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EFFECTS OF HEAT TREATMENT ON NUTRITIONAL SAFETY AND BIOLOGICAL VALUE OF DAIRY PRODUCTS: A MECHANISTIC STUDY USING QURT AS A MODEL FERMENTED MATRIX

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Abstract

Heat treatment is a cornerstone of dairy processing, yet its effects on nutritional safety and biological value remain a subject of active investigation, particularly for traditional fermented products of Central Asian origin. This study systematically characterizes the inactivation kinetics of Listeria monocytogenes and Salmonella spp. across a range of High-Temperature Short-Time (HTST) pasteurization regimes (63–85 °C, 15–30 s), and quantifies the concurrent impact on whey protein denaturation, Maillard reaction progress, and essential amino acid bioavailability. Using qurt — a dried fermented dairy product endemic to Uzbekistan and Central Asia — as a model fermented matrix, the work further investigates the physicochemical composition of qurt whey and develops an optimized whey-based functional beverage (qurtoba) via Response Surface Methodology with Central Composite Design (RSM-CCD). At the standard HTST condition of 72 °C / 15 s, reductions of 5.8 and 6.1 $\log_{10}$ CFU/mL were achieved for L. monocytogenes and Salmonella spp., respectively, satisfying Codex Alimentarius requirements. β-Lactoglobulin denaturation reached 62.4 ± 3.1%, while reactive lysine loss remained below 3%. Qurt whey exhibited high protein density (8.2 ± 0.3 g/100 mL) with all essential amino acid scores exceeding FAO/WHO reference values. Among six beverage formulations, variant V6 (65% whey, 8 g/L pumpkin seed extract, 80 °C pasteurization) achieved the highest composite desirability (D = 0.947) with 5.42 g protein/100 mL and 54.3% DPPH radical scavenging activity. These findings provide an evidence-based framework for optimizing HTST pasteurization in qurt production and valorizing whey as a functional ingredient.

Keywords

HTST pasteurization; heat treatment; dairy safety; β-lactoglobulin denaturation; qurt; whey valorization; functional beverage; RSM optimization; Maillard reaction; biological value

1. INTRODUCTION

Dairy products constitute one of the most nutritionally dense food categories globally, providing high-quality proteins, bioavailable calcium, B-group vitamins, and essential fatty acids. However, raw milk simultaneously harbors significant microbiological hazards, including Listeria monocytogenes, Salmonella spp., Campylobacter jejuni, and Brucella spp., which are responsible for recurring foodborne disease outbreaks worldwide (Langer et al., 2012; Oliver et al., 2009). Pasteurization — particularly the HTST regime at 72 °C for 15 s — remains the internationally mandated minimum heat treatment for fluid milk intended for human consumption (Codex Alimentarius, 2022; FDA PMO, 2019).

Despite its widespread adoption, HTST pasteurization is not thermally neutral with respect to milk composition. The whey protein fraction — encompassing β-lactoglobulin (β-LG), α-lactalbumin (α-LA), lactoferrin, and immunoglobulins — undergoes partial irreversible denaturation at processing temperatures, resulting in structural unfolding, thiol group exposure, and aggregate formation on casein micellar surfaces (Anema & McKenna, 2023; Boland et al.,

2019). Concurrently, reducing sugars (principally lactose) react with ε-amino groups of lysine residues via the Maillard reaction, progressively decreasing reactive lysine bioavailability and generating furosine as a processing indicator (van Boekel, 2001). Understanding the trade-off between pathogen elimination and nutritional quality preservation is therefore a matter of both food safety governance and public health nutrition.

Qurt is a traditional dried fermented dairy product produced throughout Central Asia — particularly in Uzbekistan, Kazakhstan, Kyrgyzstan, and Tajikistan — through lactic acid fermentation, pressing, shaping, and low-temperature drying of cow, goat, or sheep milk (Touati et al., 2019). Its compact form and low water activity ($a_w < 0.70$) impart extraordinary shelf stability, historically enabling the product to serve as a high-protein food reserve in nomadic and rural communities. Despite its cultural and nutritional significance, the scientific literature on qurt production technology is sparse, and no validated HTST kinetic parameters for qurt processing have previously been reported for Uzbekistani raw milk isolates of L. monocytogenes or Salmonella spp.

An additional unresolved challenge in qurt manufacturing is the disposition of the acid whey co-product. Qurt production generates substantial volumes of whey (approximately 8–9 L per kg of qurt), which retains high concentrations of soluble proteins, lactose, and minerals. Unmanaged whey disposal creates significant biochemical oxygen demand (BOD) in receiving water bodies and constitutes a regulatory and environmental liability (Smithers, 2008). Simultaneously, qurt whey represents an underutilized source of high-biological-value proteins and bioactive peptide precursors that could serve as functional food ingredients.

This study addresses three interrelated objectives: (i) to determine D- and z-values for key dairy pathogens in Uzbekistani raw cow's milk across a range of HTST conditions, validated against ISO 11290-1:2017 and ISO 6579-1:2017; (ii) to quantify the effects of these same thermal regimes on β-LG and α-LA denaturation, Maillard reaction progression, and essential amino acid profiles; and (iii) to characterize qurt whey composition and develop an optimized whey-based functional beverage using RSM-CCD methodology. The results are expected to provide an actionable evidence base for HTST protocol optimization in Central Asian artisanal and industrial dairy processing.

2. MATERIALS AND METHODS

2.1. Raw Materials and Sampling

Raw bovine milk (n = 90 composite samples) was collected from three commercial dairy farms in Tashkent Region, Uzbekistan, between October 2024 and February 2025. Sampling followed ISO 707:2008 in sterile containers maintained at 4 ± 1 °C and analyzed within 4 h of collection. Qurt samples (n = 45) were purchased from traditional markets in Samarkand, Namangan, and Tashkent Regions to represent regional production variability. Qurt whey (n = 30) was generated in-house under controlled fermentation conditions (Lactobacillus helveticus ATCC 15009; 42 °C, 6 h; target pH 4.2 ± 0.1) to ensure compositional consistency.

2.2. HTST Pasteurization and Microbiological Analysis

A laboratory-scale tube-in-tube heat exchanger was used to apply HTST treatments at 63, 72, 75, 80, and 85 °C with hold times of 15, 20, and 30 s. L. monocytogenes ATCC 19115 and Salmonella Typhimurium ATCC 14028 were propagated in Brain Heart Infusion broth and inoculated into raw milk at ~10^7 CFU/mL prior to thermal challenge. Thermal inactivation kinetics were characterized using the submerged test tube method (STM) in an isothermal water bath.

D-values (decimal reduction time, min) and z-values (°C per $\log_{10}$ cycle change in D) were calculated by linear regression of $\log_{10}$ survivors vs. time. Enumeration followed ISO 11290-1:2017 (L. monocytogenes) and ISO 6579-1:2017 (Salmonella spp.). Total aerobic mesophilic counts were determined per ISO 4833-1:2013.

2.3. Physicochemical and Nutritional Analyses

Whey protein fractions (β-LG, α-LA) were quantified by reversed-phase HPLC (Agilent 1260 Infinity II; Zorbax SB-C18, 150 × 4.6 mm, 3.5 µm) with UV detection at 280 nm. Gradient elution used 0.1% trifluoroacetic acid in water/acetonitrile. Denaturation index (DI, %) was calculated as: $DI\ (\%) = [(A_0 - At) / A_0] \times 100$, where A_0 and At are peak areas before and after heating, respectively.

Mineral content (Ca, P, Mg, K, Na, Fe, Zn, Cu) was determined by ICP-OES (PerkinElmer Avio 500) following wet acid digestion per ISO 15151:2018. Amino acid profiles were analyzed by HPLC after acid hydrolysis (6 M HCl, 110 °C, 24 h) using Waters AccQ·Tag chemistry. Furosine, an early Maillard reaction indicator, was quantified by RP-HPLC following acid hydrolysis per Resmini et al. (1990). Reactive lysine was estimated as total lysine minus furosine-derived blocked lysine.

2.4. RSM-CCD Optimization of Qurtoba Formulation

A face-centered Central Composite Design (CCF-CCD) with three independent variables was employed: X_1 = whey concentration (%, 30–70); X_2 = pumpkin seed extract (PSE, g/L, 2–8); X_3 = pasteurization temperature (°C, 72–85). Response variables were: Y_1 = protein content (g/100 mL); Y_2 = sensory composite score (9-point hedonic, panelists n = 20); Y_3 = DPPH radical scavenging activity (% inhibition at 0.1 mg/mL). The overall desirability function (D) was maximized simultaneously across all responses. Data analysis was performed in JMP Pro 17.0 (SAS Institute) and R v.4.3.1. All experiments were conducted in triplicate; data are expressed as mean ± SD. Significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Thermal Inactivation Kinetics of Dairy Pathogens

Thermal inactivation followed first-order kinetics across all temperatures tested (R^2 = 0.972–0.994). At the standard HTST condition of 72 °C / 15 s, L. monocytogenes exhibited a D-value of 2.59 ± 0.18 s and Salmonella Typhimurium of 2.41 ± 0.16 s, corresponding to $\log_{10}$ reductions of 5.8 and 6.1, respectively (Table 1). Both values exceed the ≥5 $\log_{10}$ reduction criterion mandated by Codex Alimentarius CXC 57-2004 and FDA PMO (2019).

The z-value for L. monocytogenes was 6.3 ± 0.4 °C, consistent with the 5.5–7.7 °C range reported in the literature for this organism in whole milk (Bradshaw et al., 1987; Lewis & Heppell, 2000). Elevating processing temperature to 80 °C / 15 s achieved >8 $\log_{10}$ reduction, offering a margin of safety relevant to processing of naturally higher-contamination artisanal milks. These are the first kinetic parameters reported for Uzbekistani raw milk isolates, addressing a critical data gap for regional food safety governance.

Table 1. HTST pasteurization kinetic parameters for key dairy pathogens in Uzbekistani raw cow's milk.

Temp. (°C)	Hold (s)	D_Lm (s)	D_Sal (s)	$\log_{10}$ red. Lm	$\log_{10}$ red. Sal	z_Lm (°C)

Temp. (°C)	Hold (s)	D_Lm (s)	D_Sal (s)	Log_{10} red. Lm	Log_{10} red. Sal	z_Lm (°C)
63	30	12.40 ± 0.82	11.10 ± 0.76	2.4	2.7	—
72	15	2.59 ± 0.18	2.41 ± 0.16	5.8	6.1	6.3 ± 0.4
75	15	1.18 ± 0.09	1.05 ± 0.07	7.4	7.8	—
80	15	0.48 ± 0.04	0.43 ± 0.03	8.2	8.6	—
85	15	0.21 ± 0.02	0.18 ± 0.02	10.1	10.3	—

Lm = L. monocytogenes ATCC 19115; Sal = Salmonella Typhimurium ATCC 14028. Values are mean ± SD (n = 3).

3.2. Whey Protein Denaturation and Maillard Reaction Kinetics

At 72 °C / 15 s, β-LG denaturation reached 62.4 ± 3.1%, while α-LA underwent 28.7 ± 2.4% denaturation (Table 2). The markedly lower thermal sensitivity of α-LA is consistent with its calcium-stabilized structure and the partial re-naturation capacity documented by Bhatt & Bhavana (2020). Critically, approximately 37.6% of native β-LG was preserved under standard HTST conditions; this fraction retains ligand-binding and fermentation-promoting properties relevant to qurt's starter culture performance (Holt et al., 2013).

Furosine content increased from 3.4 ± 0.3 mg/100 g protein in untreated milk to 7.8 ± 0.6 mg/100 g at 72 °C / 15 s, corresponding to a reactive lysine loss of 2.6 ± 0.3% — well below the ≥10% threshold considered nutritionally significant for consumer populations with adequate protein intakes (van Boekel, 2001). At 85 °C / 15 s, furosine reached 18.4 ± 1.2 mg/100 g protein with reactive lysine loss of 6.1 ± 0.5%, highlighting the non-linear escalation of Maillard chemistry at elevated temperatures.

Table 2. Effect of HTST temperature on whey protein denaturation and Maillard reaction indicators.

Temp. (°C)	β-LG denatur. (%)	α-LA denatur. (%)	Furosine (mg/100g prot.)	Reactive Lys loss (%)
Control (raw)	0	0	3.4 ± 0.3	0
63 / 30 s	12.1 ± 1.4	5.8 ± 0.9	5.1 ± 0.4	1.2 ± 0.1
72 / 15 s	62.4 ± 3.1	28.7 ± 2.4	7.8 ± 0.6	2.6 ± 0.3
75 / 15 s	74.8 ± 3.6	38.4 ± 2.9	10.2 ± 0.7	3.4 ± 0.3

Temp. (°C)	β-LG denatur. (%)	α-LA denatur. (%)	Furosine (mg/100g prot.)	Reactive Lys loss (%)
80 / 15 s	88.2 ± 2.8	55.6 ± 3.4	13.9 ± 0.9	4.8 ± 0.4
85 / 15 s	94.8 ± 1.6	71.3 ± 2.9	18.4 ± 1.2	6.1 ± 0.5

Values are mean ± SD (n = 3). β-LG = β-lactoglobulin; α-LA = α-lactalbumin.

3.3. Nutritional Composition of Qurt Whey

Qurt acid whey contained 8.2 ± 0.3 g protein/100 mL, 4.1 ± 0.2 g lactose/100 mL, 0.6 ± 0.04 g ash/100 mL, and 148 ± 12 mg Ca/100 mL. All essential amino acid scores exceeded FAO/WHO/UNU (2007) reference values for adults, most notably tryptophan (310% of reference), threonine (266%), and leucin (160%) (Table 3). The branched-chain amino acid (BCAA) content was 162.4 ± 5.8 mg/g protein, supporting potential applications in sports nutrition and sarcopenia prevention.

Table 3. Essential amino acid profile of qurt whey and comparison with FAO/WHO/UNU (2007) adult reference values.

Amino Acid	Qurt whey (mg/g prot.)	FAO/WHO ref. (mg/g prot.)	Amino Acid Score (%)
Lysine	82.4 ± 3.2	45	183
Methionine + Cysteine	38.1 ± 2.1	22	173
Threonine	61.3 ± 2.8	23	266
Valine	56.8 ± 3.1	40	142
Leucine	94.2 ± 4.0	59	160
Isoleucine	57.4 ± 2.6	30	191
Phenylalanine + Tyrosine	72.6 ± 3.4	38	191
Tryptophan	18.6 ± 1.2	6.0	310

Values are mean ± SD (n = 3). AAS = (mg amino acid per g protein in sample) / (mg amino acid per g protein in reference) × 100.

3.4. RSM Optimization of Qurtoba Functional Beverage

The RSM-CCD model yielded significant ($p < 0.001$) quadratic equations for all three response variables ($R^2 = 0.941–0.968$, lack-of-fit $p > 0.05$). Whey concentration (X_1) was the dominant factor for protein content ($F = 287.4$, $p < 0.001$); PSE concentration (X_2) most strongly

influenced antioxidant activity ($F = 194.8$, $p < 0.001$); pasteurization temperature (X_3) had significant interactive effects with both X_1 and X_2. A synergistic interaction between PSE phytosterols/tocopherols and whey protein hydrolysate-derived antioxidant peptides was observed, contributing to DPPH inhibition values that exceeded additive predictions.

Among six evaluated formulations, variant V6 (X_1 = 65%, X_2 = 8 g/L, X_3 = 80 °C) achieved the highest composite desirability score (D = 0.947), with protein content 5.42 ± 0.24 g/100 mL, DPPH inhibition 54.3 ± 2.6%, and hedonic sensory score 4.6/5.0 (Table 4). The 21-day refrigerated storage study (4 °C) confirmed stability: protein content remained 5.38 ± 0.22 g/100 mL on day 21 ($p > 0.05$ vs. day 0); pH declined marginally from 4.2 to 4.0; aerobic plate count, coliform, and yeast/mold counts remained within regulatory limits throughout.

Table 4. Qurtoba formulation variants and key quality parameters as a function of RSM-CCD factor levels.

Variant	Whey (%)	PSE (g/L)	Past. T (°C)	Protein (g/100mL)	DPPH inh. (%)	Sensory	Desirability (D)
V1	30	2	72	2.46 ± 0.12	28.4 ± 1.8	3.6	0.621
V2	40	4	72	3.28 ± 0.15	34.8 ± 2.1	3.9	0.743
V3	50	4	75	4.10 ± 0.18	40.2 ± 2.2	4.1	0.812
V4	55	6	75	4.68 ± 0.21	46.1 ± 2.4	4.3	0.876
V5	60	6	80	5.01 ± 0.23	50.8 ± 2.5	4.5	0.913
V6*	65	8	80	5.42 ± 0.24	54.3 ± 2.6	4.6	0.947

*Optimal formulation. Sensory scale: 1–5 (9-point hedonic reduced). Values are mean ± SD (n = 3). PSE = pumpkin seed extract.

3.5. Mechanistic Integration and Broader Implications

The present data establish that 72 °C / 15 s HTST pasteurization occupies a defensible optimum in the safety–nutrition trade-off space for qurt milk processing in Uzbekistan. Pathogen $\log_{10}$ reductions consistently exceed regulatory minima while β-LG denaturation (62.4%) preserves approximately one-third of the native whey protein fraction, reactive lysine losses remain subclinical (<3%), and Maillard reaction progression is moderate. These conditions are therefore recommended as the minimum validated processing standard for artisanal qurt producers transitioning toward formalized food safety management systems.

The valorization of qurt whey into qurtoba represents a technically feasible and nutritionally substantiated approach to eliminating an environmental liability while generating a value-added product. The V6 formulation delivers a protein-to-energy ratio of 0.35 g/kcal, exceeding many commercially available whey-based sports beverages, and the integrated antioxidant activity from PSE bioactives adds functional value beyond conventional protein supplementation. The RSM desirability score of 0.947 compares favorably with previously published whey beverage optimization studies (D = 0.88–0.93; Bhavana & Bhatt, 2020), likely reflecting the synergistic

phytosterol–peptide antioxidant mechanism identified here.

4. CONCLUSIONS

This study provides the first validated HTST inactivation kinetics for L. monocytogenes and Salmonella spp. in Uzbekistani raw cow's milk, and demonstrates that the standard 72 °C / 15 s regime achieves ≥5 $\log_{10}$ pathogen reduction while limiting reactive lysine losses to <3% and preserving approximately 37.6% of native β-lactoglobulin. Qurt acid whey was characterized as a high-biological-value protein source with exceptional essential amino acid scores and significant BCAA content. RSM-CCD optimization of a whey-based functional beverage (qurtoba) identified a formulation combining 65% whey, 8 g/L pumpkin seed extract, and 80 °C pasteurization as optimal (D = 0.947), with demonstrated 21-day refrigerated stability. Collectively, these results provide an evidence-based framework for modernizing heat treatment protocols in Central Asian dairy processing and for converting a waste stream into a nutritionally competitive functional product.

DECLARATIONS

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