (24, p. 3620). When heat coagulation times are plotted against pH, two types of curves are obtained: type A and type B (41). When heat coagulation time at 140°C is plotted against initial pH between pH 6.3 and 7.1, a type A curve shows the following:

a. heat coagulation time increases between approximately pH 6.3 and pH 6.6,
b. heat coagulation time decreases between approximately pH 6.6 and 6.9, and
c. heat coagulation time increases between approximately pH 6.9 and pH 7.1, (20).

A primary maximun heat coagulation time is observed at about pH 6.6, and a secondary maximum continues at pH greater than 7.1. A decreasing heat coagulation time is observed at pH 6.4 and below. A local heat coagulation mininum is observed at about pH 6.9. This pH will be referred to as the mininum in type A milk. A type B curve shows the following trends when heat coagulation at 140°C is plotted against initial pH:

a. heat coagulation time slowly increases from pH 6.4 to 6.9

b. heat coagulation time rapidly increases from pH 6.9 to 7.1 (20).

The type of curve obtained from a given cow's milk is positively correlated with its urea content, and it appears that dietary manipulation may play an important role by altering the levels of milk urea (24). Heat coagulation time/pH curves are interconvertible, and it is believed that serum components play a major role in determining the type of curve a sample will have (30). However, the heat stability profile is influenced by the following components:

a. whey proteins,
b. lactose,
c. caseins,
d. colloidal calcium phosphate,
e. soluble calcium phosphate,
f. detergents,
g. assay conditions such as temperature and agitation,
h. urea,
i. preheat temperature, and
j. concentration or dilution (20).

Because of the way these components affect heat coagulation, it has been possible to theorize how heat coagulation may proceed in type A milk at its minimum, or in type B milk at all pH levels, and type A outside its minimum (24). Horne and Muir (24) listed ways by which type A and type B milks may be interconverted. Type A milks may be converted to type B milks by:

a. decreasing the assay temperatures from 150°C to 120°C,
b. adding κ–casein,
c. removing colloidal calcium phosphate,
d. replacing phosphate by another anion, and
e. dialyzing type A milk against excess type B milk (24).

Type B milks may be converted to type A milks by:

a. raising the assay temperature from 130°C to 150°C,
b. forewarming milk to 80°C for 30 minutes,
c. adding β–lactoglobulin,
d. adding Ca^{2+} and/or Mg^{2+} ions, and
e. dialyzing type B milk against excess type A milk (24).

Milk is a colloidal suspension of casein micelles in a milk serum phase. Colloids are stable because a sufficiently large repulsive energy barrier exists between particles to prevent them from coagulating (34). Colloidal stability in milk is thought to be due to two constituents: 1) κ- casein and 2) colloidal calcium phosphate (24). κ– Casein with its macropeptide is the basis of the hairy micelle theory. According to this, steric interactions keep the micelles apart (29). Electrostatic components are also important in the stability of the casein micelle. Electrostatic repulsion is due mainly to the dissociated carboxyl and ester phosphate groups, which are negatively charged, on the casein molecules (50). When the macropeptide is enzymatically removed or destabilized with ethanol, coagulation does not proceed immediately if the temperature is below 20°C, implicating interactions other than electrostatic components in the stability of the micelle (24).

Combining the calculations for the van der Waals forces of attraction (dipole–dipole interactions) and forces of repulsion due to the electrical double layer between two charged particles leads to three distinct curves. These curves were independently calculated by Verwey and Overbeck, and Deryaguin and Landau, and are referred to as the DLVO theory (57). According to emulsion stability, the DLVO theory states that two colloidal particles have a potential energy, V (V+ is repulsive and V– is attractive), as a function of distance, h. The forces involved in casein micelle stability have not been quantitated

(electrostatic repulsion, van der Waals attraction, steric repulsion, and viscous resistance), but the presence of the free energy barrierVm (in a DLVO curve, by overcoming the free energy barrier there ensues an attractive potential between two charged particles which could result in coalesence), a primary mininum, (in a DLVO curve, the attractive force at an interparticle distance less than Vm), and secondary mininum (in a DLVO curve, when the distance between two sol particles is greater than Vm, there is an attractive force) are questionable. As casein micelles approach each other, steric repulsion probably dominates over van der Waals attraction. Furthermore, viscous resistance may be high because of the hairy micelle (protruding κ–casein) (51).

To understand casein micelle stability one must understand this repulsive energy barrier and, upon destabilization, what changes occur to reduce the repulsive energy barrier allowing the colloids to coagulate. These changes can either be environmental such as pH, ionic strenth, and Ca^{2+} activity; or particular such as colloidal calcium phosphate, dissociation and association of protein micelles, and dephosphorylation (50). According to Fox (21), the heat induced changes preceding coagulation include:

a. acid development,
b. precipitation of calcium phosphate,
c. Maillard reactions,
d. casein modifications, and
e. interactions of sulfhydryl groups (as whey protein denaturation).

All these reactions proceed concurrently, some being more important than others in their contribution to heat coagulation. Furthermore, products from one reaction may participate in, or influence, other reactions. Maillard browning reactions are pH dependent. Acid development due to heating in milk will slow or inhibit browning reactions (24). Whey protein denaturation on casein micelle

affects the stability of the casein micelle, but this stabilizing effect is dependent on the concentration of calcium phosphate (21).

According to Fox (21), the most important single factor in heat–induced coagulation is acidity because heat coagulation is delayed indefinitely if the pH of the heated sample is periodically adjusted to 6.7, regardless of all other heat-induced reactions taking place. However, because whey protein denaturation and calcium phosphate precipitation are complete within five minutes of heating at 140°C (heat coagulation at this temperature would occur in twenty minutes), their role, along with pH, in affecting heat stability should not be ignored (20).

pH Changes upon Milk Heating

As milk is heated, the pH decreases with increasing temperature and time; thus, electrostatic repulsion between micelles decreases (50). The rate of pH change appears to increase with increasing protein concentration (52). The longer a heated milk sample is held at 140°C, the lower the pH, at different temperatures, as the sample is cooled. The pH at each cooling temperature was determined by allowing the sample to equilibrate at that temperature for ten minutes before recording the pH. This process was repeated at 10°C intervals down to 20°C (21).

In another set of experiments, van Boekel et al. (50) state that pH does not change linearly with extended heating time. Initially, there is a rapid decrease in pH during the first two minutes of heating; then the pH decreases more slowly and linearly with time (50). Dalgleish et al. (11) report that eventually the pH decreases to the same value irrespective of the initial pH, implicating a faster rate of pH decrease in milks with high initial pH. van Boeker et al. (50) did not find that the pH tends to drop to the same low value irrespective of initial pH.

The drop in pH is thought to be due to three reactions taking place as a milk sample is heated. Fifty percent of the drop in pH is attributed to the production of organic acids, mainly formic acid, from lactose upon heating in the presence of oxygen (39). It is estimated that there is a decrease of .03 pH units per minute with a Q_{10} of approximately 1.8 (50). Lactose-free milks are more stable than normal milks in the pH range of 6.4-7.4 for both type A and type B milks. Lactose–free milk coagulates at prolonged heat coagulation times (HCT). At coagulation, milk pH decreases to 5.5-6.0 in normal milk. However, in lactose-free milk coagulation occurs at approximately pH 6.15 (corresponding to a higher HCT) (21). Hydrolytic dephosphorylation of casein with subsequent precipitation as tricalcium-diphosphate with release of H^+ is responsible for 30% of the drop in pH (39). Its importance is not well understood. The latter reaction is first order with respect to time, increasing with casein concentration. Dephosphorylation also increases with pH. The Q_{10} for this reaction is approximately 2. These two reactions (organic acid formation and hydrolysis of casein phosphate) are responsible for the gradual decline in pH observed after the first two minutes of heating at 140°C (50). Approximately twenty percent of the pH drop is due to precipitation of primary and secondary calcium phosphate as tertiary phosphate with the release of H^+ (39). These changes are reversible upon cooling depending on the severity of the heat treatment (36). The shift in calcium phosphate equilibrium is responsible for the fast initial drop in pH (50), and takes place in less than five minutes (42). Serum proteins do not significantly influence the change in pH (50).

The Q_{10} for acid development is approximately 2 whereas the Q_{10} for heat coagulation is 3 (20). With a 10°C increase in temperature, heat coagulation will proceed three times faster and acid development two times faster. However, it is thought that there are some heat–induced stabilizing changes

because milk preadjusted to pH 5.5 coagulates on heating at approximately 66°C (21). A sample preheated to 140°C for twenty minutes will coagulate at approximately 78°C at pH 5.5 (21). If milk is preheated for twenty minutes or more at 120°C, the milk will be stabilized in the pH range of 6.3-7.1. Furthermore, the preheated milk will have a type B HCT/pH profile (24).

Calcium Phosphate Precipitation

The solubility of milk serum calcium phosphate decreases with increasing temperature and decreasing pH. When milk is heated to 120°C, there is a sharp decrease in the solubility of calcium phosphate at approximately pH 6.8 which corresponds to the minimum in type A milk (10). In milk, micellar calcium is found as insoluble calcium phosphate. Casein has a number of ester phosphate and carboxylic groups available to bind calcium (36). It appears that the phosphate groups of the phosphoseryl residues in casein form part of the micellar calcium phosphate. Hence, in the micelles, the ratio of organic calcium to inorganic calcium is greater than 1, even though colloidal calcium phosphate has a structure resembling brushite (23). Despite the pH drop during heating, micellar calcium phosphate does not readily dissociate from milk casein, though it becomes more inorganic during heating as phosphoseryl residues are dephosphorylated (10).

The ionic calcium activity of milk depends on the initial pH of milk prior to heating. The pH decline during heating does not result in an increase in soluble calcium or ionic calcium activity. However, at constant temperature (i.e., 20°C) a decrease in pH is accompanied by an increase in ionic calcium activity. Only after heated milk is cooled is the ionic calcium concentration restored (50).

While in the serum, most of the calcium is complexed to citrate ions. The calcium phosphate salt is a calcium-phosphate-citrate complex: $3Ca_3(PO_4)_2 \cdot$

$CaHCitr^-$ or 2.5 $Ca_3(PO_4)_2 \cdot CaHPO_4 \cdot .5Ca_3Citr^-$ (21). It is thought that calcium phosphate precipitates upon heating as a hydroxy-apatite [$Ca_{10}(PO_4)_6(OH)_2$] (21). Citrate does not precipitate upon heating (18). The serum precipitated calcium phosphate may associate with the casein micelle (41) via carboxylic groups with the exchange of H^+. This could explain why precipitated calcium phosphate does not become a sediment. If the ζ–potential and hydration of the micelle were reduced, a surface layer of precipitated calcium phosphate on the micelle could easily destabilize the colloid (43). The precipitated calcium phosphate could also act as a sticking agent for micelles to aggregate if they stay together long enough (50).

Furthermore, ionic calcium influences the dissociation of κ–casein–β–lactoglobulin complex near the mininum pH in type A milk. As milk is heated, ionic calcium activity decreases because of calcium phosphate precipitation on micelles. Thus, the amount of calcium acting as a counterion to casein decreases, and the dissociation of κ–casein increases (50). If calcium phosphate does precipitate on the wall of the heating equipment (38), ionic calcium activity can be lowered in this way. However, a decrease in ionic calcium activity could lead to a stabilization factor with respect to κ–casein depleted micelles.

Salt bridges could lead to lasting contact between approaching micelles. Salt bridges can be formed between negatively and positively charged groups on peptide chains. Ionic calcium and colloidal calcium phosphate could mediate these linkages among peptides. The tendency to form salt bridges increases with decreasing pH (increase in ionic calcium activity). Salt bridge formation is independent of temperature in the range of 120°C and 140°C or of the pH drop occurring during heating. The latter may be attributed to the insignificant change in ionic calcium activity as a result of the pH decrease

during heating (55). When a coagulum of milk protein is formed via salt bridges, the aggregated micelles are dispersible by colloidal calcium phosphate dissolving agents (55).

Maillard Browning

Some investigators do not consider Maillard browning to be a significant factor in heat coagulation because little browning occurs during the period necessary to induce coagulation (20). However, Maillard browning may play a minor role (6). ε-Amino groups of lysine appear to be involved in a number of coagulation reactions (21, 43). About 15% of the lysine is rendered unavailable in milk heated for 20 minutes at 140°C at pH 6.7 (21). Products of Maillard browning reactions are extremely reactive, leading to polymerization reactions (32). Polymerization reactions are important in heat–induced coagulation (11, 50). Furthermore, the heat stabilizing influence of urea in artificial casein micelle systems is dependent on the presence of lactose or glucose (20). Ionic calcium activity increases when lactose is added to milk at its natural pH (31). Yet, if 5% lactose or 5% sucrose is added to lactose free milk and then heated, the calcium sensitivity of the casein isolates are not significantly different (21). Lactose addition to "normal" milk has a destabilizing effect (50), probably due to an increase in the rate of pH decline.

Effects of Heat on Whey Proteins

Whey proteins are heat labile and are completely denatured at:

a. 77.5°C when heated for 60 minutes,

b. 80°C when heated for 30 minutes, or

c. 90°C when heated for 5 minutes (21).

The thermal stability of the individual whey proteins in order of increasing stability are immunoglobulins < serum albumins < β-lactoglobulin < α-lactalbumin. Denatured whey proteins in milk will co-precipitate with casein when they are acidified, salted out, or ultracentrifuged. By sequestering calcium ions, casein fractions are thought to protect denatured whey proteins from precipitation (20). When κ–casein and β–lactoglobulin are heated together, they interact by sulfhydryl-disulfide interchange (37). Addition of κ–casein to a preheated sample of β–lactoglobulin will bind in the same way (50). The formation of the β–lactoglobulin–κ–casein complex in milk is thought to affect the heat stability of milk (44). Furthermore, the ratio of β–lactoglobulin to κ–casein may be more important in heat stability than the actual concentration of these proteins. This suggests that the surface characteristics of the micelle are critical in heat stability (52). α–Lactalbumin and β–lactoglobulin interact and form a complex which appears to associate with κ–casein (24). When heated, α–lactalbumin by itself has a similar effect on the casein micelle proteins as β–lactoglobulin. α–Lactalbumin contains no sulphydryl groups, but it does have four disulfide bonds per mole which are thought to act as active sites for complex formation with the casein micelle or β–lactoglobulin (22).

After heating milk for 1 minute at 140°C, 99% of the β–lactoglobulin and about 90% of the α–lactalbumin will have reacted. This denaturation and complexing reaction proceeds fast and is not a rate determining step in heat coagulation (50). The denatured whey protein–casein micelle complex

dissociates at pH greater than 6.8, and it is thought to be related to the minimum in the HCT/pH curve of Type A milk (24). When the pH of the milk is below 6.6 before heating, serum proteins will complex with casein micelle proteins. If the pH of the milk is greater than 6.9 before heating, serum proteins will complex with caseins in the serum phase. At pH's between 6.6 and 6.9 before heating, serum proteins will complex with both serum phase caseins and casein micelles (50). High calcium concentrations increase the association of serum proteins with casein. If calcium is sequestered, the serum proteins remain in the serum (47). Bovine serum albumin destabilizes the casein micelle (21).

Heat–Induced Changes in Casein

In the temperature range of 80°C-150°C, the following changes are noted:

a. dephosphorylation,
b. proteolysis,
c. covalent bond formation,
d. hydration,
e. zeta potential, and
f. structural changes in the casein micelle.

With increasing temperature inorganic phosphate is released (36). Dephosphorylation proceeds faster than the formation of TCA-soluble nitrogen (20). It is estimated that 12% dephosphorylation of milk casein takes place at 120°C in 90 minutes (21). Dephosphorylation in skim milk casein conforms to first order kinetics in the temperature range of 110-140°C, and it is independent of pH at 6.0-7.0 (20). Nevertheless, the effects of dephosphorylation are slow (4). Both α– and β–caseins are dephosphorylated. Dephosphorylation reduces the protein charge, which may contribute to coagulation, because of increased attractive forces between micelles. As a result, casein micelles may

become less voluminous. At constant micellar mass, a more voluminous micelle will have weaker van der Waals attraction. However, dephosphorylated micelles will bind less colloidal calcium phosphate which could cause the micelles to dissociate (50). It is known that dephosphorylated casein is much more heat labile and binds less Ca^{2+} (20).

Of the total protein nitrogen in milk, 10-20% is converted to non-protein nitrogen (NPN) in 60 minutes when heated at 135°C, and formation of NPN is linear with time (21). The electrophoretic pattern and elution profile of casein on Sephadex and Biogel are altered after heating (21). Heating whole casein or κ-casein causes the release of a macropeptide similar to the one produced when κ-casein is treated with chymosin. The glycopeptide released upon heating has a lower carbohydrate to nitrogen ratio and contains D-mannose, unlike the glycopeptide released with chymosin treatment. Thus, heat hydrolyses different bonds (21). About 20-30% of κ-casein is hydrolyzed up to the point of heat coagulation (43).

Severe heat treatment may not only promote proteolysis of casein but also polymerization of polypeptides (43). Milk, heated for 10–20 minutes at 140°C, results in the formation of covalent bonds (50). According to some investigators (50), polymerization of proteins is involved in heat coagulation because the coagulated material is not redispersible. The reactivity of some of the side chains of amino acids increases with temperature; lysine is especially reactive. At increased pH, the formation of lysinoalanine proceeds with heating (43). The heat coagulation time due to polymerization reactions is dependent on initial pH and the rate of pH decrease, but not on protein concentration. The Q_{10} is approximately 3 (55). Via intra- and intermolecular bonds, reactive groups (i.e., lysinoalanine, lanthionine or isopeptides) may combine with carbohydrates, lipids, and other residues (such as Maillard products) (43).

Intermolecular bonds could involve the κ–casein tail, the deposited serum proteins on the micelle, or the precipitated calcium phosphate associated with the micelle. However, results show that reactive groups are formed faster at higher pH's (Maillard reaction, formation of lysinoalanine, lanthionine, and isopeptides); yet, covalent bond formation among heated micelles is favored as the pH decreases. This suggests that, depending on the pH of heated milk, intramolecular bonds would be favored at high pH's and intermolecular bonds would be favored at low pH's (50).

Micellar Structure Changes upon Heating

Hydration of the micelle is pH-dependent and is affected by micelle size or calcium concentration or by micelle size and calcium concentration(43). Micellar hydration decreases with decreasing pH (9). There appears to be a positive correlation between HCT and casein micelle hydration (21). The latter is expected since casein micelle formation and stability are related to hydration. Hydrated ionic and zwitterionic species orient water to give an overall repulsive force (5). The repulsive force between charged groups is a consequence of the decreased enthalpy of water since work is required to remove water from the vicinity of the charged groups (5). Hydrophobic surfaces orient water molecules so that a co-operative attraction follows. This attractive force is entropically driven (5). The difference between the serum phase and casein micelle is less as micellar hydration increases (55), van der Waals attraction decreases, and micellar stability increases (50). Closely related to micellar hydration is the ζ–potential of the micelle, both being important in micelle stability as mentioned previously. The ζ– potential in the casein micelle is principally due to the glycopeptide region of κ–casein and the charged sections of α_s– and β–casein (21). Organic calcium and phosphate are also thought to affect the

electrokinetic potential. A decrease in organic calcium phosphate will increase the absolute value of the ζ– potential (55). The ζ– potential during heat treatment may be expected to decrease (become less negative) because of:

a. hydrolysis of κ–casein,
b. dephosphorylation of α_{s1}–, α_{s2}– and β– casein,
c. pH reduction,
d. calcium phosphate precipitation, and
e. Maillard browning (21).

However, the ζ– potential of milk heated to coagulation temperature does not change significantly implying that, at a constant pH, the electrokinetic potential of the casein micelle may increase (absolute value) (12). Therefore, the stability of micelles to heat may be evaluated in terms of hydrophobic (hydrophobic associations due to the entropic forces that bring hydrophobic groups together because of their hydrophobicity) and van der Waals interactions rather than electrostatic interactions (43).

The bonds between the molecules in a submicelle are both hydrophobic and electrostatic (51). Hydrophobic associations are partly responsible for casein micelle structure (54). At cool temperatures (< 5°C), β–casein will partly dissociate from the micelle because hydrophobic associations are weaker (13). At temperatures near boiling, hydrophobic associations are weakened or absent. This weakening is observed as soon as high temperatures are reached (50). Casein micelles seem to become more flexible at temperatures above 70°C (55). Because hydrophobic interactions are lessened, electrostatic interactions dominate (even more at high pH's); hence, the micelle may start to disassociate. Experimental data suggest that κ–casein does disassociate upon heating. β–casein should also dissociate similarly (50). However, because the turbidity of milk heated to 120°C does not significantly differ from that of milk at

20°C, this suggests that micelles keep their structural integrity at high temperatures (50).

Changes in casein micelle structure upon heating involve not only dissociation, but also association phenomena. When heated to 90°C whey proteins aggregate with little casein micelle changes (43). It is thought that in UHT processing, casein micelle aggregation is due to a combination of factors:

a. whey protein denaturation,
b. whey protein complexing with micellar surface, and
c. increase in micellar calcium (43).

During heating between 90-140°C, there is an increase in casein micelle size with an accompanied increase in number of small casein micelle particles (8). This may be due in part to the disaggregation of casein micelle and solubilization of micellar calcium. When milk is heated at 140°C, the following changes are noted at pH greater than 6.8:

a. initial association where viscosity increases and sedimentable casein increases,
b. followed by dissociation of casein micelle with observed decrease in viscosity and increase in soluble protein, and
c. coagulation with rapid increase in viscosity (43).

κ-Casein will dissociate upon heating milk and is pH-dependent, there being more solubilization at or above pH 6.9 and less solubilization at or below pH 6.5. The Q_{10} in the range of 90-120°C for the dissociation of κ–casein in skim milk at its neutral pH is 1.3 (53). β–Lactoglobulin prevents κ-casein solubilization at pH below 6.7, but not at pH greater than 6.9, when heated. β–Lactoglobulin promotes the dissociation of κ–casein after heating for 10 minutes at 90°C at pH 7.3 (45). Increased dissociation at pH >6.8 is mainly due to a change in β–lactoglobulin occurring at pH >6.8 when heated (15).

Electrostatic interactions are probably involved in κ–casein dissociation because anionic detergents intensify and cationic detergents reduce heat–induced dissociation of κ–casein regardless of the pH . Furthermore, dissociation appears to depend on some non-protein serum component (50). Increasing the negative charge on micellar surface is known to decrease hydrophobic interactions and κ-casein dissociates due to electrostatic repulsion (43). The structure of the heated micelle after cooling is different because once dissociated, the micelle is less capable of binding casein in the cold (50). Furthermore, susceptibility to ionic calcium precipitation is also changed.

Kinetics of Micellar Casein Heat Induced–Coagulation

Aspects of the polymerization behavior of small molecules are mathematically modeled using the theory of branching processes (34). According to this theory, the number of reactive sites on a monomeric unit, functionalities, determines the nature of the polymer:

a. one functionality will form a dimer,
b. two functionalities will form a polymeric chain with two unreacted groups per polymer molecule, and
c. three or more functionalities (f) will form a branched polymer, and the number of free functionalities will equal ($fx - 2x + 2$), where x is the number of monomers (34).

When one applies the theory of branching processes describing polymer condensation and gelation, heat coagulation curves of milk may be modeled (34). In this model the aggregating micelle is a trifunctional unit. The trifunctional unit is characterized by a lag stage followed by a fast precipitation and the process follows a single reaction scheme (34). In type B curves, and

type A curves above or below the minimum, single–step depletion curves are observed and the kinetic model of heat–induced coagulation is in agreement with the theory of branching process (33). In type A curves within the minimum, two-stage nitrogen depletion curves are observed. The first sign of visual coagulation corresponds to the first drop in the nitrogen depletion curve. The second drop is not visual (33). The branching process is modified to fit this behavior by making the following assumptions:

a. there exist two distinct molecules, a fast coagulating molecule and a slower one, and
b. each type of molecule is a trifunctional monomer reacting with its own kind with little or no cross reaction products (35).

As to the nature of the reactive groups, according to van Boekel et al. (51) at high temperatures two types of casein micelles can exist:

a. particle with serum proteins attached, and
b. particle with κ–casein dissociated.

At pH ≥ 7.0 the micelles are depleted; when the pH is < 6.7, casein micelles are coated with serum; if the pH is between 6.7 and 7.0, a transition zone exists. There are at least two reactions to which the particles are subject: reaction I is a salt induced coagulation, and reaction II is an unknown reaction (probably protein polymerization). Reaction II determines the actual coagulation time. However, at pH ≤ 6.35 a different situation may exist because the high ionic calcium concentration may render the casein micelles unstable. In this case, a rapid increase in aggregate size is observed, and reaction I probably predominates. van Boekel et al. (51) analyzed the changes in optical density observed during heating of milk at 140°C in terms of W, a stability factor (the higher the stability of a particle the larger the value of W). When W is plotted against time (for milks at initial pH > 6.35), W is constant for the first 10–20

minutes, and a slow coagulation is observed. This slow coagulation may be due to reaction I. Once a critical pH is reached, the casein particle becomes unstable, W rapidly decreases, and fast coagulation occurs.

Above pH 6.35, a heat coagulation–time curve may be divided into three regions. Region I (pH < 6.7) casein micelles with deposited serum proteins are subjected to both reactions I and II. The heat coagulation time depends on the initial pH, the rate at which the pH is lowered, and on the initial ionic calcium activity. The rate-determining reaction in this region is reaction II. In region II ($6.7 \leq$ pH ≤ 7.0) the depleted casein micelle coagulates prematurely due to reaction I. Region III (pH > 7.0) depleted casein micelles are not destabilized because the ionic calcium activity is low; casein micelles associate as the pH decreases during heating, and the particles then behave as in region I (50).

Theory Explaining the Minimum in a Type A Curve

The mechanism proposed by Singh and Fox (44) is based on the formation of a β–lactoglobulin and κ–casein complex. This complex is known to dissociate above pH 6.9 and is thought to make the casein micelle calcium sensitive resulting in the heat coagulation of type A milk at pH approximately 6.9. Because at pH greater than 7 micellar charge increases and ionic calcium decreases, the heat stability of the sample increases once again (24).

However, a plot for N-acetyl neuramic acid (an indicator of κ–casein) parallels that for total nonsedimentable nitrogen up to pH 6.8. As may be observed from a HCT/pH profile of a type A curve, the casein micelle becomes destabilized before the dissociation of the complex is recorded. According to Horne and Muir (24), destabilization is due to the formation of the β–lactoglobulin–κ–casein complex formation. Urea is known to increase the heat

stability of milk when a single stage coagulation occurs as in type B milk or type A milk outside the minimum (11). Within this range, urea may help by buffering milk from the acid produced during heating. Another mechanism of protection is by interacting with κ–casein. Urea is incorporated into the protein after heating via the sulfhydryl groups of proteins and cyanate (a product of urea upon heating). Outside the minimum, the reaction of urea and κ–casein is favored and formation of a cyanate–κ–casein complex proceeds. In this region the heat stability of casein micelle is positively correlated with urea levels. Inside the minimum the β–lactoglobulin–κ–casein reaction is favored and only shift when urea levels are extremely high. At pH 6.8 β–lactoglobulin dissociates (from a dimer to a monomer) and undergoes conformational changes which make available 1) the free sulfhydryl group available upon dissociation of the dimer, and 2) the four other sulfhydryl groups participating in disulfide bonds within the monomer. It is the formation of a β–lactoglobulin–κ–casein complex that makes the micelle unstable at pH 6.8 rather than the dissociation of the complex in this theory. By making the κ–casein glycopeptide rigid, the β–lactoglobulin–κ–casein complex is able to form crosslinking reactions which would neutralize the steric stabilization activity of the hairy micelle, making the micelle sensitive to calcium moderated precipitation (24).

MATERIALS AND METHODS

Materials

Milk (SM) from the Utah State University dairy farm was skimmed and vat pasteurized (63°C for 30 min) at the university dairy plant. Commercial pulpless, standard frozen orange juice (OJ) concentrate (Minute Maid®) was purchased locally. The SM/OJ blend was 85% (w/w) skim milk and 15% (w/w) reconstituted OJ. To this blend, I added .25% (w/w) microcrystalline cellulose (MCC) (Avicel® RC 591F MCC co–dried with sodium carboxymethylcellulose, FMC, Philadelphia PA) and .025% (w/w) carrageenan blend (SeaKem CM 611, FMC Marine Colloids, Philadelphia, PA). Preliminary trials included the use of other stabilizers and these were 1) TIC Gum (.5% w/w) (Belcamp, MD), Gum Colloid 1084T (a system of natural hydrocolloids), 2) Grinsted (Industrial Airport, KS) Mexpectin R5450 (.5% w/w) with FMC Sea Kem CM 611 (.02% w/w), 3) Kelcoloid-LVF (.5% w/w) (a propylene glycol alginate) by Kelco (Chicago, IL), or 4) Genu® Pectin JMJ (.25% w/w) by Hercules (Middletown, NY) with FMC Sea Kem CM 611 (.025%).

Preparation of Skim Milk/Orange Juice Blend

Stabilizer Incorporation. The stabilizer(s) was(were) blended with approximately 25% of the water necessary to reconstitute the OJ concentrate using a household blender (blending was for approximately 10 minutes). The hydrated stabilizer(s) was (were) added either to milk heated to 60°C for 30 minutes or to cold milk. Heated stabilizer/milk samples were cooled to 4°C before combining with the reconstituted orange juice. When the stabilizer(s) was(were) incorporated without heating, the hydrated stabilizer(s) was(were) mixed into the SM/OJ mix with a high speed, high shear recirculating pump.

Reconstituted Orange Juice Incorporation. SM/OJ blends of a final pH of 6.1, 6.3, or 6.5 were made. The thawed OJ concentrate (approximate pH 3.8) was adjusted to pH 4.0 or 4.5 with 1 N NaOH for two (pH 6.3, and 6.5) of the three pH treatments. The pH-adjusted OJ concentrate was diluted with the remaining 75% water (minus the volume of NaOH used) required to fully reconstitute the OJ. For the third treatment, the pH of the orange juice concetrate was not adjusted and was reconstituted with the remaining 75% water (Table 1). The reconstituded OJ was manually added to the milk to avoid protein precipitation. The SM/OJ samples (with stabilizer) were refrigerated overnight and then UHT processed with or without prior homogenization (4°C, 500 psi single stage) prior to UHT processing (Figure 1).

UHT Processing

Direct Heat Exchange. The SM/OJ drink blends were UHT processed by direct heat exchange (steam injection) using an Alfa–Laval Sterilab® UHT pilot plant. The sample blends were preheated in a plate heat exchanger to 77°C and UHT treated by direct steam injection to 140°C and held for 4 s. The sample blends were vacuum cooled (flash evaporation) to 71°C and cooled to 14°C by plate exchangers. SM/OJ blend samples were aseptically collected under an Alfa–Laval Stericab™ hyperfiltered, positive pressure air chamber (Figure 2). Blend samples were packaged in pre-sterilized, 120 ml polypropylene containers.

TABLE 1. Orange juice (OJ) preparation.

Reconstituted OJ	1	2	3
Parts OJ Conc. (pulpless)	1	1	1
Parts water	3	3	3
• stabilizer mix[1]	yes	yes	yes
• 1 N NaOH	no	yes	yes
pH adjustment	no	yes	yes
Approx. OJ conc. pH	3.8	4.0	4.5
Approx. drink pH	6.1	6.3	6.5

[1]Hydrated separately

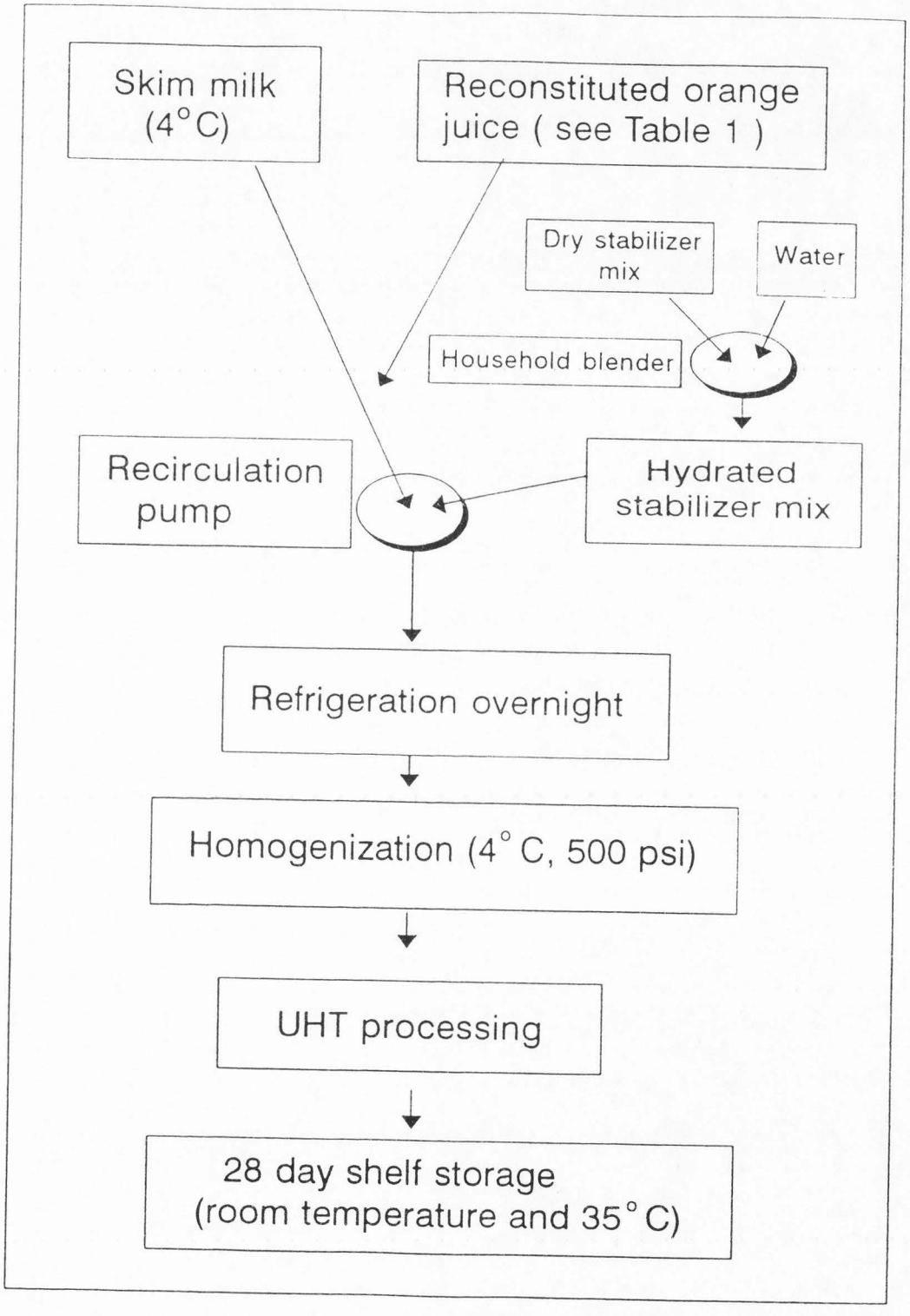


Figure 1. Flow chart for drink preparation.

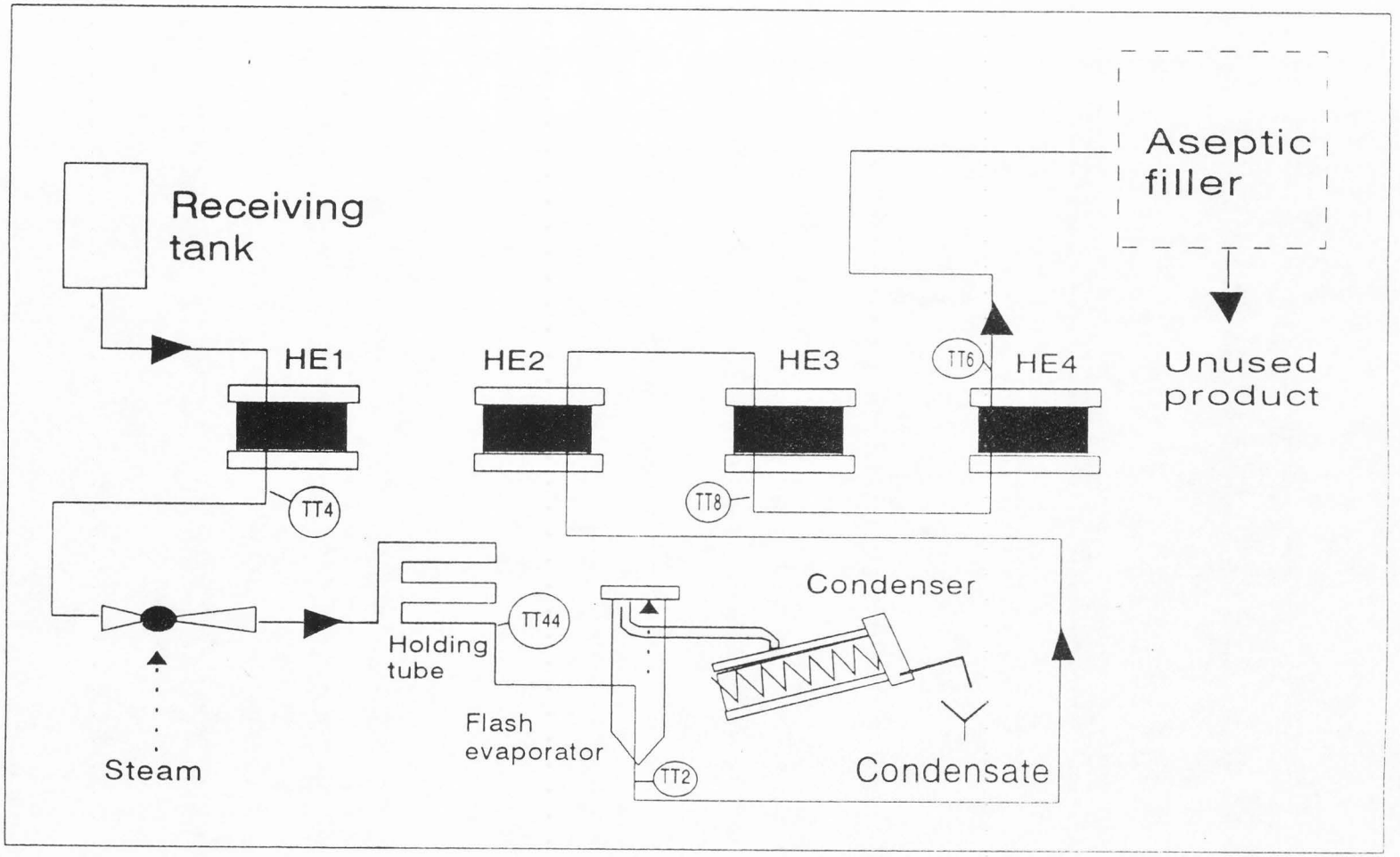


Figure 2. Direct UHT system product flow where HE = heat exchanger, and TT = temperature transmitter.

Indirect Heat Exchange. Using the above mentioned pilot plant, the system was plumbed for indirect heat exchange. Plate heat exchangers were used to preheat sample blends to 77°C and UHT-treated to 140°C and held for 4 s. The sample blends were cooled to 14°C by plate exchangers. Samples were collected as for the direct UHT processed samples.

Sample Storage and Analytical Methods

Samples were stored at room temperature (RT) and 35°C. The pH, titratable acidity, and percent top surface clearing of undisturbed samples were recorded every 7 days for a 28–day period. The pH measurements were made at RT, and measurements were made as soon as the pH meter indicated a variation of less than ± 30 mV. Percent clearing was the ratio of the height of the top surface clear zone to the total sample height multiplied by 100. The drink blends prior to and a day after UHT processing were viewed under a phase contrast microscope.

Sensory Analysis

Untrained judges (18 years and older), representing a general consumer population, participated in the sensory panels. Taste panel facilities at the university's Department of Nutrition and Food Sciences were used. Partition booths in a closed area are available with sufficient ventilation. Each booth has flworescent lights (white or red) positioned to reduce glare. The judge and taste panel coordinator communicate through a sliding metal window.

RT samples stored for 28 days were used for sensory texture evaluation. A paired comparison test was conducted under red lighting. Random numbers were used to label the samples. Forty (40) panelists compared the three pH treatments in all possible combinations (three sets per judge). Every other

participant evaluated the drinks in alternate order (Appendix A). A single serving size was 10 ml of the chilled (4°C) drink blend.

In a second evaluation, a nine point hedonic scale (9 being "most liked," 5 being "neither liked nor disliked," and 1 being "least liked") was used to determine the consumer acceptability of SM/OJ drink (pH 6.5) containing 2.5% (w/w) sugar that had been stored at room temperature for 7 days (Appendix B). The appearance, flavor, texture, and overall acceptability of the single chilled sample was rated by 83 panelists. The sample size was 10 ml.

Statistical Design

UHT Processed Test Material. A complete random block with a split–split plot design was used for analysis of variance using least squares means. The following model was used:

$$Y_{ijkl} = \mu + R_i + T_j + \varepsilon_{ij} + S_k + TS_{jk} + \gamma_{ijk} + D_l + TD_{jl} + SD_{kl} + TSD_{jkl} + \delta_{ijkl}$$

where Y_{ijkl} is the dependent variable (pH, titratable acidity, or percent clearing), μ is the overall mean of the population, and independent variables R_i, T_j, S_k, and D_l are the coefficients for the averages of replication, treatment pH, storage temperature, and day effects. Two–way interactions (TS_{jk}, TD_{jl}, and SD_{kl}) and a three–way interaction (TSD_{jkl}) are included. The data set comprised four replications (i = 1, .. 4), three treatment pH's (j = 6.1, 6.3, 6.5), two storage temperatures (k = RT, 35°C), and five 7-day intervals (k = 0, 7, 14, 21, 28). The whole plot error (ε_{ij}), subplot error (γ_{ijk}), and sub-subplot error (δ_{ijkl}) were assumed to be normal and independently distributed with zero expectation and common variance σ^2. Fisher's least significant difference was used to separate

means. Analysis of variance was performed using release 7.2 of Minitab© (State College, PA).

Sensory Test Method. Sensory evaluation data concerning texture were analyzed by tallying correct answers and calculating the significance of the results using chi–square. Hedonic ratirıg data were evuluated using frequency distributions with reported means, sample standard deviations, and 95% confidence intervals. Linear correlation between overall acceptability and appearance, flavor, or texture was determined.

RESULTS AND DISCUSSION

Drink Blend Preparation

Regardless of the stabilizer type and level, blend preparation, or UHT processing, milk proteins precipitated when blend pH was less than 6.4. FMC-Avicel® MCC was the only stabilizer with practical application in the UHT processed SM/OJ drink. The prehomogenized SM/OJ blend containing .25% FMC MCC and .025% FMC Sea Kem CM 611 (added to increase creaminess) maintained the milk proteins of the UHT–processed blend in suspension throughout the 28–day shelf storage study. Homogenization of milk (with milk fat) decreases heat stability (52). This is because casein becomes adsorbed onto the fat globule, and the fat globules then tend to behave as if they were casein micelles (52). The effect of homogenization in our product was probably to efficiently incorporate the stabilizer into the colloidal system, even though the MCC stabilizer used required low shear for incorporation (as obtained with a blender). However, because pectin was not able to perform similarly, even after prehomogenization, there may be other factors involved. Increased hydration of protein molecules may be possible by formation of a protein–polysaccharide complex. The polysaccharide may also bind water molecules and retard settling of precipitated proteins.

UHT Processing of Drink Blend

Skim milk/orange juice drink with satisfactory shelf storage must be subjected to minimal heat treatment for commercial sterility. The heat load in indirect UHT processing is more intense than in direct UHT processing due to a longer residence time of the product in the system. Direct UHT processing involves large temperature gradients when live steam is injected into the

product and vacuum evaporated (expansion cooling) (26). To ensure that the milk drink was not concentrated or diluted during processing, an energy–mass balance was calculated for each run (Appendix C). However, heat losses to the surrounding atmosphere were not considered, and the theoretical value was not large enough to remove all the steam that was injected. Furthermore, because of the presence of hygroscopic components, such as stabilizers, the removal of the steam may have been affected. In our results, it was determined that the temperature difference needed between the preheat temperature and the vacuum evaporator temperature was approximately 5.6°C (10°F), to obtain a product of the same total solids prior to UHT processing.

Fouling in indirect UHT processing increases with reduced pH milks. At pH below 6.35, UHT processed samples will coagulate (46). This was also observed in my results. I was unable to process the skim milk/orange juice blends of pH 6.3 and 6.1 by indirect UHT because the system flow was stopped within minutes. The increased deposit formation of proteins is due mainly to the heat exposure (though whey protein denaturation is unchanged) (46). Milk with initial pH above 6.35 and below normal milk pH (approximately 6.67) during indirect UHT processing will deposit mostly protein, with reduced mineral fouling, onto the heat exchanger walls (46). Flakes were seen in collected milk samples processed by indirect heat exchange with initial pH at 6.5. This could be precipitated material coming off the heat exchanger plates. However, transfer of ionic calcium to colloidal calcium may be affected during the UHT treatment. Insufficient time for transfer of ionic calcium may lead to localized high levels of ionic calcium with subsequent protein precipitation (46). Fouling with the skim milk/orange juice drink pH 6.5, processed by indirect UHT, increased with time, and eventually fluid flow was restricted, particularly in the second heat exchanger where the product was heated to sterilization

temperature. Heat processing by direct UHT was feasible, though the flow rate did drop by the end of the run.

Drink Blend pH

Treatment pH, storage temperature, day, and storage temperature-day interaction were statistically significant (Table 2). The decrease in drink pH over time in the RT samples was not statistically significant within treatment pH. There were some significant differences across days within treatment pH in samples stored at 35°C (Figure 3B). Storage temperature sometimes affected the pH of various pH treatments (Figures 3A and 3B). This was true for treatment pH 6.1 on all days after day 0 and all treatments on day 28. In these cases a higher storage temperature had a lower pH. Treatment pH 6.1 and 6.3 within storage temperature and day statistically differed only on day 21. Treatment pH 6.5 was consistently different from the other pH treatments within storage temperature and day (Figures 3A and 3B). Some of these pH decreases could be attributed to the milk buffering system reaching equilibrium.

Drink Blend Titratable Acidity

Titratable acidity is another way to determine the concentration of acids in a sample (2). However, because the acidic groups of the SM/OJ drink blend were unknown, the exact concentration of acids in the drink remained unknown. At best, we were able to determine the equivalents of base needed to reach a phenolphthalein end point. Values are reported as milliliters of NaOH (.100 ± .0001 N) to titrate ten milliliters of SM/OJ sample. Treatment pH was the only statistically significant source of variability in titratable acidity (Table 3). For the

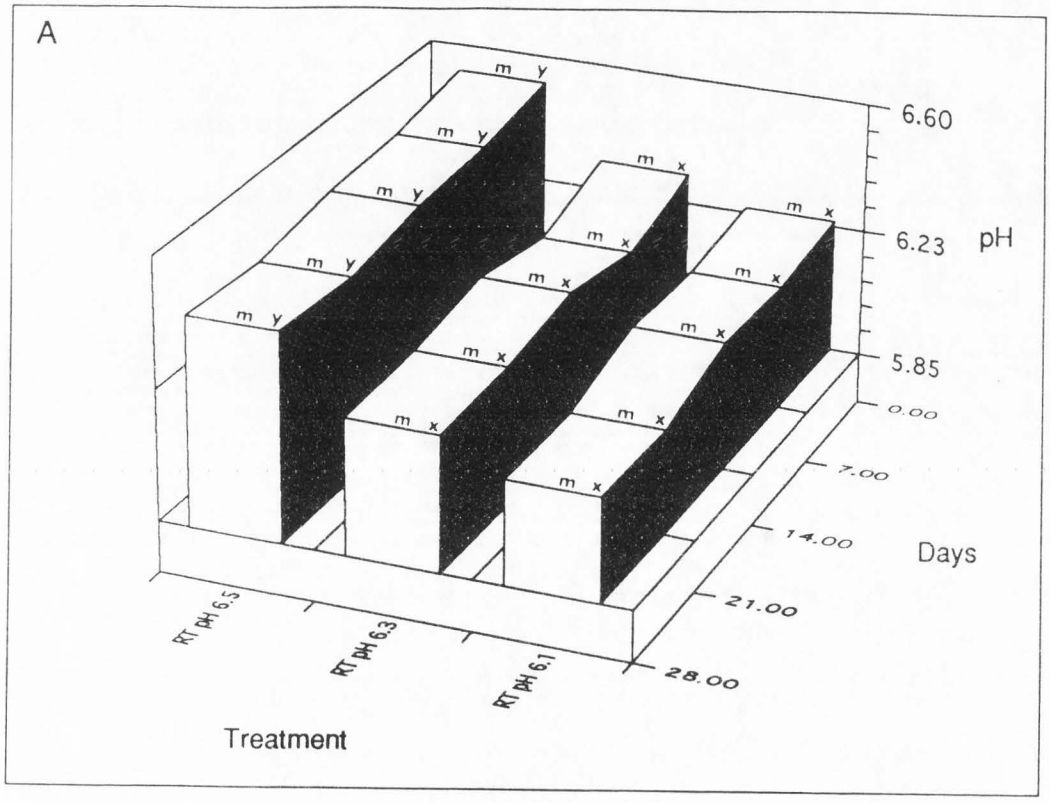
TABLE 2. Analysis of variance for the dependent variable pH[1].

Source	df	Sum of squares	MS	*F* - test
Rep.	3	.04	.01	2.80
Treatment pH (T)	2	1.68	.84	157.36**
Error (a)	6	.03	.01	
Storage Temp. (S)	1	.23	.23	67.85**
T x S	2	.00	.00	.49
Error (b)	9	.03	.00	
Days (D)	4	.32	.08	20.40**
T x D	8	.01	.00	.47
S x D	4	.07	.02	4.25**
T x S x D	8	.01	.00	.38
Error (c)	72	.29	.00	

1 Total df = 119

* $P \leq .05$

** $P \leq .01$


A
6.60
6.23
pH
5.85
0.00
7.00
14.00
21.00
28.00
Days
RT pH 6.5
RT pH 6.3
RT pH 6.1
Treatment

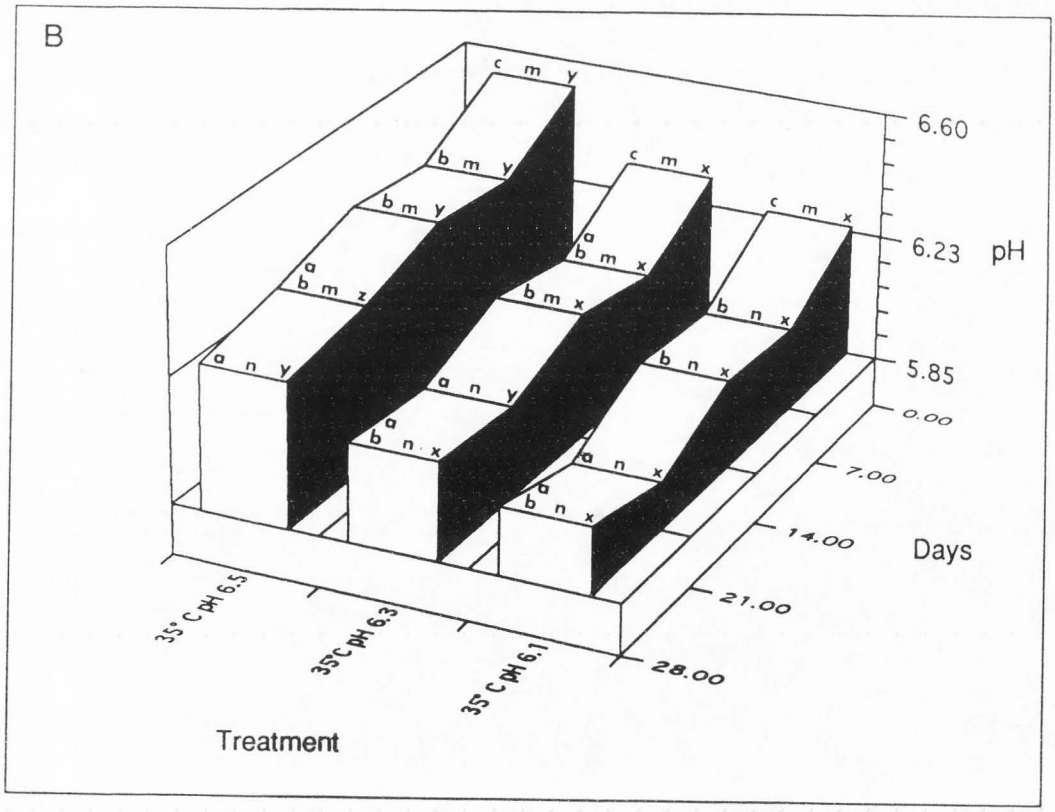

B
6.60
6.23
pH
5.85
0.00
7.00
14.00
21.00
28.00
Days
35° C pH 6.5
35°C pH 6.3
35° C pH 6.1
Treatment

TABLE 3. Analysis of variance for the dependent variable titratable acidity[1].

Source	df	Sum of squares	MS	*F* - test
Rep.	3	.71	.24	4.73
Treatment pH (T)	2	6.76	3.38	66.77**
Error (a)	6	.30	.05	
Storage Temp. (S)	1	.02	.02	2.17
T x S	2	.00	.00	.03
Error (b)	9	.08	.01	
Days (D)	4	.06	.02	1.65
T x D	8	.07	.01	.95
S x D	4	.06	.01	1.51
T x S x D	8	.03	.00	.42
Error (c)	72	.71	.01	

1 Total df = 119

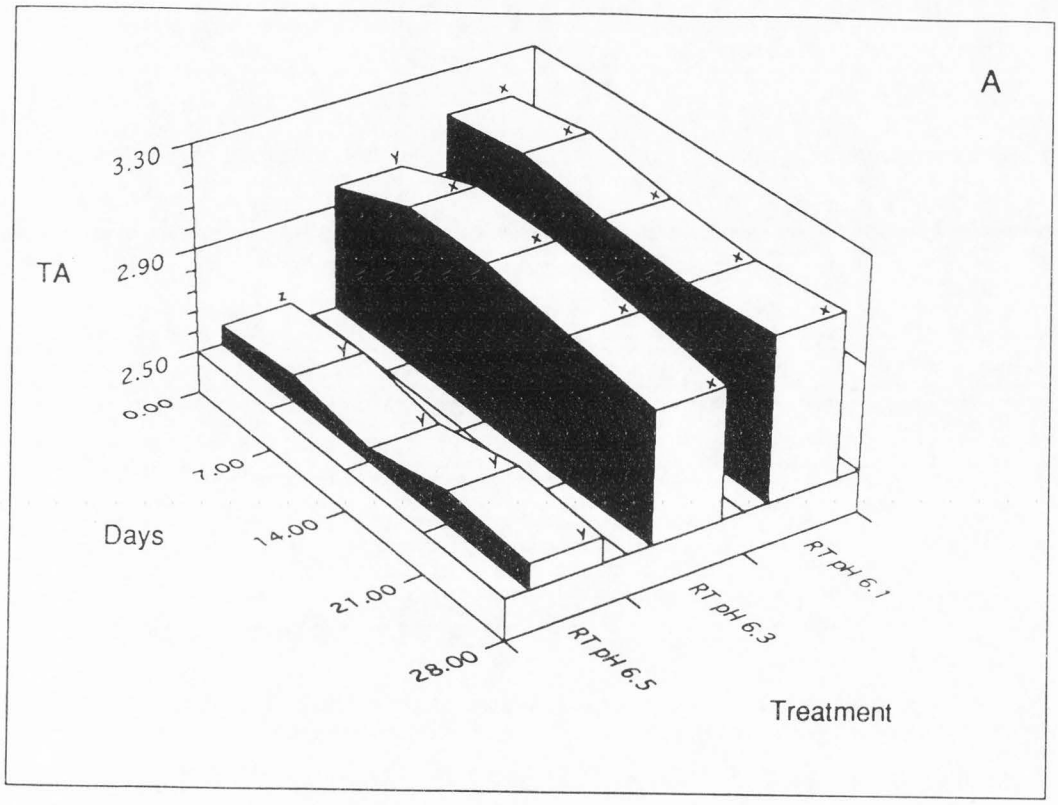
* $P \leq .05$

** $P \leq .01$

most part, titratable acidity did not differ from one time interval to the next for a treatment pH and fixed storage temperature (Figures 4A and 4B). Storage temperature did not affect the titratable acidity of a treatment pH for a given day. Yet, treatment pH 6.5 on a fixed day differed in titratable acidity from treatment pH 6.1 and pH 6.3 for each storage temperature (Figures 4A and 4B). Treatment pH 6.1 and 6.3 only differed in titratable acidity of day 0 (Figures 4A and 4B). Storage temperature did not affect the titratable acidity measurement as much as it did pH measurements. Hydrogen ion activity varies with temperature (19). Although the samples stored at 35°C were allowed to come to room temperature before pH measurements were made, and the pH meter had a temperature compensating unit, the drink was a dynamic system where an equilibrium state was not reached. This was evident because the pH continued to coast down even after the pH meter had registered pH variations of less than ±30 mV. The acidic groups in the drink blend should remain constant regardless of storage temperature; hence, tritratable acidity should not change either.

Drink Blend Percent Clearing

Treatment pH, storage temperature, day, and treatment pH-day interaction all affected percent clearing (Table 4). In pH treatment 6.1, the percent clearing was 5.2% (RT) or 7.3% (35°C) in homogenized drink blends compared to an average of 87.5% in nonhomogenized drink blends. Percent clearing varied significantly across storage temperature, within day and treatment pH, and across days, within storage temperature and treatment pH (Figures 5A and 5B). There were some differences across treatment pH within storage temperature and day: 1) at RT storage, day 21 and 28 , treatment pH 6.1 and 6.3 were


A
TA
3.30
2.90
2.50
0.00
7.00
14.00
21.00
28.00
Days
RT pH 6.5
RT pH 6.3
RT pH 6.1
Treatment

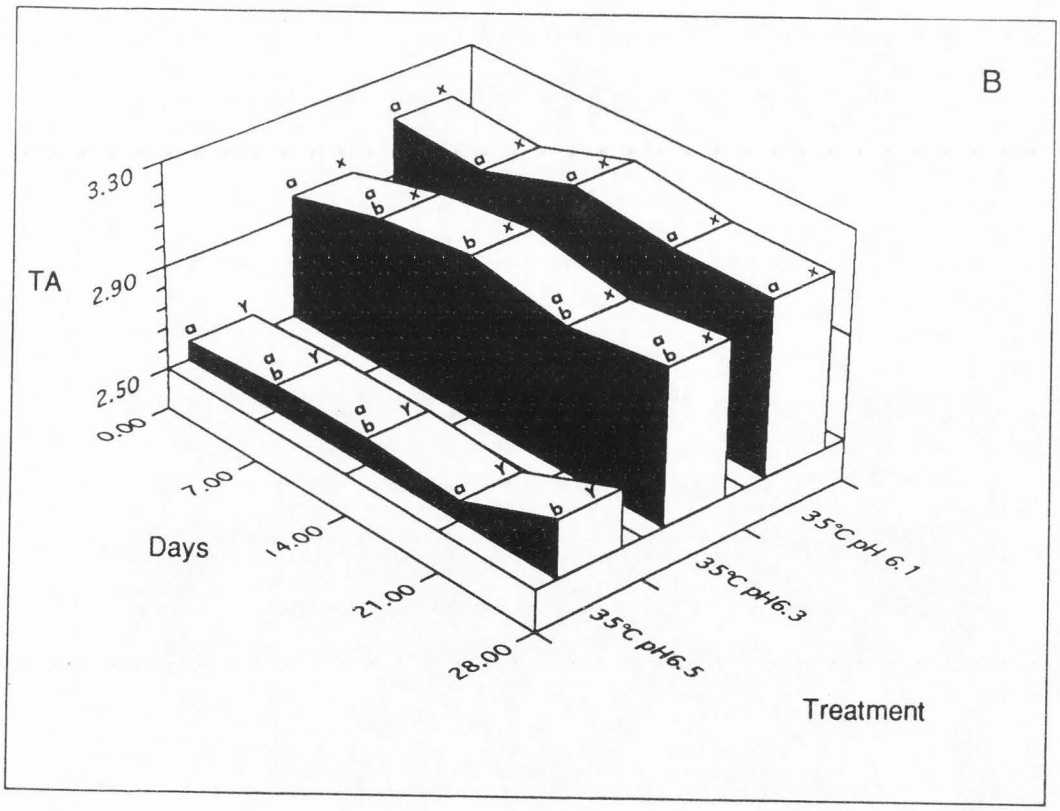

B
TA
3.30
2.90
2.50
0.00
7.00
14.00
21.00
28.00
Days
35°C pH6.5
35°C pH6.3
35°C pH 6.1
Treatment

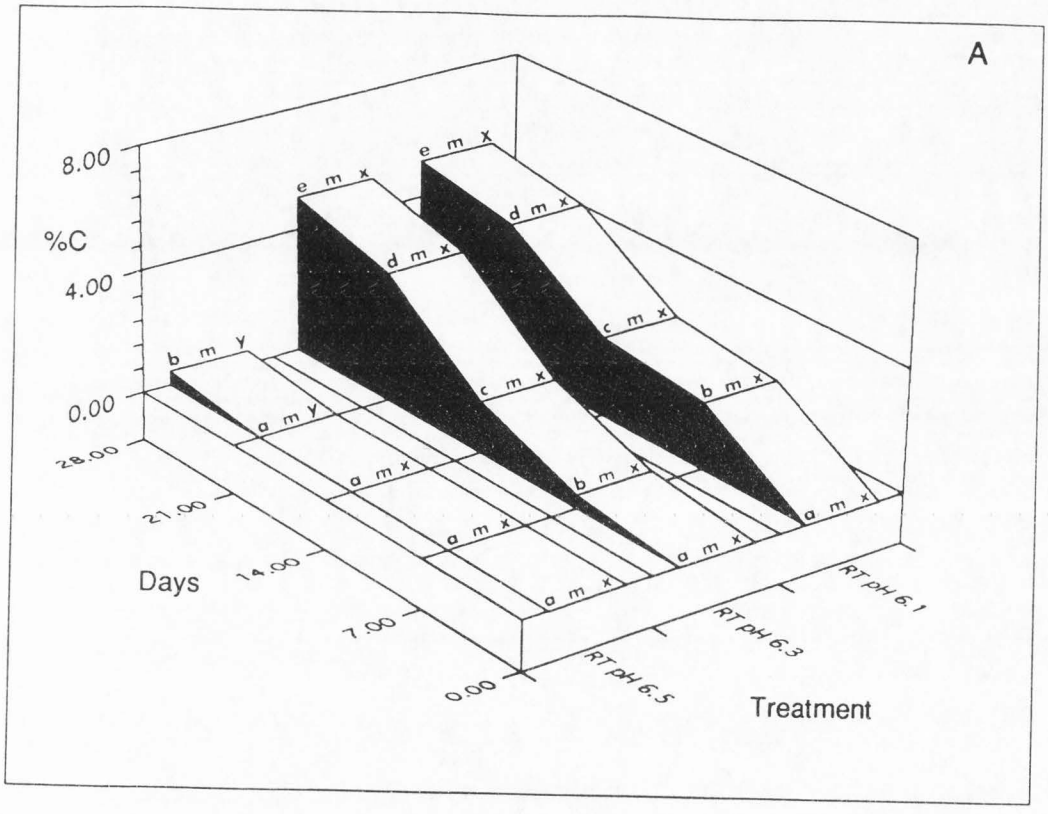
TABLE 4. Analysis of variance for the dependent variable percent clearing[1].

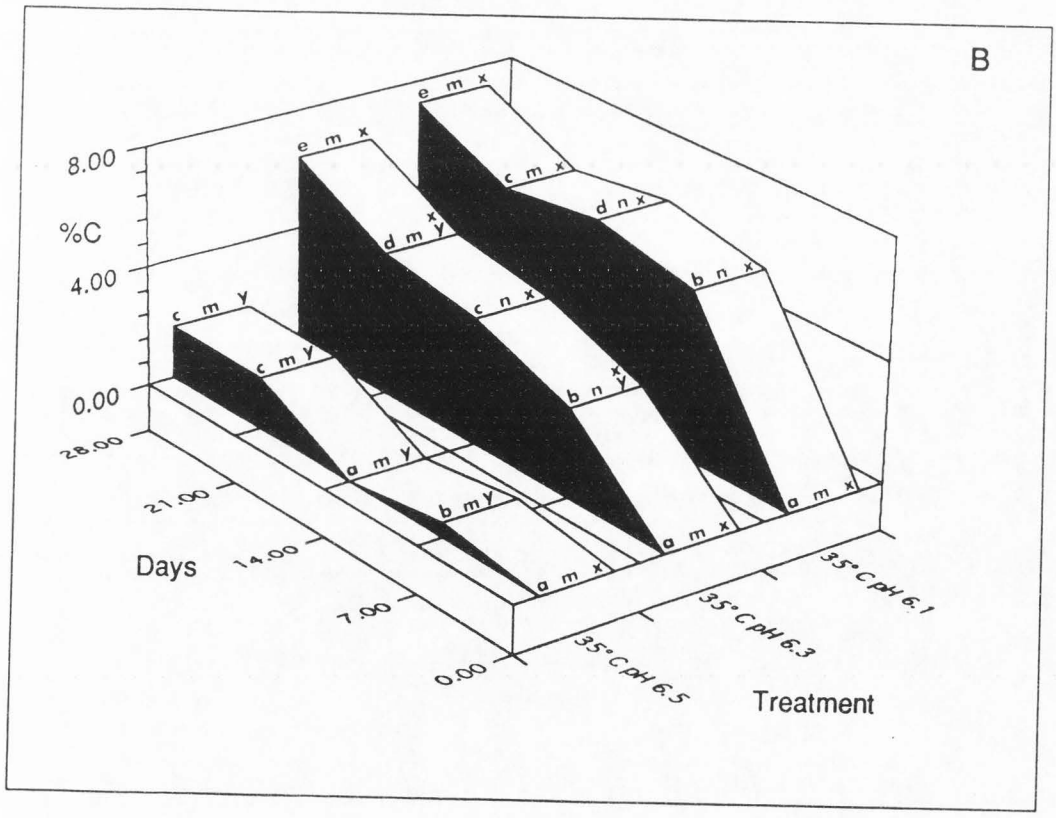
Source	df	Sum of squares	MS	*F* - test
Rep.	3	89.00	29.67	2.72
Treatment pH (T)	2	260.60	130.30	11.93**
Error (a)	6	65.51	10.92	
Storage Temp. (S)	1	57.82	57.82	45.52**
T x S	2	9.73	4.87	3.83
Error (b)	9	11.43	1.27	
Days (D)	4	263.28	65.82	26.00**
T x D	8	87.03	10.88	4.30**
S x D	4	18.44	4.61	1.82
T x S x D	8	16.16	2.02	.80
Error (c)	72	.29	.00	

1 Total df = 119

* $P \leq .05$

** $P \leq .01$


A
8.00
%C
4.00
0.00
28.00
21.00
14.00
7.00
0.00
Days
RT pH 6.5
RT pH 6.3
RT pH 6.1
Treatment


B
8.00
%C
4.00
0.00
28.00
21.00
14.00
7.00
0.00
Days
35°C pH 6.5
35°C pH 6.3
35°C pH 6.1
Treatment

significantly different from treatment pH 6.5; 2) at 35°C storage, treatment pH 6.5 differed from treatment 6.1 on all days except day 0 (for other differences see Figures 5A and 5B).

Under a phase contrast microscope (100x) differences in the size of particulates among the treatments were seen. pH treatments 6.1 and 6.3 were indistinguishable (Figures 6A to 6D). This is in accordance with our percent clearing statistical data, where treatment pH 6.1 and pH 6.3 did not differ. The size of the particles seen in treatment pH 6.1 and 6.3 were approximately 50 to 60 μm in diameter. Assuming we had spherically precipitated protein particles, at a constant temperature, the rate of sedimentation may be predicted by Stoke's Law (6):

$V = r^2 2(d_1 - d_2)g/9\eta$, where V = velocity of sedimenting particle,

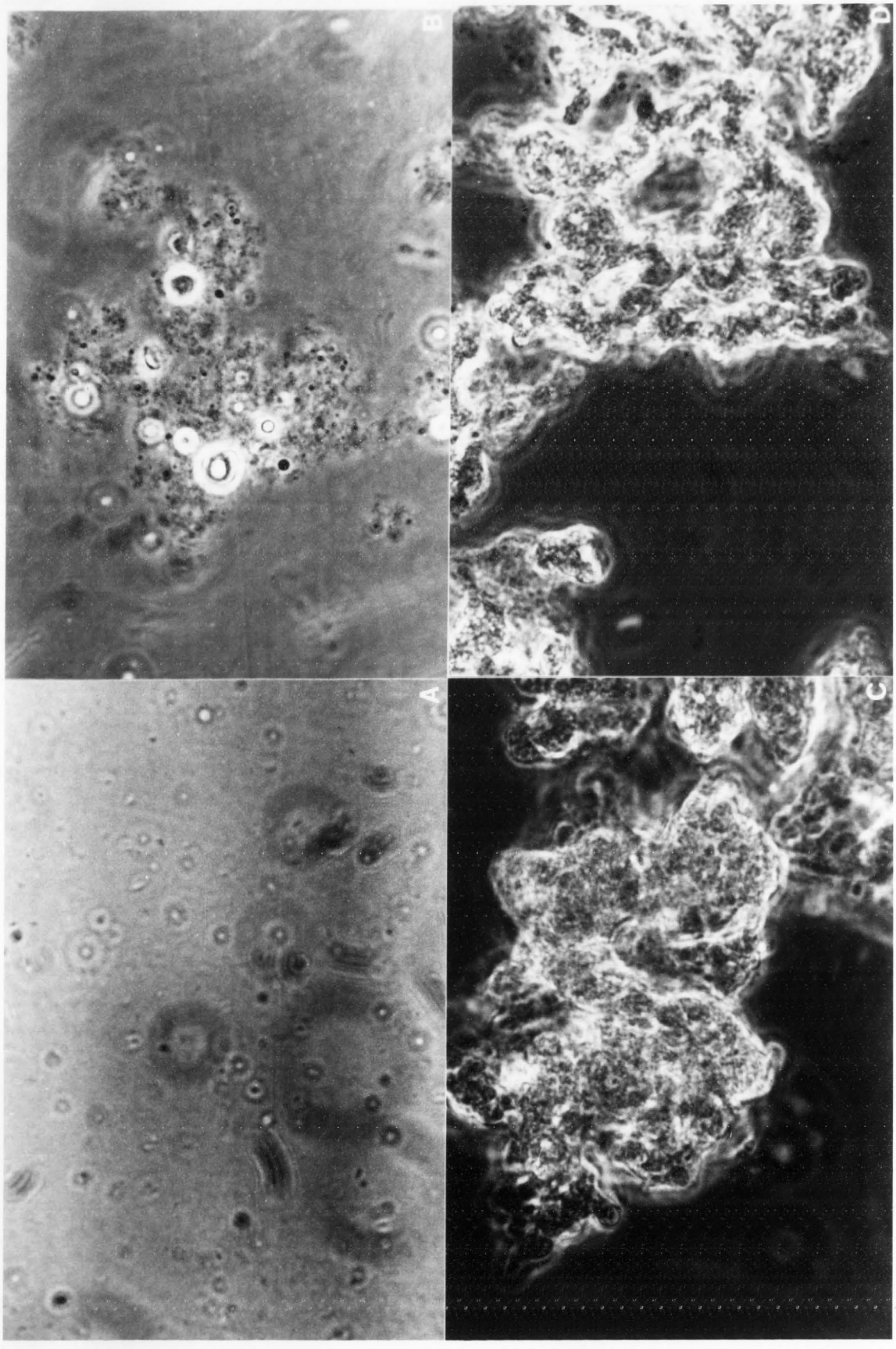
r = radius of sphere, d_1 = density of liquid phase, d_2 = density of sphere,

g = acceleration due to gravity, and η = viscosity of fluid.

If both densities and viscosity of the fluid remain constant, larger particles will become sediment faster at a given g–force. Hence, the precipitated protein spheres increased in size in this manner: treatment pH 6.1 = treatment pH 6.3 > treatment pH 6.5. Furthermore, sedimentation is opposed by diffusion. Those particles in the drink blend small enough (< 1 μm) to be disturbed by Brownian motion or convection currents will not sediment (56). At higher temperatures the viscosity of milk is reduced (3). Percent clearing at 35°C storage temperature was higher for the same treatment at room temperature storage; however, statistical analysis did not show any differences; hence, viscosity changes may have been negligible.

The differences observed are not attributed to differences in milk supply as evident in the replications in ANOVA presented in Tables 2 through 4. Furthermore,

Figure 6. Phase contrast micrographs (100x) of skim milk/orange juice drink blends. A. Blend prior to UHT processing. B. UHT processed drink blend pH 6.5. C. UHT processed drink blend pH 6.3. D. UHT processed drink blend pH 6.1.


B
D
A
C

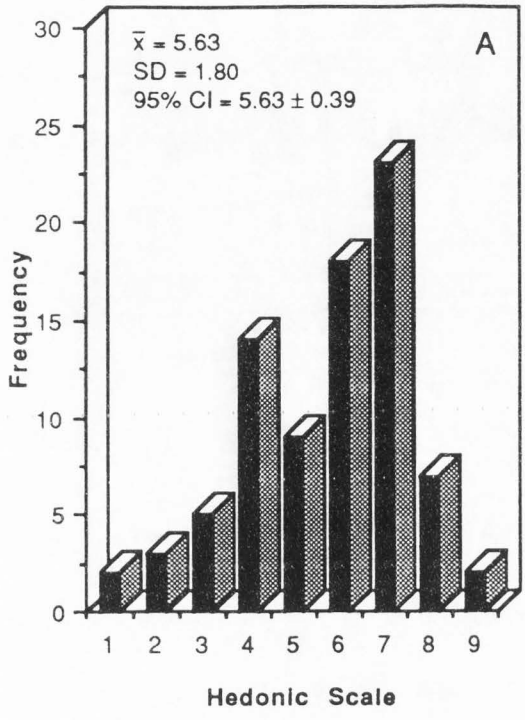
the F-test did not identify any significant three–way interactions for either pH or percent clearing.

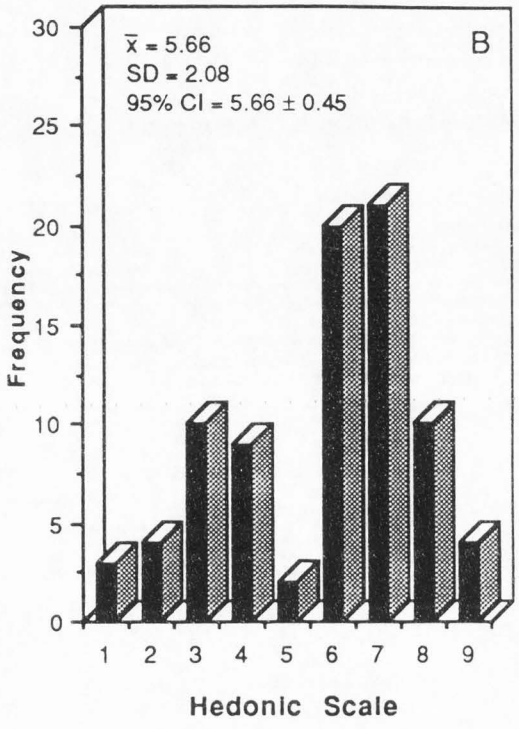
Drink Blend Sensory Evaluation

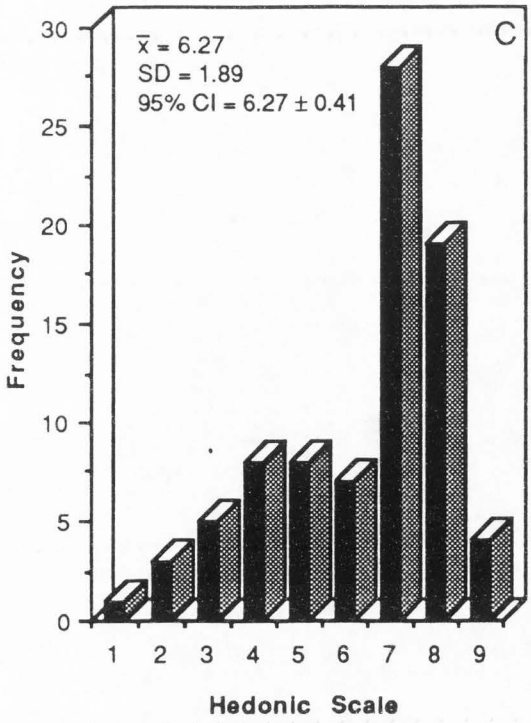
Panelists were statistically unable to perceive a textural difference among the three blends ($P \leq .05$). When comparing treatment pH 6.1 with treatment pH 6.3, 24 panelists out of 39 (one of the ballots in the first pair was inaccurately labeled and was disregarded) successfully identified the coarser sample. Out of 40 panelists, 23 and 25 panelists correctly identifed the coarser sample when comparing treatment pH 6.3 with treatment pH 6.5, and treatment pH 6.5 with treatment pH 6.1, respectively.

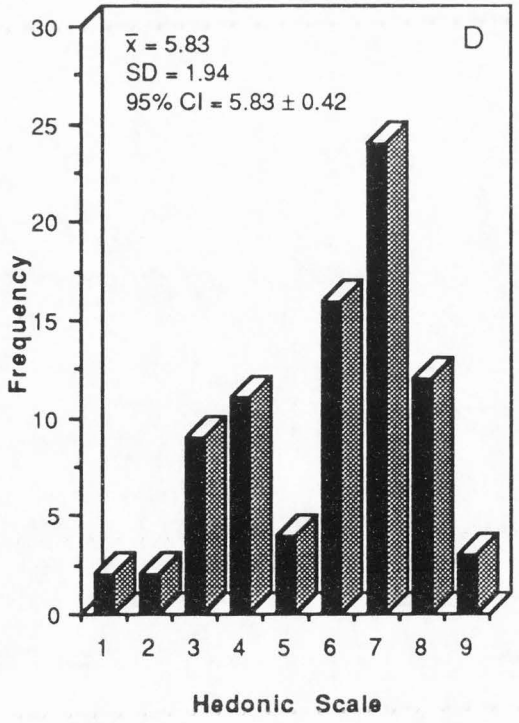
The sensory evaluation did not involve comparison with a control blend since one does not exist and was conducted to evaluate the drink blend profile. Therefore, results were not subjected to a complete statistical analysis. The histograms (Figures 7A to 7D) indicate there was a split population in the hedonic rating of the appearance, flavor, and overall acceptability of the blend. It might have been possible to avoid this split if a score of 5 (neither like nor dislike) had been omitted. This also shows that few panelists were undecided about the drink. A greater part of the population moderately liked the drink for all parameters tested as seen in the skewed distribution on the histograms. Linear correlations indicate that overall acceptability was correlated with flavor ($R^2 = .90$), appearance ($R^2 = .83$), and texture ($R^2 = .69$). Flavor would be the main factor in future efforts to improve the acceptability of the SM/OJ drink blend since panelists did indicate that the drink lacked flavor.

Figure 7. Consumer style panel (n =83) hedonic ratings of skim milk/orange juice drink. Hedonic scale ranges from 9 = like extremely, to 5 = neither like nor dislike, to 1 = dislike extremely. A. Appearance. B Flavor. C. Texture. D. Overall acceptability. (Corresponding statistical distribution is included in each histogram, where x = sample mean, SD = standard deviation, and CI = confidence interval).


$\bar{x}$ = 5.63
SD = 1.80
95% CI = 5.63 ± 0.39
A
Frequency
Hedonic Scale


$\bar{x}$ = 5.66
SD = 2.08
95% CI = 5.66 ± 0.45
B
Frequency
Hedonic Scale


$\bar{x}$ = 6.27
SD = 1.89
95% CI = 6.27 ± 0.41
C
Frequency
Hedonic Scale


$\bar{x}$ = 5.83
SD = 1.94
95% CI = 5.83 ± 0.42
D
Frequency
Hedonic Scale

CONCLUSIONS

We developed a UHT–processed SM/OJ drink that contained selected stabilizers. Addition of stabilizer(s) alone to the SM/OJ blend did not stabilize milk proteins during UHT processing or during extended storage at room temperature. Low pressure homogenization of the SM/OJ blend containing added stabilizers significantly increased milk protein stability during UHT processing and storage. The stabilizing effects of pre-homogenization may have been due to the high turbulence and cavitation of the blend at the homogenizer valve, which enhanced the interaction between stabilizer and milk proteins that would not have occurred under low–turbulence mixing. Flavor appears to be an important factor in efforts to improve the consumer acceptability of the product.

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APPENDICES

Appendix A

BALLOT FOR SENSORY TEXTURE EVALUATION

SENSORY TEXTURE EVALUATION OF AN ORANGE JUICE–SKIM MILK DRINK

Please evaluate the samples only on texture. Ignore the flavor of the drink. In this case "coarse" means the sample feels grainy or particulate, and is not smooth. The samples are presented as labeled below. Circle according to your response.

Which sample has the coarser mouthfeel?

607 820

Which sample has the coarser mouthfeel?

232 881

Which sample has the coarser mouthfeel?

738 915

SENSORY TEXTURE EVALUATION OF AN ORANGE JUICE–SKIM MILK DRINK

Please evaluate the samples only on texture. Ignore the flavor of the drink. In this case "coarse" means the sample feels grainy or particulate, and is not smooth. The samples are presented as labeled below. Circle according to your response.

Which sample has the coarser mouthfeel?

820 607

Which sample has the coarser mouthfeel?

881 232

Which sample has the coarser mouthfeel?

915 738

Appendix B

BALLOT FOR CONSUMER ACCEPTABILITY

Sensory Evaluation of an Orange Juice/Skim Milk Drink

Name:_____ Date:_____

Please evaluate the sample presented for each of the following characteristics. Check the description that best identifies the characteristic

	Appearance	Flavor	Texture	Overall Acceptability
like extremely	____	____	____	____
like very much	____	____	____	____
like moderately	____	____	____	____
like slightly	____	____	____	____
neither like nor dislike	____	____	____	____
dislike slightly	____	____	____	____
dislike moderately	____	____	____	____
dislike very much	____	____	____	____
dislike extremely	____	____	____	____

What do you think about the product? _____

_____

_____

Appendix C

DETERMINATION OF CONDITIONS AT FLASH EVAPORATOR

UHT parameters for repetition 1 were (refer to Figure 2):

TT2 = 160°F (71.1°C) TT6 = 50°F (10°C)

TT4 = 168°F (75.6°C) TT8 = 128°F (51.1°C)

vacuum pressure ≈ 4.8 psi (32.8 kPa)

enthalpy (vapor) (h_υ) at 160°F = 1130.10 Btu/lbm (2628.69 KJ/kg)

flow rate = 25.10 gallons/hour (95l/h)

h_υ at 284°F and 52.52 psi = 1175.34 Btu/lbm (2144.7 KJ/kg)

1. Determination of amount of steam needed, M_V, to raise the temperature of 1lbm of skim milk, M_{SM} , from 168°F to 284°F (refer to Figure 8A)

   C_{SM} (specific heat of skim milk) = .95 Btu/lbm °F (3.98KJ/kg K) at 20°C (assume approximately equal to that of the SM/OJ drink between 168°-284°F)

   C_W (specific heat of steam in milk) = 1.022977 Btu/lbm °F (4.283KJ/kg K)

   ΔT = temperature change

   Mass balance: $M_{SM} + M_V = M_{SM+V}$

   Energy balance: $M_{SM}C_{SM}T_1 + M_V h_\upsilon = M_{SM}C_{SM}T_2 + M_V C_W T_2$

   $M_{SM}C_{SM(\Delta T)} = M_V C_W T_2 - M_V h_\upsilon = M_V(C_W T_2 - h_\upsilon)$

   $M_V/M_{SM} = C_{SM(\Delta T)}/(C_W T_2 - h_\upsilon)$ = (.95 Btu/lbm °F) (168°F – 284°F)

   M_V/M_{SM} = (.95 Btu/lbm °F)(168 – 284)°F /(1.022977Btu/lbm °F)(284 °F) – 1175.34 Btu/lbm

   M_V/M_{SM} = 110.2/884.8 = .1245

   Therefore, for every M_{SM}, we need to remove .1245 lbm of vapor

2. Expansion conditions needed to remove all the steam added (refer to Figure 8B)

   Mass balance: $M_{SM} + M_V = M_{SM+V}$

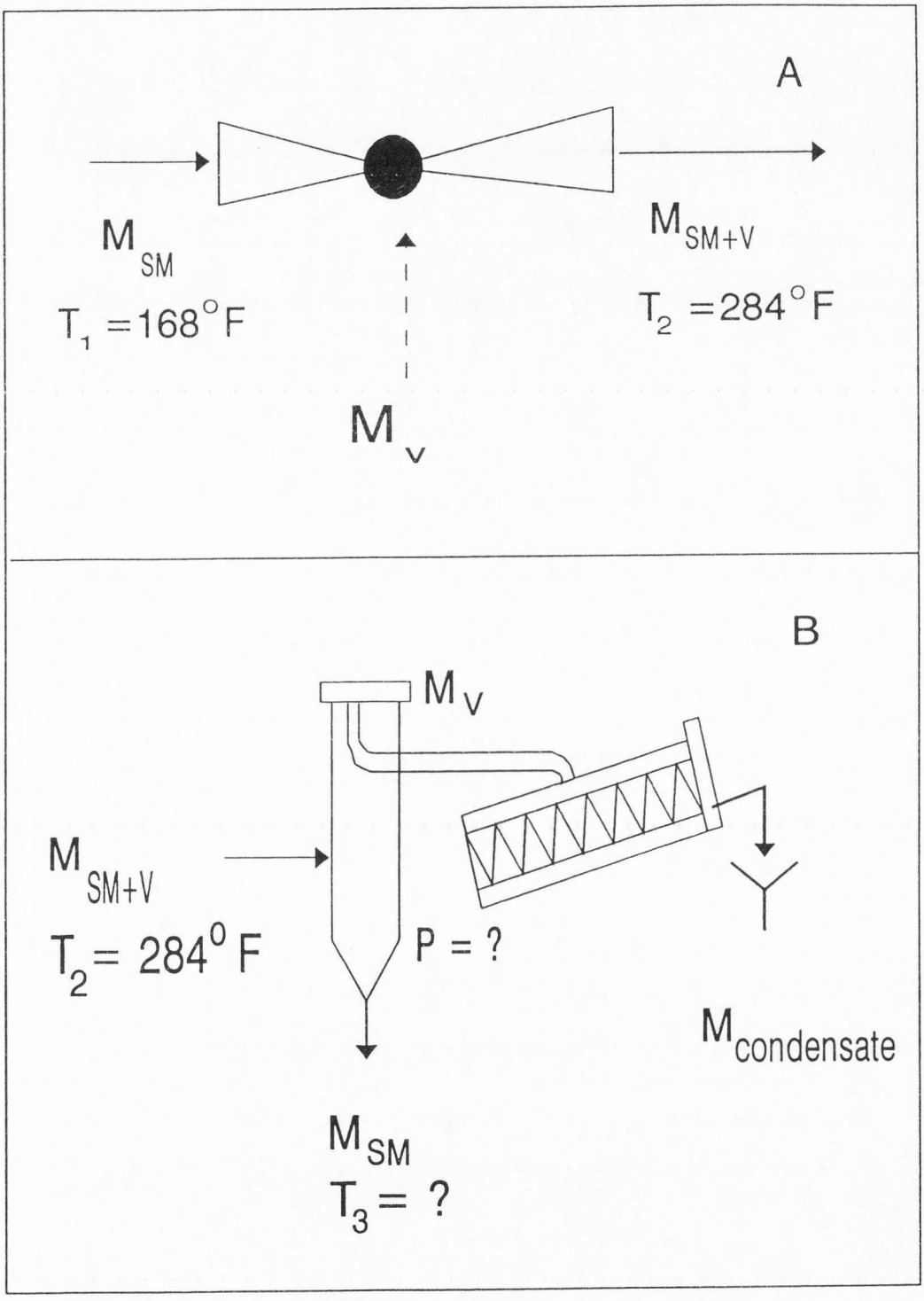

Figure 8. A. Mass flow for heating product to sterilization temperature. B. Mass flow at flash evaporator.

From the above problem we assume M_V/M_{SM} remains constant (i.e., equal to .1245).

Energy balance: $M_{SM}C_{SM}T_2 + M_V(C_WT_2 - h_{\upsilon(g)}) = M_{SM}C_{SM}T_3$

$T_3 = T_2 + .1245\ C_WT_2/C_{SM} - .1245h_{\upsilon(g)}/C_{SM}$

$T_3 = 284°F + (.1245)(1.022977\ Btu/lbm\ °F)(284°F) -$

$.1245h_{\upsilon(g)}/.95\ Btu/lbm\ °F$

$T_3 = 284°F + 38.07°F - .13h_{\upsilon(g)}/Btu/lbm°F$

$.13h_{\upsilon(g)}/Btu/lbm°F + T_3 = 322.07$ (must iterate)

From the steam tables (3), the $h_{\upsilon(g)}$–T_3 relation for which the above equation has a solution was (although conditions in the flash evaporator are for superheated vapor, they are approximately equal to saturation temperature–pressure):

$T'_3 = 174.4°F$, $h_{\upsilon(g)} = 1135.96$ Btu/lbm, and pressure at vacuum evaporator 6.66 psi

In this run, the temperature of the product leaving the flash evaporator was 160°F. From percent solid determinations, the average values were 9.36% before UHT treatment and 9.39 after UHT processing.

If:

$T_3 = T_2 + (M_V/M_{SM})(C_W/C_{SM})T_2 - (M_V/M_{SM})(h_{\upsilon(g)})/(C_{SM})$

Then by substitution:

$160°F = 284°F + (M_V/M_{SM})(1.022977\ Btu/lbm\ °F)(284°F)/(.95\ Btu/lbm\ °F)$

$- (M_V/M_{SM})(1130.1\ Btu/lbm)/(.95\ Btu/lbm\ °F)$

$160°F = 284°F + (M_V/M_{SM})(305.82°F - 1189.58°F)$ and

$M_V/M_{SM} = .14030$

Therefore, $.1245 - .14030 = -.0158$ lbm of extra vapor was removed per lbm of milk. For 110 lbm of product we would get 98.419 lb. of product after processing.

Appendix D

Dependent variable pH means

Treatment pH	day 0	day 7	day 14	day 21	day 28	means
Room temperature (RT)						
6.1	6.24	6.21	6.22	6.15	6.15	6.20
6.3	6.31	6.24	6.29	6.25	6.24	6.26
6.5	6.52	6.48	6.47	6.43	6.45	6.47
means	6.36	6.31	6.33	6.28	6.28	
35° C						
6.1	6.24	6.09	6.11	5.99	6.05	6.10
6.3	6.31	6.17	6.22	6.12	6.14	6.19
6.5	6.52	6.38	6.41	6.33	6.28	6.38
means	6.36	6.21	6.24	6.15	6.16	
Compounded means						
6.1	6.24	6.15	6.17	6.07	6.10	6.15
6.3	6.31	6.20	6.25	6.18	6.19	6.23
6.5	6.52	6.43	6.44	6.38	6.37	6.43
means	6.36	6.26	6.29	6.21	6.22	

Appendix E

Dependent variable titratable acidity means

Treatment pH	day 0	day 7	day 14	day 21	day 28	means
Room temperature (RT)						
6.1	3.13	3.18	3.13	3.10	3.13	3.13
6.3	2.98	3.10	3.10	3.05	3.00	3.05
6.5	2.58	2.60	2.55	2.63	2.60	2.59
means	2.89	2.96	2.93	2.93	2.91	
35° C						
6.1	3.13	3.10	3.23	3.18	3.18	3.16
6.3	2.98	3.08	3.13	3.05	3.10	3.07
6.5	2.58	2.60	2.60	2.58	2.73	2.62
means	2.89	2.93	2.98	2.93	3.00	
Compounded means						
6.1	3.13	3.14	3.18	3.14	3.15	3.15
6.3	2.98	3.09	3.11	3.05	3.05	3.06
6.5	2.58	2.60	2.58	2.60	2.66	2.60
means	2.89	2.94	2.95	2.93	2.95	

Appendix F

Dependent variable percent clearing means

Treatment pH	day 0	day 7	day 14	day 21	day 28	means
Room temperature (RT)						
6.1	.00	2.13	2.55	4.63	5.18	2.90
6.3	.00	.43	1.65	4.15	5.05	2.26
6.5	.00	.00	.00	.00	4.50	.09
means	.00	.85	1.40	2.93	3.56	
35° C						
6.1	.00	5.58	6.30	5.80	7.25	4.99
6.3	.00	2.93	4.15	4.70	6.40	3.64
6.5	.00	.48	.00	1.70	1.75	.79
means	.00	2.99	3.48	4.07	5.13	
Compounded means						
6.1	.00	3.85	4.43	5.21	6.21	3.94
6.3	.00	1.68	2.90	4.43	5.73	2.95
6.5	.00	.24	.00	.85	.11	.44
means	.00	1.92	2.44	3.50	4.35	