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Analysis of Chemical Changes During HTST and UHT Pasteurization Processes

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Abstract

Pasteurization is essential to ensure the microbiological safety of milk while preserving its chemical and nutritional quality. Two widely used heat treatments are high-temperature short-time (HTST; e.g., 72–78 °C for 15 s) and ultra-high temperature (UHT; ≥ 135 °C for a few seconds). HTST aims to inactivate pathogens with minimal chemical change; UHT delivers a much higher thermal load that extends ambient-temperature shelf life but accelerates heat-induced chemical reactions. This paper reviews and compares the principal chemical changes caused by HTST and UHT — protein denaturation and aggregation, enzyme inactivation, Maillard reaction progression and formation of markers (furosine, HMF, lactulose), lipid oxidation, vitamin losses, and effects on sensory compounds — by synthesizing literature and highlighting analytical indicators used to assess heat load and product quality. Key differences, practical implications for processing, and recommended analytical approaches are presented.

1. Introduction

Pasteurization is one of the most important thermal processing operations in the dairy and beverage industry, primarily aimed at ensuring microbiological safety while retaining the chemical, nutritional, and sensory quality of milk. Among the various thermal treatments used worldwide, High-Temperature Short-Time (HTST) and Ultra-High Temperature (UHT) pasteurization are the most commercially significant. HTST pasteurization typically involves heating milk to 72–78 °C for 15–20 seconds, whereas UHT processing subjects milk to much higher temperatures, generally 135–150 °C for 2–5 seconds, followed by aseptic packaging to achieve shelf stability at ambient temperatures.

Although both processes effectively inactivate pathogenic microorganisms, they differ significantly in their thermal intensity (heat load), which leads to distinct chemical transformations within the milk matrix. Milk is a complex colloidal system consisting of proteins, carbohydrates, lipids, minerals, enzymes, and vitamins. Heat treatment disrupts the equilibrium among these components, triggering reactions such as protein denaturation and aggregation, enzyme inactivation, lactose isomerization, Maillard reactions, lipid oxidation, and vitamin degradation. The extent and nature of these reactions are highly dependent on the temperature–time combination and the heating method employed. HTST pasteurization is generally considered a mild heat treatment, designed to preserve the fresh flavor and functional properties of milk while ensuring safety. In contrast, UHT treatment imposes a much higher thermal stress, resulting in more pronounced chemical changes that enhance shelf life but

may adversely affect sensory attributes and nutritional quality. Understanding these differences is crucial for process optimization, product development, regulatory compliance, and quality assurance.

In recent years, there has been growing interest in identifying chemical markers of heat treatment—such as whey protein denaturation indices, lactulose, furosine, hydroxyl methyl furfural (HMF), and fluorescence-based indicators—to reliably differentiate HTST and UHT milk. This paper presents a comparative review of chemical changes occurring during HTST and UHT pasteurization, focusing on reaction mechanisms, analytical indicators, and their implications for milk quality and processing performance.

2. Principal Chemical Reactions Induced by Heat

2.1 Protein Denaturation and Aggregation

Heat unfolds whey proteins (notably β-lactoglobulin and α-lactalbumin) and can promote their interaction with casein micelles, causing complexation, aggregation and changes in solubility and functional properties. The degree of denaturation correlates with both temperature and holding time and is considerably greater under UHT than HTST conditions. Direct-steam injection (DSI) UHT often causes different denaturation patterns versus indirect UHT due to differences in heating/cooling rates and steam condensation effects; similarly, DSI can produce lower measured denaturation for certain proteins compared with slow indirect heating methods. These denaturation differences affect rennet coagulation, emulsion stability, and susceptibility to sedimentation during storage.

2.2 Enzyme Inactivation

Alkaline phosphatase (ALP) is commonly used to confirm adequate HTST pasteurization (inactivated at standard HTST conditions), whereas lactoperoxidase and other enzymes have higher heat resistance and require higher temperatures for inactivation (often reached during UHT). Loss of enzyme activity is both a safety/quality marker and influences downstream reactions (e.g., residual lipase activity can promote hydrolytic off-flavors if not inactivated).

2.3 Maillard Reaction and Advanced Heat Markers

High temperatures accelerate non-enzymatic browning (Maillard reactions) between lactose (reducing sugar) and available amino groups (lysine residues), forming Amadori products that hydrolyze to furosine and later yield HMF and other advanced products. UHT induces significantly greater initial formation of these markers than HTST; during storage, Maillard reaction progress in UHT milk can continue and is sensitive to storage temperature and cycling. Furosine, lactulose, and HMF are established analytical indicators to distinguish heat load levels (mild: HTST; severe: UHT/sterilization).

2.4 Carbohydrate Transformations (Lactulose Formation)

Lactulose — formed by lactose isomerization under heat — is essentially absent in raw milk and below detection in properly HTST-pasteurized milk but is readily detectable in UHT milk; its concentration

rises with increasing thermal severity and storage temperature. Lactulose is therefore a reliable marker of high-temperature treatments.

2.5 Lipid Oxidation and Volatile Flavor Changes

Elevated heat and oxygen exposure can initiate lipid oxidation, creating volatile off-flavor compounds. UHT and high-severity indirect heating often generate more "cooked", "caramelized" or sulfur/eggy notes compared to HTST, and heating method (direct vs indirect) influences the volatile profile. Rapid heating (DSI) may minimize some oxidative effects but can generate distinct steam-related flavor notes.

2.6 Vitamin Stability

Fat-soluble vitamins (A, D, E) are relatively stable; certain water-soluble vitamins (e.g., vitamin C, some B vitamins) are more sensitive. Losses are generally greater with higher thermal load and extended storage after heat treatment. The magnitude of vitamin loss depends on the matrix and presence of protective components.

3. Comparative Evidence: HTST vs UHT

3.1 Protein Denaturation Patterns

Pilot-scale studies using advanced LC–MS methods show that whey protein denaturation increases with temperature and holding time; denaturation is modest under HTST but rises sharply above ~85–100 °C (typical of UHT and sterilization). Direct-steam injection (DSI) tends to produce lower measured denaturation for some whey proteins compared to indirect heat exchangers (PHE/THE), likely because steam injection provides very rapid heating and subsequent flash cooling, reducing the time proteins spend at intermediate high temperatures. These differences influence coagulation and functional properties.

3.2 Maillard Products & Heat Markers

Comparative studies indicate furosine and lactulose concentrations are substantially higher in UHT (especially indirect UHT) than in HTST milk; furosine increases even during short storage and is a sensitive indicator of the early stages of Maillard progression. Long-term monitoring of HMF and other advanced Maillard products shows greater accumulation in UHT milk stored at elevated temperatures. Thus, while HTST preserves more lysine bioavailability (lower furosine), UHT increases both early and advanced Maillard compounds.

3.3 Flavor and Sensory Outcomes

Sensory analyses show HTST milk is generally preferred by consumers to UP (ultrapasteurized) milk; UP samples—particularly DSI-UP—can present sulfuric or "eggy" notes, whereas indirect UHT may have sweeter aromatic characters. Sensory differences also evolve during storage due to ongoing

chemical reactions (e.g., Maillard), and consumer preference tends to favour the milder HTST flavor profile in the short term.

3.4 Functional and Technological Impacts

Higher denaturation in UHT reduces soluble whey fraction and can impair cheese-making and rennet-coagulation properties. HTST retains more native whey proteins and enzyme profiles favorable for some downstream processes. In beverage applications, UHT achieves shelf stability but may necessitate formulation changes (stabilizers, emulsifiers) to manage changes in turbidity, sedimentation and viscosity.

4. Analytical Methods and Indicators for Comparative Assessment

The accurate assessment of chemical changes induced by HTST and UHT pasteurization requires a combination of analytical techniques, as no single parameter can comprehensively describe heat-induced modifications in milk. Modern quality evaluation relies on chemical, enzymatic, spectroscopic, and chromatographic indicators that collectively reflect the severity of thermal treatment.

4.1 Protein-Based Indicators

Protein denaturation is one of the earliest and most sensitive indicators of heat treatment. Measurement of native and denatured whey proteins, particularly β-lactoglobulin, is commonly performed using:

- High-performance liquid chromatography (HPLC)
- Liquid chromatography–mass spectrometry (LC–MS)
- Solubility tests at pH 4.6

HTST-treated milk shows relatively low whey protein denaturation, whereas UHT-treated milk exhibits extensive unfolding and aggregation, especially in indirectly heated systems.

4.2 Enzyme Activity Assays

Residual enzyme activity is widely used for process verification:

- **Alkaline phosphatase (ALP)**: Indicator of proper HTST pasteurization
- **Lactoperoxidase and lipase**: More heat-resistant enzymes, largely inactivated only during UHT treatment

These assays help differentiate mild and severe heat treatments and ensure regulatory compliance.

4.3 Maillard Reaction Markers

Maillard reaction products are critical indicators of chemical heat damage:

- **Furosine**: Early-stage Maillard marker measured after acid hydrolysis of proteins using HPLC

- **Hydroxymethylfurfural (HMF)**: Advanced Maillard marker
- **Lactulose**: Formed by lactose isomerization and considered a definitive marker of UHT processing

HTST milk contains negligible lactulose and low furosine levels, whereas UHT milk shows significantly higher concentrations.

4.4 Spectroscopic and Fluorescence Techniques

Rapid, non-destructive methods such as:

- Front-face fluorescence spectroscopy
- FAST index (fluorescence of advanced Maillard products and soluble tryptophan)

are increasingly used for routine quality screening. These methods allow fast differentiation between HTST and UHT milk and can be coupled with chemometric analysis for improved discrimination.

4.5 Integrated Quality Assessment

A multi parametric approach combining protein denaturation, enzyme activity, Maillard markers, and spectroscopic data provides the most reliable evaluation of pasteurization severity. Such integrated analysis is particularly useful for industrial quality control and research comparisons between HTST and UHT processes.

5. Practical Implications for Process Design and Quality Control

1. **Choice of Heating Method:** If the goal is minimal sensory/chemical alteration and retention of native protein functionality (e.g., for fluid milk preferred fresh taste or cheese processing), HTST is advantageous. For ambient-stable milk products, UHT (with careful control of indirect vs direct heating) is required despite larger chemical changes.
2. **Heating Mode (Direct vs Indirect):** DSI can reduce some denaturation due to rapid heat transfer but may introduce distinct steam-derived flavor notes; indirect heating (PHE/THE) often yields higher furosine/lactulose and is more likely to promote Maillard progression.
3. **Storage Control:** UHT products are more sensitive to storage temperature fluctuations with regard to Maillard progression and flavor changes; cold chain and stable storage conditions mitigate off-flavor formation.
4. **Quality Monitoring:** Use a panel of indicators (β-LG residual, furosine, lactulose, HMF, ALP) plus rapid fluorescence screening for routine monitoring; combine with sensory panels where consumer perception is critical.

6. Limitations and Research Needs

Despite extensive research on thermal processing of milk, several limitations remain in the comparative understanding of HTST and UHT pasteurization. One major challenge is the lack of standardization

across studies, as processing conditions vary widely with respect to heating equipment (plate heat exchangers, tubular heat exchangers, direct steam injection), holding times, homogenization pressure, and milk composition. These variations complicate direct comparison of chemical changes reported in the literature. Another limitation is that many studies focus on immediate post-processing changes, while comparatively fewer investigations examine the long-term evolution of chemical reactions during storage, particularly under fluctuating temperature conditions commonly encountered in real supply chains. Maillard reaction products, lipid oxidation compounds, and protein aggregates may continue to form or evolve during storage, especially in UHT milk, influencing flavor, nutritional value, and consumer acceptance. From a nutritional perspective, further research is required to better understand the bioavailability of heat-sensitive nutrients, such as lysine and certain B-group vitamins, following HTST and UHT treatments. While chemical markers indicate heat damage, their direct correlation with nutritional outcomes and human health implications remains insufficiently explored.

Future research should also focus on mitigation strategies to reduce undesirable chemical changes during UHT processing. These may include:

- Optimization of heating and cooling rates
- Selection of direct versus indirect heating systems
- Oxygen reduction prior to heat treatment
- Use of natural antioxidants or protective processing sequences

Additionally, emerging dairy alternatives such as plant-based milk analogues require similar comparative studies, as their protein and carbohydrate compositions differ significantly from bovine milk and may respond differently to HTST and UHT processing. Overall, addressing these gaps will improve the scientific basis for selecting appropriate pasteurization technologies and enhance the balance between food safety, nutritional quality, shelf life, and sensory acceptance.

7. Conclusions

HTST and UHT differ markedly in induced chemical changes: UHT imposes a much higher thermal load, leading to greater protein denaturation/aggregation, higher levels of Maillard markers (furosine, HMF, lactulose), altered volatile profiles and potentially greater vitamin losses. HTST better preserves native proteins and sensory characteristics but sacrifices ambient shelf stability. Analytical discrimination requires a multiparametric approach; practical choices between HTST and UHT depend on the desired shelf life, sensory quality, and downstream processing needs.

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