FABIO RIBEIRO DOS SANTOS

STUDY OF DIFFERENT BARRIERS TO MICROBIOLOGY STABILIZATION OF GOAT AND SHEEP WHEY AND IMPACT ON THE PHYSICOCHEMICAL PROPERTIES AND PHYSICAL STABILITY OF PRODUCTS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Magister Scientiae*.

Orientador: Bruno Ricardo de C. Leite Júnior

Coorientadora: Alline Artigiani Lima Tribst

VIÇOSA – MINAS GERAIS
2023

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

S237s
2023

Santos, Fábio Ribeiro dos, 1993-
Study of different barriers to microbiology stabilization of goat and sheep whey and impact on the physicochemical properties and physical stability of products / Fábio Ribeiro dos Santos. - Viçosa, MG, 2023.
1 dissertação eletrônica (120 f.): il. (algumas color.).

Texto em português e inglês

.

Orientador: Bruno Ricardo de Castro Leite Júnior.
Dissertação (mestrado) - Universidade Federal de Viçosa, Departamento de Tecnologia de Alimentos, 2023.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2023.217
Modo de acesso: World Wide Web.

1. Soro de leite. 2. Queijo de leite de cabra. 3. Queijo de leite de ovelha. 4. Agentes antiinfecciosos. 5. Testes de ultrassom. I. Leite Júnior, Bruno Ricardo de Castro, 1989-. II. Universidade Federal de Viçosa. Departamento de Tecnologia de Alimentos. Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. III. Título.

CDD 22. ed. 637.3

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

FABIO RIBEIRO DOS SANTOS

STUDY OF DIFFERENT BARRIERS TO MICROBIOLOGY STABILIZATION OF GOAT AND SHEEP WHEY AND IMPACT ON THE PHYSICOCHEMICAL PROPERTIES AND PHYSICAL STABILITY OF PRODUCTS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Magister Scientiae*.

APROVADA: 28 de fevereiro de 2023

Assentimento:

Fabio Ribeiro dos Santos
Autor

Documento assinado digitalmente
gov.br BRUNO RICARDO DE CASTRO LEITE JUNIO
Data: 02/05/2023 16:11:58-0300
Verifique em https://validar.iti.gov.br

Bruno Ricardo de Castro Leite Júnior
Orientador

Dedico esse trabalho aos meus pais, José Miguel e Maria Mendes.

Com amor e gratidão.

AGRADECIMENTOS

A Deus, fonte de amor, força e inspiração.

Aos meus pais, Miguel e Maria, por toda dedicação, amor, pelo apoio incondicional e por não medirem esforços para que eu realize os meus sonhos.

Aos meus irmãos, Alisson, Wenesson, Rodrigo e Auria, com todo afeto.

Aos meus sobrinhos, Halif, Heloisa, Thalysson, Emily, Gabriel, João e Cecilia, pelo amor incondicional que vocês têm por mim.

Ao meu orientador e corientadora, Dr. Bruno Leite e Dra. Alline Tribst, todo o meu respeito, gratidão e admiração. “Uma boa cabeça e um bom coração formam sempre uma combinação formidável.” (*Nelson Mandela*)

Aos meus amigos Eulina, Ana Maria, Handray, Filipe, Renata, Eynne, Joanes, Paula, Maria Poliana, Jaudir, Barbara, Gabriel e Bruno, pela dedicação e apoio incondicional para a realização deste trabalho. “Amizade só faz sentido se traz o céu para mais perto da gente, e se inaugura aqui mesmo o seu começo.” (*Chico Xavier)*

À Universidade Federal de Viçosa, pela oportunidade de ampliar meus conhecimentos em uma excelente instituição.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pelo apoio financeiro.

Aos integrantes do LIPA, pela cooperação e companheirismo.

Aos amigos do laboratório que conheci durante essa caminhada, em especial Heloisa, Maria José, Ana Flávia, Jeferson e Flaviana, eu agradeço pela atenção, amizade e pelos valiosos momentos de convivência. “Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana.” (*Carl Jung*)

Aos professores e funcionárias da secretaria pós-graduação em Ciência e Tecnologia de Alimentos, pelo apoio e atenção.

Muito obrigado!

“Nossas dúvidas são traidoras e nos fazem perder o que, comfrequência, poderíamos ganhar, por simples medo de arriscar. “

(William Shakespeare)

RESUMO

SANTOS, Fabio Ribeiro dos, M.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Estudo de diferentes barreiras para estabilização microbiológica do soro de queijo cabra e ovelho e impacto nas propriedades físico-químicas e estabilidade física dos produtos**. Orientador: Bruno Ricardo de Castro Leite Júnior. Coorientadora: Alline Artigiani Lima Tribst.

O soro de queijo de cabra (SQC) e ovelha (SQO) são resíduos da produção de queijo e geralmente são descartados. Para criar alternativas para o uso deste resíduo, é necessário estabilizá-lo microbiologicamente. Inicialmente, avaliou-se os parâmetros cinéticos de inativação microbiana em SQC. Verificou-se que a inativação seguiu uma cinética de primeira ordem, sendo estabelecidos valores de D de 65,7 a 1,2 minutos em temperaturas de 62 a 75ºC, respectivamente. Selecionaram-se três binômios para avaliar as características microbiológicas e físico-química do soro, com e sem adição de açúcar, durante a estocagem a 7ºC. Observou-se que o binômio 75ºC/ 5 min garantiu a estabilidade microbiológica do soro por até 21 dias, independente da adição de açúcar, com menor efeito sobre a estabilidade física quando comparado à binômios mais severos (80ºC/ 1 min). Assim, visando aumentar a vida de prateleira do soro, outras barreiras foram necessárias. Desta forma, os efeitos da associação do tratamento térmico com fermentação foram estudados, incluindo o impacto da adição de protease e uso de ultrassom durante a fermentação. Verificou-se que as fermentações sob ultrassom apresentaram maior taxa de redução do pH no SQO (23-32%). A avaliação da estabilidade física mostrou que ambos os produtos tiveram separação de creme (até 60%) e formação de soro translúcido (até 80%). Para a atividade antioxidante, foi observado um aumento de 40% (SQO) e 30% (SQC), em comparação aos soros sem fermentar. Para contagens de bactérias láticas (BAL), houve redução durante o armazenamento (1,5-3,0 log UFC.mL^{-1}). Assim, os resultados demonstraram que a fonte/composição do soro, principalmente teor de gordura, afetaram a taxa de desestabilização e a perda de viabilidade de BAL, causada pela depleção de nutrientes e baixa tolerância em pH ~4,0. Por fim, avaliou-se a eficácia da nisina, adição de *Lacticaseibacillus casei* como cultura bioprotetora e acidificação direta com ácido lático (até pH 4,5, 3,5 e 2,5) como ferramentas para garantir a estabilidade de SQC e SQO durante 28 dias à 7°C. A nisina e a acidificação

em pH 3,5 e 2,5 mantiveram a contagem de bactérias totais e psicrotróficas abaixo de 1 log UFC.mL^{-1}, com pH e acidez estáveis durante o armazenamento das amostras. A inoculação com *L. casei* também foi efetiva, atingindo 7-8 log UFC.mL^{-1} e protegendo as amostras com leve (SQO) ou nenhuma acidificação (SQC) das amostras. Com relação à estabilidade física, todas as amostras foram desestabilizadas, mas as acidificadas em pH 3,5 e 4,5 tiveram maior formação de creme e sedimentos. Por outro lado, os dados de tamanho de partícula mostraram pouca diferença entre as amostras (0 e 28 dias), sugerindo que a agitação do soro foi capaz de ressuspender as partículas separadas durante a estocagem estática. Portanto, avaliação geral dos resultados apontam que as melhores soluções para estabilização de SQC e SQO são o tratamento térmico de 75ºC/5min associado ao antimicrobiano (nisina) e a cultura bioprotetora (*L. casei*); ainda assim, caso seja necessário garantir a estabilidade física dos soros no produto desenvolvido, é importante que sejam estudadas estratégias adicionais, como o uso de estabilizantes e desnate, especialmente para produtos ácidos. Os dados obtidos neste estudo fomentam novas perspectivas quanto à utilização de SQO e SQC para o desenvolvimento de novos produtos lácteos, visando promover o aumento da renda dos produtores rurais e diminuir os impactos ambientais.

Palavras-chave: Soro de queijo de cabra. Soro de queijo de ovelha. Tratamento térmico. Ultrassom. Protease. Antimicrobiano. Bioproteção. Desenvolvimento de produto.

ABSTRACT

SANTOS, Fabio Ribeiro dos, M.Sc., Universidade Federal de Viçosa, February 2023. **Study of different barriers to microbiology stabilization of goat and sheep whey and impact on the physicochemical properties and physical stability of products**. Advisor: Bruno Ricardo de Castro Leite Júnior. Co-advisor: Alline Artigiani Lima Tribst.

Goat cheese whey (GCW) and sheep cheese whey (SCW) are byproducts of cheese production and are usually discarded. To create alternatives for the use of this waste, it is necessary to stabilize it microbiologically. Initially, the kinetic parameters of microbial inactivation in GCW were evaluated. It was verified that the inactivation followed a first order kinetics, being established values of D from 65.7 to 1.2 minutes in temperatures of 62 to 75ºC, respectively. Three binomials were selected to evaluate the microbiological and physical-chemical characteristics of the whey, with and without added sugar, during storage at 7ºC. It was observed that the binomial 75ºC/5 min ensured the microbiological stability of the whey for up to 21 days, regardless of the addition of sugar, with less effect on physical stability when compared to the more severe binomials (80ºC/1 min). Thus, to increase the shelf life of the whey, other barriers were necessary. In this way, the effects of the association of heat treatment with fermentation were studied, including the impact of the addition of protease and the use of ultrasound during fermentation. It was found that the fermentations under ultrasound showed a higher rate of pH reduction in the GCW (23-32%). Physical stability evaluation showed that both products had cream separation (up to 60%) and translucent whey formation (up to 80%). For antioxidant activity, an increase of 40% (SCW) and 30% (GCW) was observed, compared to unfermented whey. For lactic bacteria (LAB) counts, there was a reduction during storage (1.5-3.0 log CFU.mL^{-1}). Thus, the results demonstrated that the source/composition of whey, mainly fat content, affected the destabilization rate and loss of viability of LAB, caused by nutrient depletion and poor tolerance at pH ~4.0. Finally, the efficacy of nisin, addition of *Lacticaseibacillus casei* as a bioprotective culture and direct acidification with lactic acid (up to pH 4.5, 3.5 and 2.5) were evaluated as tools to guarantee the stability of GCW and SCW during 28 days at 7°C. Nisin and acidification at pH 3.5 and 2.5 maintained total and psychrotrophic bacteria counts below 1 log CFU.mL^{-1}, with pH

and acidity stable during sample storage. Inoculation with *L. casei* was also effective, reaching 7-8 log CFU.mL^{-1} and protecting the samples with mild (SCW) or no acidification (GCW) of the samples. Regarding physical stability, all samples were destabilized, but those acidified at pH 3.5 and 4.5 had greater cream and sediment formation. On the other hand, the particle size data showed little difference between the samples (0 and 28 days), suggesting that shaking the whey was able to resuspend the separated particles during static storage. Therefore, a general evaluation of the results indicates that the best solutions for stabilizing GCW and SCW are the thermal treatment of 75ºC/5min associated with antimicrobial (nisin) and bioprotective culture (*L. casei*); even so, if it is necessary to guarantee the physical stability of the whey in the developed product, it is important that additional strategies be studied, such as the use of stabilizers and skimming, especially for acidic products. The data obtained in this study foster new perspectives regarding the use of GCW and SCW for the development of new dairy products, aiming to promote an increase in the income of rural producers and reduce environmental impacts.

Keywords: Goat cheese whey. Sheep cheese whey. Heat treatment. Ultrasound. Protease. Antimicrobial. Bioprotection. Product development.

LISTA DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

SUMÁRIO

1. INTRODUÇÃO

A criação de caprinos e ovinos é uma atividade agropecuária de importância social e econômica no Brasil, visto que promove emprego e renda para pequenos e médios produtores rurais. No ano de 2017, o país produziu aproximadamente 25 milhões de litros de leite de cabra e 1,6 milhões de litros de leite de ovelha (IBGE, 2017). Apesar de menor quando comparada à pecuária leiteira de bovinos, a criação de pequenos ruminantes para obtenção de leite vem crescendo no país (GUIMARÃES et al., 2022; DE OLIVEIRA et al., 2020; BRAGA LOBO et al., 2019). O leite oriundo desses animais é considerado uma alternativa sustentável, de baixo investimento inicial e de fácil manejo por mão de obra familiar, podendo melhorar a qualidade de vida do pequeno produtor. Neste sentido, muitas pequenas propriedades rurais e familiares passaram a investir na produção leiteira para fabricação de queijos artesanal (SILVA et al., 2020).

A produção artesanal de queijos de leite de pequenos ruminantes apresenta um fator específico de fragilidade: o destino dado ao soro gerado como resíduo na produção do queijo. O soro representa entre 80 e 90% do volume de leite utilizado para fabricação do queijo (HAENLEIN; PARK, 2006) e, em queijarias de pequeno porte, muitas vezes o soro é descartado em águas residuárias ou destinado para a alimentação animal, representando uma redução de renda para esses produtores (TRIBST et al., 2020) e causando impacto ambiental negativo, ainda que em pequena escala. Embora o soro de leite de vaca seja produzido em quantidades maiores para o aproveitamento industrial, incluindo desde a produção de derivados em larga escala até a realização de processos mais complexos, como separação de frações com específicas funções biológicas (PRAZERES et al., 2012), o aproveitamento do soro de queijo de ovelha e cabra é baixo ou inexistente (POLOWSKY et al., 2017; TRIBST et al., 2020). Isso se deve ao baixo conhecimento técnico e escassos recursos financeiros, visto que os envolvidos no beneficiamento desta matéria-prima são normalmente produtores rurais em pequenas propriedades (TRIBST et al., 2020).

Desta forma, desenvolver alternativas para viabilizar o uso do soro de queijo de cabra e ovelha para produção de derivados lácteos compatíveis com a realidade das produções artesanais é importante tanto para aumentar a renda dos produtores, como para reduzir o impacto ambiental destas produções, contribuindo para a sustentabilidade das mesmas (PANGHAL et al., 2018). Isso vem ao encontro da

Agenda 2030 das Organizações das Nações Unidas (ONU), que estabeleceu 17 objetivos para transformar o mundo (ONU, 2015). Entre os 17 objetivos, observa-se que a produção artesanal e sustentável de alimentos auxilia para: “erradicação da pobreza”, “fome zero e agricultura sustentável”, “saúde e bem-estar”, “trabalho decente e crescimento econômico”, “redução das desigualdades”, e “consumo e produções responsáveis” (ONU, 2015).

A primeira ação necessária para agregar valor comercial ao soro é sua estabilização microbiológica. Para tal, o processo mais comum é a realização de tratamento térmico; entretanto, este não pode afetar a estabilidade física do produto (CLAEYS et al., 2013). Assim quando o tratamento térmico não é suficiente, pode-se optar pela associação com outras barreiras como: (i) fermentação, associada ou não com ultrassom para melhoria das propriedades tecnológicas e funcionais dos soros (CARRILLO-LOPEZ et al., 2021), (ii) adição de antimicrobianos que pode inibir microrganismos resistentes à pasteurização (SILVA et al., 2018), (iii) inoculação de culturas bioprotetoras, como *L. casei,* capazes de crescer em baixas temperaturas e limitar o crescimento dos contaminantes através da competição por compostos específicos, esgotamento de nutrientes e produção de substâncias antimicrobianas (CANON et al., 2020; RAMA et al., 2019) e (iv) acidificação direta com ácido lático pode garantir o prolongamento da vida útil desejada para soro de queijo de cabra (SQC) e soro de queijo de ovelha (SQO) (RYAN: WALSH, 2016).

2. REVISÃO DE LITERARURA

2.1. Produções de leite de caprinos e ovinos no Brasil

A caprinocultura e ovinocultura brasileira têm aumentado suas atividades nos últimos anos, com aumento nos volumes de leite produzidos, ainda que estes permaneçam aquém da pecuária leiteira de bovinos (GUIMARÃES et al., 2022; DE OLIVEIRA et al., 2020; VERRUCK; PRUDENCIO, 2018). A caprinocultura leiteira no Brasil é realizada por pequenos e médios produtores rurais e, conforme dados do Anuário Leite 2018 da Embrapa, o Brasil tem uma produção anual de 270 milhões de litros de leite de caprino, volume considerado pequeno frente ao rebanho do país. Os estados responsáveis por 70% do volume produzido são Minas Gerais, Bahia, Paraíba e Pernambuco (EMBRAPA, 2018; IBGE, 2017). Isto evidencia a importância da

caprinocultura leiteira para as regiões semiáridas do Brasil, no qual a quantidade de leite de cabra produzida é quatro vezes maior do que o volume de leite bovino (SUASSUNA, 2019).

A produção de leite de ovelha no Brasil também é realizada majoritariamente por pequenos produtores. Segundo IBGE no ano de 2017 o país produziu aproximadamente 1,7 milhões de litros de leite de ovelha (IBGE, 2017). Nas regiões Sul, Sudeste e Centro-Oeste do Brasil, houve um aumento significativo na produção de ovinos leiteiros devido a vários fatores favoráveis, como solo fértil, clima ameno e topografia (BIANCHI et al., 2018).

As pequenas propriedades rurais passaram a investir na produção leiteira para fabricação de queijos artesanal, devido a sua singularidade, os queijos artesanais vêm ganhando visibilidade junto ao comércio de queijos finos no Brasil, atingindo a classe com poder aquisitivo mais elevado e com maior conhecimento de produtos com qualidade (SILVA et al., 2020: OLIVEIRA et al., 2018), e também sendo que, neste caso, a escolha da criação de pequeno ruminantes leiteiros é favorável por requerer uma área menor em comparação à criação de bovinos (CAJA et al., 2020). Nos últimos anos, o crescimento do consumo de queijos artesanais no Brasil (PENNA et al., 2021) obtidos de leites de pequenos ruminantes (RASIKA et al., 2020), impulsionou pequenos produtores de leite a realizarem o processamento deste derivado para agregação de valor ao produto final (SILVA et al., 2020).

2.2 Leite de cabra e ovelha

A composição do leite de cabra e ovelha é apresentada na Tabela 1. Essas composições podem variar de acordo com a raça, dieta, indivíduos, número de partos, estação, alimentação, manejo, condições ambientais e localidade (TIMLIN et al., 2021;WATKINS et al., 2014). De forma geral, o leite ovino tem cerca de 50% mais gordura e proteína em comparação com os leites de vaca e cabra (GARZÓN et al., 2021; MONTEIRO et al., 2019; PARK, 2007), que apresentam composições similares (PARK, 2007). Por outro lado, os leites de ovelha e cabra se destacam por apresentar similaridade em relação a conteúdo de proteínas de alta digestibilidade e hipoalergenicidade (VERRUCK et al., 2019; BALTHAZAR et al., 2017).

Em comparação ao leite de vaca, o leite de ovelha apresenta maior concentração

de vitamina D, vitaminas do complexo B, cálcio e fósforo (MOHAPATRA et al., 2019). Tem uma distribuição diferente das frações de caseína (PARK, 2007), com maior percentual de β-CN (HUPPERTZ et al., 2006) e alta concentração de ácidos linoleicos conjugados (CLAs), com impacto positivo na saúde humana (ALBENZIO et al., 2016).

Além disso, apresenta características físico-químicas específicas, como baixa estabilidade térmica devido à elevada concentração de cálcio coloidal (HUPPERTZ et al., 2006), alto ratio Ca/P, alta capacidade tamponante causada pela elevada concentração de minerais e proteínas e menor cremeação natural quando comparado ao leite de vaca, dado ao menor tamanho dos glóbulos de gordura (TRIBST et al., 2019; PARK, 2007).

Tabela 1. Composição de leite de cabra, ovelha e vaca.

Componentes	Vaca	Cabra	Ovelha
Gordura (%)	3,87 ± 0,01	3,54 ± 0,01	6,42 ± 0,04
Sólidos não gordurosos (%)	9,20 ± 0,05	8,68 ± 0,02	12,40 ± 0,00
Proteína total (%)	3,12 ± 0,04	3,30 ± 0,01	6,04 ± 0,13
Principais Proteínas (%)	2,94 ± 0,00	3,05 ± 0,00	5,79 ± 0,12
Caseína (%)	2,36 ± 0,04	2,46 ± 0,01	4,57 ± 0,13
Lactose (%)	4,59 ± 0.01	4,10 ± 0,01	4,62 ± 0,03
Cinza (%)	0,78 ± 0,02	0,88 ± 0,01	0,95 ± 0,02
Minerais (mg/L)			
Cálcio	1143 ± 7	1129 ± 7	1813 ± 9
Fósforo	653 ± 4	777 ± 10	956 ± 20
Potássio	1510 ± 24	1962 ± 26	1125 ± 23
Sódio	354 ± 0	366 ± 2	459 ± 10
Magnésio	106 ± 1	141 ± 1	184 ± 1

Fonte: GIROUX et al., 2018

O leite de cabra apresenta composição nutricional semelhante ao leite de vaca, incluindo proteínas, gordura, lactose, vitaminas e sais minerais (GETANEH et al., 2016). Entretanto, algumas diferenças específicas da espécie caprina são responsáveis por apresenta boas propriedades nutricionais e terapêuticas, reconhecidas por pesquisadores e apreciadas por consumidores em geral (GARCIA; TRAVASSOS, 2012; AMARAL et al., 2011). Dentre as principais diferenças destacam-se a ausência de aglutinina, alta quantidade de ácidos graxos de cadeia curta e média e triacilgliceróis de cadeia média (que são utilizados em tratamentos

médicos para uma série de distúrbios clínicos), maior quantidade de ácidos graxos monoinsaturados e poli-insaturados e menores tamanhos de glóbulos de gordura. Neste contexto, 28% dos glóbulos apresentam diâmetro igual ou inferior a 1,5 µm e 65% dos glóbulos com diâmetro de 3 µm (GETANEH et al., 2016), promovendo uma maior área superficial para ação enzimática, o que confere maior digestibilidade ao leite de cabra (CENACHI et al., 2011). Outro fator de destaque está relacionado às características sensoriais, especialmente o sabor e aroma típicos, que podem ser responsáveis pela aceitação ou rejeição do leite e/ou seus derivados por parte dos consumidores (PARK et al., 2017; GARCIA; TRAVASSOS, 2012; PARK, et al., 2007).

Nesse sentido, a produção leiteira de ovinos e caprinos pode ser uma estratégia de alto potencial econômico para as pequenas e médias propriedades, especialmente visando à produção de queijos artesanais. No entanto, a produção de queijos gera soro como coproduto, que representa aproximadamente 80% do volume de leite utilizado para fabricação do queijo (HAENLEIN, 2006), e que, se não for utilizado de forma adequada reduz a lucratividade da produção e pode se tornar um problema ambiental.

2.3 Soro de queijo cabra e ovelha

O soro é um resíduo obtido na etapa de dessoragem durante a fabricação de queijo. Trata-se de uma matéria-prima de alto valor nutricional, que contém 50% dos sólidos do leite, incluindo quase toda a lactose, 20% das proteínas (majoritariamente solúveis, dentre as quais algumas apresentam alto valor nutricional e biológico), aminoácidos essenciais, vitaminas e minerais, especialmente cálcio e zinco (GIROUX et al., 2018; MACEDO et al., 2018). Também apresenta uma pequena porção de caseínas e gordura (ANAND et al., 2013).

De acordo com o tipo de processo de fabricação do queijo, o soro pode ser classificado como soro doce, quando a coagulação é realizada por ação enzimática, devendo apresentar pH entre 6,0 e 6,8; ou soro ácido, quando a coagulação é realizada completa ou parcialmente por acidificação, apresentando pH inferior a 6,0 (BRASIL, 2020). Estas diferentes formas de obtenção impactam diretamente na composição de proteínas, lactose e minerais do soro (LIEVORE et al., 2015), com maior concentração de proteínas (devido à presença de glicomacropeptídeo) e lactose no soro doce e maior concentração de ácido e de minerais, especialmente cálcio e

zinco, no soro ácido (SKRYPLONEK et al., 2019).

A composição do soro de queijo também depende do leite de origem. A composição dos soros ácidos e doces obtido dos leites de ovelha, vaca e cabra são apresentados na Tabela 2. Estes dados demonstram que o teor de proteína do soro doce e ácido foi semelhante para cada espécie, mas os soros ácidos continham menos lactose e mais sólidos totais, cinzas e minerais, principalmente cálcio e fósforo, devido à desmineralização das micelas de caseína induzida pela acidificação. Como esperado, dada à composição do leite de ovelha (Tabela 1), o soro de queijo de ovelha doce ou ácido caracteriza-se por maiores teores de sólidos totais e proteína total em comparação ao soro de leite de cabra e vaca. Além disso, o soro de ovelha contém a maior proporção de β-lactoglobulina (β-LG) e a menor proporção de α-lactalbumina (α-LA).

Os soros oriundos da fabricação de queijo de cabra e ovelha são raramente processados para posterior uso como ingrediente (ANAND et al., 2013), isso pode ser explicado considerando que, na maioria dos países, a produção de leite de outros ruminantes é menor e normalmente realizada por pequenos produtores distribuídos em diferentes regiões (TRIBST et al., 2019), dificultando a logística de coleta desses soros para processamento.

Tabela 2. Composição do soro doce e ácido obtido do leite de ovelha, vaca e cabra.

Parâmetros	Soro doce			Soro ácido		
	Vaca	Cabra	Ovelha	Vaca	Cabra	Ovelha
Ph	6,00 ± 0,08	5,90 ± 0,06	6,09 ± 0,02	4,86 ± 0,04	4,80 ± 0,03	4,9 ± 0,0
Sólidos totais (%)	6,6 ± 0,0	6,1 ± 0,0	7,9 ± 0,0	6,8 ± 0,0	6,2 ± 0,0	8,2 ± 0,0
Proteína total (%)	0,9 ± 0,0	1,0 ± 0,0	1,7 ± 0,0	0,9 ± 0,0	0,1 ± 0,0	1,5 ± 0,0
Principais proteínas (%)						
albumina sérica	5,9 ± 0,0	4,2 ± 0,4	6,3 ± 0,2	6,5 ± 0,5	3,9 ± 0,2	6,3 ± 0,1
α-lactalbumina	23,1 ± 0,3	25,5 ± 0,7	13,8 ± 0,1	23,2 ± 0,0	24,7 ± 0,2	13,8 ± 0,1
β-lactoglobulina	50,3 ± 0,6	42,3 ± 1,4	56,2 ± 0,1	49,8 ± 0,6	41,6 ± 0,2	57,9 ± 0,2
IgG	15,8 ± 1,0	25,3 ± 1,2	19,3 ± 0,1	16,3 ± 0,5	26,6 ± 0,1	18,7 ± 0,4
Lactose (%)	5,9 ± 0,0	4,2 ± 0,0	5,3 ± 0,0	4,7 ± 0,0	3,9 ± 0,0	4,7 ± 0,0
Gordura (%)	0,6 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0	0,1 ± 0,0
Cinza (%)	0,6 ± 0,0	0,7 ± 0,0	0,6 ± 0,0	0,7 ± 0,0	0,8 ± 0,0	0,8 ± 0,0
Minerais (mg/L)						
Cálcio	513 ± 6	374 ± 2	362 ± 4	985 ± 2	860 ± 8	1247 ± 3
Fósforo	396 ± 1	464 ± 1	417 ± 4	569 ± 4	650 ± 2	754 ± 14
Potássio	1462 ± 13	1885 ± 26	1076 ± 2	1482 ± 15	1937 ± 13	1131 ± 10
Sódio	356 ± 1	379 ± 1	200 ± 5	368 ± 1	390 ± 1	525 ± 10
Magnésio	88 ± 1	104 ± 0	116 ± 1	100 ± 1	129 ± 1	169 ± 0

Fonte: GIROUX et al., 2018

Desta forma, os soros de queijos produzidos com leites de ovelha e cabra são destinados, na maioria das vezes, à alimentação animal ou descartados, reduzindo o ganho monetário dos pequenos produtores (DE OLIVEIRA et al., 2020; KAUR et al., 2020).

Além do prejuízo econômico, o descarte do soro em águas residuárias é poluente (MACEDO et al., 2018), porque sua elevada concentração de nutrientes favorece o crescimento de bactérias aeróbicas que consomem o oxigênio dissolvido nas águas dos rios, levando à asfixia de peixes (CARVALHO et al., 2013). Este descarte incorreto muitas vezes ocorre em pequenas propriedades devido à falta de estrutura para destinação correta do soro (DE OLIVEIRA et al., 2020). Desta forma, desenvolver alternativas viáveis para uso do soro de queijo de leite de cabra compatíveis com a realidade das produções artesanais é importante tanto para aumentar a renda dos

produtores, como para reduzir o impacto ambiental, ainda que em pequena escala, destas produções, aumentando sua sustentabilidade em diferentes eixos (PANGHAL et al., 2018). Para solucionar esse problema, o primeiro passo envolve a estabilização microbiológica e físico-química do soro. Neste sentido, o processamento térmico sob condições variadas é considerado uma etapa necessária na fabricação de produtos lácteos visando a segurança (IDF, 2022), extensão da vida útil e configuração de características texturais particulares (MOATSOU, 2023).

2.4. Processamento térmico em produtos lácteos

O processamento térmico é uma prática com uma longa história e ainda se mostra hoje em dia como um dos mais relevantes métodos de conservação usado na indústria de alimentos. O processamento térmico foi patenteado por Appert em 1810, sem qualquer conhecimento sobre microbiologia (SMELT; BRUL, 2014). Apenas no final do século XIX que Louis Pasteur “inventou” o processo de aquecer líquidos, tais como, vinho e cerveja a temperaturas relativamente baixas (55 ºC) durante um curto espaço de tempo, com o objetivo de evitar a deterioração destes produtos. Este método foi denominado pasteurização (processo térmico a temperaturas geralmente inferiores a 100 ºC) e é atualmente aplicado em uma vasta gama de produtos, incluindo o leite, com o objetivo de reduzir o número de microrganismos presentes, inativando os microrganismos patogênicos e reduzindo os deterioradores, para garantir a segurança e aumentar o tempo de vida útil do alimento (COSTARD et al., 2017; SMELT; BRUL, 2014).

Apesar de a pasteurização ser efetiva para inativar a maioria das bactérias, vírus, leveduras, bolores e protozoários, existe um grupo de microrganismos termodúricos que podem sobreviver ao processo (RAMESH; AMUTHAVALLI, 2021). Além deles, os esporos bacterianos não são inativados pela pasteurização (TIMMERMANS et al., 2022). Assim, para adequada extensão da vida útil e garantia da segurança do produto, é necessário associar refrigeração antes e após o tratamento térmico em produtos lácteos (FELLOWS, 2018; SHASHI et al., 2018; SOUZA et al., 2013).

Para que a inativação microbiana em processos de pasteurização seja descriminada, alguns modelos cinéticos são utilizados. O modelo mais simples que pode ser ajustado a uma curva de inativação microbiana é a cinética de primeira ordem, descrita pelo modelo de Bigelow, através dos parâmetros D e z (AUGUSTO et

al., 2011). O valor D é o tempo necessário para reduzir um ciclo logarítmico (ou seja, 90%) de uma população de microrganismos a uma temperatura constante. Já o valor z, é a mudança de temperatura necessária para alterar o valor D em 10 vezes.

Tais parâmetros são úteis para mensurar o nível de resistência de um microrganismo ao tratamento térmico em uma faixa de temperatura (LINDSAY et al., 2021; WRAY, 2015). A Tabela 3 mostra os valores D e z de várias bactérias importantes na fabricação de laticínios e derivados lácteos.

Tabela 3 - Valores D e z de bactérias não formadoras de esporos importantes na fabricação de laticínios e derivados lácteos.

Bactéria	Temp. de processo aplicado (°C)	Valor D (min)	Valor z (ºC)	Matriz láctea	Referência
E. coli	64	0,385	1,69	Mistura de sorvete	DESMARCHELIE; FEGAN (2003); MCKELLAR, 1996
	76	0,00195	2,80	Leite	JOHNSON et al, (1990); READ et al. (1961);
	76	0,00265	---	Achocolatado	READ et al. (1961)
S. aureus	70	0,10	3,53	Leite	PEARCE et al. (2012); ICMSF (1996)
	75	0,02	---	Leite	ICMSF (1996)
L. monocytogenes	68	0,19	6,71	Manteiga	DE RAMOS (1998)
	72	0,03	---	Leite	SUTHERLAND; PORRITT (1997)
Salmonella	60	11,00	6,10	Leite desnatado	MAÑAS et al. (2001)
	65	1,30	6,10	Leite desnatado	MAÑAS et al. (2001)
	80	3,50	14,75	leite em pó integral	MAÑAS et al. (2001)
	85	3,54	15,02	leite em pó integral desnatado	WEI et al., (2020)

Fonte: Adaptada de Lindsay et al. (2021)

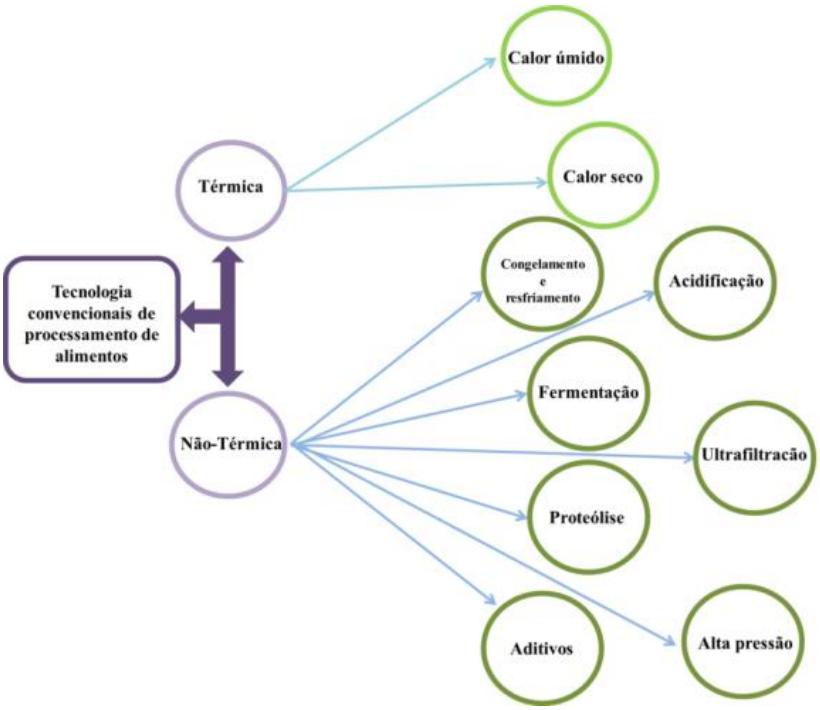
Apesar de importante no ponto de vista microbiológico, o processo térmico é limitado e pode causar efeitos negativos nos alimentos por meio de alterações nas propriedades sensoriais, como cor, textura e sabor (BARBA et al., 2012). Além disso, pode afetar o valor nutricional, como redução de alguns compostos bioativos, indução

de inativação enzimática, perda de vitaminas, oxidação lipídica e desnaturação de proteínas, resultando em alimentos de pior qualidade quando comparado aos alimentos *in natura* (MORENO-VILET et al., 2018; CHOUDHARY et al., 2012). Neste contexto, muitas vezes faz-se necessário associar barreiras ao crescimento microbiano para garantir a obtenção de um produto com características sensoriais, nutricionais e vida de prateleira adequada sem a necessidade de aplicação de temperaturas extremas.

2.5 Principais barreiras tecnológicas utilizadas para a conservação de leite e produtos lácteos

A aplicação da tecnologia de barreira ou obstáculos é um método para garantir a segurança dos alimentos (LIAO et al., 2020). Esta tecnologia combina dois ou mais métodos de conservação de alimentos, visando controlar o crescimento de microrganismos, tornando os alimentos seguros para consumo e prolongando sua vida útil (DENG et al., 2020). A conservação de quase todos os alimentos é baseada na aplicação combinada de várias tecnologias (KHAN et al., 2017). Muitas técnicas de conservação de alimentos, como pasteurização, congelamento, resfriamento, redução da atividade de água, acidificação, antimicrobiana e fermentação têm sido usadas para controlar o crescimento microbiano em alimentos (Figura 1) (NEGI, 2012). No entanto, a contaminação de alimentos e a deterioração microbiana é um problema que ainda precisa ser controlado de forma eficaz.

Figura 1 – Principais tecnologias convencionais de processamento de alimentos usadas na indústria alimentícia



Fonte: Khan et al. (2017).

A utilização da tecnologia de obstáculos requer cuidados na aplicação e é preciso saber quais serão os efeitos da aplicação dos obstáculos sobre constituintes dos alimentos e sobre a aceitação sensorial. Por exemplo, as tecnologias térmicas convencionais podem afetar a cor do alimento, que é um parâmetro crítico de qualidade, e também reduzir seu valor nutricional (ROOBAB et al., 2021: BERDEJO et al., 2019). A aplicação do ozônio, apesar dos benefícios já identificados, ainda é pouco utilizada por causa do alto custo e também pela baixa eficácia contra esporos, vírus e cistos. Outro exemplo a ser citado são as micro-ondas cuja utilização não é bem aceita por consumidores, dificultando a comercialização, também apresenta alto custo, além disso, não apresenta aquecimento eficiente em toda a embalagem contendo o alimento (LIAO et al., 2020). Dentre tecnologias citadas, a tecnologia de ultrassom se destaca devido à sua operação simples, fácil controle, bons efeitos nas propriedades físico-químicas e microbiológicas dos alimentos tendo grande potencial para ser usado em produções artesanais, bem como para ser aplicado em produtos fermentados, facilitando o crescimento e a proliferação de células microbianas e redução do tempo de processo durante a fermentação (PEREIRA; VICENTE, 2010: OJHA et al., 2016). Para a produção artesanal o uso da associação de barreiras é dificultado tanto pela falta de acesso às novas tecnologias, pois muitas vezes são de

alto custo, como também pela dificuldade com a operacionalização. As barreiras passíveis de serem aplicadas nas produções artesanais são em sua maioria tecnologias convencionais do processamento de alimentos, redução da atividade de água, temperatura tanto para aquecimento, resfriamento ou congelamento, acidificação, aplicação de bioconservantes ou microbiota competitiva como o *Lactcaseibacillus casei*, aplicação de conservantes naturais como a nisina, que é um bacteriocina de amplo espectro metabolizado por *Lactococcus lactis* (KHAN et al., 2017; CHEN; HOOVER, 2006).

2.5.1 Fermentação e acidificação

A conservação pela redução do pH tem como objetivo manter o pH do produto ≤ 4,6 visando a inibição do crescimento da maioria das espécies de bactérias, incluindo as esporuladas, com consequente melhora na conservação dos alimentos. Ela pode ser alcançada por acidificação direta, na qual um ácido é misturado ao produto até alcançar o pH desejado (KAUR; KUHAD, 2019), ou por fermentação, processo no qual o crescimento de microrganismos específicos utiliza os açúcares disponíveis no meio como fonte de energia, resultando na produção de ácidos (DIEZ-GUTIÉRREZ et al., 2020).

Antigamente, a preservação do alimento acontecia através de sua fermentação natural, entretanto, com a produção em larga escala e com o avanço na biotecnologia, técnicas de fermentação mais aprimoradas foram inseridas, garantindo a padronização da qualidade do produto final (MISHRA et al., 2017; ROSS et al., 2002). Os principais ácidos desenvolvidos nos processos de fermentação são o ácido láctico, por bactérias ácidas lácticas, e o ácido acético, por bactérias acéticas. Além dos ácidos, alguns processos de fermentação podem resultar na produção de álcool, que também tem um papel de conservação dos alimentos (ROBLES et al., 2019).

A fermentação pode apresentar mudanças nas características sensoriais e nutricionais, devido à transformação de substratos e formação de produtos bioativos ou biodisponíveis (SUO et al., 2021: MARCO et al., 2017). Como exemplo desse processo em produtos lácteos fermentados, tem-se o leite fermentado que é resultado do processo de redução do teor de lactose e pH do leite por meio do metabolismo de culturas do gênero *Lactobacillus sp.* e da espécie *Streptococcus thermophilus* e/ou outras bactérias láticas. Tais microrganismos convertem a lactose em ácido lático, que

aumenta a vida útil do produto e modifica sua textura e o sabor (SILVA, 2019). Além disso, eles também produzem metabólitos que conferem aroma e sabor, modificando as características sensoriais dos produtos de forma desejável. Estes microrganismos específicos devem ser viáveis, ativos e abundantes no produto final durante seu prazo de validade (BARBOZA; BELO, 2017; CASAROTTI et al., 2014).

A acidificação direta também é usada na conservação de alimentos. Exerce função nas propriedades físicas, biológicas e sensoriais nos alimentos (MACHADO et al., 2011). Diferentes ácidos (por exemplo, ácidos cítrico, ascórbico, lático, fumárico e málico) são economicamente viáveis para produtos lácteos, como por exemplo, promovendo a coagulação das proteínas do leite, reduzindo o tempo de coagulação e simplificando o método de obtenção da massa de queijo, quando comparada com o processo de acidificação por fermentação lática (HORBAN et al., 2017). O ácido láctico é bastante utilizado na acidificação direta, e está relacionado ao controle de microrganismos patogênicos, sabor, controle do pH, características sensoriais e melhor retenção de água (MACHADO et al., 2011).

É importante ressaltar que, apesar da acidificação ser uma barreira interessante para limitar o crescimento microbiano, ela precisa ser associada com outras ferramentas, como pasteurização para inibição da carga nativa do produto e refrigeração, de forma a garantir a segurança e uma vida útil adequada ao produto (PEREIRA et al., 2021; LEISTNER; GORRIS, 1995).

2.5.2 Nisina e cultura bioprotera *L casei*

A nisina é uma bacteriocina, isto é, peptídeo com atividade antimicrobiana produzida por *Lactococcus lactis subsp. lactis* (HAYES et al., 2019). Apresenta baixa toxicidade e tem atividade contra muitas bactérias Gram-positivas, incluindo algumas bactérias lácticas, patógenos como *Listeria* e *Staphylococcus*, e as bactérias formadoras de esporos, como *Bacillus* e *Clostridium* (OKUDA et al., 2013; BARTOLONI et al., 2004; FERREIRA; LUND, 1996).

A nisina é utilizada para conservação de alimentos como alternativa aos conservantes sintéticos (OGAKI et al., 2015; CHEN; HOOVER, 2003), sendo que uma das suas primeiras aplicações foi realizada visando evitar o estufamento tardio em queijos causado por *Clostridium spp*. (GALVEZ et al., 2008). Para exemplificar, SAAD et al. (2019) avaliaram o impacto da nisina no controle do crescimento de bactérias

deteriorantes do leite e verificaram um efeito inibitório nas contagens bacterianas totais, bactérias psicotrópicas com extensão da vida útil das amostras tratadas. PELINCAN e HASTAOĞLU (2020) investigaram o efeito da nisina no crescimento de cepas de *Staphylococcus aureus* no queijo branco fresco e verificaram que a nisina inibiu o crescimento de todas as cepas avaliadas. Apesar do uso amplo na conservação de alimentos, o efeito inibitório da nisina sobre *Staphylococcus* varia entre as espécies. LEITE JUNIOR e TRIBST (2022) avaliaram o efeito da nisina sobre espécies de bactérias lácticas com atividade fermentativa e/ou probiótica (*Lactobacillus helveticus*, *Lacticaseibacillus rhamnosus* e *Lacticaseibacillus casei*) adicionadas em soro de queijo de ovelha e de cabra e observaram que a nisina inibiu o crescimento de *Lactobacillus helveticus*, reduziu a população de *Lacticaseibacillus rhamnosus* e a contagem de bactérias lácticas após o período de armazenamento, indicando que o uso de nisina deve ser cauteloso em produtos onde o crescimento de bactérias lácticas é desejado.

As culturas bioprotetoras são bactérias ou fungos capazes de produzir substâncias antimicrobianas, incluindo os ácidos orgânicos, tais como ácido láctico, acético e outros ácidos, peptídeos, reuterina, e outros compostos (LASSOIS et al., 2008; MAGNUSSON; SCHNÜRER, 2001). As bactérias lácticas se destacam neste grupo de culturas bioprotetoras dada a variedade de compostos antimicrobianos que produzem.

Nos últimos anos, muitos pesquisadores descobriram que as cepas de BAL podem inibir os crescimentos de microrganismos patogênicos (BROSNAN et al., 2012; MUHIALDIN; HASSAN, 2011). Entre as bactérias lácticas o *Lacticaseibacillus casei* se destaca pela capacidade em inibir microrganismos deteriorantes em alimentos, como *E. coli* (PANAGOU, 2006), *Salmonella enterica* (YADAV et al., 2017) e *Listeria monocytogenes* (WU et al., 2022).

2.5.3 Tecnologia de Ultrassom em produtos lácteos

O ultrassom é uma tecnologia nova e promissora em produtos lácteos, visando minimizar o tempo de processamento, aumentar a qualidade, melhorar a eficácia do processamento e garantir a segurança dos alimentos e a extensão da sua vida útil (MARTINI, 2013; AWAD et al., 2012). A tecnologia de ultrassom é segura, econômica e sustentável, apresentando assim vantagens importantes sobre outras tecnologias

inovadoras. O ultrassom é definido como ondas sonoras de frequências além do limiar de audição humana, geralmente superiores a 20 kHz. Com base na frequência e quantidade de energia ou intensidade do som, a tecnologia de ultrassom na indústria de alimentos é classificada em dois tipos: ultrassom de alta intensidade e baixa frequência ou ultrassom de baixa intensidade e alta frequência. O ultrassom de baixa frequência e alta intensidade mostra grande potencial para uma ampla gama de aplicações no processamento de laticínios, uma vez que sua alta potência é suficiente para gerar cavitação e produzir efeitos físicos, químicos e bioquímicos desejáveis nos alimentos. Esses efeitos são usados para modificar as propriedades físico-químicas e melhorar a qualidade dos alimentos durante o processamento (SFAKIANAKIS et al., 2015, SHERSHENKOV; SUCHKOVA, 2015)

CARRILLO-LOPEZ et al. (2021) relataram que o uso de ultrassom (US) foi capaz de aumentar o rendimento do queijo Panela para 24,29% com 10 min de sonicação, quando comparado ao controle 20,33% sem o uso do US e diminuiu efetivamente as contagens de bactérias coliformes totais ~ 5 log UFC.mL^{-1} para < 1 log UFC.mL^{-1}. Os resultados mostraram que o ultrassom melhorou o rendimento e as qualidades microbiológica do queijo Panela.

ABESINGHE et al. (2019) também observaram que o ultrassom (20 KHz, 180 W, 270 W e 450 W) é capaz de reduzir o tempo de fermentação e melhorar a qualidade, estabilidade e emulsificação dos produtos lácteos fermentados. Tais autores observaram que o processo pode ser benéfico quando usado como pré-tratamento (antes da inoculação), mas pode ser prejudicial quando aplicado durante a fermentação. Neste caso, os principais problemas relatados são a formação de grandes agregados proteicos e a redução da firmeza.

3. REFERÊNCIAS

ABESINGHE, A. M. N. L. et al. Effects of ultrasound on the fermentation profile of fermented milk products incorporated with lactic acid bacteria. **International Dairy Journal**, v. 90, p. 1-14, 2019.

ALBENZIO, M, et al, Nutritional properties of small ruminant food products and their role on human health, **Small Ruminant Research,** v, 135, p, 3-12, 2016.

ANAND, S,; SOM NATH, K,; CHENCHAIAH, M, Whey and whey products, **Milk and Dairy Products in Human Nutrition: Production, Composition and Health**, p, 477-

497, 2013.

AUGUSTO, P, E,; TRIBST, A, A,; CRISTIANINI, M, Thermal inactivation of Lactobacillus plantarum in a model liquid food, **Journal of Food Process Engineering,** v, 34, n, 4, p, 1013-1027, 2011.

AWAD, T. S. et al. Applications of ultrasound in analysis, processing and quality control of food: A review. **Food research international**, v. 48, n. 2, p. 410-427, 2012.

BALTHAZAR, C, et al, Sheep milk: physicochemical characteristics and relevance for functional food development, **Comprehensive reviews in food science and food safety,** v, 16, n, 2, p, 247-262, 2017.

BARTOLONI, A. et al. In-vitro activity of nisin against clinical isolates of Clostridium difficile. **Journal of chemotherapy**, v. 16, n. 2, p. 119-121, 2004.

BARBOZA, Jéssica Costa Alves; BELO, Renata França Cassimiro. Análise de leites fermentados comercializados como alimentos funcionais probióticos. **Revista Brasileira de Ciências da Vida**, v. 5, n. 1, 2017.

BROSNAN, Brid et al. Rapid identification, by use of the LTQ Orbitrap hybrid FT mass spectrometer, of antifungal compounds produced by lactic acid bacteria. **Analytical and Bioanalytical Chemistry**, v. 403, n. 10, p. 2983-2995, 2012.

BONATSOU, Stamatoula; PARAMITHIOTIS, Spiros; PANAGOU, Efstathios Z. Evolução de consórcios de leveduras durante a fermentação de azeitonas pretas naturais de Kalamata após dois tratamentos iniciais de acidificação. **Fronteiras em microbiologia** , v. 8, p. 2673, 2018.

BERDEJO, Daniel et al. Exploiting the synergism among physical and chemical processes for improving food safety. **Current Opinion in Food Science**, v. 30, p. 14-20, 2019.

BIANCHI, A, E, et al, Effect of the addition of protected fat from palm oil to the diet of dairy sheep, **Revista Brasileira de Zootecnia,** v, 47, 2018.

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento, Portaria n° 80,de 13 de agosto de 2020, Regulamento técnico para fixação de identidade e qualidade de soro de leite e o soro de leite ácido,Diário Oficial da República Federativa do Brasil,Brasília, 13 ago, 2020, Disponivel em: <https://www,gov,br/agricultura/pt-br/assuntos/suasa/regulamentos-tecnicos-de-identidade-e-qualidade-de-produtos-de-origem-animal-1/rtiq-leite-e-seus-derivados> Acessado em: 15/12/2022.

BRAGA LOBO, Raimundo Nonato. Opportunities for investment into small ruminant breeding programmes in Brazil. **Journal of Animal Breeding and Genetics**, v. 136,

n. 5, p. 313-318, 2019.

BARBA, Francisco J.; ESTEVE, María J.; FRÍGOLA, Ana. High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: a review. **Comprehensive Reviews in Food Science and Food Safety**, v. 11, n. 3, p. 307-322, 2012.

CASAROTTI, Sabrina N.; CARNEIRO, Bruno M.; PENNA, Ana Lúcia B. Evaluation of the effect of supplementing fermented milk with quinoa flour on probiotic activity. **Journal of Dairy Science**, v. 97, n. 10, p. 6027-6035, 2014.

CAJA, G, et al, Sensing solutions for improving the performance, health and wellbeing of small ruminants, **Journal of Dairy Research,** v, 87, n, S1, p, 34-46, 2020.

CANON, F, et al, Understanding the mechanisms of positive microbial interactions that benefit lactic acid bacteria co-cultures, **Frontiers in Microbiology,** v, 11, p, 2088, 2020.

CARRILLO-LOPEZ, L, M, et al, Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties, **Ultrasonics Sonochemistry,** v, 73, p, 105467, 2021.

CARVALHO, F,; PRAZERES, A, R,; RIVAS, J, Cheese whey wastewater: Characterization and treatment, **Science of the total environment,** v, 445, p, 385-396, 2013.

CENACHI, D, B, et al, Aspectos composicionais, propriedades funcionais, nutricionais e sensoriais do leite de cabra: Uma revisão, **Revista do Instituto de Laticínios Cândido Tostes,** v, 66, n, 382, p, 12-20, 2011.

CHEN, A. H; HOOVER, D. G. Bacteriocins and their food applications. **Comprehensive reviews in food science and food safety**, v. 2, n. 3, p. 82-100, 2003.

CHOUDHARY, Ruplal et al. Ultraviolet pasteurization for food industry. International Journal of Food Science and Nutrition Engineering, v. 2, n. 1, p. 12-15, 2012.

CLAEYS, W, L, et al, Raw or heated cow milk consumption: Review of risks and benefits, **Food control,** v, 31, n, 1, p, 251-262, 2013.

COSTARD, S, et al, Outbreak-related disease burden associated with consumption of unpasteurized cow's milk and cheese, United States, 2009–2014, **Emerging infectious diseases,** v, 23, n, 6, p, 957, 2017.

DA SILVA, D, F, et al, Physical and functional properties of cheese powders affected by sweet whey powder addition before or after spray drying, **Powder Technology,** v,

323, p, 139-148, 2018.

DA SILVA DUARTE, V, et al, Comparative evaluation of cheese whey microbial composition from four Italian cheese factories by viable counts and 16S rRNA gene amplicon sequencing, **International Dairy Journal,** v, 104, p, 104656, 2020.

DE OLIVEIRA, I, K, C, P, et al, Proximate composition determination in goat cheese whey by near infrared spectroscopy (NIRS), **PeerJ,** v, 8, p, e8619, 2020.

DESMARCHELIER, P. M. et al. Enteropathogenic Escherichia coli. **Foodborne microorganisms of public health significance**, n. Ed. 6, p. 267-310, 2003.

DE MATOS, R. Esteves. Heat resistance of Listeria monocytogenes in dairy products as affected by the growth medium. **Journal of applied Microbiology**, v. 84, n. 2, p. 234-239, 1998.

DENG, Zhen-Shan et al. Insights into non-symbiotic plant growth promotion bacteria associated with nodules of Sphaerophysa salsula growing in northwestern China. **Archives of microbiology**, v. 202, n. 2, p. 399-409, 2020.

DIEZ-GUTIÉRREZ, Lucía et al. Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. **Journal of Functional Foods,** v. 64, p. 103669, 2020.

DO AMARAL, D, S,; DO AMARAL, D, S,; DE MOURA NETO, L, G, Tendências de consumo de leite de cabra: enfoque para a melhoria da qualidade, **Revista Verde de Agroecologia e Desenvolvimento Sustentável,** v, 6, n, 1, p, 5, 2011.

EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária,Anuário do leite, 2018, Disponível em: <https://www,embrapa,br/busca-depublicacoes/-/publicacao/1094149/anuario-leite-2018indicadores-tendencias-eoportunidades-para-quem-vive-no-setor-leiteiro> Acesso em: 17/12/2022.

FERREIRA, M. A. S. S.; LUND, B. M. The effect of nisin on Listeria monocytogenes in culture medium and long-life cottage cheese. **Letters in Applied Microbiology**, v. 22, n. 6, p. 433-438, 1996.

FELLOWS, Peter J. **Tecnologia do Processamento de Alimentos-: Princípios e Prática**. Artmed Editora, 2018.

GALVEZ, A, et al, Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria, **Critical reviews in biotechnology,** v, 28, n, 2, p, 125-152, 2008.

GARZÓN, A. et al. Derivation of multivariate indices of milk composition, coagulation properties, and curd yield in Manchega dairy sheep. **Journal of Dairy Science**, v. 104,

n. 8, p. 8618-8629, 2021.

GARCIA, R, V,; TRAVASSOS, A, E, R, Aspectos gerais sobre o leite de cabra: uma revisão, **Revista do Instituto de Laticínios Cândido Tostes,** v, 67, n, 386, p, 81-88, 2012.

GETANEH, G, et al, Review on goat milk composition and its nutritive value, **J, Nutr, Health Sci,** v, 3, n, 4, p, 401-410, 2016.

GIROUX, H, J,; VEILLETTE, N,; BRITTEN, M, Use of denatured whey protein in the production of artisanal cheeses from cow, goat and sheep milk, **Small Ruminant Research,** v, 161, p, 34-42, 2018.

GUIMARÃES, V, P, et al, The future of small ruminants in Brazil: Lessons from the recent period and scenarios for the next decade, **Small Ruminant Research,** v, 209, p, 106651, 2022.

HAENLEIN, G, F,; PARK, Y, W, **Handbook of milk of non-bovine mammals**, Blackwell, 2006, ISBN 0813820510.

HAYES, K.; COTTER, L.; O'HALLORAN, F. In vitro synergistic activity of erythromycin and nisin against clinical Group B Streptococcus isolates. **Journal of applied microbiology**, v. 127, n. 5, p. 1381-1390, 2019.

HORBAN, M. A.; SILVA, A.A.; MAYDL, M. P.; CASTELLA. R.; LUNELLI.C. E.

Produção de poli (ácido lático) a partir do soro do leite. **The Journal of Engineering and**

Exact Sciences, v. 3, n. 8, p. 1136-1150, 2017.

HUPPERTZ, T,; KELLY, A,; FOX, P, High pressure-induced changes in ovine milk, 1, Effects on the mineral balance and pH, **Milchwissenschaft-milk Science International,** v, 61, n, 3, p, 285-288, 2006.

IBGE, Instituto Brasileiro de Geografia, Censo Agropecuário 2017, Disponível em <https://sidra,ibge, gov,br/pesquisa/censo-agropecuario/censo-agropecuario-2017>, Acesso em 05/12/2022.

(ICMSF),"International Committee on Microbiological Specification for Foods", 1996. Microorganisms in Foods: Their significance and method of enumeration. 2 Ed. University of th Toronto Press, Toronto, Canada, p: 564-790.

International Dairy Federation (IDF). Heat treatment of milk (Bulletin of the IDF n° 516/2022), Brussels, Belgium. Disponível em < https://shop.fil-idf.org/collections/publications/products/bulletin-of-the-idf-n-515-2022-heat-treatment-of-milk>, Acesso em 05/01/2023.

JUNIOR, Bruno Ricardo de Castro Leite; TRIBST, Alline Artigiani Lima. Use of nisin and bioprotective lactic cultures to extend the shelf life of sheep and goat cheese whey. **Food Bioscience**, v. 50, p. 102096, 2022.

JOHNSON, Eric A.; NELSON, John H.; JOHNSON, Mark. Microbiological safety of cheese made from heat-treated milk, part II. microbiology. Journal of Food Protection, v. 53, n. 6, p. 519-540, 1990.

KAUR, N, et al, Recent developments in purification techniques and industrial applications for whey valorization: A review, **Chemical Engineering Communications,** v, 207, n, 1, p, 123-138, 2020.

KHAN, I, et al, Hurdle technology: A novel approach for enhanced food quality and safety–A review, **Food Control,** v, 73, p, 1426-1444, 2017,

KAUR, Amandeep; KUHAD, Ramesh Chander. Valorization of rice straw for ethanol production and lignin recovery using combined acid-alkali pre-treatment. **BioEnergy Research**, v. 12, n. 3, p. 570-582, 2019.

LEISTNER, L,; GORRIS, L, G, Food preservation by hurdle technology, **Trends in food science & technology,** v, 6, n, 2, p, 41-46, 1995.

LIEVORE, P, et al, Chemical characterisation and application of acid whey in fermented milk, **Journal of food science and technology,** v, 52, n, 4, p, 2083-2092, 2015,

LINDSAY, D, et al, Heat induced inactivation of microorganisms in milk and dairy products, **International Dairy Journal,** v, 121, p, 105096, 2021,

LASSOIS, Ludivine; DE BELLAIRE, L. de Lapeyre; JIJAKLI, M. Haïssam. Biological control of crown rot of bananas with Pichia anomala strain K and Candida oleophila strain O. **Biological Control**, v. 45, n. 3, p. 410-418, 2008.

LIAO, Xinyu et al. Intervenções de barreira baseadas em plasma frio: novas estratégias para melhorar a segurança alimentar. **Food Engineering Reviews** , v. 12, n. 3, pág. 321-332, 2020.

MUHIALDIN, Belal J.; HASSAN, Zaiton. Screening of lactic acid bacteria for antifungal activity against Aspergillus oryzae. **American Journal of Applied Sciences**, 2011.

MAGNUSSON, Jesper; SCHNÜRER, Johan. Lactobacillus coryniformis subsp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound. **Applied and environmental microbiology**, v. 67, n. 1, p. 1-5, 2001.

MARCO, Maria L. et al. Health benefits of fermented foods: microbiota and beyond. **Current opinion in biotechnology**, v. 44, p. 94-102, 2017.

MAÑAS, Pilar; BARSOTTI, Laura; CHEFTEL, J. Claude. Microbial inactivation by pulsed electric fields in a batch treatment chamber: effects of some electrical parameters and food constituents. **Innovative Food Science & Emerging Technologies**, v. 2, n. 4, p. 239-249, 2001.

MARTINI, Silvana; MARTINI, Silvana. An overview of ultrasound. **Sonocrystallization of fats**, p. 7-16, 2013.

MACEDO, A, et al, Integration of Membrane Processes for By-Product Valorization to Improve the Eco-Efficiency of Small/Medium Size Cheese Dairy Plants, **Foods,** v, 10, n, 8, p, 1740, 2021,

MACEDO, A,; MONTEIRO, J,; DUARTE, E, A contribution for the valorisation of sheep and goat cheese whey through nanofiltration, **Membranes,** v, 8, n, 4, p, 114, 2018.

MAGALHÃES, I, S, et al, Ultrasound-assisted enzymatic hydrolysis of goat milk casein: Effects on hydrolysis kinetics and on the solubility and antioxidant activity of hydrolysates, **Food Research International,** v, 157, p, 111310, 2022.

MISHRA, S, S, et al, Technological innovations in processing of fermented foods an overview, **Fermented Foods**, p, 21-45, 2017.

MOATSOU, Golfo. Heat treatment of goat milk–A review. **International Dairy Journal**, p. 105569, 2022.

MOHAPATRA, A,; SHINDE, A, K,; SINGH, R, Sheep milk: A pertinent functional food, **Small ruminant research,** v, 181, p, 6-11, 2019.

MONTEIRO, A, et al, Goat and sheep milk as raw material for yoghurt, **Milk Production, Processing and Marketing**, p, 13, 2019.

MORENO-VILET, L.; HERNÁNDEZ-HERNÁNDEZ, H. M.; VILLANUEVA-RODRÍGUEZ, S. J. Current status of emerging food processing technologies in Latin America: Novel thermal processing. **Innovative Food Science & Emerging Technologies**, v. 50, p. 196-206, 2018.

MCKELLAR, R. C. Influence of ice-cream mix components on the thermal stability of bovine milk γ-glutamyl transpeptidase and Listeria innocua**. International dairy journal**, v. 6, n. 11-12, p. 1181-1189, 1996.

NEGI, Pradeep Singh. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. **International journal of food microbiology**, v. 156, n. 1, p. 7-17, 2012.

OJHA, Kumari Shikha et al. Ultrasound assisted diffusion of sodium salt replacer and effect on physicochemical properties of pork meat. **International journal of food**

science & technology, v. 51, n. 1, p. 37-45, 2016.

ONU, Organização das Nações Unidas,Sustainable Development Goals, Retrieved from Sustainable Development Goals website: Disponível em: <https://www,undp,org/sustainable-development-goals>Acesso em: ,15/12/2022, 2015.

OKUDA, Ken-ichi et al. Effects of bacteriocins on methicillin-resistant Staphylococcus aureus biofilm. **Antimicrobial agents and chemotherapy**, v. 57, n. 11, p. 5572-5579, 2013.

OGAKI, Mayara Baptistucci; FURLANETO, Márcia Cristina; MAIA, Luciana Furlaneto. Revisão: Aspectos gerais das bacteriocinas. **Brazilian Journal of Food Technology**, v. 18, p. 267-276, 2015.

PANAGOU, Efstathios Z. Greek dry-salted olives: Monitoring the dry-salting process and subsequent physico-chemical and microbiological profile during storage under different packing conditions at 4 and 20 C. **LWT-Food Science and Technology**, v. 39, n. 4, p. 323-330, 2006.

PANGHAL, A, et al, Whey valorization: current options and future scenario–a critical review, **Nutrition & Food Science**, 2018.

PARK, Y, Rheological characteristics of goat and sheep milk, **Small Ruminant Research,** v, 68, n, 1-2, p, 73-87, 2007.

PARK, Y,; JEANJULIEN, C,; SIDDIQUE, A, Factors affecting sensory quality of goat milk cheeses: A review, **J Adv Dairy Res,** v, 5, n, 185, p, 2, 2017.

PELİNCAN, Özlem; HASTAOĞLU, Emre. The effects of nisin on the growth of milk-derived Staphylococcus aureus strains in the cheese**. Harran Tarım ve Gıda Bilimleri Dergisi**, v. 24, n. 3, p. 310-316, 2020.

PEARCE, L. E. et al. Pasteurization of milk: the heat inactivation kinetics of milk-borne dairy pathogens under commercial-type conditions of turbulent flow**. Journal of dairy science**, v. 95, n. 1, p. 20-35, 2012.

PENNA, A, L, B,; GIGANTE, M, L,; TODOROV, S, D, Artisanal Brazilian Cheeses—History, Marketing, Technological and Microbiological Aspects, **Foods,** v, 10, n, 7, p, 1562, 2021.

PEREIRA, R. N.; VICENTE, A. A. Environmental impact of novel thermal and non-thermal technologies in food processing. **Food Research International**, v. 43, n. 7, p. 1936-1943, 2010.

PEREIRA, Tarciara Fonseca et al. A Tecnologia de Pasteurização pelo Método

Alternativo: Capacitação e Novas Perspectivas para a Agricultura Familiar. **Saber Humano: Revista Científica da Faculdade Antonio Meneghetti,** v. 11, n. 19, p. 213-226, 2021.

RAMA, G, R, et al, Potential applications of dairy whey for the production of lactic acid bacteria cultures, **International Dairy Journal,** v, 98, p, 25-37, 2019.

RAMESH, T.; AMUTHAVALLI, A. Microbial Biotechnology of biofertilizers-An overview. **Recent Trends in Modern Microbial Technology**, v. 1, p. 86, 2021.

ROBLES, Alicia et al. Determination and identification of organic acids in wine samples. Problems and challenges**. TrAC Trends in Analytical Chemistry**, v. 120, p. 115630, 2019.

ROOBAB, Ume et al. Impact of high-pressure treatments on enzyme activity of fruit-based beverages: an overview. International Journal of Food Science & Technology, v. 57, n. 2, p. 801-815, 2022.

READ JR, R. B.; SCHWARTZ, Charles; LITSKY, Warren. Studies on the thermal destruction of Escherichia coli in milk and milk products. **Applied microbiology**, v. 9, n. 5, p. 415-418, 1961.

ROSS, R, P,; MORGAN, S,; HILL, C, Preservation and fermentation: past, present and future, **International journal of food microbiology,** v, 79, n, 1-2, p, 3-16, 2002.

RYAN, M, P,; WALSH, G, The biotechnological potential of whey, **Reviews in Environmental Science and Bio/Technology,** v, 15, n, 3, p, 479-498, 2016.

SAAD, M, A,; OMBARAK, R, A,; ABD RABOU, H, S, Effect of nisin and lysozyme on bacteriological and sensorial quality of pasteurized milk, **Journal of advanced veterinary and animal research,** v, 6, n, 3, p, 403, 2019.

SHASHI, S, et al, Food cold chain management: From a structured literature review to a conceptual framework and research agenda, **The International Journal of Logistics Management**, 2018.

SILVA, Jaqueline Ferreira. **Caracterização microbiológica e pós-acidificação de leites**

fermentados comercializados em Barra do Garças-MT. Trabalho de Conclusão de Curso

(Engenharia de Alimentos) – Instituto de Ciências Exatas e da Terra. Universidade Federal de

Mato Grosso, campus Universitário Do Araguaia, Barra do Garças – MT, 2019.

SILVA, C, C,; SILVA, S, P,; RIBEIRO, S, C, Application of bacteriocins and protective

cultures in dairy food preservation, **Frontiers in microbiology,** v, 9, p, 594, 2018,

SILVA, M, A, P, D, et al, Available in: <https://www,milkpoint,com,br/artigos/producao/producaoartesanal-de-queijos-alternativa-para-pequenos-produtores-de-leite-219626/>, Access in: March 25, 2022, 2020,

SHERSHENKOV, Boris; SUCHKOVA, Elena. The direct microbial synthesis of complex bioactive compounds as perspective way of milk whey utilization**. Energy Procedia**, v. 72, p. 317-321, 2015.

SKRYPLONEK, K,; DMYTRÓW, I,; MITUNIEWICZ-MAŁEK, A, Probiotic fermented beverages based on acid whey, **Journal of dairy science,** v, 102, n, 9, p, 7773-7780, 2019,

SMELT, J,; BRUL, S, Thermal inactivation of microorganisms, **Critical reviews in food science and nutrition,** v, 54, n, 10, p, 1371-1385, 2014.

SUO, Xiangshu et al. Effect of culturing lactic acid bacteria with varying skim milk concentration on bacteria survival during heat treatment. **Journal of Food Engineering**, v. 294, p. 110396, 2021.

SOUZA, M, et al, Emprego do frio na conservação de alimentos, **Enciclopédia Biosfera,** v, 9, n, 16, 2013.

SFAKIANAKIS, Panagiotis; TOPAKAS, Evangelos; TZIA, Constantina. Comparative study on high-intensity ultrasound and pressure milk homogenization: Effect on the kinetics of yogurt fermentation process. **Food and bioprocess technology**, v. 8, n. 3, p. 548-557, 2015.

SUN, Yaru et al. Mesopic fermentation contributes more to the formation of important flavor compounds and increased growth of Lactobacillus casei Zhang than does high temperature during milk fermentation and storage. **Journal of Dairy Science**, 2022.

SUASSUNA, J, Caprinos, uma pecuária necessária no Semiárido nordestino, Disponível em:<https://www,gov,br/fundaj/pt-br/destaques/observa-fundaj-itens/observa-fundaj/artigos-de-joao-suassuna/caprinos-uma-pecuaria-necessaria-no-semiarido-nordestino#:~:text=Cabra%20Murciana%3A%20importante%20alternativa%20para,do%20per%C3%ADodo%20colonial%20do%20Brasil,>Acesso em: 11/12/2022.

SUTHERLAND, P.; PORRITT, R. Dissemination and ecology of Listeria monocytogenes in Australian dairy factory environments. **Food Australia,** v. 48, n. 4, p. 172-178, 1996.

TIMMERMANS, Rian; NIEROP GROOT, Masja; MATSER, Ariette. Liquid Food Pasteurization by Pulsed Electric Fields. In: **Pulsed Electric Fields Technology for the Food Industry**. Springer, Cham, 2022. p. 299-323.

TRIBST, A,; FALCADE, L,; DE OLIVEIRA, M, Strategies for raw sheep milk storage in smallholdings: Effect of freezing or long-term refrigerated storage on microbial growth, **Journal of dairy science,** v, 102, n, 6, p, 4960-4971, 2019.

TRIBST, A, A, L, et al, Manufacture of a fermented dairy product using whey from sheep's milk cheese: An alternative to using the main by-product of sheep's milk cheese production in small farms, **International Dairy Journal,** v, 111, p, 104833, 2020.

TRIBST, A, A, L, et al, Impact of extended refrigerated storage and freezing/thawing storage combination on physicochemical and microstructural characteristics of raw whole and skimmed sheep milk, **International dairy journal,** v, 94, p, 29-37, 2019.

VERRUCK, S, et al, Dairy foods and positive impact on the consumer's health, **Advances in food and nutrition research,** v, 89, p, 95-164, 2019.

VERRUCK, S,; PRUDENCIO, E, S, INOVAÇÃO NA TECNOLOGIA DE DERIVADOS DO LEITE DE CABRA, 2018.

WATKINS, P, et al, Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat, **Meat Science,** v, 96, n, 2, p, 1088-1094, 2014.

WU, Mengjie et al. Potential antimicrobial activities of probiotics and their derivatives against Listeria monocytogenes in food field: A review. **Food Research International**, p. 111733, 2022.

WRAY, E, Reducing microbial spoilage of beer using pasteurisation, In: (Ed,), **Brewing Microbiology**: Elsevier, 2015, p,253-269.

YADAV, Ashok Kumar et al. Adhesion of lactobacilli and their anti-infectivity potential. **Critical reviews in food science and nutrition**, v. 57, n. 10, p. 2042-2056, 2017.

4. OBJETIVOS

4.1 Objetivo geral

Avaliar a eficiência da combinação de tratamento térmico com outras barreiras (fermentação assistida por US, redução de pH por acidificação direta, adição de nisina e inoculação de *L. casei* como cultura bioprotetora) na extensão da vida útil de soros de queijo de ovelha e cabra e determinar o impacto dos diferentes processos nas características físico-químicas, microbiológicas e na estabilidade física dos produtos.

4.2 Objetivos específicos

- Determinar condições de tratamentos térmicos que promova a estabilização do soro doce de queijo de cabra: obtenção de 5, 6 e 7 reduções decimais da população microbiana total e inativação de pelo menos 90% na atividade coagulante das proteases;
- Avaliar os parâmetros de fermentação SQC e SQO sob US, com ou sem proteases, e caracterizar os produtos obtidos quanto à contagem de BAL, características físico-químicas, estabilidade estrutural e atividade antioxidante *in vitro*;
- Avaliar a eficácia da nisina, da adição de *Lacticaseibacillus casei* como cultura bioprotetora e da adição direta de ácido lático (até pH 4,5, 3,5 e 2,5) como ferramentas para garantir a estabilidade física e microbiológica de SQC e SQO por 28 dias a 7°C.

CAPÍTULO 1

Kinetic parameters of microbial thermal death in goat cheese whey and growth of surviving microorganisms under refrigeration

Estudo publicado na revista **Journal of Food Process Engineering**. SANTOS, Fábio Ribeiro dos; LEITE JÚNIOR, Bruno Ricardo de Castro; TRIBST, Alline Artigiani Lima. Kinetic parameters of microbial thermal death in goat cheese whey and growth of surviving microorganisms under refrigeration. **Journal of Food Process Engineering**, p. e14191, 2022.

Kinetic parameters of microbial thermal death in goat cheese whey and growth of surviving microorganisms under refrigeration

Running Title: *Kinetics of inactivation in goat cheese whey*

Fábio Ribeiro dos Santos[1], Bruno Ricardo de Castro Leite Júnior[1], Alline Artigiani Lima Tribst[2]

Affiliations:

[1]Department of Food Technology (DTA), Federal University of Viçosa (UFV), University Campus, 36570-900, Viçosa, MG, Brazil,

[2]Center for Food Studies and Research (NEPA), University of Campinas (UNICAMP), Albert Einstein, 291, 13083-852, Campinas, SP Brazil,

*Author for Correspondence, Albert Einstein, 291, Postal code: 13083-852, Campinas, São Paulo, Brazil, Phone: +55 19 3521-2176, E-mail: tribst@unicamp,br

Abstract:
Whey is a byproduct of cheese production and, in smallholdings, is usually destined for animal feed or improperly disposed. To create alternatives for whey use, microbiological stabilization is the first step. Therefore, this study evaluated the kinetic parameters of the native microbiota thermal death (62-85 ºC) in goat cheese whey (GCW) and the growth of survivors after thermal processing performed at three different time-temperatures. The results showed that the come-up time inactivated 1.0-1.8 log CFU/mL (≤71 ºC), 3.8 log CFU/mL (75 ºC) and more than 8 log CFU/mL (≥80 ºC). The inactivation data at 62, 65, 68, 71, and 75 ºC were fitted to the Bigelow model, from which D values of 65.7, 31.0, 11.7, 5.4 and 1.2 min, respectively, were determined. Using a first-order equation, the z value was calculated as 7.5 ºC (R2 = 0.99). Processes time-temperatures were selected to achieve 2.5 (71 ºC / 20 min), 0.5 (75 ºC / 5 min) and <0.1 log CFU/mL (80 ºC / 1 min) survivors. During the shelf life, microbiological growth was observed in all samples, reaching total bacterial counts (TBC) and psychrotrophic bacteria (TPC) >3 log CFU/mL between 14 and 28 days, with a predominance of non-acidifying psychrotrophic bacteria. From the results, the time-temperature of 75 ºC/ 5 min was established as the best option for GCW processing, ensuring counts <2 log CFU/mL for up to 21 days.
Keywords: Goat cheese whey; Pasteurization in batch; Microbiological inactivation; Kinetic parameters; Shelf Life; Artisanal production,

Practical Application:
This study showed that the time-temperatures 71 ºC / 20 min, 75 ºC / 5 min, or 80 ºC / 1 min, applied in a batch pasteurization process, were able to inactivate more than 5 log CFU/mL of the native microbiota of goat cheese whey. However, the shelf life of the product at 7 ºC was limited to 21 days due to the growth of the surviving population, even for samples that reached < 0.1 log CFU/mL immediately after heating. Therefore, if extended shelf life is needed, additional barriers to microbial growth should be used. These data can be readily applied by artisanal goat cheese producers as a way to stabilize whey for later use, either in the development of whey beverages or for the use of whey as an ingredient in products.

1. Introduction

Goat milk is mainly used to produce artisanal cheese (Silva et al., 2020). Production occurs commonly in smallholdings that manage the entire production chain, from herd management to cheese sales (Aldalur et al., 2019). Cheese whey is a byproduct of cheese production and represents 80-90% of the volume of processed milk (Macedo et al., 2021). Cheese whey produced by enzymatic coagulation is called sweet cheese whey (pH > 6.0), while that with a pH below 5.8, obtained from casein coagulated by fermentation or direct acidifying is named acid cheese whey (Zotta et al., 2020; Pires et al., 2021). In addition, there is another byproduct obtained from the production of whey cheese (such as Ricotta fresca – Pala et al., 2016), called “second whey cheese” (Macedo et al., 2021), which is traditionally acidic and has a lower protein and fat content in relation to the primary whey (Pires et al., 2021).

Currently, cow sweet or acid cheese whey is stabilized by spray-drying (da Silva et al., 2018) or by concentration in membranes (Macedo et al., 2021) and used as an ingredient in various foods, such as dairy products and baked goods, whereas second cheese whey is less explored (Pires et al., 2021). On the other hand, goat cheese whey (GCW) is rarely processed for subsequent use as an ingredient due to the lower volume produced and the spreading of producers in different regions (Anand et al., 2013), which makes the transportation of whey to the industrial processing unfeasible.

Therefore, GCW is commonly used as animal feed or discarded, reducing the monetary gain of small producers (de Oliveira et al., 2020; Kaur et al., 2020). The processing of whey for the production of beverages or other ready-to-use foods, such as vinegar (Zotta et al., 2020), would be an alternative for the use of sweet and acid cheese whey, even on an artisanal scale. Nevertheless, the producers’ usual low technical knowledge makes this difficult (Tribst, Falcade, & De Oliveira, 2019). In addition to economic damage, the disposal of whey in wastewater is a pollutant (Macedo et al., 2018). Thus, developing alternatives for the use of GCW compatible with the reality of artisanal production is important both to increase the income of producers and to reduce the environmental impact of these productions, which is in line with the goals number 6 (clean water and sanitation), 8 (decent work and economic growth), 12 (responsible consumption and production) and 14 (life below water) of the 2030 Agenda for Sustainable Development (UNDP, 2015).

Initially, to fill this gap, it is necessary to determine the kinetic parameters for heat treatment of the GCW using a batch process, normally available in artisanal production

(Giribaldi et al., 2017). Such treatment should target the thermal, physical, and physicochemical stability of the whey, ensuring that it has a shelf life adjusted to the necessary time between production and consumption (Fellows, 2000). To the best of our knowledge, these pieces of information about sweet goat cheese whey, especially considering the native microbial load, are not available in the scientific literature.

The high availability of lactose and the pH close to neutrality make the sweet whey perishable (Zotta et al., 2020). Its native microbiota can have microorganisms with different resistances to heat treatment, such as starter cultures added to the cheese to start fermentation (Zotta et al., 2020), contaminants resulting from deficiency in hygiene and/or excessive manipulation in cheese manufacture, and thermoduric microorganisms, including sporulated microorganisms from milk that survived to pasteurization (Pala et al., 2016). Therefore, for practical applications, it is relevant to evaluate the impact of processing conditions on the real microbiota found in a product, rather than on specific cultures intentionally inoculated into the product (Tribst & Leite Júnior, 2022).

Although important for microbiological safety, heat treatment can lead to denaturation, aggregation, and, consequently, destabilization and sedimentation of whey proteins (Dumitraşcu et al., 2013), which is undesirable for whey beverage production (Tribst et al., 2020). According to Zhao et al. (2020), goat whey proteins are completely denatured when treated at 85 °C for 30 min.

The development of commercial products from goat cheese whey is considerably scarce compared to those from cows. Most of the studies have addressed this issue have focused on: (i) the concentration of whey through membrane processes (Macedo et al., 2021), using ultrafiltration and nanofiltration (Macedo et al., 2021b), or (ii) the formulation of dairy beverages mixing goat milk and cheese whey (Chaves de Lima et al., 2017; da Silveira et al 2015). Although interesting for scientific knowledge, these solutions are not desirable by artisanal producers due to the costs and difficulty of operating the membranes for the concentration of whey and the need of using part of the milk produced for the manufacture of beverages instead of making cheeses. For this reason, in Brazil, about 50% of the cheese whey is disposed (Magalhães et al., 2011; Cedeño et al., 2018).

Considering the importance of this theme for artisanal production and the observed knowledge gaps, the objectives of this study were: (i) to study a wide range of temperatures (62 - 85 ºC) to determine the kinetic parameters of microbial

inactivation by discontinuous processes, (ii) select three time-temperature conditions compatible with artisanal production and desired product quality (microbiological and physicochemical stability), and (iii) evaluate how these selected heating conditions, with different levels of residual population, would impact the growth of survivors during samples storage at 7 ºC.

2. Material and Methods

2.1 Goat cheese whey

Goat cheese whey (0.95 ± 0.05% protein, 4.14 ± 0.05% lactose, 0.60 ± 0.12% fat, and 6.39 ± 0.17% total solids) was obtained in the process of fresh cheese such as Feta (Ammar, Khalel, & Mostafa, 2014) produced with chymonsin and inoculated with a pool of starter cultures containing Streptococcus thermophilus, Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris (Capril do Bosque artisanal cheese factory, Joanópolis, Brazil). The whey was filtered through food grade cheesecloth to retain any cheese curds and stored at 1°C until processed (48 h).

2.2 Thermal processing of whey and kinetic parameters of thermal death curves

Thermal inactivation of the native whey microbiota was performed simulating heating in an artisanal cheese factory, i.e., using batch processing and slow pasteurization (Giribaldi et al., 2017). A volume of 800 mL of whey was added to a 1 L glass beaker. A T-type thermocouple (Omega Engineering Inc, USA), with a standard limit error of 0.75%, was inserted in the center of the beaker, and the sample was heated in a boiling water bath under stirring until reaching the process temperature. Then, the beaker was transferred to another water bath set at the fixed process temperature, and the time counting was started. Aliquots of 10 mL were taken at predetermined time intervals and immediately transferred to test tubes immersed in cold water. Temperatures were recorded at 5 s intervals during all experiments. Table 1 shows the process temperature and residence time for sampling. Surviving mesophilic microorganisms were counted in plate count agar incubated at 35 °C/48 h (Tribst et al., 2019). Processes were carried out in triplicate and counted in duplicate.

Table 1. Process temperature and residence time for taking aliquots

Temperature (ºC)	Residence time for taking aliquots
62	0, 7.5, 15, 22.5, and 30 min
65	0, 5, 10, 15, 20, 25, and 30 min
68	0, 2, 4, 6, 10, 15, 20, 25, and 30 min
71	0, 1, 3, 5, 7, 10, 15, 20, 25, and 30 min
75	0, 1, 3, 5, 7, 10, 15, 20 min
80	0, 1, 3, 5, 7, 10, 15, 20 min
85	0, 1, 3, 5, 7, 10 min

The decimal reduction time (D) values were obtained as the slope of the thermal inactivation curves (Augusto et al., 2011), following equation 1 [Eq. (1)]. The thermal coefficient (z) value was obtained from the slope of the thermal death time curve (log[D] versus temperature), following equation 2 [Eq. (2)] (Pflug, 1988).

$$\frac{N_t}{N_0} = 10^{-\frac{t}{D}} \;\; \therefore \;\; D = \frac{t}{(Log\, N_0 - N_t)} \qquad \text{Eq, (1)}$$

Where: N0 is the initial number of microbial cells (CFU/mL), Nt is the number of microbial cells (CFU/mL) at time t (min), t is the processing time (min) at fixed temperature (T), and D is the decimal reduction time (min) at fixed temperature (T).

$$D_2 = D_1 \cdot 10^{\left(\frac{T_1 - T_2}{z}\right)} \;\; \therefore \;\; z = \frac{T_1 - T_2}{\log D_2 - \log D_1} \qquad \text{Eq, (2)}$$

Where: D1 and D2 are the decimal reduction time (min) at fixed temperatures, T1 and T2 (°C), respectively, and z is the number of degrees Celsius required to reduce D by a factor of 10.

2.3 Impact of heat treatment on the shelf life of whey

For this step, three time-temperature combinations were chosen: 71 °C / 20 min, 75 °C / 5 min, and 80 °C / 1 min, aiming to assess how different levels of residual counts (~2.5, 0.5 and <0.1 log CFU/mL) would impact the growth and spoilage of the whey during its storage. Moreover, considering that most commercial whey beverage is sweetened, the rejection of unsweetened fermented milk by Brazilian consumers (Tribst et al., 2020), and the potential contamination of sugar with resistant bacteria

(Thompson, 2009), for each time-temperature combination, samples were prepared with sugar addition (3 or 6%), and one was kept unsweetened (pure GCW), as a control.

After, the samples were processed following the procedure described in Section 2.1. After the residence time, the beaker containing the entire volume (800 mL) was transferred to a cold water bath for rapid temperature reduction.

Then, the samples were divided into 5 glass flasks (120 mL), 1 tube (10 mL), and 4 tubes (8 mL) previously sterilized. Each process was carried out in triplicate. Samples were stored at 7 °C and evaluated after 0, 7, 14, 21, and 28 days. The storage temperature was chosen considering the deficiency of cold chain distribution in Brazil (Sadhu, 2018) and the usual temperatures of domestic refrigerators, equipment commonly used in artisanal cheese factories, consumers, and artisanal cheese shops.

2.3.1. Microbiological and physicochemical characterization

Samples were serially diluted in saline solution (0.85% NaCl) and plated in plate count agar (PCA) for total bacteria count (TBC) and psychrotrophic bacteria count (TPC), as described by Tribst et al. (2019). The TBC counts were determined after incubation at 35 °C/ 48 h, and the psychrotropic counts were determined after incubation at 7 °C / 10 days (Tribst et al., 2019). The counts of each sample were performed in duplicate.

Titratable acidity, pH (AOAC, 1999), and stability to ethanol (Tribst et al., 2019) were determined in triplicate. The percentage of sedimentation and creaming in each sample was measured after 28 days of storage. The sedimentation was the ratio between sediments formed in the base of the tube and the total volume (8 mL) of the sample (Kubo et al., 2013), and creaming was the ratio between the cream separated at the top of the tube and the total volume of the sample. Furthermore, at day 0, the residual activity of rennet was measured in triplicate (using the 10 mL sample), following the method described by Júnior. (2014), replacing the enzyme solution with whey and carrying the reaction by 120 min.

2.4 Statistical analysis

The differences in kinetic parameters of thermal death curves and in microbiological and physicochemical parameters of the whey were evaluated by using the analysis of variance (ANOVA) and the Tukey test at a 95% confidence level (XLSTAT software, version 2015.2.02, Microsoft, Inc., USA). The results are expressed as the mean ± standard deviation.

3. Results and Discussion

3.1 Kinetic parameters of the thermal death curves

The initial characterization of the GCW showed a TBC of 8.17 ± 0.11 log CFU/mL, pH of 5.65 ± 0.01, and initial acidity of 0.16 ± 0.01 g lactic acid/100 mL. The high initial microbial count observed was expected, since the whey was obtained from cheese inoculated with a pool of starter cultures containing Streptococcus thermophilus, Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris.

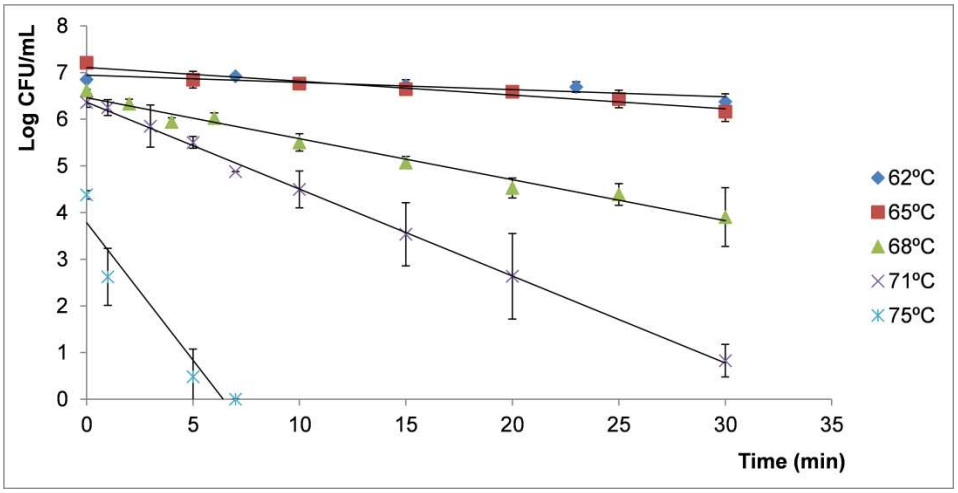
Table 2 shows the come-up time and D values of microbial inactivation in goat cheese whey processed at temperatures between 62 and 85 ºC. The come-up time ranged from 140 to 516 seconds, and the higher the temperature was, the longer the rise time. All studied processes caused microorganism reduction at the come-up time, being greater the higher the temperature ($p<0.05$) and reaching reductions up to 8.17 log CFU/mL. The come-up time, i.e., the time spent for samples reaching the set temperature for thermal processing, is directly related to the energy transfer rate, being lower in equipment with higher efficiency (Pitarch et al., 2021). Thus, the relatively high come-up times of the studied processes were expected because batch pasteurization, typically used in artisanal production, is less efficient than the continuous processing applied in the food industry.

Table 2. Kinetic parameters of the microbial thermal death curve in goat cheese whey

Set Temperature (ºC)	Real Temperature (ºC)	Come-up time		D-value	
		Time (s)	Inactivation [log(CFU/mL)]	D-value (min)	R^2
62.0	61.6 ± 1.3	140 ± 2	1.32 ± 0.06^{e}	65.7 ± 11.9^{a}	0.89 - 0.90
65.0	65.5 ± 0.8	246 ± 4	0.96 ± 0.06^{f}	31.0 ± 3.9^{b}	0.85 - 0.97
68.0	67.7 ± 1.0	221 ± 2	1.55 ± 0.03^{d}	11.7 ± 2.1^{c}	0.89 - 0.99
71.0	70.7 ± 0.6	329 ± 11	1.81 ± 0.11^{c}	5.4 ± 0.1^{d}	0.96 - 0.97
75.0	74.7 ± 0.7	435 ± 4	3.79 ± 0.09^{b}	1.2 ± 0.4^{e}	0.97 - 0.98
80.0	80.0 ± 0.7	399 ± 13	8.06 ± 0.17^{a}	Inactivated come-up time	
85.0	85.1 ± 0.8	516 ± 8	8.17 ± 0.00^{a}	Inactivated come-up time	

$^{a-f}$ Different superscript letters indicate significant differences among processes ($p<0,05$),

The microbial inactivation showed a linear death curve (Figure S1 – Supplementary file), justifying the adjustment of the Bigelow model to the data. The D values for each temperature (Table 2) were determined from the slope of each thermal death curve according to Eq. 1. The Bigelow model is based on the first-order kinetics between the logarithm of the survivors and the processing time (Augusto et al., 2011) and showed a good fit to the experimental data for almost all samples, except for the one processed at 62 ºC due to the low inactivation at this temperature. As expected, the increase in temperature between 62 and 75 ºC reduced the D values ($p<0.05$), which refers to the time required to cause inactivation of 1 log in the population (Pflug et al., 1998) of native microorganisms in GCW. The D values were used to calculate the z value (z = 7.5 ºC, R2 = 0.99) from the slope of the thermal death time curve (log [D]) versus temperature (Eq. 2). This value indicates the temperature variation required to change the D value by 1 log (Pflug et al., 1998).



Supplementary File – Figure S1: Thermal death curve of native microbial load in goat cheese whey

D and z values are needed to obtain equivalent thermal processes when it is desired to make changes in the time-temperature applied for food processing (Augusto et al., 2011). In the processes performed at 80 and 85 ºC, D values could not be determined because the microbial population was inactivated at come-up time, i.e., before reaching the temperature set for processing.

The linearity of microbial inactivation at each temperature (da Silva Duarte et al., 2020; Gabriel et al., 2020) and the absence of shoulders and/or tails (Den Besten et al., 2018) suggest that the microorganisms found in the native population of goat cheese whey have a similar inactivation profile. This hypothesis is supported by the good adjustment (R2 = 0.99) of the linear regression used to determine the z-value (Evelyn & Silva, 2015; van Boekel, 2022). Whereas the GCW was obtained from a cheese inoculated with ~7 log CFU/mL of starter culture containing *Streptococcus thermophilus, Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris*, it is possible to infer that at least one of these species had a higher thermal resistance, governing the results of thermal inactivation at the relative high temperatures observed. It is also worth mentioning that gram-positive cocci-shaped bacteria are the bacteria with the highest thermal resistance among vegetative cells (Jay et al., 2005), which is compatible with the D values observed for the different studied temperatures and previously described in the literature for other cocci-shaped bacteria (Bensalah, Delorme, & Renault, 2009).

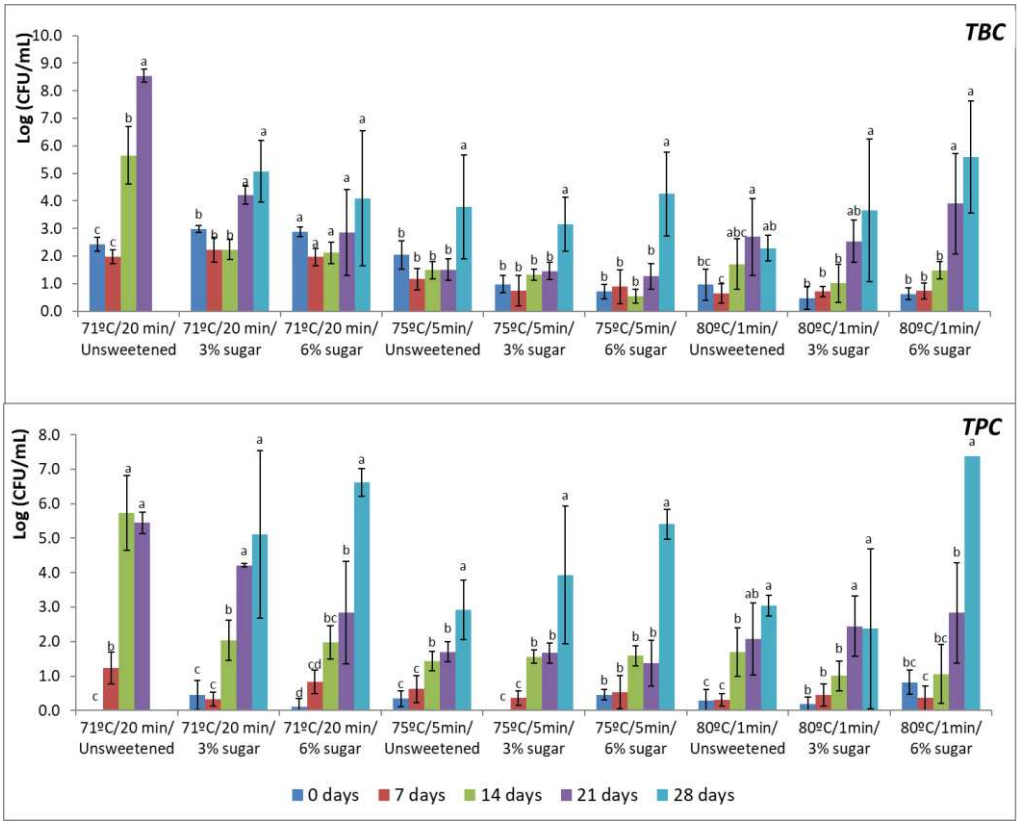
Regarding the physical stability of the GCW, it was visually observed that the process at 85 ºC caused intense protein destabilization, explained by the denaturation

of whey proteins, especially β-lactoglobulin, which is the main protein constituent of goat cheese whey (Giroux et al., 2018).

Based on the results of microbial inactivation (Fig S1 – Supplementary file), time-temperatures capable of resulting in different levels of microbiological inactivation were chosen: 71 ºC/ 20 min (5.5 decimal reduction), 75 ºC/ 5 min (7.9 decimal reduction) and 80 ºC/ 1 min (8.2 decimal reduction). These processing conditions were evaluated to determine how different levels of survivors could grow and alteration the GCW during refrigerated storage.

The residual proteolytic activity was <1.7% for the mildest time-temperature (71ºC/20 min) (Table S1 – Supplementary File). This proves that all time-temperatures applied were able to guarantee sufficient inactivation of rennet, resulting in a negligible GCW proteolysis during refrigerated storage. These results corroborate the study by Belenkaya et al. (2018), who estimated the inactivation of recombinant chymosin at temperatures ranging from 50 to 60 ºC, depending on the enzyme source.

Figure 1. Enumeration of total (TBC) and psychrotrophic (TPC) bacteria during goat cheese whey storage at 7ºC.



Legend: a-c Lower case superscripts show significant differences assessed by the Tukey test ($p < 0,05$) in each parameter measured on different days of storage for the same sample.

3.2 Impact of heat treatment on the shelf life of whey

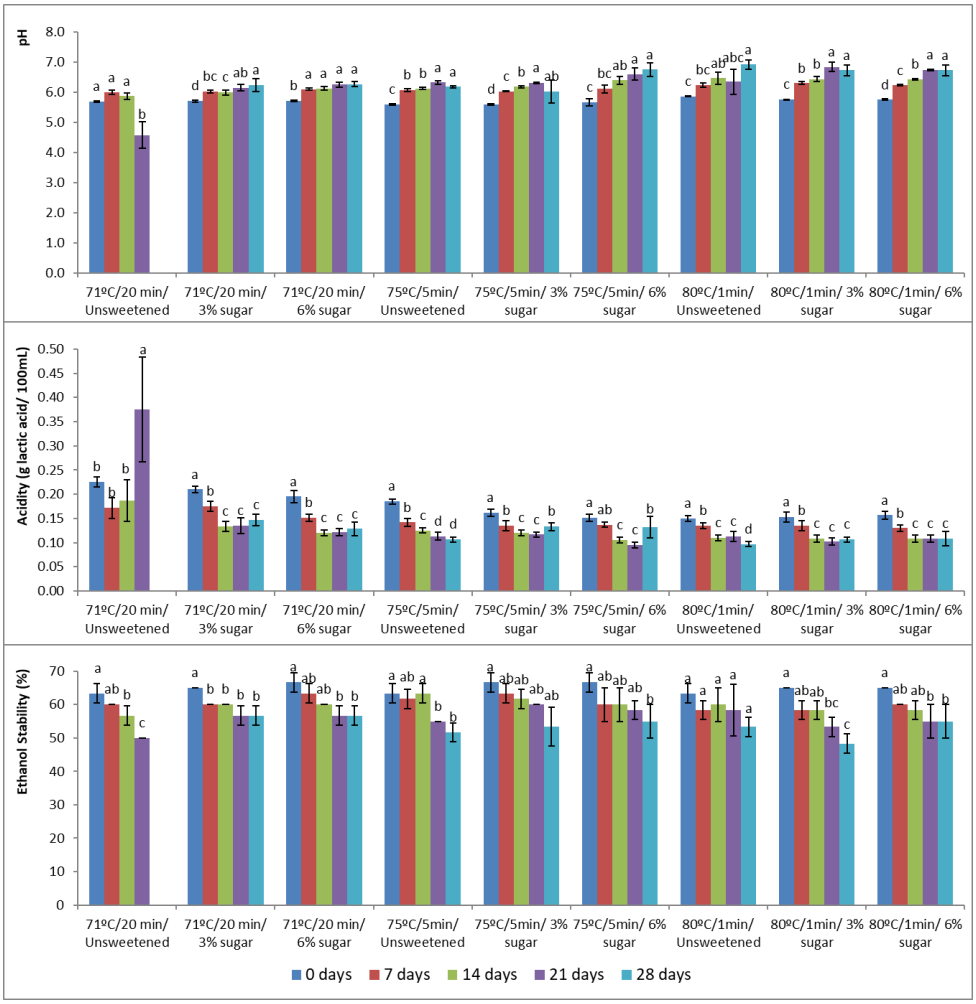
Unsweetened GCW and sweetened (with 3 and 6% sugar) whey samples were processed at the selected time-temperatures for subsequent evaluation of the shelf life at 7 ºC. The microbiological growth results (Figure 1) showed that TBC and TPC counts remained stable until 7 days of storage for all samples ($p>0.05$).

After 14 days of storage, the unsweetened GCW processed at 71 ºC / 20 min showed counts of ~ 6 log CFU/ mL of mesophilic and psychrotrophic, resulting in a slight reduction in alcohol stability ($p<0.05$), but without affecting pH and acidity (Figure 2). After 21 days, however, acidification and intense loss of stability ($p<0.05$) were observed for this sample, which reached TBC ~ 8 cycles log CFU/mL. These results show that the application of a heat treatment with ~ 2 log CFU/mL of survivors can result in a short shelf-life product due to the growth of the survivors, even under refrigeration. According to Rauh & Xiao (2022), heat-treated dairy products stored under refrigeration have a short shelf life due to microbial growth.

For samples added with 3 or 6% of sugar, there was no increase in the mesophilic population up to 14 days; however, there was an increase ($p<0.05$) in the count of psychrotrophic microorganisms (0.8 - 1.4 cycles log CFU/mL) for almost all samples, except those with 3% sugar processed at 80 ºC / 1 min or those with 6% sugar processed at 75ºC/ 5 min or 80 ºC / 1 min (Figure 1).

Regardless of microbiological growth, significant increases in pH (ΔpH 0.3–0.7) and reductions in acidity (0.04-0.08 g lactic acid/ 100 mL whey) were observed during storage for all samples with added sugar, suggesting the action of proteolytic enzymes that can release basic peptides and contribute to the formation of bitter-tasting compounds and protein sedimentation (Juven et al., 2012). These enzymes may be endogenous (such as plasmin – Zhang et al., 2018), produced by psychrotrophic microorganisms that were present in raw milk, since these enzymes are not inactivated by the pasteurization process (Zhang et al., 2018) or produced by the surviving microorganisms at the beginning of the growth phase. Despite the changes in pH and acidity, no changes in alcohol stability of the samples were observed ($p>0.05$), as shown in Figure 2.

Figure 2. Physicochemical parameters of heat-processed goat cheese whey during shelf life at 7ºC



Legend: a-c Lower case superscripts show significant differences assessed by the Tukey test ($p < 0,05$) in each parameter measured on different days of storage for the same sample.

After 21 days of storage, all samples (sweetened and unsweetened) processed at 75 ºC / 5 min maintained low total mesophilic and psychrotrophic counts (≤ 2 log CFU/mL), demonstrating the effectiveness of this time-temperature in the microbiological control of whey. On the other hand, other samples showed an increase in TBC and TPC, reaching counts up to 4 log CFU/mL of TBC and TPC, even with non-significant growth ($p>0.05$). This fact is explained by the high standard deviation of the measures, which commonly occurs when analyzing the growth of autochthonous microorganisms found in different samples (Quigley et al., 2013) from artisanal cheese factories.

Finally, after 28 days of storage, most samples reached counts of 4-5 log CFU/mL of TBC and > 4 log CFU/mL of TPC (Figure 1), but with virtually no physicochemical changes compared to 21 days of storage for most samples. Even so, the high microbiological growth suggests that the samples may not be adequate for consumption. At this time, the physical stability of the samples was determined through measurements of sedimentation and cremeation (data not shown). Sedimentation <0.1% and cremeation between 5 and 6.7% ($p < 0.05$) were observed, indicating that the different levels of microbiological growth and physicochemical changes did not change the macroscopic physical stability of the product. The high percentage of creaming can be explained by the use of whole whey (non-skimmed) and its low concentration of casein (Du, Zhou, Lyu, Liu, & Ding, 2022), making it impossible to maintain the emulsified fat.

Comparing the initial counts of pure GCW and samples added with sugar, it was observed that supplementation with sugar did not cause an increase in TBC and TPC (<0.5 log CFU/mL), suggesting that the sugar did not increase the load of microorganisms resistant to thermal treatment in the samples. This is important because sugar can be contaminated during its production; thus, its addition in formulations can reduce the efficacy of heat treatment and increase the growth capacity of the residual population (Thompson, 2009). Moreover, in samples processed at 71 ºC / 20 min, sugar inhibited the growth of acidifying microorganisms that rapidly deteriorated the unsweetened sample (pure GCW), suggesting an inhibitory effect (Costa et al., 2019). However, for the other tested time-temperatures conditions, there was no clear effect of the sugar, possibly because the acidifying population sensitive to it was inactivated at the higher temperatures studied.

The general evaluation of these results suggests that, except for the unsweetened sample (pure GMW) processed at 71 ºC / 20 min, there was a growth predominant of psychrotrophic non-acidifying bacteria. Similar behavior was previously observed in pasteurized goat milk (Tan et al., 2020) and in pasteurized sheep cheese whey (Tribst & Leite Júnior, 2022). It is interesting to note that even very low populations (<1 log CFU/mL), such as those observed after processing at 80 ºC / 1 min, were able to grow and deteriorate the GCW after 21 or 28 days of storage at 7 ºC, as observed in milk by Masiello et al. (2017), probably due to the absence of competing microorganisms (Canon et al., 2020). Thus, if a GCW shelf life of more than 21 days is desired, it is necessary to associate other barriers to microbial growth, such

as acidification (Fellows, 2000), the use of antimicrobials (Anumudu et al., 2021) and/or the addition of bioprotective cultures (Canon et al., 2020).

Furthermore, the physicochemical analysis data suggest the occurrence of proteolysis by the action of preexisting proteases in milk and/or produced by microorganisms during growth under refrigeration (Quigley et al., 2013), with the production of compounds of basic character, resulting in increased pH, reduced acidity and small reduction of alcohol stability for most samples (Tan et al., 2020).

4. Conclusions

Among the time-temperatures evaluated for microbiological stabilization of goat cheese whey using batch processing, the time-temperature of 75 ºC/ 5 min was able to ensure the microbiological stability of goat cheese whey for at least 21 days (TBC and TPC <2 log CFU/mL), regardless of the addition of sugar. On the other hand, after 28 days of storage, the processed samples showed counts greater than 4-5 log CFU/mL for TBC and up to 8 log CFU/mL of TPC, with physicochemical alterations compatible with the growth of a non acidifying and proteolytic psychrotrophic microbiota. Conversely, the application of more intense time-temperatures is not recommended due to a drastic reduction in the thermal stability of whey proteins. From these results, it was concluded that thermal pasteurization is able to guarantee a limited shelf life for GCW (21 days), showing the need of additional barriers, such as the use of antimicrobials, bioprotective cultures or acidification, if an extended shelf life is required.

Acknowledgments

The authors would like to thank the Capril do Bosque smallholding for the milk donation. This work was supported by the São Paulo Research Foundation (FAPESP, project no. 2020/10930-9) and by the National Council for Scientific and Technological Development with the productivity grants of B. R. C. Leite Júnior (306514/2020-6) and A. A. L. Tribst (305050/2020-6) and CAPES (code 001) for the master's scholarship granted to F.R. dos Santos.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

F.R. dos Santos: Investigation; methodology and writing – review and editing. B.R.C. Leite Júnior: Conceptualization; funding acquisition; project administration; software; supervision; writing – review and editing. A.A.L. Tribst: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; validation; writing – original draft; writing – review and editing.

Compliance with ethics requirements

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

Readers can access the data supporting the conclusions of this study on the following link: https://doi,org/10,25824/redu/XVVPFJ

5. References

Aldalur, A., Bustamante, M. Á., & Barron, L. J. R. (2019). Effects of technological settings on yield, curd, whey, and cheese composition during the cheese-making process from raw sheep milk in small rural dairies: Emphasis on cutting and cooking conditions. Journal of dairy science, 102(9), 7813-7825. https://doi.org/10.3168/jds.2019-16401

Ammar, E.-T., Khalel, A. E., & Mostafa, M. (2014). Effect of type of milk on the properties of traditional feta cheese. Journal of Food and Dairy Sciences, 5(5), 315-https://doi.org/327. 10.21608/jfds.2014.52960

Anand, S., Som Nath, K., & Chenchaiah, M. (2013). Whey and whey products. Milk and Dairy Products in Human Nutrition: Production, Composition and Health, 477-497. https://doi.org/10.1002/9781118534168.ch22

Anumudu, C., Hart, A., Miri, T., & Onyeaka, H. (2021). Recent advances in the application of the antimicrobial peptide nisin in the inactivation of spore-forming bacteria in foods. Molecules, 26(18), 5552. https://doi.org/10.3390/molecules26185552

AOAC, I. (1999). Official Methods of Analysis. 16th ed. AOAC Int., Washington, DC.

Augusto, P. E., Tribst, A. A., & Cristianini, M. (2011). Thermal inactivation of Lactobacillus plantarum in a model liquid food. Journal of Food Process Engineering,

34(4), 1013-1027. https://doi.org/10.1111/j.1745-4530.2009.00529.x

Bensalah, F., Delorme, C., & Renault, P. (2009). Characterisation of thermotolerant cocci from indigenous flora of ‘leben’in algerian arid area and DNA identification of atypical Lactococcus lactis strains. Current Microbiology, 59(2), https://doi.org/139-146. 10.1007/s00284-009-9411-1

Belenkaya, S., Rudometov, A., Shcherbakov, D., Balabova, D., Kriger, A., Belov, A.... Elchaninov, V. (2018). Biochemical Properties of Recombinant Chymosin in Alpaca (Vicugna pacos L.). Applied Biochemistry and Microbiology, 54(6), 569-https://doi.org/576. 10.1134/S0003683818060054

Cedeño, M. M., Tamayo, L. D. Y., & Ramírez-Cárdenas, L. (2018). Elaboración de una bebida utilizando subproductos de la industria láctea. Enfoque UTE, 9(2), 59-69. https://doi.org/10.29019/enfoqueute.v9n2.295

Campagnollo, F. B., Margalho, L. P., Kamimura, B. A., Feliciano, M. D., Freire, L., Lopes, L. S.... Schaffner, D. W. (2018). Selection of indigenous lactic acid bacteria presenting anti-listerial activity, and their role in reducing the maturation period and assuring the safety of traditional Brazilian cheeses. Food Microbiology, 73, 288-297. https://doi.org/10.1016/j.fm.2018.02.006

Canon, F., Nidelet, T., Guédon, E., Thierry, A., & Gagnaire, V. (2020). Understanding the mechanisms of positive microbial interactions that benefit lactic acid bacteria cocultures. Frontiers in Microbiology, 2088. https://doi.org/10.3389/fmicb.2020.02088

Costa, G. M., Paula, M. M., Barão, C. E., Klososki, S. J., Bonafé, E. G., Visentainer, J. V.... Pimentel, T. C. (2019). Yogurt added with Lactobacillus casei and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. International Dairy Journal, 97, 139-148. https://doi.org/10.1016/j.idairyj.2019.05.007

da Silva, D. F., Ahrné, L., Larsen, F. H., Hougaard, A. B., & Ipsen, R. (2018). Physical and functional properties of cheese powders affected by sweet whey powder addition before or after spray drying. Powder Technology, 323, 139-148. https://doi.org/10.1016/j.powtec.2017.10.014

da Silva Duarte, V., Carlot, M., Pakroo, S., Tarrah, A., Lombardi, A., Santiago, H.... Giacomini, A. (2020). Comparative evaluation of cheese whey microbial composition from four Italian cheese factories by viable counts and 16S rRNA gene amplicon sequencing. International Dairy Journal, 104, 104656. https://doi.org/10.1016/j.idairyj.2020.104656

da Silveira, E. O., Neto, J. H. L., da Silva, L. A., Raposo, A. E., Magnani, M., & Cardarelli, H. R. (2015). The effects of inulin combined with oligofructose and goat cheese whey on the physicochemical properties and sensory acceptance of a probiotic chocolate goat dairy beverage. LWT-Food Science and Technology, 62(1), 445-451. https://doi.org/10.1016/j.lwt.2014.09.056

de Lima, E. D. L. C., de Moura Fernandes, J., & Cardarelli, H. R. (2017). Optimized fermentation of goat cheese whey with Lactococcus lactis for production of antilisterial bacteriocin-like substances. LWT, 84, 710-716. https://doi.org/10.1016/j.lwt.2017.06.040

de Oliveira, I. K. C. P., Salles, H. O., Dos Santos, K. M. O., Veras, G., & Buriti, F. C. A. (2020). Proximate composition determination in goat cheese whey by near infrared spectroscopy (NIRS). PeerJ, 8, e8619. https://doi.org/10.7717/peerj.8619

Den Besten, H. M., Wells-Bennik, M. H., & Zwietering, M. H. (2018). Natural diversity in heat resistance of bacteria and bacterial spores: impact on food safety and quality. Annual Review of Food Science and Technology, 9, 383-410. https://doi.org/10.1146/annurev-food-030117-012808

Du, Q., Zhou, L., Lyu, F., Liu, J., & Ding, Y. (2022). The complex of whey protein and pectin: Interactions, functional properties and applications in food colloidal systems–A review. Colloids and Surfaces B: Biointerfaces, 210, 112253. https://doi.org/10.1016/j.colsurfb.2021.112253

Dumitraşcu, L., Moschopoulou, E., Aprodu, I., Stanciu, S., Râpeanu, G., & Stănciuc, N. (2013). Assessing the heat induced changes in major cow and noncow whey proteins conformation on kinetic and thermodynamic basis. Small Ruminant Research, 111(1-3), 129-138. https://doi.org/10.1016/j.smallrumres.2012.12.019

Evelyn, E., & Silva, F. V. (2015). Thermosonication versus thermal processing of skim milk and beef slurry: modeling the inactivation kinetics of psychrotrophic Bacillus cereus spores. Food research international, 67, 67-74. https://doi.org/10.1016/j.foodres.2014.10.028

Fellows, P. (2000). Principles and practice. Food processing technology, 2nd ed., ed. Ellis Horwood, Chickester, UK, 369-380.

Gabriel, A. A., Bayaga, C. L. T., Magallanes, E. A., Aba, R. P. M., & Tanguilig, K. M. N. (2020). Fates of pathogenic bacteria in time-temperature-abused and Holder-pasteurized human donor-, infant formula-, and full cream cow's milk. Food Microbiology, 89, 103450. https://doi.org/10.1016/j.fm.2020.103450

Giribaldi, M., Antoniazzi, S., Gariglio, G. M., Coscia, A., Bertino, E., & Cavallarin, L. (2017). A preliminary assessment of HTST processing on donkey milk. Veterinary sciences, 4(4), 50. https://doi.org/10.3390/vetsci4040050

Giroux, H. J., Veillette, N., & Britten, M. (2018). Use of denatured whey protein in the production of artisanal cheeses from cow, goat and sheep milk. Small Ruminant Research, 161, 34-42. https://doi.org/10.1016/j.smallrumres.2018.02.006

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Milk, fermentation, and fermented and nonfermented dairy products. Modern Food Microbiology, https://doi.org/149-173. 10.1007/0-387-23413-6_7

Júnior, B. R. d. C. L., Tribst, A. A. L., & Cristianini, M. (2014). Proteolytic and milk-clotting activities of calf rennet processed by high pressure homogenization and the influence on the rheological behavior of the milk coagulation process. Innovative food science & emerging technologies, 21, 44-49. https://doi.org/10.1016/j.ifset.2013.11.006

Juven, B., Gordin, S., Rosenthal, I., & Laufer, A. (1981). Changes in refrigerated milk caused by Enterobacteriaceae. Journal of dairy science, 64(9), 1781-1784. https://doi.org/10.3168/jds.S0022-0302(81)82766-X

Kaur, N., Sharma, P., Jaimni, S., Kehinde, B. A., & Kaur, S. (2020). Recent developments in purification techniques and industrial applications for whey valorization: A review. Chemical Engineering Communications, 207(1), 123-138. https://doi.org/10.1080/00986445.2019.1573169

Kubo, M. T. K., Augusto, P. E., & Cristianini, M. (2013). Effect of high pressure homogenization (HPH) on the physical stability of tomato juice. Food research international, 51(1), 170-179. https://doi.org/10.1016/j.foodres.2012.12.004

Macedo, A., Azedo, D., Duarte, E., & Pereira, C. (2021). Valorization of goat cheese whey through an integrated process of ultrafiltration and nanofiltration. Membranes, 11(7), 477b. https://doi.org/10.3390/membranes11070477

Macedo, A., Bilau, J., Cambóias, E., & Duarte, E. (2021). Integration of Membrane Processes for By-Product Valorization to Improve the Eco-Efficiency of Small/Medium Size Cheese Dairy Plants. Foods, 10(8), 1740. https://doi.org/10.3390/foods10081740

Macedo, A., Monteiro, J., & Duarte, E. (2018). A contribution for the valorisation of sheep and goat cheese whey through nanofiltration. Membranes, 8(4), 114. https://doi.org/10.3390/membranes8040114

Magalhães, K. T., Dragone, G., de Melo Pereira, G. V., Oliveira, J. M., Domingues, L.,

Teixeira, J. A., ... & Schwan, R. F. (2011). Comparative study of the biochemical changes and volatile compound formations during the production of novel whey-based kefir beverages and traditional milk kefir. Food Chemistry, 126(1), 249-253. https://doi.org/10.1016/j.foodchem.2010.11.012

Masiello, S., Kent, D., Martin, N., Schukken, Y., Wiedmann, M., & Boor, K. (2017). Longitudinal assessment of dairy farm management practices associated with the presence of psychrotolerant Bacillales spores in bulk tank milk on 10 New York State dairy farms. Journal of dairy science, 100(11), 8783-8795. https://doi.org/10.3168/jds.2017-13139

Pala, C., Scarano, C., Venusti, M., Sardo, D., Casti, D., Cossu, F.... Marras, M. (2016). Shelf life evaluation of ricotta fresca sheep cheese in modified atmosphere packaging. Italian journal of food safety, 5(3). https://doi.org/10.4081/ijfs.2016.5502

Panghal, A., Patidar, R., Jaglan, S., Chhikara, N., Khatkar, S. K., Gat, Y., & Sindhu, N. (2018). Whey valorization: current options and future scenario–a critical review. Nutrition & Food Science. https://www.emerald.com/insight/content/doi/10.1108/NFS-01-2018-0017/full/html

Pflug, I. J. (1988). Selected papers on the microbiology and engineering of sterilization processes. Minneapolis: Environmental Sterilization Laboratory, 5th ed.

Pires, A.F., Marnotes, N.G., Rubio, O.D., Garcia, A.C., & Pereira, C.D. (2021). Dairy By-Products: A Review on the Valorization of Whey and Second Cheese Whey. Foods, 10(5), 1067. https://doi.org/10.3390/foods10051067

Pitarch, J. L., Vilas, C., de Prada, C., Palacín, C. G., & Alonso, A. A. (2021). Optimal operation of thermal processing of canned tuna under product variability. Journal of Food Engineering, 304, 110594. https://doi.org/10.1016/j.jfoodeng.2021.110594

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2013). The complex microbiota of raw milk. FEMS microbiology reviews, 37(5), 664-698. https://doi.org/10.1111/1574-6976.12030

Rauh, V., & Xiao, Y. (2022). The shelf life of heat-treated dairy products. International Dairy Journal, 125, 105235. https://doi.org/10.1016/j.idairyj.2021.105235

Sadhu, S.P. (2018). Effect of cold chain interruptions on the shelf-life of fluid pasteurised skim milk at the consumer stage. Brazilian Journal of Food Technology. 21:e2017064. http://dx.doi.org/10.1590/1981-6723.06417.

Samaržija, D., Zamberlin, Š., & Pogačić, T. (2012). Psychrotrophic bacteria and their negative effects on milk and dairy products quality. Mljekarstvo: časopis za

unaprjeđenje proizvodnje i prerade mlijeka, 62(2), 77-95.

Silva, M. A. P. d., Santos, G. d. O., Medeiros, J. S., Teixeira, P. C., & Carmo, R. M. d. (2020). Available in: <https://www.milkpoint.com.br/artigos/producao/producaoartesanal-de-queijos-alternativa-para-pequenos-produtores-deleite-219626/>. Access in: March 25, 2022.

Tan, S. F., Chin, N. L., Tee, T. P., & Chooi, S. K. (2020). Physico-chemical changes, microbiological properties, and storage shelf life of cow and goat milk from industrial high-pressure processing. Processes, 8(6), 697. https://doi.org/10.3390/pr8060697

Thompson, S. (2009). Microbiological spoilage of high-sugar products Compendium of the microbiological spoilage of foods and beverages (pp. 301-324): Springer. https://doi.org/10.1007/978-1-4419-0826-1_11

Tribst, A., Falcade, L., & De Oliveira, M. (2019). Strategies for raw sheep milk storage in smallholdings: Effect of freezing or long-term refrigerated storage on microbial growth. Journal of dairy science, 102(6), 4960-4971. https://doi.org/10.3168/jds.2018-15715

Tribst, A. A. L., Falcade, L. T. P., Carvalho, N. S., Júnior, B. R. d. C. L., & de Oliveira, M. M. (2020). Manufacture of a fermented dairy product using whey from sheep's milk cheese: An alternative to using the main byproduct of sheep's milk cheese production in small farms. International Dairy Journal, 111, 104833. https://doi.org/10.1016/j.idairyj.2020.104833

Tribst, A.A.L., Leite Júnior, B.R.C. (2022). Heat treatment design for the valorization of sheep cheese whey in artisanal Production. Research, Society and Development, v. 11, n. 9, e20911931776, 2022. http://dx.doi.org/10.33448/rsd-v11i9.31776

UNDP. (2015). Sustainable Development Goals. Retrieved from Sustainable Development Goals website: https://www.undp.org/sustainable-development-goals

van Boekel, M. (2022). Kinetics of heat-induced changes in dairy products: Developments in data analysis and modeling techniques. International Dairy Journal, 126, 105187. https://doi.org/10.1016/j.idairyj.2021.105187

Zhang, C., Bijl, E., & Hettinga, K. (2018). Destabilization of UHT milk by protease AprX from Pseudomonas fluorescens and plasmin. Food Chemistry, 263, 127-134. https://doi.org/10.1016/j.foodchem.2018.04.128

Zhao, X., Cheng, M., Zhang, X., Li, X., Chen, D., Qin, Y.... Wang, C. (2020). The effect of heat treatment on the microstructure and functional properties of whey protein from goat milk. Journal of dairy science, 103(2), 1289-1302.

https://doi.org/10.3168/jds.2019-17221
Zotta, T., Solieri, L., Iacumin, L., Picozzi, C., & Gullo, M. (2020). Valorization of cheese whey using microbial fermentations. Applied Microbiology and Biotechnology, 104(7), 2749-2764. https://doi.org/10.1007/s00253-020-10408-2

CAPÍTULO 2

Impact of ultrasound and protease addition on the fermentation profile and final characteristics of fermented goat and sheep cheese whey

Estudo submetido na revista **Journal of Food Science and Technology**. Manuscript Number: JFST-D-22-01665, 2022. SANTOS, Fábio Ribeiro dos; LEITE JÚNIOR, Bruno Ricardo de Castro; TRIBST, Alline Artigiani Lima. Impact of ultrasound and protease addition on the fermentation profile and final characteristics of fermented goat and sheep cheese whey.

Impact of ultrasound and protease addition on the fermentation profile and final characteristics of fermented goat and sheep cheese whey

Fabio Ribeiro dos Santos, Bruno Ricardo de Castro Leite Junior[1], Alline Artigiani Lima Tribst[2*]

Affiliations:

[1]Department of Food Technology (DTA), Federal University of Viçosa (UFV), University Campus, 36570-900, Viçosa, MG, Brazil,

[2]Center for Food Studies and Research (NEPA), University of Campinas (UNICAMP), Albert Einstein, 291, 13083-852, Campinas, SP Brazil,

* Corresponding Author, Albert Einstein, 291, Postal code: 13083-852, Campinas, São Paulo, Brazil, Phone: +55 19 3521-2176, E-mail: tribst@unicamp.br

Abstract

Goat (GCW) and sheep cheese whey (SCW) are cheese by-products that can be fermented to develop a new product. However, the limited nutrient availability for lactic acid bacteria (LAB) growth and the low stability of whey are challenges. This work evaluated the addition of protease and/or ultrasound-assisted fermentation as tools to improve GCW and SCW fermentation and the final quality of the products. Results showed that the US/protease increased by 23 – 32% pH decline rate (for SCW only) and modified the separation of cream (≤ 60% for GCW) and whey (≤ 80% for both whey sources, with higher values for GCW) during storage, explained by changes in the microstructure protein, fat globules, and their interactions. Furthermore, the whey source/composition (mainly lower fat content in SCW) affected the destabilization rate and the LAB viability loss (1.5 – 3.0 log CFU/mL), caused by nutrient depletion and low tolerance at pH ~4.0. Finally, exploratory results showed that fermentation under sonication (with/without protease) resulted in 24 – 218% higher antioxidant activity *in vitro* than unfermented samples. Therefore, fermentation associated with proteases/sonication can be an interesting strategy to modify GWC and SCW, and the final process chosen depends on the desired changes in whey.

Keywords: sonication, artisanal production, commercial enzyme, fermented whey drink, physical structure, LAB viability, storage

1. Introduction

Goat and sheep farming focused on milk production is a growing rural activity in smallholdings and the milk obtained is used mainly for the manufacture of artisanal cheese. The whey obtained as by-product in these cheese factories is commonly underutilized or discarded, reducing the income of artisanal producers and causing a negative environmental impact (Macedo et al. 2018; Tribst et al. 2020).

Fermentation of whey to produce dairy beverages or whey drinks is considered a feasible, simple, and inexpensive option for direct use of whey, as it does not require preprocessing, such as concentration or drying (Zotta et al. 2020), which would be incompatible with artisanal size production (Tribst et al. 2020).

According to Martí-Quijal et al. (2021) and Zotta et al. (2020), fermented whey products have advantages compared to non-fermented products, such as: (i) better sensory acceptance due to lactic acid and aroma compounds production (ii) lower allergenicity due to partial hydrolysis of β-lactoglobulin, (iii) presence of peptides with probable biological activity due to the proteolytic activity of lactic acid bacteria (LAB) during their growth and potential production of lactobionic acid and exopolysaccharides that have antioxidant properties), and (iv) lower susceptibility to deterioration and/or pathogens growth due to low pH.

However, cheese whey can have nutritional deficiencies, especially regarding protein content, that may hinder/ limit LAB growth (Castro et al. 2013), resulting in acidification rates lower than those observed for milk fermentation (Rama et al. 2019). This effect may be minimized by proteases added to whey prior or during fermentation, because protein hydrolysis into short peptide chains and/or essential amino acids favors the growth of LAB (Chourasia et al. 2022). Moreover, hydrolysis can decrease the allergenicity of whey proteins by reducing the β-lactoglobulin content (Chourasia et al. 2022) and improves the nutritional quality of whey by increasing the peptides concentration with biological activity, such as ACE inhibitory and antioxidant peptides (Magalhães et al., 2022).

Ultrasound (US) is a simple and inexpensive technology and has recently been studied as a tool to improve fermentation processes (Umego et al. 2021). Previous results have shown that ultrasound reduces the fermentation time due to activity enhancing of starter culture (Herrera-Ponce et al. 2022), explained by increased cell permeability and temporary pores formation on cells under US (Gholamhosseinpour & Hashemi 2018) that improves the mass transfer of substrates into the cells and the

removal of by-products from cell metabolism (Herrera-Ponce et al. 2022). Furthermore, acoustic cavitation in US-assisted fermentation acts as catalyst for various reactions due to the generation of highly reactive radicals, substrate homogenization, chemical dissolution, and breakdown of cell clusters (Umego et al. 2021). Finally, in addition to mass transfer improvement, the US-assisted reaction may potentiate enzymatic reactions due to: (i) changes in the substrate able to cause great exposure to enzyme attack and (ii) partial unfold of the enzyme, with exposure of active sites entrapped in the hydrophobic core and/or stabilization of the enzyme structure (Magalhães et al. 2022).

Despite this, to the best of our knowledge, no previous research has evaluated the impact of US-assisted fermentation of goat (GCW) and sheep (SCW) cheese whey. Therefore, this research aimed to fill this gap by studying GCW and SCW fermentation parameters under US, with or without proteases, and characterizing the obtained products regarding LAB count, physicochemical characteristics, structural stability, and in vitro antioxidant activity.

2. Material and Methods

2.1 Goat (GCW) and sheep cheese whey (SCW)

GCW (1.01% fat, 0.68% protein, and 3.23% lactose) was obtained from a fresh cheese inoculated with starter culture Lacticaseibacillus casei (BGP 93, Sacco Brasil Comércio de Alimentos Ltda, São Paulo-SP, Brazil) produced in the laboratory of Federal University of Viçosa (Viçosa, Brazil). SCW (0.18% fat, 0.95% protein, and 4.72% lactose) was obtained from an artisanal cheese inoculated with Lactococcus lactis subsp. Lactis, Lactococcus lactis subsp. cremoris (R-704, Chr. Hansen Indústria e Comércio, Valinhos - SP, Brazil), produced in São Clemente artisanal cheese factory (Ouro Preto, Brazil). Whey was stored at 1ºC up to processing (24h).

.2.2 GCW and SCW fermentation process

A volume of 5.4L of each whey were pasteurized at 75 ºC/ 5 min to reach microbial counts lower than 102 UFC/mL (dos Santos et al., 2021; Tribst & Leite Júnior, 2022) and cold to 43 ºC. Then, each whey was inoculated with ~106 CFU/mL yogurt culture (Streptococcus thermophilus and Lactobacillus bulgaricus, Y472e Code, Sacco Brasil - Campinas, Brazil) and divided in two parts of equal volume and one of these parts was added with 1% of protease solution (1%, v/v) (Alcalase®, Novozymes Latin America - Araucaria, Brazil). Finally, each sample was again divided in two parts and

one part was traditionally fermented in a thermostatic bath at 43 ºC (Solidsteel, Piracicaba, São Paulo) and the other one was fermented at the same temperature in an ultrasonic bath (Unique, USC 2800 A model, Indaiatuba, Brazil) with internal dimensions of 30 × 24 × 15 cm, equipped with five transducers of 25 kHz arranged below the vat and nominal potential of 450 W and volumetric potential of 23.8 W/L. Each process was carried out in triplicate and fermentation was carried out in a sterilized borosilicate bottle.

The fermentation was evaluated by measuring the pH decline at 30-min intervals up to reach pH 4.60 ± 0.05. The results were modeled by a modified Gompertz equation adapted by de Brabandere & Baerdemaeker (1999) to describe the pH decline in fermentation (Eq. 1).

$$pH = pH_0 + (pH_\infty - pH_0) exp\left\{-exp\left[\frac{\mu e}{(pH_0 - pH_\infty)}(\lambda - t) + 1\right]\right\} \quad Eq, 1$$

After fermentation, the bottles containing the whey samples were refrigerated at 7 °C and evaluated after 1, 14, and 28 days of storage.

2.3. pH, LAB counts, physical stability, microstructure, and in vitro antioxidant activity of the fermented whey

After 1, 14, and 28 days of fermentation, the pH of the GCW and SCW samples was measured in triplicate and lactic acid bacteria (LAB) counts were determined in duplicate, following the methodology described by Tribst et al. (2020).

To access the physical stability of the whey, sterilized borosilicate tubes were filled with 10 mL of each fermented whey and kept at 7ºC. After 1, 3, 7, 21, and 28 days of storage, the whey destabilization was measured using a digital pachymeter considering: (i) the occurrence of cream separation, as a dense phase in the top of the tube, (ii) whey separation, as the translucent phase, and (iii) sedimentation, as a dense phase in the bottom of the tube (Tribst et al. 2020). Illustrative images of the tube samples were taken on all stability assessment days.

In addition, to get insights about the sample's microstructure, after 1, 14, and 28 days of storage, a drop of each sample was placed on a microscope slide and images were obtained after optical microscopically magnification with an objective lens of 40x (Anatomic Opton®, TIM-18, Brasil), according to the described Tribst et al. (2020).

In vitro antioxidant activity was measured after 1 and 28 days of storage by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity (Magalhães et al. 2022). The stock solution containing ABTS (7 mM) and potassium persulfate (2.45 mM) (1:1 ratio), was prepared and stored (12 - 16h) in the dark at 4 °C. Then, the solution was diluted with distilled water to reach an absorbance at 734 nm of 0.700 ± 0.02. Subsequently, 150 µL of the fermented sample (diluted 1:100) was added to 2.85 mL of the diluted ABTS radical solution. After incubation in the dark at room temperature for 1h, the absorbance was read at 734 nm. The ABTS radical scavenging activity was calculated using equation 2 (Eq. 2).

$$Antioxidant\ activity\ (\%) = \left(\frac{Abs_{0\ min} - Abs_{60\ min}}{Abs_{0\ min}}\right) \times 100 \quad Eq, 2$$

Where: Abs $_{0min}$ is the absorbance of the sample at time 0 and Abs$_{60min}$ is the absorbance of the sample after 60 min of reaction.

2.4 Statistical evaluation

The impacts of the different processing conditions (traditional and US-assisted fermentation carried out with/without protease) on the GCW and SCW fermentation parameters and on pH, LAB count, stability parameters and in vitro antioxidant activity were evaluated by using the analysis of variance (ANOVA) and the Tukey test at a 95% confidence level. The same tests were used to compare the same product throughout the shelf life.

3. Results and Discussion

3.1 Fermentation profile of goat and sheep cheese whey

The pH decline curves obtained for each sample during fermentation (Supplementary File – Figure S1). Fermentation time was mainly influenced by the whey source, with sheep cheese whey being fermented 25% slower than GCW. In addition, US-assisted fermentation slightly delayed the total fermentation time in GCW, and the addition of protease improved the fermentation time in SCW. The obtained data had a good adjustment to the modified Gompertz model (R2>0.95), showing that the pH decline of the samples could be used to indirectly assess bacterial growth through the parameters of Equation 1 (de Brabandere & Baerdemaeker 1999), as shown in Table 1.

The parameter λ represents the lag phase of the pH decline, i.e., the sum of the time spent for LAB culture adaptation to the medium with the time taken to produce the

minimum acid concentration capable of inducing a pH reduction in the samples of whey, and the parameter μ represents the maximum rate of pH decline (de Brabandere & Baerdemaeker, 1999; Tribst et al. 2018; Tribst et al. 2020b). The results showed that US-assisted fermentation and the addition of protease resulted in parameters similar to those observed for traditional fermentation of GCW ($p>0.05$). On the other hand, for SCW, it was observed that although both interventions negatively impacted the lag phase, delaying it by 41 – 70% ($p < 0.05$), they led to significantly higher rates of pH decline (23 – 32%), when compared to traditional fermentation. Considering the faster fermentation observed for GCW, it was concluded that the yogurt culture had no difficulty of growth in this whey and the tested interventions were unnecessary. On the contrary, the longer fermentation of SCW indicates that the culture had some barriers to grow, justifying the observed impact of the US-assisted processing and protease.

Supplementary File – Figure S1. pH decline of goat (GCW) and sheep cheese whey (SCW) during fermentation carried out using traditional and ultrasound (US) assisted processes, added or not by protease.

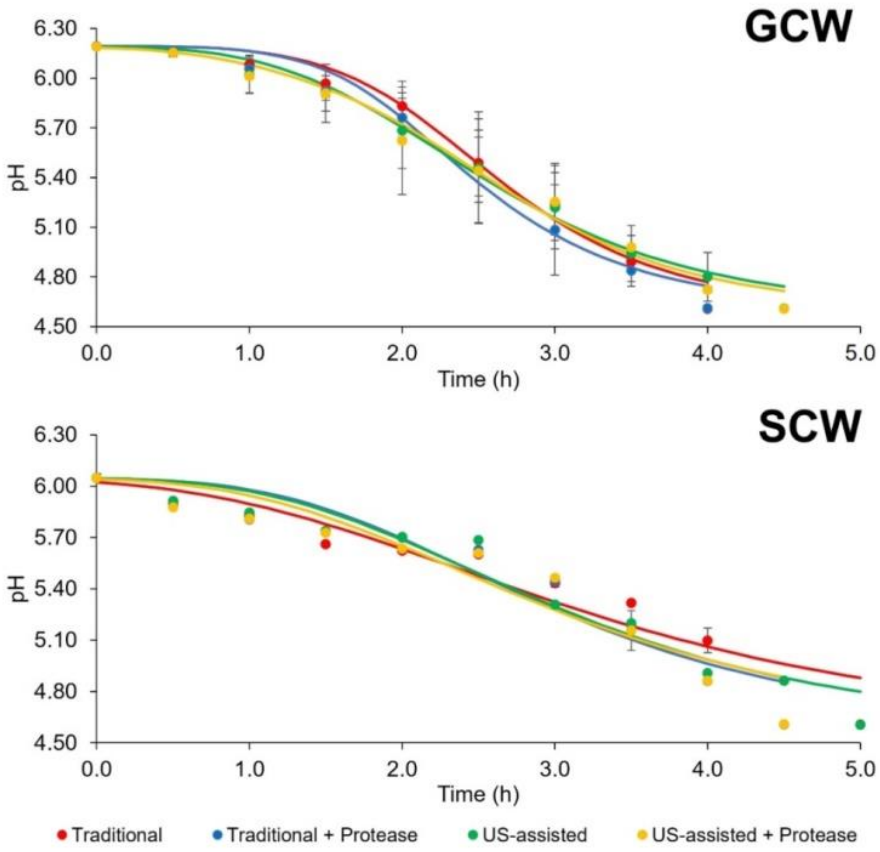


Table 1. arameters of the modified Gompertz equation adapted to pH decrease data of goat and sheep cheese whey during fermentation**.**

Whey source	Fermentation process	$pH_{initial}$	**λ (h)	*** μ (h^{-1})	R^2
*Goat	Traditional	6.19 ± 0.01[a]	1.54 ± 0.38[a]	-0.78 ± 0.10[ab]	0.987
	Traditional + Protease	6.19 ± 0.01[a]	1.43 ± 0.29[a]	-0.81 ± 0.19[a]	0.983
	US-assisted	6.19 ± 0.01[a]	1.27 ± 0.40[a]	-0.64 ± 0.07[b]	0.988
	US-assisted + Protease	6.19 ± 0.01[a]	1.27 ± 0.64[a]	-0.68 ± 0.11[ab]	0.989
Sheep	Traditional	6.05 ± 0.02[a]	0.68 ± 0.12[c]	-0.31 ± 0.01[c]	0.959
	Traditional + Protease	6.05 ± 0.02[a]	1.16 ± 0.20[a]	-0.42 ± 0.03[a]	0.953
	US-assisted	6.05 ± 0.02[a]	1.10 ± 0.11[ab]	-0.40 ± 0.01[ab]	0.972
	US-assisted + Protease	6.05 ± 0.02[a]	0.96 ± 0.13[b]	-0.38 ± 0.02[b]	0.947

Legend of Table 1: * Samples were identified by the whey source and process of fermentation, **λ = lag phase time (h); ***μ= maximum pH decline rate (h^{-1}), [a-c] Lower case superscripts indicate significant differences evaluated by the Tukey test ($p < 0,05$) among the processes for each whey source.

In symbiotic fermentation using yogurt culture, *S. thermophilus* is the microorganism that has the fastest growth in the beginning of the process, due to its capability of growing at high pH (Tribst et al. 2018). Thus, increase in the lag phase caused by protease and US suggests that these interventions had a negative impact on the growth of this microorganism. On the other hand, in the pH decay between 5.7 and 5.0, characterized by the symbiotic growth of S. thermophilus and L. bulgaricus (de Brabandere & Baerdemaeker 1999), the US-assisted reaction and the protease had a positive influence. The protease effect can be explained by the partial hydrolysis of proteins into peptides and/or amino acids after 60-90 min of reaction, improving the availability of essentials amino acids (Magalhães et al. 2022) for S. thermophilus, which is produced exclusively by *L. bulgaricus* in traditional fermentation (Tamime & Robinson 2007). The effects of US can be explained mainly by its ability to increase cell permeability (Gholamhosseinpour & Hashemi 2018), improving: (i) mass transfer of substrates to cells, including the metabolites produced by S. thermophilus that are important for L. bulgaricus growth and vice-versa (Tamime & Robinson 2007), (ii) the removal of by-products from cell metabolism (Herrera-Ponce et al. 2022), and (iii) the excretion of enzymes that hydrolyze nutrients necessary for bacteria growth, such as β-galactosidase (Ewe et al. 2012). On the other hand, the absence of improvements when protease and US were combined, compared to those of individual interventions, released that the effects of US and protease were not additive and that the expected

positive impact of US on enzyme performance (Magalhães et al. 2022) was insufficient to alter the growth of the microorganism and, consequently, the pH decline.

3.2. pH and LAB count of fermented goat and sheep cheese whey

During the storage of the samples, a continuous reduction in the pH was observed, independent on the whey source and the use of US-assisted fermentation and protease addition (Table 2). These results suggested that LAB remained producing lactic acid even during storage at 7ºC (Wei et al. 2017) causing post-acidification, as related by other authors (Tribst et al., 2020). The comparison between the different samples showed that the only consistent observation was the higher pH of the sample added with protease and fermented without US ($p<0.05$), possibly due to peptides with basic character formed from protein hydrolysis (Juven et al. 1981).

Table 2. pH and lactic acid bacteria (LAB) count measured during the shelf life at 7°C of goat (GCW) and sheep cheese whey (SCW) fermented through traditional and ultrasound (US) assisted processes, added or not by protease.

Whey source	Fermentation process	Ph			Lactic acid bacteria count (log CFU/mL)		
		1 day	14 days	28 days	1 day	14 days	28 days
Goat	Traditional	4.61 ± 0.01^{Aa}	4.15 ± 0.02^{Db}	4.01 ± 0.01^{Bc}	7.56 ± 0.14^{Aa}	6.82 ± 0.34^{Ab}	4.88 ± 0.21^{BCc}
	Traditional + Protease	4.61 ± 0.01^{Aa}	4.41 ± 0.01^{Bb}	4.22 ± 0.04^{Ac}	6.55 ± 0.29^{Ca}	6.79 ± 0.20^{Aa}	5.54 ± 0.51^{ABb}
	US-assisted	4.61 ± 0.01^{Aa}	4.21 ± 0.03^{Cb}	3.99 ± 0.02^{Bc}	7.49 ± 0.36^{Aba}	7.24 ± 0.71^{Aa}	6.00 ± 0.39^{Ab}
	US-assisted + Protease	4.61 ± 0.01^{Aa}	4.76 ± 0.04^{Ab}	3.59 ± 0.02^{Cc}	7.06 ± 0.24^{Ba}	7.19 ± 0.33^{Aa}	4.29 ± 0.98^{Cb}
Sheep	Traditional	4.67 ± 0.01^{Ba}	4.25 ± 0.03^{Db}	4.08 ± 0.00^{Cc}	6.34 ± 0.44^{Aba}	4.87 ± 0.16^{Ab}	4.88 ± 0.18^{Bb}
	Traditional + Protease	4.63 ± 0.01^{Ca}	4.53 ± 0.01^{Ab}	4.27 ± 0.01^{Ac}	6.95 ± 0.65^{Aa}	3.68 ± 0.22^{Bc}	5.33 ± 0.24^{Ab}
	US-assisted	4.71 ± 0.01^{Aa}	4.35 ± 0.00^{Cb}	3.98 ± 0.01^{Dc}	5.91 ± 0.34^{Ba}	4.39 ± 1.08^{ABb}	3.82 ± 0.33^{Cb}
	US-assisted + Protease	4.60 ± 0.00^{Da}	4.46 ± 0.02^{Bb}	4.21 ± 0.01^{Bc}	5.98 ± 0.15^{Ba}	4.72 ± 0.08^{Ab}	4.96 ± 0.27^{ABb}

Legend of Table 2: $^{A-D}$ Capital superscripts indicate significant differences evaluated by the Tukey test ($p < 0,05$) among the processes for each whey source at each day, $^{a-c}$ Lower case superscripts indicate significant differences evaluated by the Tukey test ($p < 0,05$) for the same sample at different days.

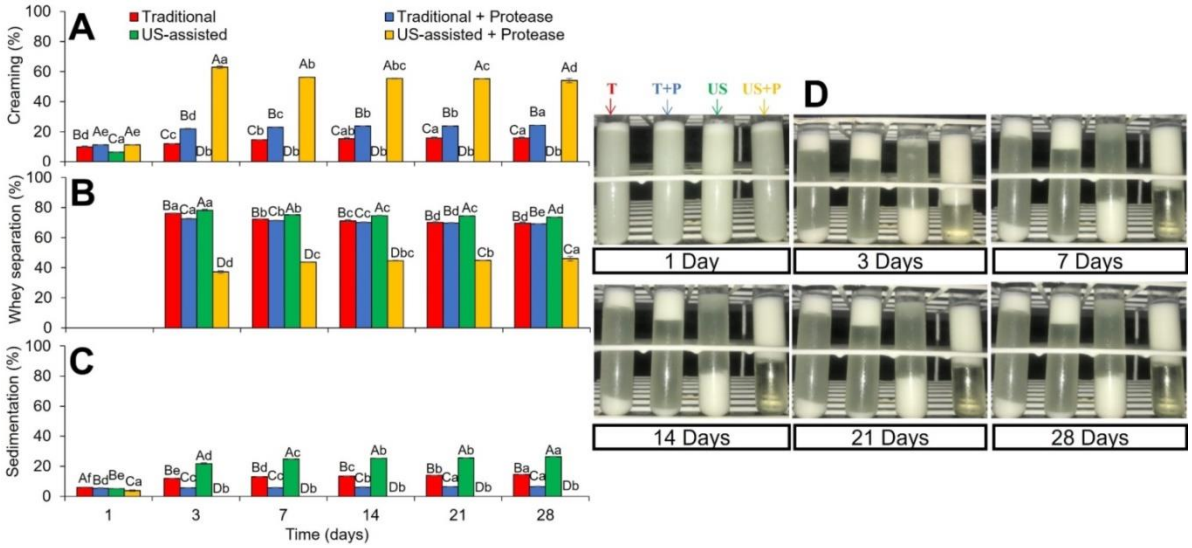
With respect to LAB counts (Table 2), GCW samples had ~10^7 CFU/mL and SCW had a population 1 log smaller, suggesting that the longer fermentation of SCW occurred due to culture inhibition at the end of acidification (Tamime & Robinson 2007). After 14 days, LAB counts remained equal in fermented GCW while a further decreased ($p<0.05$) of at least 1.26 log CFU/mL was observed for SCW samples. On the other hand, at the end of storage, the GWC showed reductions in LAB counts similar to those observed in SWC after 14 days, while the counts of most SCW samples remained unchanged. Furthermore, minor and unspecific differences were observed between samples produced using the same whey, suggesting that whey composition/characteristics were more important for the survival of LAB during storage than the interventions studied during fermentation.

Although post-acidification is considered an important cause of viability loss of yogurt microorganisms (Undugod & Nilmini 2019), the comparison of the results in Table 2 suggests that the reductions in LAB counts were not exclusively due to pH reduction, since GCW and SCW samples had similar pH at days 1 and 14, but counts in SCW were approximately 1 and 2.5 log CFU/mL lower, respectively. These results indicated that, although LAB culture was stressed by nutrient restriction and cumulation of inhibitory substances (Rama et al. 2019) in the storage of both whey sources, the characteristics of SCW (including composition, salt balance, or presence of antimicrobials) were more harmful to yogurt culture. Moreover, the overall evaluation of these results highlighted that storage at 7 ºC was insufficient to maintain the LAB viability for 28 days, emphasizing the need to increase the LAB count at the end of fermentation and/or store the fermented whey at lower temperature to reduce the microorganism's metabolism during storage (Tribst et al. 2020).

3.3. Physical stability and microstructure of fermented goat and sheep cheese whey

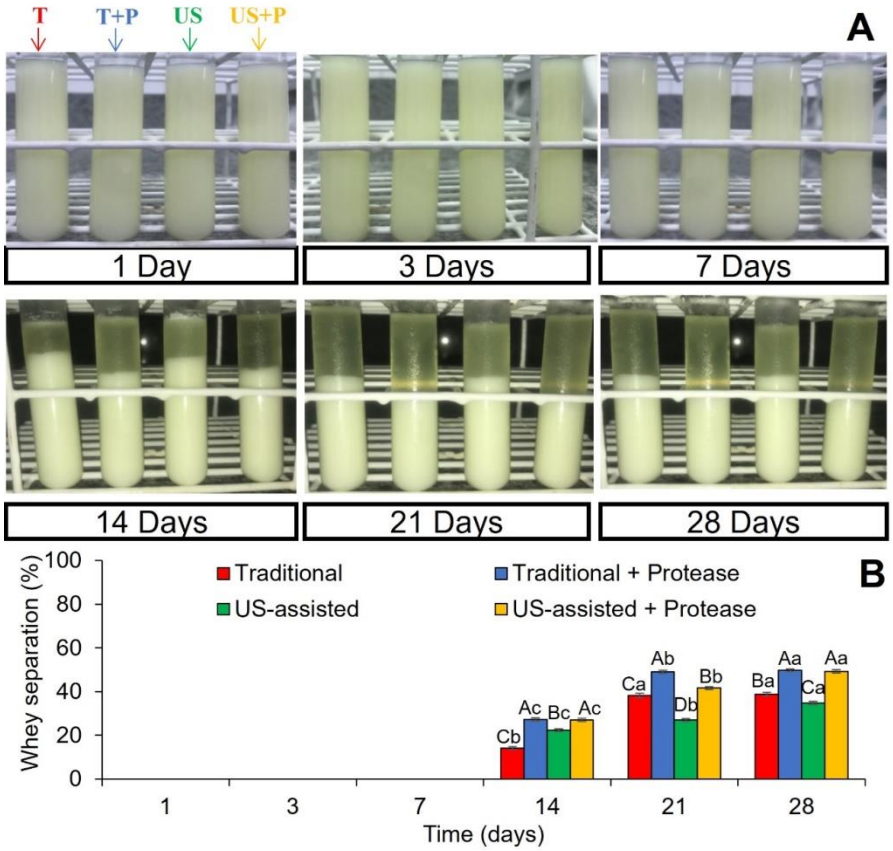
The evaluation of the physical stability of fermented GCW (Figure 1) and SCW (Figure 2) showed that both products were destabilized during storage, but in different ways. The micrographs of proteins in the samples obtained after 1, 14, and 28 days of storage help to explain the observed phenomena (Figure 3).

Figure 1. Whey destabilization during the shelf life at 7°C of goat cheese whey (GCW) fermented through traditional and ultrasound (US) assisted processes, added or not by protease.



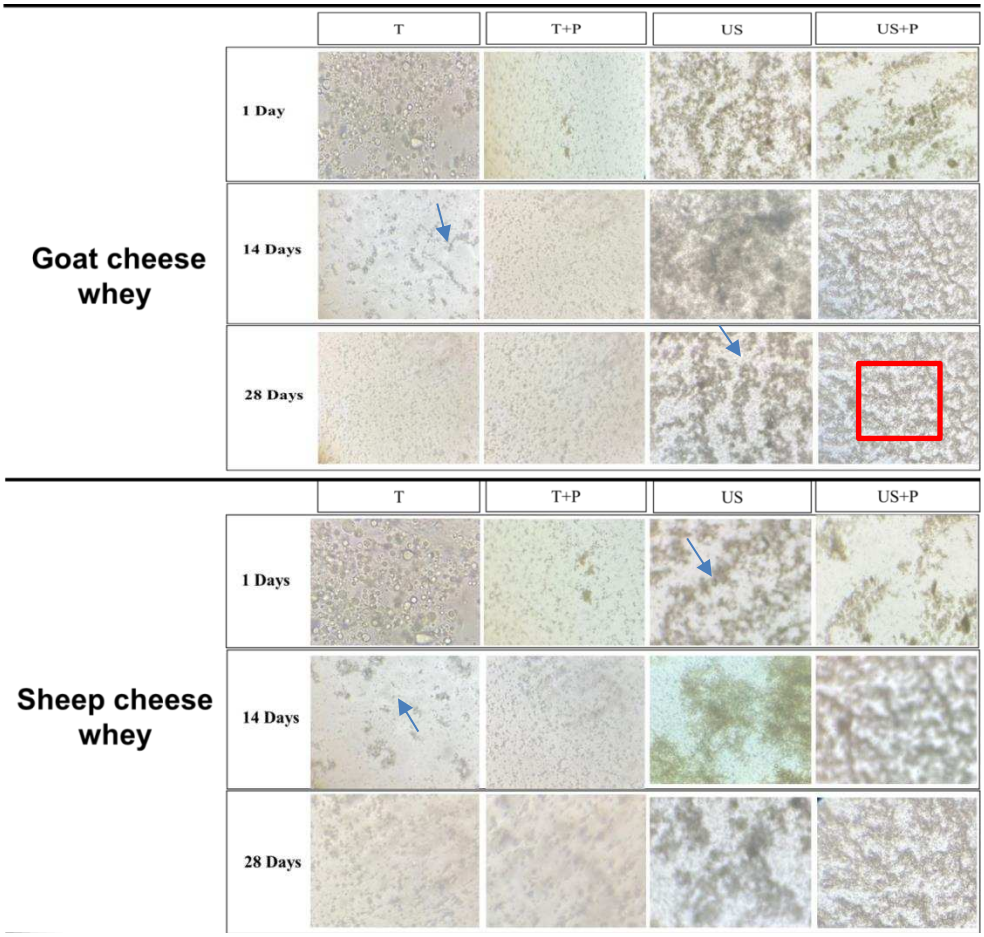
Legend of Figure 1: Cream separation (A); Whey separation (B), Sedimentation (C) and Illustrative images of GCW during storage (D). Fermentation process: T: Traditional; T+P: Traditional + Protease; US: US-assisted; US+P: US-assisted + Protease. A-D Capital letters indicate significant differences evaluated by the Tukey test ($p < 0.05$) among the processes at each day. a-c Lower case superscripts indicate significant differences evaluated by the Tukey test ($p < 0.05$) for the same sample at different days.

Figure 2. Whey destabilization during the shelf life at 7°C of sheep cheese whey (SCW) fermented through traditional and ultrasound (US) assisted processes, added or not by protease.



Legend of Figure 2: Illustrative images of SCW during storage (A); Whey separation (B), Fermentation process: T: Traditional; T+P: Traditional + Protease; US: US-assisted; US+P: US-assisted + Protease, A-D Capital letters indicate significant differences evaluated by the Tukey test ($p < 0,05$) among the processes at each day, a-c Lower case superscripts indicate significant differences evaluated by the Tukey test ($p < 0,05$) for the same sample at different days.

Figure 3. Microscopic observation (40x of magnification) of goat (GCW) and sheep cheese whey (SCW) fermented through traditional and ultrasound (US) assisted processes, added or not by protease.



Legend of Figure 3. The blue arrows identify protein agglomerates formed in the samples and the red square shows the most organized structure observed among the samples. Fermentation process: T: Traditional; T+P: Traditional + Protease; US: US-assisted; US+P: US-assisted + Protease.

For GCW, an intense destabilization was observed after 3 days (Figure 1D), with cream separation (Figure 1A), whey separation (Figure 1B), and sedimentation (Figure 1C). These levels of destabilization remained almost unchanged up to 28 days, which can be explained by the absence of a strong protein network that could contract with the consequent water release (Laiho et al., 2017) and by the minimal hydrophobic protein attraction under refrigeration, with maintenance of protein aggregation only through weaker interactions (Liyanaarachchi & Vasiljevic 2018).

Interestingly, the fermentation conditions, i.e., protease addition and US, impacted the way that GCW destabilized. In the traditional fermented sample, destabilization resulted in a proportional creaming and sedimentation (~10%), with a layer of whey between them. This separation profile means that the low protein content of GCW associated with their destabilization, caused by pasteurization followed by acidification that proximate them to the isoelectric point, make it impossible to maintain the stability of the suspension (Tribst et al. 2020). As a consequence, fat globules separated from the system and, due to its low density (Walstra et al. 2006), migrated to the top of the tube, while the whey protein agglomerates (Figure 3, after 14 days of storage) sedimented and a whey translucent material containing soluble nutrients was formed between them.

For the sample added with protease, smaller sediments were formed compared to those observed in traditional fermentation. This is explained by the partial hydrolysis of proteins that resulted in less agglomeration (Figure 3) and greater solubility (Bustamante et al., 2021) compared to control. On the other hand, the larger cream suggests that part of these hydrolysates interacted with the fat globules (da Capela et al. 2022), forming a thick and dense layer at the top of the tube ($p<0.05$). The sample fermented under US had an opposite behavior, without cream formation and with dense sedimentation ($p<0.05$). Considering that US can reduce fat globule size (da Capela et al. 2022), altering protein structures (Zhao et al. 2014), and favoring interactions between these compounds (Zhao et al. 2014), the main hypothesis to explain the phenomenon is the formation of protein-fat complex, with the protein governing the physical behavior of the structure, leading to dense sedimentation. This hypothesis is corroborated by the micrographs of US-fermented samples (Figure 3). Conversely, the sample added with protease and fermented under US showed the thickest cream ($p<0.05$), suggesting that the partially hydrolyzed protein interacted with small fat globules forming a light (Figures 1A and 1D) and more continuous structure (Figure 3), with better water holding capacity than other samples, resulting in less whey separation.

In contrast to GCW, destabilization of SCW resulted only in whey separation at the top of the tube after 14 days of storage, followed by a continuous separation up to 28 days, with higher values (~50%) for protease-added samples and lower separation for sample fermented under US ($p<0.05$). Figure 4 showed that the microstructure of SCW

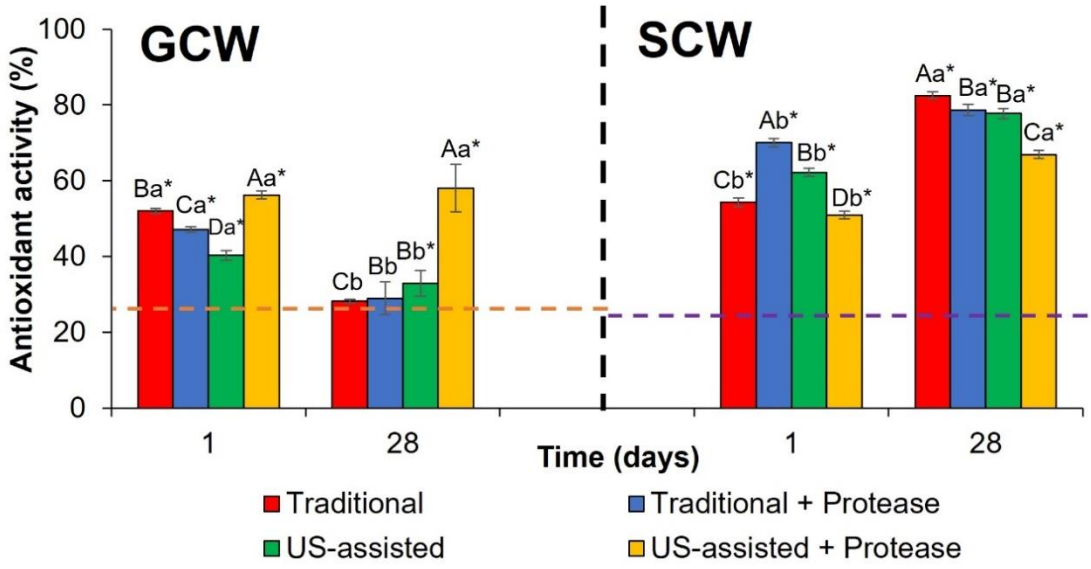
protein processed under different conditions were similar to those observed for GCW. Therefore, the differences observed in the destabilization of goat and sheep cheese whey can be attributed to the differences of whey composition, since SCW had almost no fat (0.18% vs. 1.01% in GCW), preventing cream separation, and high concentration of protein (0.95 vs. 0.68% in GCW), slightly favoring water holding capacity (Sánchez et al. 2020). Finally, regarding the greater whey separation in SCW added with proteases, maybe the reduction of protein agglomerates due to partial hydrolysis resulted in smaller structures with better accommodation, reducing the amount of water between them. The overall assessment of the samples physical stability emphasized the importance of developing strategies to ensure whey stabilization through the addition of stabilizers (Arab et al. 2022) associated with skimming or fat size reduction (Tribst et al. 2020b).

3.4. *In vitro* antioxidant activity of fermented goat and sheep cheese whey

After production, all samples showed higher in vitro antioxidant activities of the ABTS radical compared to the respective unfermented whey (Figure 4), with higher values for SCW. In addition, for SCW samples, an increase in the antioxidant activity was observed during storage ($p<0.05$). In contrast, for GCW, a reduction in antioxidant activity was observed for all samples ($p<0.05$) during storage, except for the sample produced under US and added with protease.

The *in vitro* antioxidant activity of fermented whey may be explained by the composition of whey proteins, mainly β-lactoglobulin, which has a large amount of sulfur-containing amino acids, principally cysteine (Ma et al. 2018). As observed for the SCW, the presence of lactic acid cultures and protease contributed to increase the scavenging ABTS radicals during storage, due to increased protein hydrolysis, allowing the release of amino acid residues in the polypeptide chain, which is related to improvements in antioxidant properties (Magalhães et al. 2022). However, this hypothesis was not confirmed for GCW, probably due to: (i) intense hydrolysis of proteins by Alcalase and by enzymes from LAB, releasing amino acids or peptides without antioxidant activity and (ii) low reaction with ABTS radicals. Therefore, these results should be considered only exploratory and further investigations, including in vivo assays, are necessary to affirm the biological benefits of fermented whey.

Figure 4. *In vitro* antioxidant activity (ABTS assay) of goat (GCW) and sheep cheese whey (SCW) fermented through traditional and ultrasound (US) assisted processes, added or not by protease.



Legend of Figure 4: [A-D] Capital letters indicate significant differences evaluated by the Tukey test ($p < 0.05$) among the processes for each whey source at each day. [a-c] Lower case superscripts indicate significant differences evaluated by the Tukey test ($p < 0.05$) for the same sample at different days. Fermentation process: T: Traditional; T+P: Traditional + Protease; US: US-assisted; US+P: US-assisted + Protease. Orange and purple dashed line represents the antioxidant activity (%) of unfermented GCW (26.36 ± 1.28) and SCW (24.44 ± 0.95), respectively. *: indicate significant differences evaluated by the Tukey test ($p < 0.05$) of each sample in relation to the respective unfermented whey.

4. Conclusions

US-assisted fermentation and the addition of proteases could be interesting interventions to improve the pH decline rate in SCW fermentation, which proved to be more difficult to ferment than GCW, probably due to limited nutrients. Moreover, these interventions directly affected the way the samples destabilized during storage, due to the

reduction in the fat globule size caused by US and proteolysis caused by protease; although none of them were able to avoid phase separation during whey storage. In contrast, interventions were insufficient to guarantee higher levels of LAB viability during storage, to avoid post-acidification and to improve the in vitro antioxidant activity of the samples. Further studies should access the impact of these interventions, associated with the addition of stabilizers and nutritional supplementation/storage at low temperature, on the structure and LAB viability in sheep and goat cheese whey.

5. References

Arab M, Yousefi M, Khanniri E, Azari M, Ghasemzadeh-Mohammadi V and Mollakhalili-Meybodi N (2022). A comprehensive review on yogurt syneresis: effect of processing conditions and added additives. Journal of Food Science and Technology, 1-10. doi: 10.1007/s13197-022-05403-6

Bustamante SZ, González JG, Sforza S and Tedeschi T (2021). Bioactivity and peptide profile of whey protein hydrolysates obtained from Colombian double-cream cheese production and their products after gastrointestinal digestion. LWT [online]. 145, 111334. doi: 10.1016/j.lwt.2021.111334

Capela APd, Tribst AAL, Augusto PED. and Leite Júnior BRdC (2022). Use of physical processes to maximize goat milk cream hydrolysis: Impact on structure and enzymatic hydrolysis. Food Research International. 156, 111343. 10.1016/j.foodres.2022.111343

Castro WF, Cruz AG, Rodrigues D, Ghiselli G, Oliveira CAF, Faria JAF and Godoy HT (2013). Short communication: Effects of different whey concentrations on physicochemical characteristics and viable counts of starter bacteria in dairy beverage supplemented with probiotics. Journal of Dairy Science [online]. 96(1), 96–100. doi: 10.3168/jds.2012-5576

Chourasia R, Phukon LC, Abedin MM, Padhi S, Singh SP. and Rai AK., (2022). Whey valorization by microbial and enzymatic bioprocesses for the production of nutraceuticals and value-added products. Bioresource Technology Reports. 101144. doi: 10.1016/j.biteb.2022.101144

De Brabandere AG and De Baerdemaeker JG (1999). Effects of process conditions on the pH development during yogurt fermentation. Journal of Food Engineering. 41(3-4), 221–227. doi: 10.1016/s0260-8774(99)00096-5

de Santos, FR, Junior Leite, BRC, Tribst, AL (2021). Thermal death kinetics of microbial load in goat's cheese whey. In: Anais do 14 SLACA - Simpósio Latino-Americano De Ciência De Alimentos, 2021, Campinas. Anais eletrônicos... Campinas, Galoá, 2021. Disponível em: <https://proceedings.science/slaca/slaca-2021/papers/thermal-death-kinetics-of-microbial-load-in-goat---s-cheese-whey>. Acesso em: 22 set. 2022.

Ewe, JA, Abdullah WNW, Bhat R, Karim A and Liong MT (2012). Enhanced growth of lactobacilli and bioconversion of isoflavones in biotin-supplemented soymilk upon ultrasound-treatment. Ultrasonics sonochemistry, 19(1), 160-173. doi: 10.1016/j.ultsonch.2011.06.013

Gholamhosseinpour A and Hashemi SMB (2018). Ultrasound pretreatment of fermented milk containing probiotic Lactobacillus plantarum AF1: Carbohydrate metabolism and antioxidant activity. Journal of Food Process Engineering [online]. 42(1), e12930. doi: 10.1111/jfpe.12930

Herrera-Ponce AL, Salmeron-Ochoa I, Rodriguez-Figueroa JC, Santellano-Estrada E, Garcia-Galicia IA. and Alarcon-Rojo AD (2021). High-intensity ultrasound as pre-treatment in the development of fermented whey and oat beverages: effect on the fermentation, antioxidant activity and consumer acceptance. Journal of Food Science and Technology. 59(2), 796-804. doi: 10.1007/s13197-021-05074-9

Juven BJ, Gordin S, Rosenthal I and Laufer A (1981). Changes in Refrigerated Milk Caused by Enterobacteriaceae. Journal of Dairy Science [online]. 64(9), 1781–1784. doi: 10.3168/jds.s0022-0302(81)82766-x

Laiho S., Williams RPW, Poelman A, Appelqvist I and Logan A (2017). Effect of whey protein phase volume on the tribology, rheology and sensory properties of fat-free stirred yoghurts. Food Hydrocolloids [online]. 67, 166–177. doi: 10.1016/j.foodhyd.2017.01.017

Liyanaarachchi WS and Vasiljevic T (2018). Caseins and their interactions that modify heat aggregation of whey proteins in commercial dairy mixtures. International Dairy Journal. 83, 43–51. doi: 10.1016/j.idairyj.2018.03.006

Ma S, Wang C and Guo M (2018). Changes in structure and antioxidant activity of β-lactoglobulin by ultrasound and enzymatic treatment. Ultrasonics Sonochemistry. 43, 227–236. doi: 10.1016/j.ultsonch.2018.01.017

Macedo A, Monteiro J and Duarte E (2018). A Contribution for the Valorisation of Sheep and Goat Cheese Whey through Nanofiltration. Membranes. 8(4), 114. doi: 10.3390/membranes8040114

Magalhães IS, Guimarães ADB, Tribst AAL, Oliveira EBd and Leite Júnior BRdC, (2022). Ultrasound-assisted enzymatic hydrolysis of goat milk casein: Effects on hydrolysis kinetics and on the solubility and antioxidant activity of hydrolysates. Food Research International. 157, 111310. doi: 10.1016/j.foodres.2022.111310

Martí-Quijal FJ, Khubber S, Remize F, Tomasevic, I, Roselló-Soto E and Barba FJ (2021). Obtaining antioxidants and natural preservatives from food by-products through fermentation: A review. Fermentation, 7(3), 106. doi: 10.3390/fermentation7030106

Rama GR, Kuhn D, Beux S, Maciel MJ and Volken de Souza CF (2019). Potential applications of dairy whey for the production of lactic acid bacteria cultures. International Dairy Journal. 98, 25–37. doi: 10.1016/j.idairyj.2019.06.012

Sánchez L, Pérez MD and Parrón JA (2020). HPP in dairy products: Impact on quality and applications. In: Present and Future of High Pressure Processing. Elsevier. pp. 245–272. doi: 10.1016/b978-0-12-816405-1.00011-x

Tamime AY (2007). Tamime and Robinson's yoghurt: Science and technology. 3rd ed. Cambridge: Woodhead Pub.

Tribst AAL and Júnior BRDCL (2022). Heat treatment design for the valorization of sheep cheese whey in artisanal production. Research, Society and Development, 11(9), e20911931776-e20911931776. DOI: https://doi.org/10.33448/rsd-v11i9.31776

Tribst, AAL, Falcade LTP, Carvalho NS., Junior BRdCL. and de Oliveira MM (2020b). Using stirring and homogenization to improve the fermentation profile and physicochemical characteristics of set yogurt from fresh, refrigerated and frozen/thawed sheep milk. LWT [online]. 130, 109557. doi: 10.1016/j.lwt.2020.109557

Tribst AAL, Falcade LTP, Carvalho NS, Leite Júnior BRdC. and Oliveira MMd (2020). Manufacture of a fermented dairy product using whey from sheep's milk cheese: An

alternative to using the main by-product of sheep's milk cheese production in small farms. International Dairy Journal [online]. 111, 104833. 10.1016/j.idairyj.2020.104833

Tribst AAL, Ribeiro LR, Leite Junior BRdC, de Oliveira MM and Cristianini M (2018). Fermentation profile and characteristics of yoghurt manufactured from frozen sheep milk. International Dairy Journal [online]. 78, 36–45. doi: 10.1016/j.idairyj.2017.10.005

Umego EC, He R, Huang G, Dai C and Ma H (2021). Ultrasound-assisted fermentation: Mechanisms, technologies, and challenges. Journal of Food Processing and Preservation [online]. 45(6). doi: 10.1111/jfpp.15559

Undugoda LJS and Nilmini A H L (2019). Effect of lactic acid microbial ratio of yoghurt starter culture in yoghurt fermentation and reduction of post acidification. J. Food Ind. Microbiol, 5(1). ISSN: 2572-4134

Walstra P, Wouters JTM, and Geurts TJ (2006). Dairy science and technology (2nd ed.). Boca Raton, London, New York: CRC Taylor & Francis Group, 782p.

Wei Z, Zhang W, Wang Y and Wang J (2017). Monitoring the fermentation, post-ripeness and storage processes of set yogurt using voltammetric electronic tongue. Journal of Food Engineering. 203, 41–52. doi: 10.1016/j.jfoodeng.2017.01.022

Zhao L, Zhang S, Uluko H, Liu L, Lu J, Xue H, Kong F and Lv J (2014). Effect of ultrasound pretreatment on rennet-induced coagulation properties of goat’s milk. Food Chemistry. 165, 167–174. doi: 10.1016/j.foodchem.2014.05.081

Zotta T, Solieri L, Iacumin L, Picozzi C and Gullo M (2020). Valorization of cheese whey using microbial fermentations. Applied Microbiology and Biotechnology. 104(7), 2749–2764. doi: 10.1007/s00253-020-10408-2

CAPÍTULO 3

Simple strategies to extend the shelf life of sheep and goat cheese whey under refrigeration: nisin, bioprotective culture, and acidification

Artigo sendo preparado para submissão. SANTOS, Fábio Ribeiro dos; LEAL, Damaris Moura, AUGUSTO, Pedro Esteves Duarte, LEITE JÚNIOR, Bruno Ricardo de Castro; TRIBST, Alline Artigiani Lima. Simple strategies to extend the shelf life of sheep and goat cheese whey under refrigeration: nisin, bioprotective culture, and acidification.

Simple strategies to extend the shelf life of sheep and goat cheese whey under refrigeration: nisin, bioprotective culture, and acidification

Fabio Ribeiro dos Santos[1], Damaris Moura Leal[2], Pedro Esteves Duarte Augusto[3], Bruno Ricardo de Castro Leite Junior[1], Alline Artigiani Lima Tribst[2]*

Affiliations:

[1]Department of Food Technology (DTA), Federal University of Viçosa (UFV), University Campus, 36570-900, Viçosa, MG, Brazil.

[2] Núcleo de Estudos e Pesquisas em Alimentação (NEPA). Universidade Estadual de Campinas (UNICAMP), Albert Einstein, 291, 13083-852, Campinas, SP Brazil.

[3] Université Paris-Saclay, CentraleSupélec, Laboratoire de Génie des Procédés et Matériaux, Centre Européen de Biotechnologie et de Bioéconomie (CEBB), 3 rue des Rouges Terres 51110 Pomacle, France.

* Corresponding Author. Albert Einstein, 291. Postal code: 13083-852, Campinas, São Paulo, Brazil, Phone: +55 19 3521-2176. E-mail: tribst@unicamp.br

Abstract

Sheep's cheese whey (SCW) and goat's cheese whey (GCW) is a by-product of cheese production and, on small farms, is not used properly due to small volumes and lack of knowledge on how to process it. Thus, the whey is either destined for animal feed or disposed of, reducing the income of these producers and causing environmental concerns. Microbiological stabilization with consequent shelf life extension is the first step to adding value to these products. Considering the need for simple solutions to be applied in artisanal production, we evaluated the effectiveness of nisin, the addition of *Lacticaseibacillus casei* as a bioprotective culture, and direct acidification with lactic acid (up to pH 4.5, 3.5, and 2.5) as tools to ensure the stability of SCW and GCW for 28 days at 7°C. The results showed that nisin and acidification at pH 3.5 and 2.5 maintained total and psychrotrophic bacteria counts below 1 log CFU/ml, with stable pH and acidity during sample storage. The inoculation with *L. casei* was also effective, reaching 7-8 log CFU/ml and protecting the samples with slight (SCW) or no acidification (GCW) of the samples. Regarding physical stability, all samples were destabilized, but those acidified at pH 3.5 and 4.5 had higher cream and sediment formation. On the other hand, particle size data showed little difference between the samples (0 and 28 days), suggesting that agitation of the whey was able to resuspend the separated particles during static storage. The final evaluation of the results of this phase of the study highlighted the addition of nisin and inoculation of *L. casei* as effective barriers to prevent spoilage of SCW and GCW, while acidification needs to be evaluated carefully due to the high amount of acid (6.6-7.7 v/v 40% lactic acid) required to reach pH 2.5, the low physical stability observed at pH 3.5 and 4.5, and vulnerability of the samples to contamination at pH 4.5.

Keywords: Sheep cheese whey; Goat cheese whey; Antimicrobials; Bioprotection; lactic acid; Hurdle

1. Introduction

Small ruminants' livestock, such as sheep and goats, are important to smallholders for economic and social reasons, improving their income and social insertion,and requiring a smaller area compared to cattle breeding (CAJA et al., 2020).In 2018, 458 million sheep and goats were used for milk production worldwide (PULINA et al., 2018), with a great percentage in underdeveloped and developing countries (SEJIAN et al., 2021). The milk obtained is mainly used for cheese manufacture (Macedo et al. 2021), which results in an expressive volume of whey. Moreover, this cheese production is mainly obtained through artisanal processing (Macedo et al. 2021), whose whey, due to the lack of structure and/or technical knowledge (Santos et al. 2022, Tribst & Leite Júnior, 2022), is commonly destined for animal feed or discharged, reducing the income of artisanal producers, and causing negative environmental impact (Kaur et al. 2020; de Oliveira et al. 2020).

To study alternatives for goat and sheep whey stabilization, therefore, can represent positive social, economic, and environmental impacts. Previous work from our research group demonstrated that heat treatment up to 75°C/ 5 min was insufficient to guarantee an adequate shelf life for those co-products, due to the residual growth of thermoduric and psychrotrophic populations (Leite Júnior & Tribst, 2022; Santos et al. 2022). Although more severe thermal processes could be efficient to inactive those microorganisms, increasing the processing temperature is not feasible due to the thermal sensibility of whey proteins, especially β-lactoglobulin (Pan et al. 2022), leading to intense sedimentation (Dumitraşcu et al. 2013).

Therefore, additional barriers must be included in the whey processing for proper stabilization. Considering the scarce equipment availability and low level of technification in small producers, these barriers need to be simple, cheap, and easy to be applied. To meet these requirements, alternatives such as inoculation with competitive culture for bioprotection (Rama et al. 2019), the addition of nisin (Silva et al. 2018), and acidification (Ryan & Walsh, 2016), are highlighted.

For instance, *L. casei* was described as a potential bioprotective culture, due to its ability to grow at low temperatures (Ben Said et al. 2019), competing with undesirable microflora for specific compounds, nutrient depletion, and production of antimicrobial

substances (Rama et al. 2019; Canon et al. 2020). In addition, the milk population that survives after pasteurization are commonly sporulated microorganisms sensitive to nisin (Silva et al. 2018). Therefore, nisin addition can be an effective approach for whey stabilization. Finally, those microorganisms may have low acid tolerance, due to the high pH of milk, and direct acidification with lactic acid can ensure the desired shelf life extension (Ryan & Walsh, 2016). However, any of those alternatives were evaluated before.

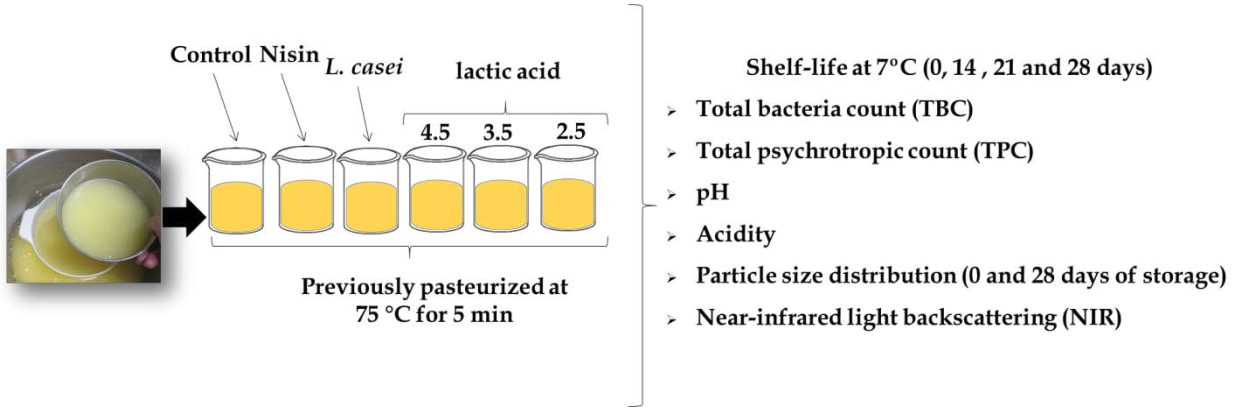
Consequently, the objective of this work was to evaluate the three strategies for physical and microbiological stability of sheep whey and goat whey for 28 days at 7°C: nisin addition, inoculation of *Lacticaseibacillus casei* as a bioprotective culture, and acidification using lactic acid (up to pH 4.5, 3.5, and 2.5).

2. Material and Methods

2.1 Goat and sheep whey

Goat whey (1.24 ± 0.1 % protein, 4.11 ± 0.1 % lactose, and 1.1 ± 0.0 % fat) was obtained from the Capril do Bosque artisanal cheese factory (Joanópolis, Brazil). Sheep whey (2.3 ± 0.1 % protein, 5.2 ± 0.0 % lactose, and 1.2 ± 0.2 % fat) was obtained from the Rima artisanal cheese factory (Porto Feliz, Brazil). Whey samples were stored at 1ºC up to processing (24h) Figure 1.

Figure 1. Flowchart of the experiment



2.2. Acidification and bioprotection using nisin and *Lacticaseibacillus casei*

Sheep and goat whey were pasteurized at 75 °C for 5 min to reach a total bacterial count <2 log CFU/mL (Santos et al. 2022; Tribst & Leite Júnior, 2022), and cooled to 7 °C.

Nisin (Nisaplin ®, Dupont, Wilmington, USA) treatment was conducted with concentration of 12.5mg/L (Nieva et al., 2022)(NIEVA et al., 2022). *Lacticaseibacillus casei* (BGP1, Sacco Brasil, Campinas, Brazil) was inoculated with 10^7 CFU/mL. Acidification was conducted by adding sterilized lactic acid to reach pH 4.5, 3.5, and 2.5, using acid solutions of 20 % (to achieve pH 4.5 and 3.5) or 40 % (to achieve pH 2.5). A control treatment consisted of a pasteurized sample with no addition of additives or cultures.

Immediately after preparation, the samples were divided into three sterilized glass flasks for microbiological and physicochemical evaluation at days 0, 14, and 28 days. The remained volume was used to fill two sterilized borosilicate glass tubes (12 mm inner diameter and 30 mm high) for measuring the near-infrared (NIR) light backscattering (% BS).

Each whey was processed in triplicate and, after complete preparation, the samples were stored at 7 ºC/ 28 days for evaluating the microbiological, physical, and physicochemical stability.

2.3. Bacterial counts, pH, and acidity

For microbiological evaluation, samples were serially diluted in saline solution (0.85% NaCl) and plated in plate count agar (PCA) for psychrotrophic bacteria count (TPC) and total bacteria count (TBC). The TBC counts were determined after incubation at 35 °C/ 48 h, and the psychrotrophic counts were determined after incubation at 7 °C / 10 days (Tribst et al., 2019). For the sample added with *L. casei*, lactic acid bacteria count was determined in MRS agar after incubation at 35ºC/ 48 h (Tribst et al. 2020).

2.4. Particle size distribution

The particle size determination of whey samples (0 and 28 days of storage) was carried out using a Mastersizer® laser diffraction particle size analyzer (Malvern

Instruments Ltd, England) equipped with a 300 RF (Reverse Fourrier) lens and He-Ne laser (λ= 633 nm). Samples were diluted in distilled water up to 14% of obscuration (~14%). Particle size distribution analysis was based on a polydisperse model using the following conditions: refractive index of fluid (water) of 1.33 and real refractive index of 1.349. The size distribution was characterized by the diameter below 10 ($d_{0.1}$) and 50% ($d_{0.5}$) of the volume of particles were found. The mean particle diameter was evaluated by the particle surface area ($D_{3,2}$ – parameter more influenced by smaller particles) and the specific surface area was also analyzed, considering the total area of the particles divided by their total weight (Tribst et al. 2019).

2.4. Near-infrared light backscattering

The physical stability of whey samples was also monitored using a near-infrared light backscatter at 880 nm from the bottom to the top of the tube (Turbiscan MA2000, Formulaction, France). The measures were carried out after 0, 1, 2, 4.5, 6.5, 24, 30, and 48 hours of whey samples production and then after 7, 14, 21, and 28 days. During this period, the samples were statically stored in borosilicate tubes at 7 ^{o}C to avoid perturbations (Tribst et al., 2020).

The backscattered profiles between 1 and 45 mm (full sample) were collected and analyzed using the Turbisoft 2.0 software (Leite Júnior et al. 2017). The cream separation phenomenon was measured by the % of backscattering (BS) at the creaming peak (%BS maximum) and the height of the cream in the tubes. The sedimentation was characterized by the height of sediments formed on the tube base. The %BS in the half tube height was also measured to track the reduction in backscatter caused by destabilization (both by creaming and sedimentation). These results were modelled considering different mathematical functions for better interpretation. Illustrative images of the samples in borosilicate tubes were taken at the end of shelf life.

2.5. Experimental design, statistical evaluation, and regressions

Each process was carried out in triplicate. Microorganism counts, particle size determination, and NIR backscattering were performed in duplicate for each processing replicate, totaling six measurement repetitions for each process condition. The titratable

acidity and pH (AOAC, 1999) were determined in triplicate, resulting in nine measurements for each process.

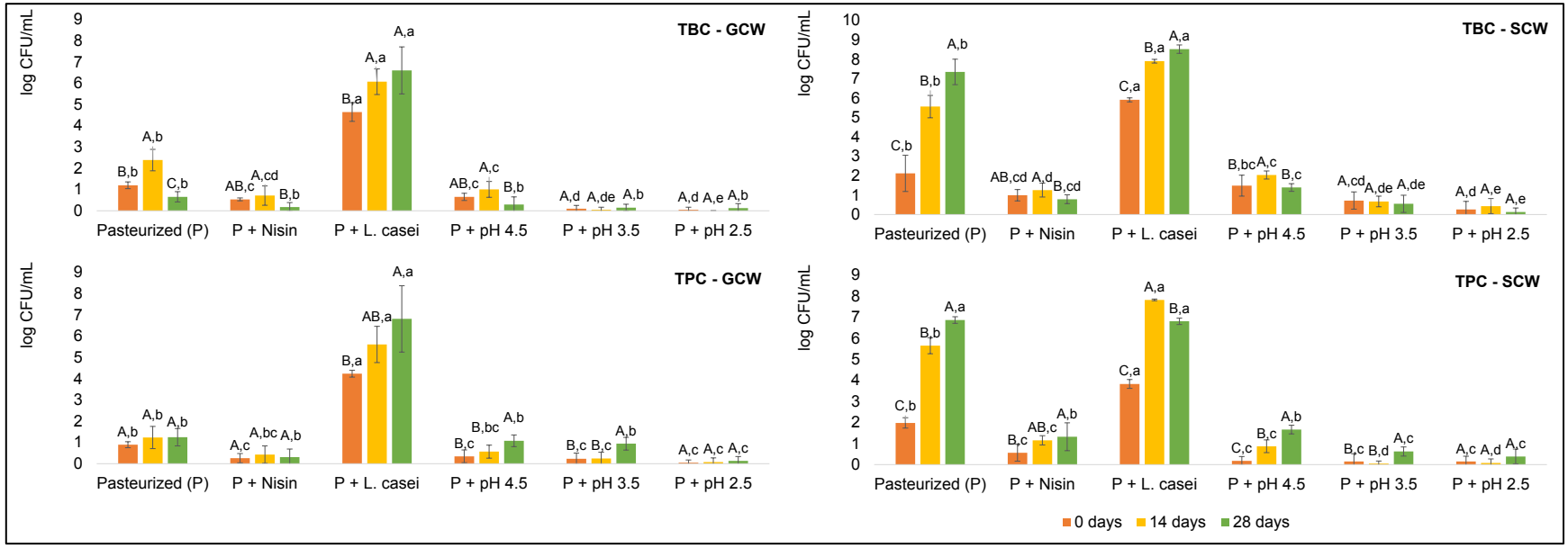
The results were expressed as mean ± standard deviation and evaluated through analysis of variance (ANOVA) and the Tukey test at a 95% confidence level (XLSTAT software, version 2015.2.02, Microsoft, Inc., USA).

Different mathematical functions were applied to describe the variation of physical stability over storage, according to the observed behaviours. Each function was fitted to the experimental data by nonlinear regression, using a generalized reduced gradient algorithm implemented in the 'Solver' tool of software Excel 365 (Microsoft, USA). The parameters of each model was iteratively adjusted to the experimental data by minimizing the sum of squared errors (SSE), considering all the process and analyses replicates. Different initial guesses of each parameter was evaluated to detect possible convergence to local optima. Model accuracy was evaluated by the coefficient of correlation (R^2) and the adjusted coefficient of correlation (adj-R^2).

3. Results

The mesophilic and psychrotrophic bacteria grew substantially in pasteurized sheep whey, reaching ~6 log CFU/mL and ~7 log CFU/mL after 14 and 28 days of storage (Figure 2), respectively ($p<0.05$). On the other hand, only TBC grew in pasteurized goat whey, achieving a maximum of 3 log CFU/mL after 14 days of storage ($p<0.05$).

Figure 2. Total bacterial count (TBC) and total psychrotrophic counts (TPC) during the storage of pasteurized goat (GCW) and sheep (SCW) whey associated with nisin, *L. casei* as bioprotective culture or low pH as strategies of stabilization.



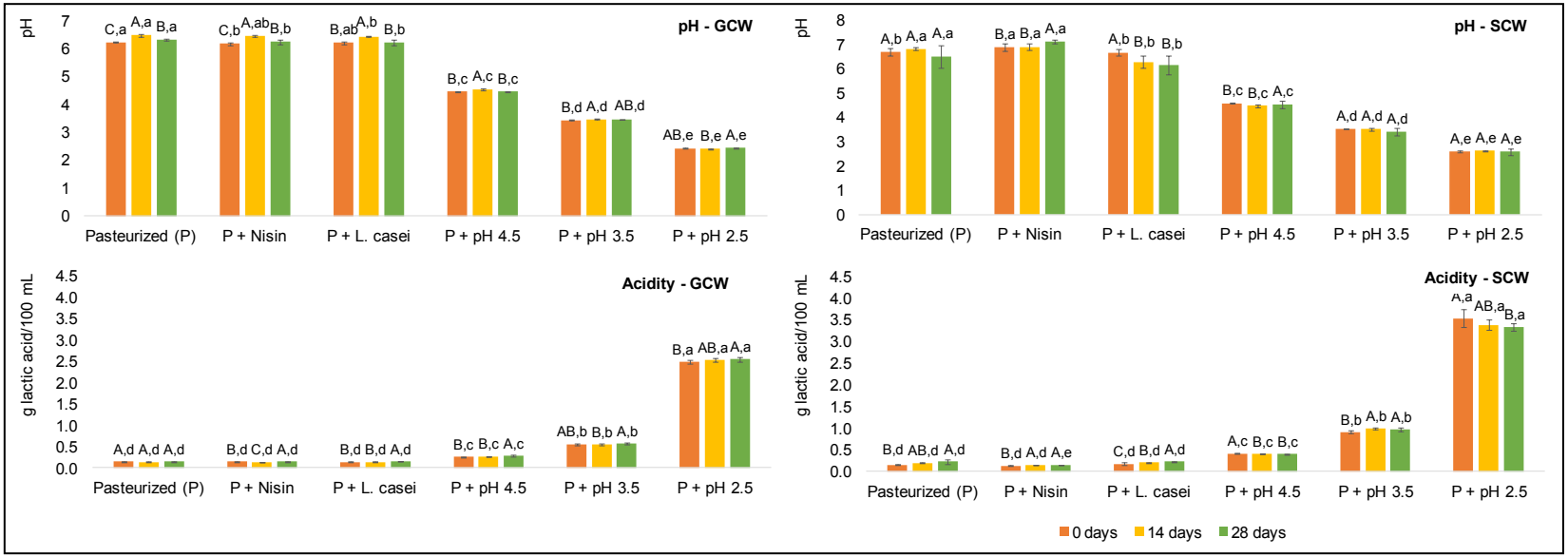
[A-C] means differences between the same sample measured at 0,14, and 28 days of storage ($P<0.05$). a-e means differences between samples produced by different processes on the same day and using the same whey ($P<0.05$).

The addition of nisin and acidification at pH ≤3.5 resulted in TBC and TPC < 1 log CFU/mL (Figure 2), even when a significant population increase was observed. For samples acidified to pH 4.5, a slight growth ($p<0.05$) was detected in sheep whey, reaching TBC and TPC of ~2 log CFU/mL, whereas counts < 1 log CFU/mL were observed for goat whey.

Finally, samples inoculated with *L. casei* showed growth of 2-3 log CFU/mL during storage, with final counts of 7-8 log CFU/mL ($p<0.05$). It is important to note that the counts achieved on MRS agar incubated at 7 and 37ºC were similar to those determined in TPC (up to 6.7 log CFU/mL in goat whey and sheep whey) and TBC (up to 6.6 log CFU/mL in goat whey and up to 8.5 log CFU/mL in sheep whey), respectively. Moreover, the colonies and cellular characteristics observed by microscopy (non-sporulated gram positive short rods) were homogenous (data non-showed). This showed that *L. casei* was able to grow under adverse conditions of LAB (Lacumin et al. 2021; Leite Junior & Tribst, 2022), preventing the growth of native load found in pasteurized sheep and goat whey.

Acidified samples had great differences in acidity, with ~0.40, 0.95 and 3.5 g lactic acid/ 100g of sheep whey and ~0.25, 0.55 and 2.5 g lactic acid/ 100g of goat whey at pH 4.5, 3.5 and 2.5, respectively ($p<0.05$). Conversely, acidified samples maintained stable pH and acidity during storage ($p>0.05$). On the other hand, control sample and those added with nisin or inoculated with *L. casei* had similar pH and acid values, with little variation during storage (< 5%, even when significant) - Figure 2.

Figure 3. pH and acidity evaluated during the storage of pasteurized goat (GCW) and sheep cheese whey (SCW) associated with nisin, *L. casei* as bioprotective culture or low pH.



A-C means differences between the same sample measured at 0,14, and 28 days of storage (P<0.05). a-e means differences between samples produced by different processes on the same day and using the same whey (P<0.05).

The physical stability evaluation during storage showed that each whey destabilized in a different pattern. The samples images at the end of storage illustrate the observed phenomena (Figure 4) and the different parameters associated with the stability over storage are presented in Figures 5 (sheep) and 6 (goat) - sedimentation rate (defined as % of tube height), %BS in the half tube height, and cream separation (% BS maximum and cream height).

Figure 4. Illustrative images obtained after 28 days of storage at 7ºC of pasteurized goat and sheep cheese whey associated with nisin, *L. casei* as bioprotective culture or low pH

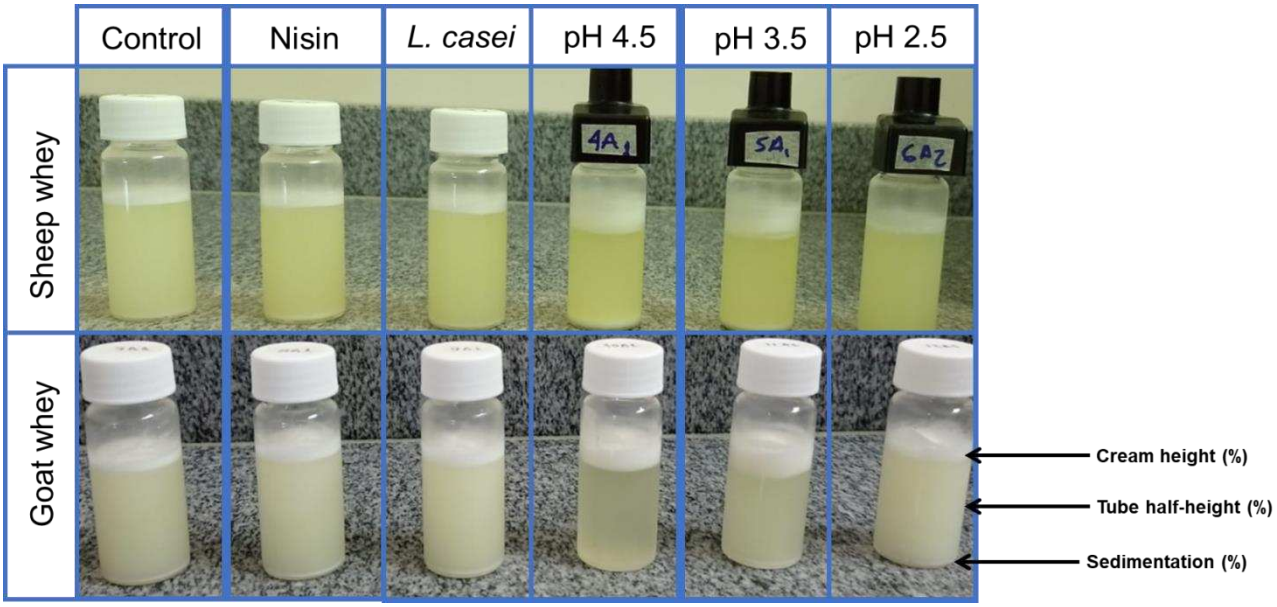


Figure 5. Physical stability of pasteurized sheep cheese whey (SCW) during the storage, associated with nisin, *L. casei* as bioprotective culture or low pH as strategies of stabilization. Sedimentation, cream height and backscattering at the half tube height and maximum cream value. Dots represent the average of experimental values and vertical bars the associated standard deviations. Curves are the proposed models.

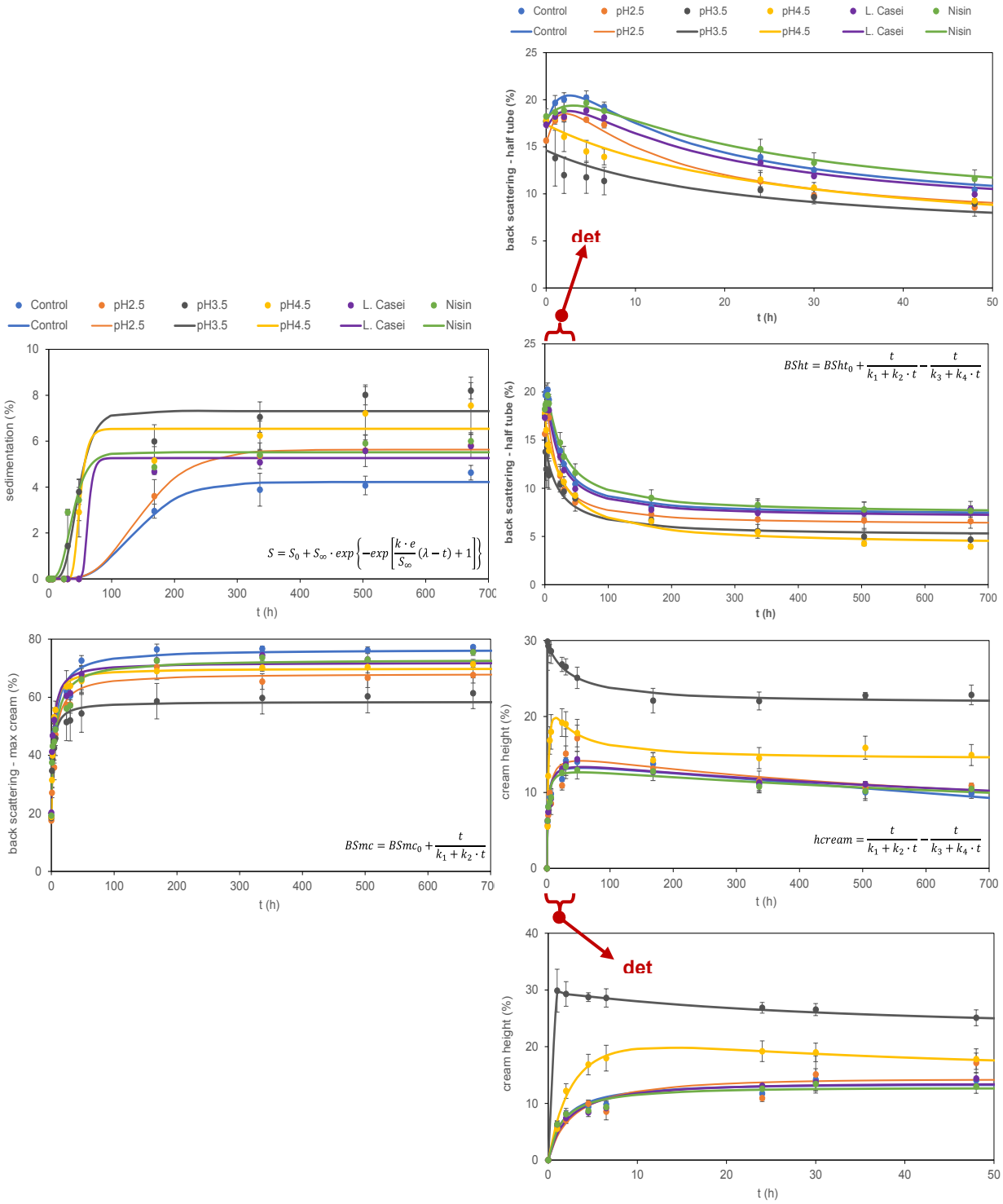
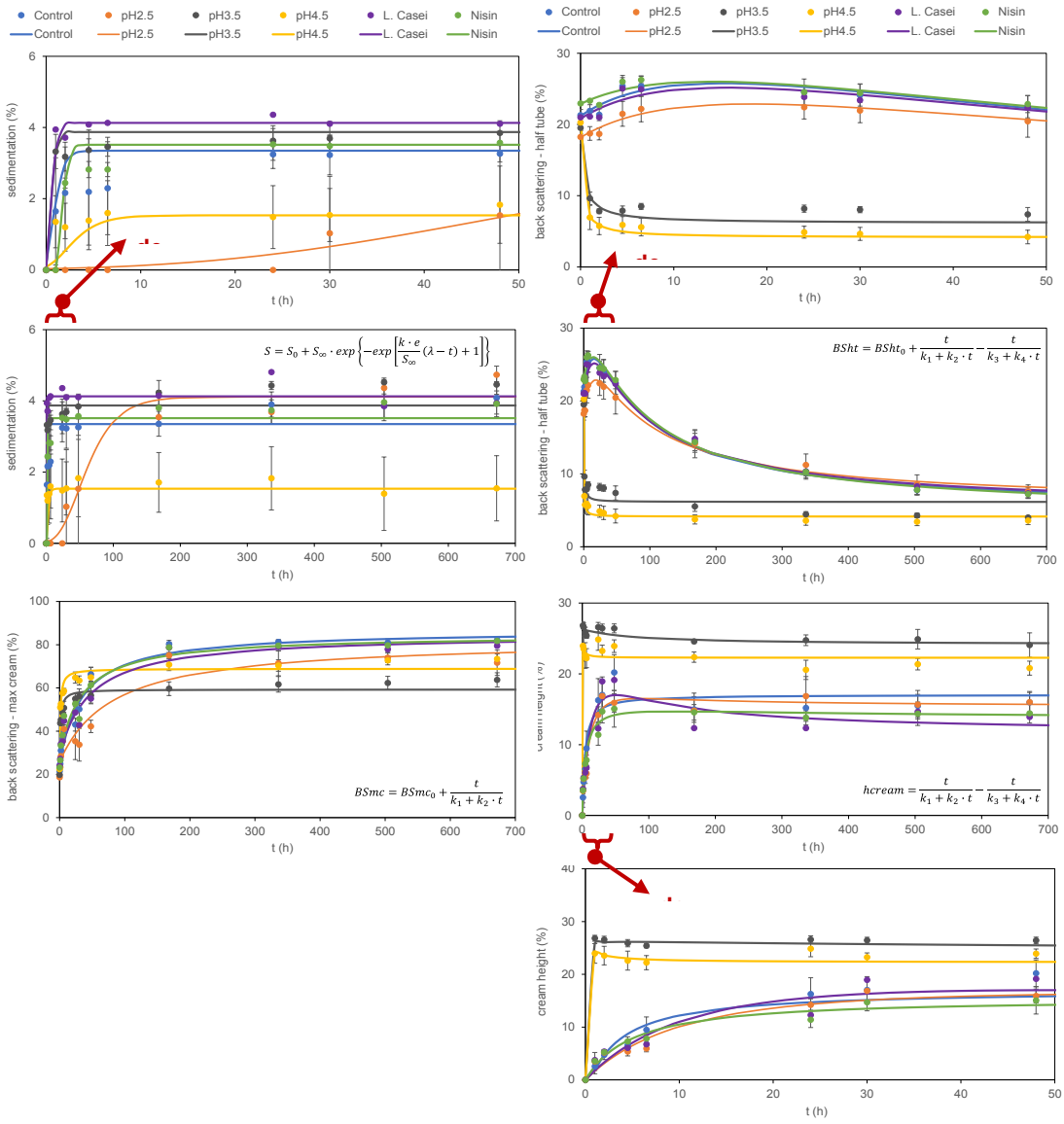


Figure 6. Physical stability of pasteurized goat cheese whey (GCW) during the storage, associated with nisin, *L. casei* as bioprotective culture or low pH as strategies of stabilization. Sedimentation, cream height and backscattering at the half tube height and maximum cream value. Dots represent the average of experimental values and vertical bars the associated standard deviations. Curves are the proposed models.



According to the presented behaviours, each parameter was modelled considering a mathematical function – whose equation, curves and parameters are presented in Figures 4 and 5 and Tables 1 and 2.

Table 1. Modelling the physical stability of pasteurized sheep cheese whey (SCW) during the storage, associated with nisin, *L. casei* as bioprotective culture or low pH as strategies of stabilization: sedimentation, cream height and back scattering at the half tube height and maximum cream value.

Physical stability parameter	Model	Parameters	Treatments					
			Control	Nisin	*L. casei*	pH 4.5	pH 3.5	pH 2.5
Sedimentation (%)	$S = S_0 + S_\infty \cdot exp\left\{-exp\left[\frac{k \cdot e}{S_\infty}(\lambda - t) + 1\right]\right\}$	S_∞ (%)	4.22	5.52	5.26	6.54	7.31	5.63
		k (%·h^{-1})	0.03	0.13	0.34	0.30	0.16	0.04
		λ (h)	68.36	16.73	54.50	38.36	24.31	72.83
		R^2	0.98	0.94	0.99	0.94	0.95	0.98
		adj-R^2	0.97	0.92	0.98	0.91	0.93	0.97
Cream height (%)	$\frac{h_{cream}}{} = \frac{t}{k_1 + k_2 \cdot t} - \frac{t}{k_3 + k_4 \cdot t}$	k_1 (%$^{-1}$·h)	0.12	0.12	0.15	0.05	0.001	0.18
		k_2 (%$^{-1}$)	0.07	0.07	0.07	0.01	0.03	0.06
		k_3 (%$^{-1}$·h)	139.49	132.63	98.31	0.07	4.11	63.80
		k_4 (%$^{-1}$)	0.25	0.39	0.01	0.03	0.44	0.01
		R^2	0.89	0.90	0.84	0.93	0.96	0.82
		adj-R^2	0.83	0.84	0.75	0.89	0.94	0.72
Back scattering at the half tube height (%)	$BSht = BSht_0 + \frac{t}{k_1 + k_2 \cdot t} - \frac{t}{k_3 + k_4 \cdot t}$	$BSht_0$ (%)	17.57	17.99	17.03	17.35	14.62	15.52
		k_1 (%$^{-1}$·h)	0.06	0.15	0.15	651.46	651.46	0.05
		k_2 (%$^{-1}$)	0.01	0.02	0.03	3631.57	3631.33	0.01
		k_3 (%$^{-1}$·h)	0.08	0.18	0.22	2.11	2.34	0.07
		k_4 (%$^{-1}$)	0.01	0.02	0.02	0.08	0.10	0.01
		R^2	0.98	0.97	0.97	0.97	0.82	0.98
		adj-R^2	0.96	0.96	0.96	0.95	0.71	0.96
Backscattering at maximum cream value (%)	$BSmc = BSmc_0 + \frac{t}{k_1 + k_2 \cdot t}$	$BSmc_0$ (%)	25.67	27.36	25.44	18.39	20.79	19.57
		k_1 (%$^{-1}$·h)	0.13	0.17	0.08	0.05	0.07	0.11
		k_2 (%$^{-1}$)	0.02	0.02	0.02	0.02	0.03	0.02
		R^2	0.95	0.90	0.93	0.97	0.83	0.94
		adj-R^2	0.93	0.86	0.88	0.96	0.76	0.92

Table 2. Modelling the physical stability of pasteurized goat cheese whey (GCW) during the storage, associated with nisin, *L. casei* as bioprotective culture or low pH as strategies of stabilization: sedimentation, cream height and back scattering at the half tube height and maximum cream value.

Physical stability parameter	Model	Parameters	Treatments					
			Control	Nisin	*L. casei*	pH 4.5	pH 3.5	pH 2.5
Sedimentation (%)	$S = S_0 + S_\infty \cdot exp\left\{-exp\left[\frac{k \cdot e}{S_\infty}(\lambda - t) + 1\right]\right\}$	S_∞ (%)	3.35	3.52	4.13	1.53	3.87	4.11
		k (%·h^{-1})	1.96	3.34	4.34	0.29	4.44	0.05
		λ (h)	0.03	1.23	0.12	0.14	0.12	17.95
		R^2	0.75	0.90	0.87	0.31	0.81	0.87
		adj-R^2	0.40	0.73	0.65	0.24	0.52	0.67
Cream height (%)	$h_{cream} = \frac{t}{k_1 + k_2 \cdot t} - \frac{t}{k_3 + k_4 \cdot t}$	k_1 (%$^{-1}$·h)	0.15	0.32	0.23	0.003	0.001	0.21
		k_2 (%$^{-1}$)	0.05	0.06	0.01	0.02	0.04	0.01
		k_3 (%$^{-1}$·h)	0.06	116.06	0.40	0.01	31.35	0.32
		k_4 (%$^{-1}$)	0.25	0.39	0.01	0.03	0.44	0.01
		R^2	0.85	0.93	0.86	0.94	0.99	0.91
		adj-R^2	0.57	0.77	0.60	0.81	0.95	0.73
Back scattering at the half tube height (%)	$BSht = BSht_0 + \frac{t}{k_1 + k_2 \cdot t} - \frac{t}{k_3 + k_4 \cdot t}$	$BSht_0$ (%)	0.15	0.27	0.34	0.00	0.00	0.18
		k_1 (%$^{-1}$·h)	0.01	0.01	0.01	0.00	0.00	0.01
		k_2 (%$^{-1}$)	0.17	0.32	0.47	0.02	0.03	0.21
		k_3 (%$^{-1}$·h)	0.01	0.01	0.01	0.06	0.08	0.01
		k_4 (%$^{-1}$)	21.20	22.90	20.83	20.25	19.47	18.19
		R^2	0.96	0.98	0.95	0.95	0.87	0.92
		adj-R^2	0.88	0.94	0.84	0.83	0.61	0.76
Back scattering at maximum cream value (%)	$BSmc = BSmc_0 + \frac{t}{k_1 + k_2 \cdot t}$	$BSmc_0$ (%)	0.58	0.61	0.84	0.02	0.03	1.45
		k_1 (%$^{-1}$·h)	0.02	0.02	0.02	0.02	0.03	0.02
		k_2 (%$^{-1}$)	25.50	28.48	29.09	23.53	20.82	25.87
		R^2	0.96	0.94	0.92	0.93	0.90	0.87
		adj-R^2	0.91	0.85	0.81	0.82	0.76	0.70

Sedimentation presented a sigmoidal behaviour, with the typical initial lag phase (time needed to observe the phenomena) and plateau. Consequently, sedimentation data was adjusted to the Gompertz Model (Gompertz, 1825; Winsor, 1932), whose parameters represent the time needed to start sedimentation (λ, lag phase), the sedimentation rate (k) and the maximum sedimentation (S_∞).

The maximum cream backscattering showed a downward concave shape behaviour, with a quick initial growth followed by a plateau. Therefore, a mathematical function similar to those proposed by Peleg (PELEG, MOR, 1980; PELEG, MICHA, 1988) was used to describe it. In this model, the inverse of k_1 represents the variation rate (i.e., as smaller k_1, as faster is the increase in backscattering), while the inverse of k_2 represents the plateau (i.e., as smaller k_2, as higher values of backscattering are achieved during storage). Moreover, $BSmc_0$ represents the initial value of backscattering.

Both the backscattering in the half tube height and cream height showed a complex behaviour, with an initial increase and then a decrease, reaching a plateau. Consequently, a composed model was applied, comprising two functions similar to those proposed by Peleg (Mor Peleg, 1980; Micha Peleg, 1988) - the first term being positive, representing the phenomena of increasing the values, and the second being negative, representing the phenomena of decreasing values. Therefore, in those values, k_1 and k_3 represents the kinetic parameter (the inverse of the velocity) of the first and second phenomena, respectively. Similarly, k_2 and k_4 represent the plateau of the first (the inverse of maximum achieved value) and second (the inverse of the minimum achieve value) phenomena, respectively.

The adjustment of models to the observed data was evaluated by the coefficient of correlation (R^2) and adjusted coefficient of correlation (adj-R^2), as shown on Tables 1 and 2. It can be seen that the model adjustment varied from very well to poor descriptions, which can be associated with the dispersion of experimental data, limited number of experimental points during storage and, mainly, variations between the two evaluated whey sources. Even so, the curves presented on Figures 4 and 5 clearly show the obtained models could well describe the observed behaviours, being a tool to compare the treatments and infers about the associated mechanisms. Consequently, the model parameters (Table 1 and 2) were used to qualitatively discuss the obtained data.

For both whey, results showed that samples at pH 4.5 and 3.5 were those with greater differences in most evaluated parameters compared to the control. Specifically for sheep cheese whey, it was observed sedimentation at least 54% higher; values 60% (k_1) and 97% (k_3) lower in cream height; k_1 and k_2 more than 1000 times higher in the backscatter measured at the half tube height, and 47% lower k_1 in the maximum backscatter measured in cream. In goat cheese whey, the main differences were observed in cream height (k_1 and k_3) and initial backscattering of maximum cream value (BS_{mc0}) of samples at pH 4.5 and 3.5 compared with the control, even with a different behaviour between them. Conversely, other studied process conditions (addition of nisin, inoculation of *L. casei* and acidification to pH 2.5) had most of the evaluated parameters quite similar to the control sample.

The particle size distribution over 28 days of storage is presented in Table 2. The results showed that goat whey particles were not affected by storage, keeping the shape of particles distribution (monomodal and irregular for control, nisin and *L. casei* samples and monomodal/bimodal regular for acidified whey).

Table 3. Particle size distribution of pasteurized goat (GCW) and sheep cheese whey (SCW) associated with nisin, *L. casei* as bioprotective culture or low pH (determined after 0 and 28 days of storage at 7ºC.

Whey origin	Time	Processing	**Bell Curve Shape**	**Specific surface area**	**D [3, 2] - Surface weighted mean**	**d (0.1)**	**d (0.5)**
Goat	0 days	TT	Irregular*	2.64 ± 0.05 ^{A,ab}	2.27 ± 0.04 ^{A,de}	1.20 ± 0.01 ^{A,d}	2.52 ± 0.04 ^{A,e}
		TT+nis	Irregular	2.61 ± 0.03 ^{A,b}	2.29 ± 0.02 ^{A,d}	1.21 ± 0.00 ^{B,d}	2.58 ± 0.02 ^{A,d}
		TT+Lc	Irregular	2.67 ± 0.02 ^{B,a}	2.24 ± 0.01 $_{A,e}$	1.20 ± 0.00 ^{A,d}	2.51 ± 0.02 ^{A,e}
		TT+pH4.5	Regular	2.45 ± 0.07 ^{A,d}	2.45 ± 0.07 ^{A,b}	1.39 ± 0.04 ^{A,b}	2.87 ± 0.09 ^{A,b}
		TT+pH3.5	Regular	2.09 ± 0.01 ^{A,e}	2.86 ± 0.01 ^{A,a}	1.53 ± 0.00 ^{A,a}	3.56 ± 0.03 ^{B,a}
		TT+pH2.5	Irregular	2.51 ± 0.03 ^{A,c}	2.38 ± 0.03 ^{A,c}	1.24 ± 0.01 ^{A,c}	2.76 ± 0.07 ^{A,c}
	28 days	TT	Irregular	2.61 ± 0.06 ^{A,b}	2.29 ± 0.05 ^{A,d}	1.20 ± 0.01 ^{A,c}	2.55 ± 0.05 ^{A,d}
		TT+nis	Irregular	2.59 ± 0.04 ^{A,b}	2.31 ± 0.03 ^{A,d}	1.22 ± 0.01 ^{A,c}	2.61 ± 0.02 ^{A,d}
		TT+Lc	Irregular	2.73 ± 0.05 ^{A,a}	2.19 ± 0.04 ^{B,e}	1.21 ± 0.05 ^{A,c}	2.46 ± 0.05 ^{B,e}
		TT+pH4.5	Regular	2.41 ± 0.02 ^{A,d}	2.48 ± 0.02 ^{A,b}	1.39 ± 0.02 ^{A,b}	2.93 ± 0.03 ^{A,b}
		TT+pH3.5	Regular	2.10 ± 0.02 ^{A,e}	2.84 ± 0.03 ^{A,a}	1.46 ± 0.03 ^{B,a}	3.64 ± 0.09 ^{A,a}
		TT+pH2.5	Irregular	2.49 ± 0.05 ^{A,c}	2.40 ± 0.05 ^{A,c}	1.23 ± 0.01 ^{B,c}	2.79 ± 0.08 ^{A,c}
Sheep	0 days	TT	Regular	2.14 ± 0.06 ^{A,a}	2.80 ± 0.08 ^{B,d}	1.41 ± 0.05 ^{A,b}	3.63 ± 0.08 ^{B,c}
		TT+nis	Irregular	2.02 ± 0.04 ^{A,b}	2.96 ± 0.07 ^{B,c}	1.40 ± 0.03 ^{B,b}	3.95 ± 0.10 ^{B,b}
		TT+Lc	Regular	2.15 ± 0.03 ^{A,a}	2.79 ± 0.04 ^{B,d}	1.42 ± 0.02 ^{A,b}	3.57 ± 0.05 ^{B,c}
		TT+pH4.5	Irregular	1.68 ± 0.08 ^{B,d}	3.57 ± 0.31 ^{A,a}	1.50 ± 0.08 ^{A,a}	4.86 ± 3.23 ^{A,a}
		TT+pH3.5	Irregular	1.86 ± 0.00 ^{B,c}	3.21 ± 0.00 ^{A,b}	1.32 ± 0.00 ^{A,c}	4.79 ± 0.01 ^{A,a}
		TT+pH2.5	Regular	2.16 ± 0.03 ^{A,a}	2.77 ± 0.05 ^{B,d}	1.30 ± 0.02 ^{A,c}	3.84 ± 0.10 ^{A,b}
	28 days	TT	Irregular	1.93 ± 0.10 ^{B,c}	3.09 ± 0.21 ^{A,b}	1.31 ± 0.05 ^{B,c}	4.46 ± 0.31 ^{A,b}
		TT+nis	Irregular	1.24 ± 0.05 ^{B,e}	4.82 ± 0.07 ^{A,a}	1.78 ± 0.01 ^{A,a}	9.61 ± 0.08 ^{A,a}
		TT+Lc	Irregular	1.88 ± 0.01 ^{B,d}	3.18 ± 0.03 ^{A,b}	1.33 ± 0.01 ^{B,bc}	4.69 ± 0.21 ^{A,b}
		TT+pH4.5	Regular	2.08 ± 0.02 ^{A,b}	2.87 ± 0.02 ^{B,cd}	1.36 ± 0.01 ^{B,b}	3.93 ± 0.05 ^{B,b}
		TT+pH3.5	Regular	2.16 ± 0.06 ^{A,a}	2.77 ± 0.08 ^{B,d}	1.33 ± 0.04 ^{A,bc}	3.82 ± 0.09 ^{B,b}
		TT+pH2.5	Regular	2.04 ± 0.03 ^{B,b}	2.93 ± 0.04 ^{A,c}	1.32 ± 0.04 ^{A,bc}	4.12 ± 0.04 ^{B,b}

The specific surface area, D [3,2], d (0.1) and d (0.5) were maintained ($p>0.05$) for most of the samples, with slight variations < 5% for different ones ($p<0.05$). Furthermore, an overall comparison of samples showed that control sample and those added with nisin or inoculated with *L. casei* samples had 8-20% high surface area, ~10% lower D [3,2], ~15% lower d (0,1) and ~10% lower d (0.5) than samples at pH 4.5 and 3.5, while goat whey at pH 2.5 showed parameters with intermediate values between those observed for neutral whey and samples with pH 4.5-3.5.

On the contrary, the sheep whey had the opposite behavior, with large changes in the shape of the particle size distribution during storage and, consequently, in the evaluated parameters (Table 3). Interestingly, samples with neutral pH change the distribution of particle size from monomodal to bimodal after 28 days of storage, with a consequent 10-39% reduction of specific surface area, 10-62% increase of D[3,2] and 63-246% increase of d(0,5) ($p<0.05$). On the other hand, samples at pH near to isoelectric point showed an opposite performance, with particle size distribution changing from bimodal to monomodal, leading to an increase of specific surface area and a reduction in D[3,2] and d(0,5).

Furthermore, the comparison of sheep whey processed by different strategies showed that, on day 0, the samples had similar behavior to the observed for goat whey at days 0 and 28, with larger particles for samples at pH 4.5 and 3.5, evidenced by D[3,2] and d(0.5) values, while an opposite behavior was observed after 28 days.

4. Discussion

Sheep and goat cheese whey obtained in smallholdings are commonly discharged or destined for animal feeding (Tribst et al. 2020), reducing its profitable value due to the lack of technical knowledge on how to process it (Santos et al. 2022; Tribst & Leite Júnior, 2022).

To establish proper processing, the first step is to stabilize the whey microbiologically; however, previous results have shown that pasteurization at 75ºC/ 5 min was insufficient to guarantee the stability of sheep and goat whey (Tribst & Leite Junior, 2022; Santos et al. 2022), while more intense heating leads to undesirable destabilization of protein (Pan et al. 2022). These results explain the goal of the present work that focused on establishing barrier options to be associated with thermal treatment for guaranteeing sheep and goat whey stability during refrigerated storage.

The microbiological evaluation (Figure 2) confirmed the undesirable growth of mesophilic and psychrotrophic bacteria in whey subjected exclusively to pasteurization, with the most expressive growth in the sheep whey. In addition, the low counts of mesophilic and psychrotrophic microorganisms, observed in samples added with nisin or acidified to pH≤3.5, showed these alternatives were adequate to control the growth of native microbial load in sheep and goat whey during 28 days at 7ºC. For sheep whey acidified to pH 4.5, the observed marginal growth (~ 1 log CFU/mL) was likely insufficient to threaten product safety and/or quality (Osman et al. 2014, Datta & Deeth, 2001), making this low level of acidification a feasible alternative. Finally, the high TPC, TBC, and LAB counts of the samples inoculated with *L. casei* suggested this bioprotective microorganism was able to grow in sheep and goat whey, preventing the growth of unwanted/unknown microorganisms and reaching counts that make the product eligible to be classified as a probiotic (Kowalczyk et al. 2022).

The microorganisms commonly found in cheese whey includes cheese starters cultures (Lo et al. 2016), cheese contaminants microorganisms (Pala et al. 2016), and thermoduric/sporulated microorganisms capable of surviving in milk pasteurization (Lo et al. 2016, Ribeiro-Júnior et al. 2018; da Silva Duarte et al. 2020). Therefore, after the intense whey pasteurization (75ºC / 5 min), probably only sporulated microorganisms were able to survive (Santos et al. 2022; Tribst & Leite Júnior, 2022), being sequentially inhibited by the antimicrobial activity of nisin (Silva et al. 2018), unfavorable pH≤4.5 (Leite Junior & Tribst, 2022) or due to competition with *L. casei* - due to nutrient depletion caused by the fast growth of the bioprotective culture and its antimicrobial production (Rama et al. 2019; Canon et al. 2020). Therefore, all the here studied strategies were considered adequate for the microbial stabilization of whey, and the final choice of the barrier should be made according to the consequences on the physical stability of the whey, the final physicochemical characteristics of the product and other aspects such as legislation and availability.

With respect to alterations in pH and acidity (Figure 3), the neutral samples showed a small variation during storage, whose values would not jeopardize the product stability nor sensory characteristics (Tribst et al. 2020). For acidified samples, it was observed that an expressive increase in samples acidity was necessary to achieve the desirable pH, mainly pH 2.5, which is associated to the high buffering capacity of whey protein solution at pH below 3.5 (Salaün et al. 2005), requiring the

addition of large volumes of acid to cause a pH drop between pH 3.5 and 2.5. The final consequence of this behavior is the prohibitive amount of lactic acid needed to reach pH 2.5 (6.6-7.7 v/v 40% lactic acid), diluting the product unacceptably and likely impacting the sensory profile of the product, making it unpalatable. Therefore, acidification at pH 2.5 is not proposed here for cheese whey stabilization.

Regarding the physical stability of the samples, different rates and final intensities of destabilization were observed. Results from near-infrared backscattering (Figure 5 and 6/Table 1 and 2) and particle size (Table 3) helped to explain the sedimentation and cream separation at the end of storage, illustrated in Figure 3.

The backscattering measured during the storage time at the center of the tube presents a first increase and then a decrease until a plateau value, reflecting two mechanisms that can be associated to the creaming of low-density phase (Hillbrick et al. 1999; Walstra & Jenness, 1984) and sedimentation of a high-density phase (Dalgleish & Robson, 1985). A two-stage function could well describe that behaviour, and the evaluation of each phenomena separated demonstrate creaming was faster than sedimentation (thus resulting in the observed behaviour for the backscattering at half tube height).

Protein sedimentation occurred in all the treatments but presented differences among whey source and stabilization strategies. All the treatments resulted in a sigmoidal sedimentation for sheep whey, increasing the sedimentation amount (S_∞) and velocity (increasing both rate k and reducing the lag phase, λ). For instance, while the control treatment presented 68 h of lag phase and maximum sedimentation of 4% (Table 1), acidification at pH 3.5 presented a 65% shorter lag phase and a sedimentation 70% higher. Goat whey sedimentation was smaller than sheep, presenting smaller plateau values, which can be related with the difference in protein concentration – the sheep whey protein content was almost twice the goat whey concentration, as described in the methodology section. However, a faster phenomenon and a different behaviour were observed: instead of a sigmoidal behaviour (presented only be the acidification at pH 2.5), a quick sedimentation occurred in goat whey – presenting a downward concave shape behaviour, explaining the poor adjustment of the sigmoidal model.

Therefore, the evaluated treatments reduced both whey stability in relation to protein sedimentation, but with different impacts. The acidification at pH 3.5 presented

higher sedimentation in both whey sources, while at pH 4.5 resulted in higher sedimentation for sheep whey and smaller for goat whey. Although the acidification at pH 2.5, the addition of nisin and the inoculation of *L. casei* resulted in an increase in sedimentation from 5-30%, the overall result can be considered acceptable and easy to overcome through the addition of stabilizers (Arab et al. 2022) – although the acidification at pH 2.5 has limited applicability due to its high acidity, as discussed.

Creaming presented a first increase and then a decrease in cream height until a plateau value, in relation to storage time. Two mechanisms affected the cream height, justifying the two-terms model here adopted (Tables 1 and 2). In the beginning of storage, the fraction with small density start to separate from the continuous phase, resulting in a quick increase in cream height (Hillbrick et al. 1999; Walstra & Jenness, 1982). The observed high velocity is explained by the weak structure of the colloidal suspension, attributed to the lack of protein or stabilizers (Arab et al. 2022) that would be able to increase the viscosity of the system and reduces the phase separation (Mehra et al., 2022). Subsequently, the interactions among particles in this light phase (Walstra et al. 2006) make the cream layer more compact, slightly reducing the thickness of the separated layer until the plateau value. While the first mechanism represents big changes in cream height, the second represents only a small reduction, with a slight impact on the cream backscattering (whose behaviour, therefore, is represented by a single term equation) – Figures 5 and 6, Tables 1, and 2.

The acidification at pH 3.5 and 4.5 resulted in higher cream separation (cream heights) for both whey sources when compared to other treatments and control. Again, the treatments of acidification at pH 3.5 and 4.5 promoted higher alterations in whey along the storage, which is explained by the destabilization caused by pasteurization followed by acidification that brings them closer to the isoelectric point, makes it impossible to maintain suspension stability (Tribst et al. 2020). This hypothesis is corroborated by the stability results obtained for samples at pH 2.5, where pH reduction below isoelectric point (Mohebrad et al., 2019) allows the increase of protein solubility and, consequently, the system stability (Kotchabhakdi & Vardhanabhuti 2020).

While the goat whey sedimentation was smaller and faster than sheep, the cream formation was faster in sheep (evaluated by the inverse of k1 in the backscattering at maximum cream value – Tables 2). This suggests there is the formation of the protein-fat complex, with the fat governing the physical behaviour of

the structure. Fat globules, probably aggregated with part of the protein, separated from the system due to their low density (Walstra et al. 2006), and migrated to the top of the tube, while whey protein clumps sedimented and a translucent whey material containing soluble nutrients was formed between them (as highlighted in Figure 6).

Despite the intense changes observed in the static experiment measured by the % of light backscattering, the minor differences in particle size data suggested that particle interactions capable of causing physical destabilization of the samples during the static storage were disrupted by the stirring performed during the measurement of the particle size. This reflects the aggregation/agglomeration observed in both cheese whey is mainly a result of weak force interactions among proteins and fat, also suggesting the destabilization can be reverted by stirring. Even so, the slightly larger particles for goat (0 and 28 days) and sheep whey (0 days) samples close to the isoelectric point suggest that the protein and/or protein-fat aggregates formed in these conditions (KIEŁCZEWSKA et al., 2020)were stronger than in other samples, probably because the low surface charge of proteins reduced their solvation and favors the maintenance of aggregates (Moatsou, 2022), even under intense agitation promoted by light scattering equipment.

Those results suggest that although all samples had been destabilized during storage (Figure 5, Table 1), the proximity to the isoelectric point of the samples at pH 4.5 and 3.5 allowed the formation of more stable aggregates (Tribst et al. 2020; (RYAN, K. N.; ZHONG; FOEGEDING, 2013) that was not disrupted during stirring. Conversely, the samples with pH reduction to 2.5 allowed the partial recovery of protein repulsion, allowing the solvation of the proteins and leading to an intermediate performance between control sample and those close to the isoelectric point. This explains why the destabilization of samples at pH 4.5 and 3.5 was faster and more intense than the observed for samples at pH greater than 6.2 or at 2.5 (Figure 5, Table 1).

The opposite behavior of sheep whey particle size after 28 days of storage was unexpected, but perhaps it is explained by the activity of microbial proteases that are commonly found in sheep milk due to the long storage under refrigeration (Tribst et al. 2018), which favors the growth of proteolytic psychrotrophic microorganisms (CAPODIFOGLIO et al., 2016). These enzymes, which have optimum pH near to neutrality, probably catalyzed the partial hydrolyze of whey protein, favoring the

interactions of these protein fragments (DEETH; BANSAL, 2018), with consequent aggregation.

The overall evaluation of the results showed that the addition of nisin and inoculation of *L. casei* as barriers was effective in preventing the deterioration of sheep whey and goat whey, while acidification needs to be evaluated carefully due to the high amount of acid (6 .6-7.7 v/v lactic acid 40%) required to reach pH 2.5, the low stability observed at pH 3.5 and 4.5 and the vulnerability of samples to contamination at pH 4.5. And the physical stability of the samples emphasized the importance of developing strategies to ensure whey stabilization through the addition of stabilizers. These results can be directly applied to improve the artisanal production of sheep and goat cheese, bringing benefits to the product.

5. Conclusions

This study showed that the barriers studied to prevent the growth of deteriorating microorganisms in goat and sheep whey, the inoculation of the *L. casei* culture, and the addition of nisin was the best alternatives to prolong the shelf life of the sera, as nisin was able to to keep the mesophilic and psychrotrophic bacteria counts below <1 log CFU/mL and *L. casei* was able to grow under adverse LAB conditions. On the other hand, the biggest challenge was to maintain the physical stability of the sera, all samples were destabilized. In view of this, it is necessary to develop a strategy to guarantee the physical stabilization of goat and sheep whey throughout its shelf life.

Acknowledgments

The authors would like to thank the Rima and Capril do Bosque for the sheep whey and goat whey. This work was supported by the São Paulo Research Foundation (FAPESP, project no. 2020/10930-9) and by the National Council for Scientific and Technological Development with the productivity grants of Bruno Ricardo de Castro Leite Júnior (306514/2020-6) and Alline Artigiani Lima Tribst (305050/2020-6), and CAPES (code 001) for the master's scholarship granted to Fábio Ribeiro dos Santos.

Conflict of interests

The authors declare no conflict of interest.

Funding

This work was supported by the São Paulo Research Foundation (FAPESP, project no. 2020/10930-9) and by the National Council for Scientific and Technological Development with the productivity grants of B. R. C. Leite Júnior (306514/2020-6) and A. A. L. Tribst (305050/2020-6) and CAPES (code 001) for the master's scholarship granted to F.R. dos Santos

References

ALBENZIO, M. et al. Nutritional properties of small ruminant food products and their role on human health. **Small Ruminant Research,** v. 135, p. 3-12, 2016.

AMMAR, E.-T.; KHALEL, A. E.; MOSTAFA, M. Effect of type of milk on the properties of

traditional feta cheese. **Journal of Food and Dairy Sciences,** v. 5, n. 5, p. 315-327, 2014.

ANUMUDU, C. et al. Recent advances in the application of the antimicrobial peptide nisin in the inactivation of spore-forming bacteria in foods. **Molecules,** v. 26, n. 18, p. 5552, 2021.

AOAC, I. Official Methods of Analysis. 16th ed. AOAC Int., Washington, DC.. 1999.

AUGUSTO, P. E.; TRIBST, A. A.; CRISTIANINI, M. Thermal inactivation of Lactobacillus plantarum in a model liquid food. **Journal of Food Process Engineering,** v. 34, n. 4, p. 1013-1027, 2011.

BIANCHI, A. E. et al. Effect of the addition of protected fat from palm oil to the diet of dairy sheep. **Revista Brasileira de Zootecnia,** v. 47, 2018.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Portaria n° 80, de 13 de agosto de 2020. Regulamento técnico para fixação de identidade e qualidade de soro de leite e o soro de leite ácido.Diário Oficial da República Federativa do Brasil, Brasília, 13 ago. 2020. Disponivel em: <https://www.gov.br/agricultura/pt-br/assuntos/suasa/regulamentos-tecnicos-de-identidade-e-qualidade-de-produtos-de-origem-animal-1/rtiq-leite-e-seus-derivados> Acessado em: 15/12/2022. 2020.

CAJA, G. et al. Sensing solutions for improving the performance, health and wellbeing of small ruminants. **Journal of Dairy Research,** v. 87, n. S1, p. 34-46, 2020.

CANON, F. et al. Understanding the mechanisms of positive microbial interactions

that benefit lactic acid bacteria co-cultures. **Frontiers in Microbiology**, p. 2088, 2020a.

CANON, Fanny et al. Understanding the mechanisms of positive microbial interactions that benefit lactic acid bacteria co-cultures. Frontiers in Microbiology, v. 11, p. 2088, 2020.

CAPODIFOGLIO, E. et al. Lipolytic and proteolytic activity of Pseudomonas spp. isolated during milking and storage of refrigerated raw milk. **Journal of dairy science,** v. 99, n. 7, p. 5214-5223, 2016.

CARRILLO-LOPEZ, L. M. et al. Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties. **Ultrasonics Sonochemistry,** v. 73, p. 105467, 2021.

CENACHI, D. B. et al. Aspectos composicionais, propriedades funcionais, nutricionais e sensoriais do leite de cabra: Uma revisão. **Revista do Instituto de Laticínios Cândido Tostes,** v. 66, n. 382, p. 12-20, 2011.

CLAEYS, W. L. et al. Raw or heated cow milk consumption: Review of risks and benefits. **Food control,** v. 31, n. 1, p. 251-262, 2013.

COSTA, G. M. et al. Yoghurt added with Lactobacillus casei and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. **International Dairy Journal,** v. 97, p. 139-148, 2019.

DA SILVA DUARTE, V. et al. Comparative evaluation of cheese whey microbial composition from four Italian cheese factories by viable counts and 16S rRNA gene amplicon sequencing. **International Dairy Journal,** v. 104, p. 104656, 2020.

DE OLIVEIRA, I. K. C. P. et al. Proximate composition determination in goat cheese whey by near infrared spectroscopy (NIRS). **PeerJ,** v. 8, p. e8619, 2020.

DEETH, H. C.; BANSAL, N. **Whey proteins: from milk to medicine**. Academic Press, 2018. ISBN 0128121254.

DEN BESTEN, H. M.; WELLS-BENNIK, M. H.; ZWIETERING, M. H. Natural diversity in heat resistance of bacteria and bacterial spores: impact on food safety and quality. **Annual Review of Food Science and Technology,** v. 9, p. 383-410, 2018.

DU, Q. et al. The complex of whey protein and pectin: Interactions, functional properties and applications in food colloidal systems–A review. **Colloids and Surfaces B: Biointerfaces,** v. 210, p. 112253, 2022.

EVELYN, E.; SILVA, F. V. Thermosonication versus thermal processing of skim milk

and beef slurry: modeling the inactivation kinetics of psychrotrophic Bacillus cereus spores. **Food Research International,** v. 67, p. 67-74, 2015.

FELLOWS, P. Principles and practice. **Food processing technology, 2nd ed., ed. Ellis Horwood, Chickester, UK**, p. 369-380, 2000.

GABRIEL, A. A. et al. Fates of pathogenic bacteria in time-temperature-abused and Holder-pasteurized human donor-, infant formula-, and full cream cow's milk. **Food Microbiology,** v. 89, p. 103450, 2020.

GETANEH, G. et al. Review on goat milk composition and its nutritive value. **J. Nutr. Health Sci,** v. 3, n. 4, p. 401-410, 2016.

GIROUX, H. J.; VEILLETTE, N.; BRITTEN, M. Use of denatured whey protein in the production of artisanal cheeses from cow, goat and sheep milk. **Small Ruminant Research,** v. 161, p. 34-42, 2018.

HAENLEIN, G. F.; PARK, Y. W. **Handbook of milk of non-bovine mammals**. Blackwell, 2006. ISBN 0813820510.

HUPPERTZ, T.; KELLY, A.; FOX, P. High pressure-induced changes in ovine milk. 1. Effects on the mineral balance and pH. **Milchwissenschaft-milk Science International,** v. 61, n. 3, p. 285-288, 2006.

JUVEN, B. et al. Changes in refrigerated milk caused by Enterobacteriaceae. **Journal of Dairy Science,** v. 64, n. 9, p. 1781-1784, 1981.

KIEŁCZEWSKA, K. et al. The effect of high pressure treatment on the dispersion of fat globules and the fatty acid profile of caprine milk. **International Dairy Journal,** v. 102, p. 104607, 2020.

KUBO, M. T. K.; AUGUSTO, P. E.; CRISTIANINI, M. Effect of high pressure homogenization (HPH) on the physical stability of tomato juice. **Food research international,** v. 51, n. 1, p. 170-179, 2013.

LIEVORE, P. et al. Chemical characterisation and application of acid whey in fermented milk. **Journal of food science and technology,** v. 52, n. 4, p. 2083-2092, 2015.

MOHAPATRA, A.; SHINDE, A. K.; SINGH, R. Sheep milk: A pertinent functional food. **Small ruminant research,** v. 181, p. 6-11, 2019.

MONTEIRO, A. et al. Goat and sheep milk as raw material for yoghurt. **Milk Production, Processing and Marketing**, p. 13, 2019.

NIEVA, S. et al. Fruit and vegetable smoothies preservation with natural antimicrobials

for the assurance of safety and quality. **LWT,** v. 154, p. 112663, 2022.

ONU. Organização das Nações Unidas.Sustainable Development Goals. Retrieved from Sustainable Development Goals website: Disponível em: <https://www.undp.org/sustainable-development-goals>Acesso em: .15/12/2022. 2015.

PANGHAL, A. et al. Whey valorization: current options and future scenario–a critical review. **Nutrition & Food Science**, 2018.

PARK, Y. Rheological characteristics of goat and sheep milk. **Small Ruminant Research,** v. 68, n. 1-2, p. 73-87, 2007.

PELEG, M. Linearization of relaxation and creep curves of solid biological materials. **Journal of Rheology,** v. 24, n. 4, p. 451-463, 1980.

PELEG, M. An empirical model for the description of moisture sorption curves. **Journal of Food science,** v. 53, n. 4, p. 1216-1217, 1988.

PENNA, A. L. B.; GIGANTE, M. L.; TODOROV, S. D. Artisanal Brazilian Cheeses—History, Marketing, Technological and Microbiological Aspects. **Foods,** v. 10, n. 7, p. 1562, 2021.

PFLUG, I. J. Selected papers on the microbiology and engineering of sterilization processes. Minneapolis: Environmental Sterilization Laboratory, 5th ed. 1988.

PITARCH, J. L. et al. Optimal operation of thermal processing of canned tuna under product variability. **Journal of Food Engineering,** v. 304, p. 110594, 2021.

POLOWSKY, P. et al. Flavor and Sensory Characteristics of Non-Bovine Species Milk and Their Dairy Products. **Handbook of Milk of Non-Bovine Mammals**, p. 595-623, 2017.

PRAZERES, A. R.; CARVALHO, F.; RIVAS, J. Cheese whey management: A review. **Journal of environmental management,** v. 110, p. 48-68, 2012.

PULINA, G. et al. Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. **Journal of dairy science,** v. 101, n. 8, p. 6715-6729, 2018.

QUIGLEY, L. et al. The complex microbiota of raw milk. **FEMS microbiology reviews,** v. 37, n. 5, p. 664-698, 2013.

RAMA, G. R. et al. Potential applications of dairy whey for the production of lactic acid bacteria cultures. **International Dairy Journal,** v. 98, p. 25-37, 2019.

RASIKA, D. et al. Probiotics and prebiotics in non-bovine milk. In: (Ed.). **Advances in**

Food and Nutrition Research: Elsevier, v.94, 2020. p.339-384. ISBN 1043-4526.

RAUH, V.; XIAO, Y. The shelf life of heat-treated dairy products. **International Dairy Journal,** v. 125, p. 105235, 2022.

RYAN, K. N.; ZHONG, Q.; FOEGEDING, E. A. Use of whey protein soluble aggregates for thermal stability—A hypothesis paper. **Journal of Food Science,** v. 78, n. 8, p. R1105-R1115, 2013.

RYAN, M. P.; WALSH, G. The biotechnological potential of whey. **Reviews in Environmental Science and Bio/Technology,** v. 15, n. 3, p. 479-498, 2016.

S, A.; K, S. N.; M, C. Whey and whey products. Milk and Dairy Products in Human Nutrition: Production, Composition and Health. 2013:477-97. 10. Kilara A, Vaghela M. Whey proteins. Proteins in food processing: **Elsevier**;2018. p. 93-126., 2018.

SAMARŽIJA, D.; ZAMBERLIN, Š.; POGAČIĆ, T. Psychrotrophic bacteria and their negative effects on milk and dairy products quality. **Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka,** v. 62, n. 2, p. 77-95, 2012.

SEJIAN, V. et al. Opportunities, Challenges, and Ecological Footprint of Sustaining Small Ruminant Production in the Changing Climate Scenario. **Agroecological Footprints Management for Sustainable Food System**, p. 365-396, 2021.

SILVA, C. C.; SILVA, S. P.; RIBEIRO, S. C. Application of bacteriocins and protective cultures in dairy food preservation. **Frontiers in microbiology,** v. 9, p. 594, 2018.

SILVA, M. A. P. D. et al. Produção artesanal de queijos: alternativa para pequenos produtores de leite. MilkPoint. Disponível em: <https://www.milkpoint.com.br/artigos/producao/producaoartesanal-de-queijos-alternativapara-pequenos-produtores-de-leite-219626/> . Acesso em 17/12/2022., 2020.

SMELT, J.; BRUL, S. Thermal inactivation of microorganisms. **Critical reviews in food science and nutrition,** v. 54, n. 10, p. 1371-1385, 2014.

SUASSUNA, J. Caprinos, uma pecuária necessária no Semiárido nordestino. Disponível em:<https://www.gov.br/fundaj/pt-br/destaques/observa-fundaj-itens/observa-fundaj/artigos-de-joao-suassuna/caprinos-uma-pecuaria-necessaria-no-semiarido-nordestino#:~:text=Cabra%20Murciana%3A%20importante%20alternativa%20para,do%20per%C3%ADodo%20colonial%20do%20Brasil.>Acesso em: 11/12/2022., 2019.

TAN, S. F. et al. Physico-chemical changes, microbiological properties, and storage shelf life of cow and goat milk from industrial high-pressure processing. **Processes,** v. 8, n. 6, p. 697, 2020.

THOMPSON, S. Microbiological spoilage of high-sugar products. In: (Ed.). **Compendium of the microbiological spoilage of foods and beverages**: Springer, 2009. p.301-324.

TRIBST, A.; FALCADE, L.; DE OLIVEIRA, M. Strategies for raw sheep milk storage in smallholdings: Effect of freezing or long-term refrigerated storage on microbial growth. **Journal of dairy science,** v. 102, n. 6, p. 4960-4971, 2019.

TRIBST, A. A. L. et al. Manufacture of a fermented dairy product using whey from sheep's milk cheese: An alternative to using the main by-product of sheep's milk cheese production in small farms. **International Dairy Journal,** v. 111, p. 104833, 2020.

TRIBST, A. A. L. et al. Impact of extended refrigerated storage and freezing/thawing storage combination on physicochemical and microstructural characteristics of raw whole and skimmed sheep milk. **International dairy journal,** v. 94, p. 29-37, 2019.

VAN BOEKEL, M. Kinetics of heat-induced changes in dairy products: Developments in data analysis and modelling techniques. **International Dairy Journal,** v. 126, p. 105187, 2022.

VERRUCK, S.; PRUDENCIO, E. S. Inovação na tecnologia de derivados do leite de cabra. 2018.

WATKINS, P. et al. Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat. **Meat Science,** v. 96, n. 2, p. 1088-1094, 2014.

5. CONCLUSÕES FINAIS

Os resultados obtidos nas condições deste estudo possibilitaram concluir que, entre os tratamentos térmicos avaliados para estabilização microbiológica do soro de queijo de cabra, o binômio de 75°C/5 min foi capaz de garantir sua estabilidade microbiológica por 21 dias a 7ºC (CBT e CBP <2 log UFC/ml^{-1}). Entretanto, não foi possível estabelecer tratamentos térmicos capazes de promover maior tempo de vida de prateleira, uma vez que o aumento de temperatura de processo se mostrou proibitivo dada a instabilidade térmica do soro em temperaturas iguais ou superiores a 80 ºC. Assim, com a necessidade da extensão da vida de prateleira do soro de queijo de cabra e ovelha para tempo superior a 21 dias, foi necessário associar outras barreiras ao tratamento térmico dos soros, como fermentação, acidificação direta, uso de cultura bioprotetora e antimicrobiano.

Para os ensaios de fermentação, foram estudadas além do processo convencional, a adição de protease e a realização do processo assistido por ultrassom, com o intuito de aumentar a velocidade do processo e melhorar a estabilidade do produto obtido. Os resultados mostraram que fermentação assistida por ultrassom foi capaz de aumentar a taxa de queda do pH no SQO. Em contrapartida, esse processo afetou negativamente a estabilidade física das amostras de SQO e SQC, devido à fonte/composição dos soros, principalmente teor de gordura afetou a taxa de desestabilização. Os níveis de viabilidade BAL também foram afetados, havendo uma diminuição durante o armazenamento, provavelmente causada pela depleção de nutrientes e baixa tolerância em pH ~4,0. Por fim, outras barreiras foram estudadas como: adição da nisina, adição de *Lacticaseibacillus casei* como cultura bioprotetora e acidificação direta com ácido lático (até pH 4,5, 3,5 e 2,5), entre as quais se destacaram a adição de nisina e a inoculação de *L. casei* como barreiras eficazes para evitar a deterioração microbiológica de SQC e SQO, com menor impacto em termos de estabilidade física do que o observado para amostras acidificadas até pH 4,5 ou 3,5 e sem descaracterizar o produto pelo excesso de ácido adicionado, como ocorreu para amostra acidificada até pH 2,5. Porém, vale ressaltar que todas as amostras foram desestabilizadas ao longo da estocagem a 7ºC, sendo requeridos novos estudos para avaliar alternativas de estabilização física dos soros, como realização de dessoragem ou uso de agentes de corpo, caso o produto precise apresentar estabilidade em repouso.

De forma geral, a partir dos conhecimentos produzidos neste estudo, é possível concluir que o tratamento térmico associado com uma barreira adicional como, a adição de nisina ou a inoculação de *L. casei*, representa uma alternativa eficaz para evitar a deterioração dos soros oriundos de queijo produzido com leite de pequenos ruminantes (SQO e SQC) e que estudos futuros devem avaliar o impacto da adição de estabilizantes para melhorar a estabilidade física do soro de queijo de ovelha e cabra processados.