UNIVERSIDADE FEDERAL DA BAHIA

FACULDADE DE FARMÁCIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS

JOSELENE CONCEIÇÃO NUNES NASCIMENTO

EFEITO DO ULTRASSOM ASSOCIADO À PASTEURIZAÇÃO DE CREME DE LEITE NA INATIVAÇÃO DE *Staphylococcus aureus* RESISTENTE À METICILINA E OUTROS MICRORGANISMOS INDICADORES

SALVADOR

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Tese submetida ao Programa de Pós-Graduação em Ciência de Alimentos (nível Doutorado) da Faculdade de Farmácia da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de Doutor em Ciência de Alimentos.

Prof. Dra. Marion Pereira da costa
Orientador

Prof. Dr. Bruno Nicolau Paulino
Prof. Dr. José Givanildo da Silva
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SALVADOR
2024

Dados internacionais de catalogação-na-publicação
(SIBI/UFBA/Biblioteca Universitária Reitor Macedo Costa)

Nascimento, Joselene Conceição Nunes.
Efeito do ultrassom associado à pasteurização de creme de leite na inativação de Staphylococcus aureus resistente à meticilina e outros microrganismos indicadores / Joselene Conceição Nunes Nascimento. - 2024.
211 f.: il.

Orientadora: Profa. Dra. Marion Pereira da Costa.
Coorientador: Prof. Dr. Bruno Nicolau Paulino.
Coorientador: Prof. Dr. José Givanildo da Silva.
Tese (doutorado) - Universidade Federal da Bahia, Faculdade de Farmácia, Salvador, 2024.

1. Alimentos funcionais. 2. Alimentos - Análise. 3. Creme de leite - Microbiologia. 4. Creme de leite - Contaminação. 5. Contaminação microbiana. 6. Staphylococcus aureus. 7. Segurança alimentar. I. Costa, Marion Pereira da. II. Universidade Federal da Bahia. Faculdade de Farmácia. III. Título.

CDD - 664.07
CDU - 664

UNIVERSIDADE FEDERAL DA BAHIA
FACULDADE DE FARMÁCIA
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TERMO DE APROVAÇÃO

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Aprovada em 28 de maio de 2024.

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Dedico este trabalho,

À Profa. Janice Izabel Druzian (in memoriam), cuja memória e legado perdurável seguem iluminando e enriquecendo as vidas de todos que tiveram o privilégio de cruzar seu caminho. Sua sabedoria e dedicação ecoam através de nossos corações e inspiram cada passo que damos.

Meus agradecimentos,

A Deus, que em seu plano para minha vida, além de me proporcionar a oportunidade de vivenciar esta experiência e concretizar este trabalho, me forneceu a coragem e manteve meu equilíbrio espiritual nas adversidades desta jornada.

À minha família, e especialmente minha irmã, sou eternamente grata pelo suporte incondicional em todas as fases da minha vida e pela compreensão que tiveram nos dias em que o cansaço se começava a sentir.

À minha mãe, que, mesmo já não estando entre nós, sempre acreditou em mim, no meu potencial, e cuja memória nunca me deixou desistir.

Aos meus afilhados, Laura e Bryan, cujas brincadeiras inocentes, me ajudou a distrair e desanuviar das situações de maior ansiedade...

À minha orientadora, Professora Marion Pereira da Costa, cuja perspicácia e sentido crítico refinaram profundamente este trabalho. Sua orientação, paciência e profundo conhecimento foram essenciais para o meu desenvolvimento acadêmico. Estou honrada em tê-la como mentora.

Aos meu Coorientadores, Professores Bruno Nicolau Paulino e José Givanildo, meu sincero agradecimento pela sua dedicação e insights valiosos.

Agradeço aos meus colegas de trabalho —Bianca, Rejane, Ingrid, Selma, Anailton, Janaina, William, Andrei — pelas pausas cheias de descontração. Um agradecimento especial a Cristiane, por compartilhar sua alquimia na preparação de meios de cultura, a Adriana, pelas risadas que nos lembravam que há mais na vida além da academia e a Arlete pelos mimos semanais.

À Professora Mariana, gratidão pelas liberações concedidas durante os momentos de trabalho, permitindo-me dedicar tempo aos estudos.

À equipe dos Laboratórios de Inspeção e Tecnologia de Leites e Derivados (LaITLácteos) pela ajuda no desenvolvimento desse projeto, em especial a Iuri, Madian, Catherine; Ao Laboratório de Controle de Qualidade de Produtos Farmacêuticos (CQFAR), pela concessão de suas dependências para o desenvolvimento das análises microbiológicas e a todos no LAPAAC, agradeço pela colaboração imprescindível e pelo ambiente de apoio mútuo.

A todos os docentes e servidores que cruzei nos corredores da Faculdade de Farmácia e da Escola de Medicina Veterinária — Prof. Ederlan, Prof.Henrique, Prof.Ricardo, Profa.Carolina, Prof. Edimar, Prof.Izabel, Profa. Alini, Profa. Alaise, Profa. Mara, Profa.Eliete e Priscila (PGALi) — obrigada pelas palavras de consolo e encorajamento.

Aos meus amigos e sócios, Adriana Short e Milson Short, agradeço a paciência e compreensão durante minhas ausências.

À Universidade Federal da Bahia e à Faculdade de Farmácia, onde trabalho há quase 20 anos, expresso minha profunda gratidão pelo ambiente enriquecedor e estimulante que tanto

contribuiu para meu desenvolvimento acadêmico e pessoal. Esta instituição não só me proporcionou uma excelente formação, mas também me presenteou com amizades que levarei por toda a vida.

Aos amigos e colegas que a Faculdade de Farmácia me deu, um agradecimento especial: Leonardo Maciel, Adriana Barros, Mariana Barros (Bali), Fabiana Pacheco, Danilo Vilas Boas, Juliana Ranzan, Renata Quartiere, Ivo Henrique, Joseane Alencar, Raissa Bahia, Aurora, Lorena, Marcos Silva, Pedro (LAFAP), Gildeon, Talita, Vanessa Costa. Cada um de vocês enriqueceu minha jornada de maneiras únicas, oferecendo apoio, risadas e momentos inesquecíveis. Obrigada por fazerem parte dessa importante etapa da minha vida.

Aos meus queridos Cleonice, Rosalvo, Mari, Camila e Gabriela pela gentileza e cuidado.

Agradeço também àqueles que, ao tentarem me desafiar, inadvertidamente me fortaleceram. Ensinaram-me a lutar com mais cuidado e a descobrir habilidades que a comodidade e a política da boa vizinhança haviam ocultado. Obrigada por fazerem parte, mesmo que indiretamente, do meu crescimento pessoal e profissional.

Por fim, deixo aqui meus agradecimentos especiais...

À Clicia Capibaribe Leite, expresso minha imensa gratidão por ter aberto as portas do Laboratório de Microbiologia de Alimentos e por me introduzir ao fascinante mundo da microbiologia. Obrigada por compartilhar seu vasto conhecimento e por ser uma mentora tão inspiradora.

Ao Professor Bruno Nicolau Paulino, minhas palavras mal conseguem expressar a gratidão que sinto. Sua ajuda foi além do acadêmico, envolvendo paciência, ensinamentos, muitas gargalhadas, conversas motivadoras, e, acima de tudo, uma amizade genuína. Obrigada por cada incentivo e por toda a dedicação. Sem dúvida, minha experiência no doutorado não teria sido a mesma sem sua presença marcante e inspiradora.

Estendo aqui meus agradecimentos a Joseane Alencar ...é um prazer e uma honra caminhar ao lado de alguém tão respeitável e dedicada....

E não menos importante, à minha amiga Biane Philadelpho, que desempenhou os papéis de terapeuta e cúmplice nas escapadas para um "chá" que sempre me reconduziam ao foco. Sua amizade é um verdadeiro porto seguro, uma fonte constante de apoio e compreensão.

À minha querida amiga Miraildes Calazans, agradeço pelos almoços, cafés, e longas conversas repletas de cuidado e atenção. Sua companhia e apoio foram essenciais para tornar os dias mais leves e cheios de alegria. Obrigada por cada momento compartilhado e por sua amizade sincera.

"Se vi mais longe, foi porque estava sobre

ombros de gigantes"

Isaac Newton

RESUMO

O interesse dos consumidores por alimentos seguros tem se intensificado ao longo dos anos, motivando estudos aprofundados sobre a segurança dos alimentos. No contexto dos produtos lácteos, o creme de leite é um produto de destaque. No entanto, devido à sua composição nutritiva, o creme pode fornecer um ambiente propício para a proliferação de microrganismos patogênicos se não for manuseado ou processado adequadamente. Este estudo comparou a eficácia da pasteurização rápida e lenta e da termossonicação na redução de *Staphylococcus aureus* resistente à meticilina (MRSA) e microrganismos indicadores em amostras de creme de leite. Amostras de creme cru (35% de gordura) foram obtidas de uma fábrica de laticínios em São Sebastião do Passé, Bahia, Brasil. Foram utilizadas cinco cepas de *Staphylococcus aureus* resistente a β-lactâmicos (MRSA) associadas à pecuária no Brasil. As culturas de MRSA foram preservadas em meio de cultura enriquecido com glicerol e revitalizadas antes do experimento. Para a contaminação artificial, um inóculo de MRSA foi adicionado às amostras de creme, resultando em uma concentração final de 6,82 log UFC/mL. Os tratamentos térmicos incluíram pasteurização rápida (72°C por 15 segundos) seguida de sonicação por durações de 3, 5 e 10 segundos (US1, US2 e US3) e pasteurização lenta (65°C por 40 minutos) seguida de sonicação por 5, 10 e 15 minutos (US4, US5 e US6), com intensidades, durações e temperaturas variadas, usando um processador ultrassônico VC 505 (Vibra-Cell, Sonics & Materials, Connecticut, EUA). A eficácia dos tratamentos foi avaliada por contagens microbianas imediatamente após o processamento e ao longo de um período de 30 dias de armazenamento refrigerado, seguindo as diretrizes da APHA. As análises incluíram contagens de *Staphylococcus aureus*, bactérias aeróbias mesófilas, *Enterobacteriaceae*, bolores e leveduras. A eficiência da inativação microbiana foi calculada em log UFC/mL. Os resultados demonstraram que tanto a pasteurização tradicional quanto a termossonicação reduziram efetivamente as concentrações de MRSA. Após a pasteurização rápida (IP-72), as contagens foram reduzidas para 2,00 log UFC/mL e para 2,39 log UFC/mL com a pasteurização lenta (IP-65). Ambos os tratamentos mostraram um aumento significativo nas contagens durante o armazenamento. A termossonicação reduziu as contagens para 4,14 log UFC/mL (US1) e 4,24 log UFC/mL (US2). Durante o armazenamento, as contagens aumentaram significativamente em US1 a US3, enquanto o tratamento US6 manteve os níveis abaixo de 3 log UFC/mL, sem variações. Em relação aos microrganismos indicadores, houve variabilidade na eficácia dos diferentes tratamentos aplicados. Os resultados demonstraram que tanto a pasteurização convencional quanto a termossonicação foram eficazes na redução das concentrações de MRSA. Destaca-se que a termossonicação apresentou uma eficácia superior, especialmente na manutenção de níveis reduzidos de microrganismos ao longo de um período prolongado, enfatizando as vantagens do ultrassom na otimização de processos tecnológicos associados à segurança de alimentos.

***Palavras-chave**: Staphylococcus aureus resistente à meticilina. Termossonicação. Segurança de Alimentos. Produtos Lácteos.*

ABSTRACT

Consumer interest in safe foods has intensified over the years, prompting in-depth studies on food safety. In the context of dairy products, cream is a standout product. However, due to its nutritious composition, cream can provide a conducive environment for the proliferation of pathogenic microorganisms if not handled or processed adequately. This study compared the efficacy of rapid and slow pasteurization and thermosonication in reducing methicillin-resistant *Staphylococcus aureus* (MRSA) and indicator microorganisms in cream samples. Raw cream samples (35% fat) were obtained from a dairy factory in São Sebastião do Passé, Bahia, Brazil. Five strains of β-lactam-resistant *Staphylococcus aureus* (MRSA) associated with livestock in Brazil were used. MRSA cultures were preserved in a glycerol-enriched culture medium and revitalized before the experiment. For artificial contamination, an MRSA inoculum was added to the cream samples, resulting in a final concentration of 6.82 log CFU/mL. Thermal treatments included rapid pasteurization (72°C for 15 seconds) followed by sonication for durations of 3, 5, and 10 seconds (US1, US2, and US3) and slow pasteurization (65°C for 40 minutes) followed by sonication for 5, 10, and 15 minutes (US4, US5, and US6), with varying intensities, durations, and temperatures, using a VC 505 ultrasonic processor (Vibra-Cell, Sonics & Materials, Connecticut, USA). The efficacy of the treatments was assessed by microbial counts immediately after processing and over a 30-day refrigerated storage period, following APHA guidelines. Analyses included counts of *Staphylococcus aureus*, mesophilic aerobic bacteria, *Enterobacteriaceae*, molds, and yeasts. Microbial inactivation efficiency was calculated in log CFU/mL. The results demonstrated that both traditional pasteurization and thermosonication effectively reduced MRSA concentrations. After rapid pasteurization (IP-72), counts were reduced to 2.00 log CFU/mL and to 2.39 log CFU/mL with slow pasteurization (IP-65). Both treatments showed a significant increase in counts during storage. Thermosonication reduced counts to 4.14 log CFU/mL (US1) and 4.24 log CFU/mL (US2). During storage, counts significantly increased in US1 to US3, while the US6 treatment-maintained levels below 3 log CFU/mL, without variations. Regarding indicator microorganisms, there was variability in the efficacy of the different treatments applied. The results demonstrated that both conventional pasteurization and thermosonication were effective in reducing MRSA concentrations. Notably, thermosonication showed superior efficacy, especially in maintaining reduced microbial levels over a prolonged period, emphasizing the advantages of ultrasound in optimizing technological processes associated with food safety.

Keywords*: Methicillin-resistant Staphylococcus aureus. Thermosonication. Food Safety. Dairy products.*

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ANVISA	Agência Nacional de Vigilância Sanitária
APCs	Células Apresentadoras de Antígenos
APHA	*American Public Health Association*
BHI	*Brain Heart Infusion*
CA-MRSA	*Staphylococcus aureus* Resistente a Meticilina Associado à Comunidade
DP	Desvio Padrão
DRBC	*Dichloran Rose Bengal Chloramphenicol Agar*
DVA	Doenças Veiculadas por Alimentos
ECP	Estafilococos Coagulase-Positivos
EROs	Espécie reativa de oxigênio
FAO	Organização das Nações Unidas para Alimentação e Agricultura
FDA	Food and Drug Administration
HA-MRSA	*Staphylococcus aureus* Resistente a Meticilina Associado a Cuidados de Saúde
HTST	High Temperature/Short Time
IBGE	Instituto Brasileiro de Geografia e Estatística
IN	Instrução Normativa
kHz	Kilohertz
LA-MRSA	*Staphylococcus aureus* Resistente a Meticilina associado à Pecuária
LTLT	Low Temperature Long Time
MFGM	Milk Fat Globule Membrane
MAPA	Ministério da Agricultura Pecuária e Abastecimento
MRSA	*S. aureus* Resistente a Meticilina
MHz	Megahertz
NMP	Número Mais Provável
OMS	Organização Mundial da Saúde
PBS	Solução Salina Tamponada com Fosfato
PBP	*Penicilin Binding Protein*
PCA	*Plate Count Agar*

RDC	Resolução da Diretoria Colegiada
RTIQ	Regulamento Técnico de Identidade e Qualidade
SEs	*Staphylococcal enterotoxin*
SFP	*Staphylococcal food poisoning*
TSST-1	Síndrome do Choque Tóxico tipo 1
UFC	Unidade Formadora de Colônia
UHT	*Ultra-high Temperature*
US	Ultrassom
W/cm^2	Watts por centímetro quadrado
VRBGA	*Violet Red Bile Glucose Agar*

SUMÁRIO

INTRODUÇÃO

A demanda crescente dos consumidores por alimentos seguros, de alta qualidade nutricional e sensorial, e com benefícios funcionais é impulsionada por uma conscientização cada vez maior sobre os riscos associados aos alimentos contaminados ou de baixa qualidade (Dash *et al*., 2022; Whang *et al*., 2024). Esta preocupação está fundamentada nos dados oficiais que revelam as severas consequências das Doenças Veiculadas por Alimentos (DVA), as quais representam uma ameaça real e global, com impactos significativos na saúde pública e na economia (FDA, 2019).

A Organização Mundial da Saúde (OMS) estima que anualmente cerca de 600 milhões de pessoas são afetadas por doenças decorrentes do consumo de alimentos contaminados, resultando em aproximadamente 420.000 óbitos, enquanto nos Estados Unidos, cerca de 48 milhões de casos são registrados a cada ano (CDC, 2022; FDA, 2019; OMS, 2022). No Brasil, apesar dos esforços regulatórios, as DVA continuam a representar um desafio significativo, frequentemente causadas por microrganismos patogênicos como Salmonella, *Escherichia coli* e *Staphylococcus aureus*. Esses dados reforçam a importância crucial de garantir a segurança e a qualidade dos alimentos para proteger a saúde pública e promover o bem-estar global (Brasil, 2024).

Na categoria de produtos lácteos, o creme de leite se destaca por suas propriedades funcionais e sabor distinto, tornando-se um item de particular relevância (Calvo; Juarez; Fontecha, 2022; Najmitdinova, 2023; Vidal; Saran Netto, 2018). Contudo, sua susceptibilidade à contaminação microbiana é elevada devido à sua rica composição nutricional. Embora métodos convencionais de processamento e preservação, tais como a pasteurização térmica e a refrigeração, sejam amplamente empregados, a persistência de microrganismos indicadores e patogênicos sublinha a necessidade de vigilância contínua. (Abolghait *et al*., 2020; Shoaib *et al*., 2023).

O *S. aureus* é uma espécie notável dentro do gênero *Staphylococcus*, conhecida por sua ampla presença no ambiente e no corpo humano. No contexto da segurança de alimentos na cadeia produtiva de leite e derivados, *Staphylococcus aureus* se destaca como um dos principais agentes causadores de DVA (Chen et *al*., 2022; Kansaen *et al*., 2023; Li *et al*., 2022; Shoaib *et al*., 2023). No Brasil, *S. aureus* foi responsável por cerca de 170 surtos de DVA entre 2013 e 2022, consolidando-se como um dos principais patógenos relacionados à segurança dos alimentos no país (Brasil, 2024). A contaminação do leite e dos produtos lácteos por *S. aureus* muitas vezes tem sua origem nos próprios animais ou no ambiente da fazenda, reconhecidos

como fontes conhecidas de transmissão de doenças (Garcia; Osburn; Cullor, 2019). Algumas espécies de estafilococos são inofensivas, atuando como comensais, enquanto outras são patogênicas, capazes de desencadear uma variedade de doenças (Bencardino; Amagliani; Brandi, 2021; Shoaib *et al*., 2023). Este microrganismo pode representar sérios riscos à saúde, caso não sejam implementadas medidas adequadas de higiene e controle de qualidade durante as etapas de produção e armazenamento. Além disso, o surgimento de cepas bacterianas resistentes, como o *Sthapylococcus aures* Resistente a Meticilina (*Methicillin-resistant Staphylococcus aureus*, MRSA), tem colocado em questão a eficácia dos métodos convencionais de controle, impulsionando a busca por novas tecnologias de processamento (Aaliya *et al*., 2021; Chen *et al*., 2022; Dash *et al*., 2022; Shoaib *et al*., 2023).

Diante desse cenário, o processamento por ultrassom (US) tem recebido considerável atenção na indústria de laticínios. A tecnologia de US de baixa frequência e alta intensidade tem sido cada vez mais explorada para a inativação de microrganismos em diversos contextos (Martínez-Moreno *et al*., 2020; Balthazar *et al*., 2019; Nascimento *et al*., 2023; Rathnakumar *et al*., 2023). Essa tecnologia fundamenta-se na cavitação acústica e no *streaming* acústico, envolvendo o uso de ondas sonoras de alta frequência que produzem efeitos mecânicos, térmicos e químicos nos microrganismos, resultando em sua inativação ou destruição (Guimarães *et al*., 2021; Rathnakumar *et al*., 2023; Scudino *et al*., 2020). Somar US e pasteurização, é possível potencializar os efeitos de inativação dos microrganismos, permitindo a redução da temperatura e/ou do tempo de tratamento térmico necessário (Nascimento *et al*., 2023). Isso é particularmente relevante para alimentos sensíveis ao calor, nos quais a aplicação de altas temperaturas durante a pasteurização pode resultar em alterações indesejáveis nas propriedades sensoriais e nutricionais (Balthazar *et al*., 2019; Soltani Firouz; Farahmandi; Hosseinpour, 2019). Essa abordagem integrada, conhecida como termossonicação, tem sido objeto de estudos e pesquisas em diversos setores da indústria de laticínios, buscando desenvolver processos de tratamento mais eficientes e sustentáveis (Martínez-Moreno *et al*., 2020).

A integração da termossonicação em processos de tratamento promete não apenas melhorar a eficiência da inativação de patógenos como o MRSA em produtos lácteos, mas também proporcionar uma solução mais robusta para garantir a segurança e a qualidade dos alimentos. Assim, este estudo visa aprimorar a inativação do MRSA em creme de leite, contribuindo para práticas mais seguras na indústria de laticínios, ao passo que oferece tranquilidade aos consumidores que confiam na integridade de seus produtos alimentícios e assegurando a sustentabilidade econômica a longo prazo.

2 OBJETIVOS

2.1 Objetivo geral

- ✓ O objetivo central deste estudo foi comparar a eficácia da pasteurização, rápida e lenta, e da termossonicação na redução das contagens de de *Staphylococcus aureus* resistente à meticilina (MRSA) em amostras de creme de leite.

2.2 Objetivos específicos

- ✓ Avaliar a eficácia da termossonicação na redução das contagens de MRSA em creme de leite, comparando-a com a pasteurização convencional;
- ✓ Quantificar a redução das contagens de MRSA em amostras tratadas com diferentes combinações de pasteurização (rápida e lenta) e termossonicação;
- ✓ Identificar os tempos de exposição à sonicação (5, 10 segundos/minutos, 25 minutos) mais eficazes para a inativação de MRSA no creme de leite;
- ✓ Determinar o impacto da pasteurização convencional e da termossonicação na inativação de outros microrganismos indicadores presentes no creme de leite;
- ✓ Investigar a influência do armazenamento sob condições controladas na sobrevivência de MRSA em diferentes intervalos de tempo (dias 0, 15 e 30).
- ✓ Avaliar os efeitos do armazenamento sob condições controladas na sobrevivência de microrganismos indicadores em diferentes intervalos de tempo (dias 0 e 30).

3 FUNDAMENTAÇÃO TEÓRICA

3.1 Creme de leite: aspectos gerais, tecnologia e legislação

O consumo de produtos lácteos é essencial para a saúde e amplamente apreciado em todo o mundo. O leite, em particular, destaca-se pelo seu alto valor nutricional, sendo uma fonte rica em proteínas, carboidratos, lipídeos, minerais, vitaminas e compostos bioativos. Esses nutrientes são fundamentais para o desenvolvimento e o funcionamento adequado do sistema imunológico e outras funções vitais do organismo (Bernardo; Rosário; Conte-Junior, 2021; Scudino *et al*.,2020). Em resposta às demandas atuais dos consumidores, que buscam produtos mais naturais e mais palatáveis, com menos aditivos e provenientes de sistemas de produção sustentáveis, a indústria láctea tem adaptado seus métodos de produção e processamento para atender essas expectativas (Carrilho-Lopez *et al*., 2021; Dash *et al*., 2022; Whang *et al*., 2024). Além de fornecerem nutrição, esses alimentos são valorizados por sua versatilidade culinária, tornando-se elementos indispensáveis nas mesas familiares, refletindo diretamente no aumento da demanda global por esses produtos (Najmitdinova, 2023).

A produção e consumo de produtos lácteos têm uma influência significativa na economia e na saúde da população mundial. A projeção do crescimento da produção global de leite prevê que alcance 1.060 milhões de toneladas até 2031, com um ritmo de crescimento anual de 1,8% (FAO, 2022). O Brasil, uma das principais nações produtoras de leite, demonstra seu protagonismo no cenário global através de números expressivos. Em 2021, o país foi o terceiro maior produtor mundial de leite, ficando atrás apenas dos Estados Unidos e da Índia (FAO, 2022). A produção leiteira no Brasil foi estimada em 34,6 bilhões de litros para 2022. Dados recentes do Instituto Brasileiro de Geografia e Estatística (IBGE) destacam que, no quarto trimestre de 2023, a produção nacional de leite cru aumentou 2,2% em comparação ao mesmo período de 2022, atingindo 6,46 bilhões de litros. Na Bahia, a produção estimada para 2022 foi de 1,27 bilhão de litros, e a aquisição de leite cru no quarto trimestre de 2023 chegou a 133,4 milhões de litros, uma ligeira queda de 1,7% em relação ao mesmo período do ano anterior (IBGE, 2022a;2022b; 2024).

Dentro deste cenário de produção de lácteos, o creme de leite assume um papel fundamental, sendo um dos produtos mais exportados pelo Brasil. Entre 2013 e 2022, o país exportou, em média, 6,6 mil toneladas de creme de leite, evidenciando sua importância tanto no mercado interno quanto externo (Leite; Stock, 2023). A versatilidade culinária e o valor

nutricional do creme de leite o tornam uma opção popular nas mesas brasileiras e em receitas ao redor do mundo (Najmitdinova, 2023).

Conforme estabelecido pelo Regulamento Técnico de Identidade e Qualidade (RTIQ) (Brasil, 1996), o creme de leite é definido como o produto relativamente rico em gordura, obtido do leite por meio de processos tecnológicos adequados, apresentando-se como uma emulsão de gordura em água. Para ser classificado como tal, o produto deve conter no mínimo 10% de gordura láctea, enquanto aqueles com teores de gordura entre 10% e 19,9% são categorizados como creme de leite leve (Brasil, 1996).

Reconhecido na indústria alimentícia por suas propriedades funcionais e sabor distinto, o mercado oferece uma variedade significativa de cremes de leite, destacando-se as variações no conteúdo calórico, o qual é influenciado pelos teores de gordura (Calvo; Juarez; Fontecha, 2022). Além da gordura láctea, o creme de leite é uma fonte rica em proteínas, lactose, ácidos orgânicos, sais minerais e vitaminas como A, E, B1, B2 e C. Sua composição específica o torna um ingrediente versátil na indústria alimentícia, utilizado para conferir cremosidade, sabor e textura a uma variedade de produtos (Najmitdinova, 2023). Além disso, sua elevada concentração de gordura o torna indispensável na produção de manteiga e na modulação do teor lipídico de diversos produtos lácteos (Vidal; Saran Netto, 2018).

3.2 Características e composição dos glóbulos de gordura no creme de leite: análise das tecnologias de fabricação

Os lipídeos presentes no creme, assim como no leite, estão contidos na forma de glóbulos de gordura. Esses glóbulos de gordura do leite são partículas lipídicas envoltas por uma superfície coberta por uma camada de filme fino de 10–20 nm, esférico, com diâmetro de cerca de 0,1–15 μm, conhecida como membrana do glóbulo de gordura do leite (MFGM, do inglês "*milk fat globule membrane*") (Nguyen *et al*., 2015; Fox, 2011; Sun; Roos; Miao, 2024; Whang *et al*., 2024; Wiking *et al*., 2022). A MFGM, é formada por triacilgliceróis envoltos por uma bicamada de fosfolipídeos, proteínas, colesterol, glicolipídeos e vitaminas. Esta estrutura desempenha um papel crucial na estabilidade e funcionalidade dos glóbulos de gordura, protegendo-os contra enzimas lipolíticas e facilitando sua interação com outros componentes do leite (Whang *et al*., 2024; Wiking *et al*., 2022).

As propriedades físico-químicas do creme estão sujeitas a uma série de fatores, os quais incluem o estado dos glóbulos de gordura e da MFGM, a concentração dos glóbulos de gordura, a temperatura do creme e os métodos de manipulação, como tratamento térmico e mecânico

(Sun; Roos; Miao). Dentro dos glóbulos de gordura, os triglicerídeos compõem a maior fração da composição lipídica, representando até 98% da massa total. Os ácidos graxos saturados, como o ácido palmítico e o ácido esteárico, predominam, seguidos pelos ácidos graxos monoinsaturados e poliinsaturados. Além disso, A composição lipídica é influenciada por fatores como a raça da vaca e sua alimentação, estágio de lactação, qualidade microbiológica do leite e derivados, tratamento térmico, homogeneização, entre outros processos de manipulação do leite e derivados (Fox, 2011)

O MFGM, também se caracteriza por ter uma alta proporção de proteínas em sua composição. De fato, as proteínas podem constituir de 25% a 70% do peso do MFGM. Essa significativa presença proteica desempenha um papel crucial em diversas funções biológicas e na estabilidade estrutural dos glóbulos de gordura, impactando diretamente a qualidade e as propriedades dos produtos lácteos (Nguyen *et al*., 2015; Wiking *et al*., 2022). As proteínas mais predominantes na MFGM incluem butirofilina, xantina oxidase, ácido periódico Schiff 6 e 7(também conhecido como lactadherina), cluster de diferenciação 36 e mucina 1. Essas proteínas podem estar ligadas de forma periférica ou integral à MFGM, o que é crucial para entender sua sensibilidade ao aquecimento e aos processos de manipulação, influenciando diretamente a integridade e as características finais dos produtos lácteos (Wiking *et al*., 2022).

Além de suas funções biológicas e nutricionais, a MGGL apresenta propriedades tecnológicas relacionadas ao seu elevado teor de fosfolipídios e outros materiais de superfície ativa (Nguyen *et al*., 2015). A fabricação do creme de leite é um processo detalhado que abrange várias etapas tecnológicas visando assegurar a qualidade, consistência e segurança do produto (Tabela 1). Esse processo engloba a separação entre a fase creme e a fase leite, a padronização do creme para alcançar o teor de gordura desejado e o tratamento térmico para prolongar sua vida útil. Adicionalmente, em alguns casos específicos, é aplicado o processo de homogeneização para melhorar a estrutura do produto ou aumentar sua vida útil (Tabela 1) (Vidal; Saran Netto, 2018).

Tabela 1 – Etapas de fabricação do creme de leite e suas respectivas finalidades

Etapa	Descrição	Finalidade
Separação Centrífuga	Utilização de um separador centrífugo para isolar os glóbulos de gordura do leite através da força centrífuga	Segregar a gordura do leite, formando a base do creme de leite
Padronização da Gordura	Ajuste do teor lipídico do creme para alcançar as especificações do produto final	Definir a consistência, textura e características funcionais do creme de leite
Tratamento Térmico	Aplicação de calor por meio de pasteurização ou esterilização	Inativar microrganismos patogênicos e desativar enzimas que podem prejudicar a qualidade e estabilidade do produto
Homogeneização	Redução do tamanho dos glóbulos de gordura por meio de pressão alta	Melhorar a textura, a viscosidade e a estabilidade do creme, evitando a separação de gordura
Resfriamento e Envasamento	Resfriamento rápido do creme seguido de envasamento em condições assépticas	Retardar o crescimento microbiano e preservar a qualidade e segurança do creme de leite durante a distribuição e armazenamento

Fonte: Vidal; Saran Neto (2018)

Na etapa de Separação, ocorre a formação de creme. Isso ocorre devido à menor densidade dos glóbulos de gordura do leite em comparação com o soro do leite. Essa diferença de densidade faz com que os glóbulos de gordura subam para a superfície, formando uma camada cremosa sobre o leite desnatado que fica abaixo. Este processo é acelerado em baixas temperaturas devido à aglutinação a frio (Wiking *et al*., 2022). Posteriormente, o creme é padronizado para ajustar o teor de gordura de acordo com as especificações do produto. Esse processo envolve a adição de leite desnatado ou integral ao creme para alcançar a concentração desejada de gordura. A padronização é fundamental para assegurar a consistência do creme de leite, influenciando diretamente seu sabor, textura e comportamento durante o cozimento (Vidal; Saran Netto, 2018).

O creme padronizado é submetido a tratamento térmico, que é essencial para controlar o crescimento de bactérias patogênicas ou deteriorantes e inativar enzimas que degradam o produto (Lalwani; Ramsingh; Mahmood, 2024; Wiking *et al*., 2022). Além de controlar microrganismos, o tratamento térmico também é crucial para a inativação da lipoproteína lipase. Esta enzima é responsável pela hidrólise dos triacilglicerídeos, processo que libera ácidos graxos livres e pode resultar em um sabor rançoso. A inativação dessa enzima durante o processamento térmico impede a degradação dos lipídios, preservando a qualidade sensorial do

produto. Isso é fundamental para evitar o desenvolvimento de sabores indesejáveis, garantindo a aceitação do creme pelos consumidores (Wiking *et al*., 2022).

Dependendo do produto e das especificações de qualidade desejadas, o tratamento térmico pode variar em intensidade e duração. Por exemplo, a pasteurização, um processo tradicional de aquecimento, submete o produto a temperaturas entre 60 e 80 °C, eliminando microrganismos prejudiciais e preservando suas características organolépticas (Aaliya *et al*., 2021; Dash *et al*., 2022; Najmitdinova, 2023). Esse processo pode ser conduzido em baixa temperatura por um longo período (*Low Temperature Long Time*, LTLT), como 63 °C por 30 minutos, ou em alta temperatura por um curto período (*High Temperature/Short Time*, HTST), como 72-75 °C por 15-20 segundos (Lalwani; Ramsingh; Mahmood, 2024).

Os cremes tratados por temperatura ultra-alta (*Ultra-high Temperature*, UHT) são expostos a temperaturas extremamente elevadas, entre 135-150 °C, por um período muito breve de 2-5 segundos. Esta técnica elimina praticamente todos os microrganismos, garantindo uma maior estabilidade do produto e permitindo o armazenamento por longos períodos à temperatura ambiente, sem a necessidade de refrigeração (Scudino *et al*., 2020). No entanto, embora o tratamento UHT seja eficaz na eliminação de bactérias vegetativas e na destruição da maioria dos esporos, enzimas microbianas com atividade lipolítica podem permanecer ativas após esse processo devido à sua alta estabilidade ao calor. Este fenômeno evidencia a resistência de certas enzimas aos métodos convencionais de pasteurização e esterilização, exigindo condições de tratamento ainda mais rigorosas para garantir a completa inativação (Wiking *et al*., 2022). Por outro lado, a pasteurização, que utiliza uma faixa de temperatura mais baixa, embora seja suficiente para eliminar a maioria dos microrganismos nocivos, não consegue erradicar completamente todos os microrganismos patogênicos ou eliminar bactérias formadoras de esporos resistentes ao calor (Gao *et al*.,2014). Assim, o creme pasteurizado não alcança o mesmo grau de estabilidade microbiológica que o tratamento UHT. Consequentemente, o creme pasteurizado é mais suscetível a variações microbiológicas durante o armazenamento e possui uma vida útil relativamente mais curta (Aaliya *et al*., 2021).

O aquecimento geralmente é aplicado em conjunto com a homogeneização, seja antes ou após o aquecimento final. A combinação de temperatura com a homogeneização tem um impacto notável na cobertura proteica da MFGM após a homogeneização (Wiking *et al*., 2022). Durante a homogeneização, os glóbulos de gordura são quebrados em partículas menores e mais uniformes para prevenir ou minimizar a separação do creme (Vidal; Saran Netto, 2018). Esse processo envolve forçar o creme através de pequenos orifícios sob alta pressão. A homogeneização evita a separação da gordura do restante do líquido, melhorando a textura,

consistência e paladar do creme de leite. Além disso, estabiliza o produto, distribuindo uniformemente a gordura por todo o creme (Fox, 2011; Wilbey, 2011). Após a homogeneização, o creme é rapidamente resfriado a temperaturas seguras para retardar o crescimento microbiano e preservar a qualidade. Em seguida, é envasado em condições esterilizadas para evitar contaminação e está pronto para distribuição.

Cada uma dessas etapas é crucial para garantir que o creme de leite possua as características desejadas em termos de textura, sabor e propriedades culinárias, além de cumprir com os padrões de segurança estabelecido pelas legislações vigentes.

3.3 Padrões microbiológicos do creme de leite

Os padrões microbiológicos do creme de leite são regulados pela Portaria 146/1996 do Ministério da Agricultura Pecuária e Abastecimento (MAPA). No entanto, tanto o creme de leite pasteurizado quanto o UHT têm padrões microbiológicos definidos pelas legislações do Ministério da Saúde, através da Agência Nacional de Vigilância Sanitária (ANVISA), em sua Resolução Resolulçao da Diretoria Colegiada (RDC) n.º 724/22 e na Instrução Normativa (IN) nº 161/2022 (Brasil, 1996, 2022a, 2022b).

De acordo com os critérios microbiológicos da Portaria n.º 146/1996, o creme de leite pasteurizado deve apresentar contagens de microrganismos aeróbios mesófilos de 10^4 a 10^5 UFC/g, coliformes totais entre 10 a 100 Número Mais Provável por grama (NMP/g), máximo de 10 NMP/g de coliformes a 45 °C, e estafilococos coagulase positiva entre 10 a 100UFC/g (Brasil, 1996). Por sua vez, a RDC n.º 724/22 e IN n.º 161/2022 estabelecem que gorduras lácteas e cremes de leite pasteurizado devem apresentar no máximo 10 NMP/g de *Escherichia coli*, estafilococos coagulase positiva entre 10 a 100 Unidades Formadoras de Colônias por grama (UFC/g), e ausência de *Salmonella* (Brasil, 200a, 2022b)

As variações nos padrões microbiológicos entre as normativas refletem atualizações nas técnicas de detecção e controle de qualidade, bem como a compreensão da relevância de certos patógenos em produtos lácteos. Enquanto a Portaria 146/1996 do MAPA estabelece limites para coliformes totais e coliformes a 45 °C, indicando preocupação com contaminações gerais e condições higiênicas durante o processo de fabricação, a RDC 724/22 e a Instrução Normativa nº 161/2022 da ANVISA focam na ausência de *Salmonella* e no controle de *Escherichia coli* (Brasil, 1996, 2022a, 2022b).

Diante das variações normativas e do avanço nos métodos de detecção, a importância de microrganismos indicadores e patogênicos deve ser considerada. A qualidade e a segurança

do leite durante o processamento refletem diretamente o estado higiênico das fazendas, tanto individualmente quanto em grupo. As análises microbiológicas são essenciais para identificar a presença de microrganismos que podem afetar a qualidade e segurança do leite (Bardalles *et al*., 2024; Dash *et al*., 2022). No entanto, além dos parâmetros microbiológicos, os aspectos físico-químicos e a contagem de células somáticas também são cruciais para a avaliação da qualidade do leite. A legislação vigente requer que estes parâmetros sejam rigorosamente monitorados para garantir a conformidade com os padrões de qualidade e segurança (Bardalles *et al*., 2024).

Microrganismos indicadores e patogênicos estão presentes em diversos ambientes, incluindo ar, água e solo, enquanto os vírus se replicam dentro das células de organismos hospedeiros (Dash *et al*.,2022). Essa ubiquidade os torna ferramentas valiosas para monitorar a contaminação ambiental e a eficácia das práticas de higiene em vários contextos, especialmente na indústria de laticínios. Por exemplo, a presença de certos microrganismos, como bactérias do grupo dos coliformes no leite, pode indicar falhas nos processos de pasteurização ou contaminação pós-processamento. Estes microrganismos são frequentemente utilizados como indicadores de contaminação porque sua presença sugere que outras patógenos potencialmente prejudiciais também podem estar presentes (Bardales *et al*., 2024; Dash *et al*., 2022).

Coliformes, parte da família *Enterobacteriaceae*, são bactérias anaeróbias facultativas, gram-negativa e não esporuladas, que fermentam a lactose produzindo ácido e gás em 24 a 48 horas em temperaturas entre 35ºC e 45ºC (APHA, 2001; Silva *et al*., 2021). A presença de coliformes sinaliza possíveis contaminações por patógenos de origem fecal, como *Escherichia coli*. *Escherichia coli*, especificamente, é uma bactéria comensal da microbiota intestinal de animais endotérmicos e destaca-se entre os coliformes termotolerantes.Com diversos patotipos conhecidos por seu mecanismo de virulência, como *Escherichia coli* enteropatogênica, *Escherichia coli* enterotoxigênica, *Escherichia coli* enteroinvasiva, *Escherichia coli* enterohemorrágica ou produtora da toxina Shiga, *Escherichia coli* enteroagregativa e *Escherichia coli* de aderência difusa, é frequentemente implicada em surtos de origem alimentar (Bernardo; Rosário; Conte-Junior, 2021; FDA, 2013; APHA, 2001; Silva *et al*., 2021).

Fungos (bolores e leveduras), como indicadores de contaminação, são essenciais para o monitoramento da qualidade em laticínios. Eles sinalizam condições inadequadas de armazenamento ou falhas nos processos produtivos, particularmente em ambientes úmidos e quentes. Embora geralmente não sejam patogênicos, a presença de fungos em produtos lácteos pode indicar contaminação ambiental e resultar em deterioração acelerada, afetando negativamente a aparência, sabor e textura dos produtos. Isso compromete a qualidade, reduz o

valor comercial e aumenta os riscos à segurança alimentar, especialmente em produtos com longos prazos de validade (Dash *et al*., 2022).

Microrganismos indicadores são essenciais porque sua presença pode sugerir que outros patógenos potencialmente prejudiciais também estejam presentes. No contexto da cadeia produtora de leite e seus derivados, *Staphylococcus aureus* (*S.aureus*) é um dos principais causadores de doenças de origem alimentar (Chen *et al*., 2022; Kansaen *et al*., 2023; Li *et al*., 2022; Shoaib *et al*., 2023). Além dele, patógenos como *Listeria monocytogenes, Campylobacter, Salmonella, Yersinia*, e *Escherichia coli* produtoras de toxina Shiga também representam riscos substanciais (Dash *et a*l., 2022).

As duas normativas, tanto a Portaria 146/1996 do MAPA quanto a RDC 724/22 e a Instrução Normativa nº 161/2022 da ANVISA, estabelecem limites semelhantes para estafilococos coagulase positiva, variando de 10 a 100 UFC/g (Brasil, 1996, 2022a, 2022b). Essa consistência destaca a preocupação contínua com este microrganismo patogênico, que serve como um indicador de manipulação pós-contaminação e de condições higiênicas inadequadas na produção de produtos lácteos, como o creme de creme de leite. Nosso estudo enfoca especialmente o *S.aureus*, pois este patógeno pode produzir toxinas termoestáveis que persistem mesmo após o tratamento térmico dos produtos lácteos, representando um risco significativo para a saúde pública (Chen *et al*., 2022; Kansaen *et al*., 2023; Li *et al*., 2022; Shoaib *et al*., 2023).

3.4 *Staphylococcus aureus*: desafios na cadeia produtiva de laticínios

O *S. aureus* é uma espécie notável dentro do gênero *Staphylococcus*, conhecida por sua ampla presença no ambiente e no corpo humano. Algumas espécies de estafilococos são comensais inofensivos, enquanto outras são patogênicas, capazes de causar várias doenças. O gênero *Staphylococcus* é incrivelmente diversificado, compreende mais de 81 espécies e várias subespécies identificadas até o momento (Bencardino; Amagliani; Brandi, 2021; Shoaib *et al*., 2023).

O gênero *Staphylococcus* é classificado em grupos com base na produção da coagulase, uma enzima que coagula o plasma. Esta distinção é crucial para compreender a patogenicidade e orientar as estratégias de tratamentos (Cavaiuolo *et al*., 2023; Kansaen *et al*., 2023). Os Estafilococos coagulase-positivos (ECP), incluindo *S. aureus*, destacam-se pela sua capacidade de sobrevivência em diferentes condições ambientais (Bencardino; Amagliani; Brandi, 2021). Além de *S. aureus*, outras espécies comuns de ECP incluem *S. schleiferi* subsp *coagulans*, *S.*

lutrae, S. hyicus, S. intermedius, S. argenteus, S. schweitzeri e *S. delphini*. Dentre esses, o *S. aureus* se destaca por sua ubiquidade e virulência, reconhecido como um patógeno oportunista transmitido por alimentos (Cavaiuolo *et al*., 2023; Kansaen *et al*., 2023; Shoaib *et al*., 2023).

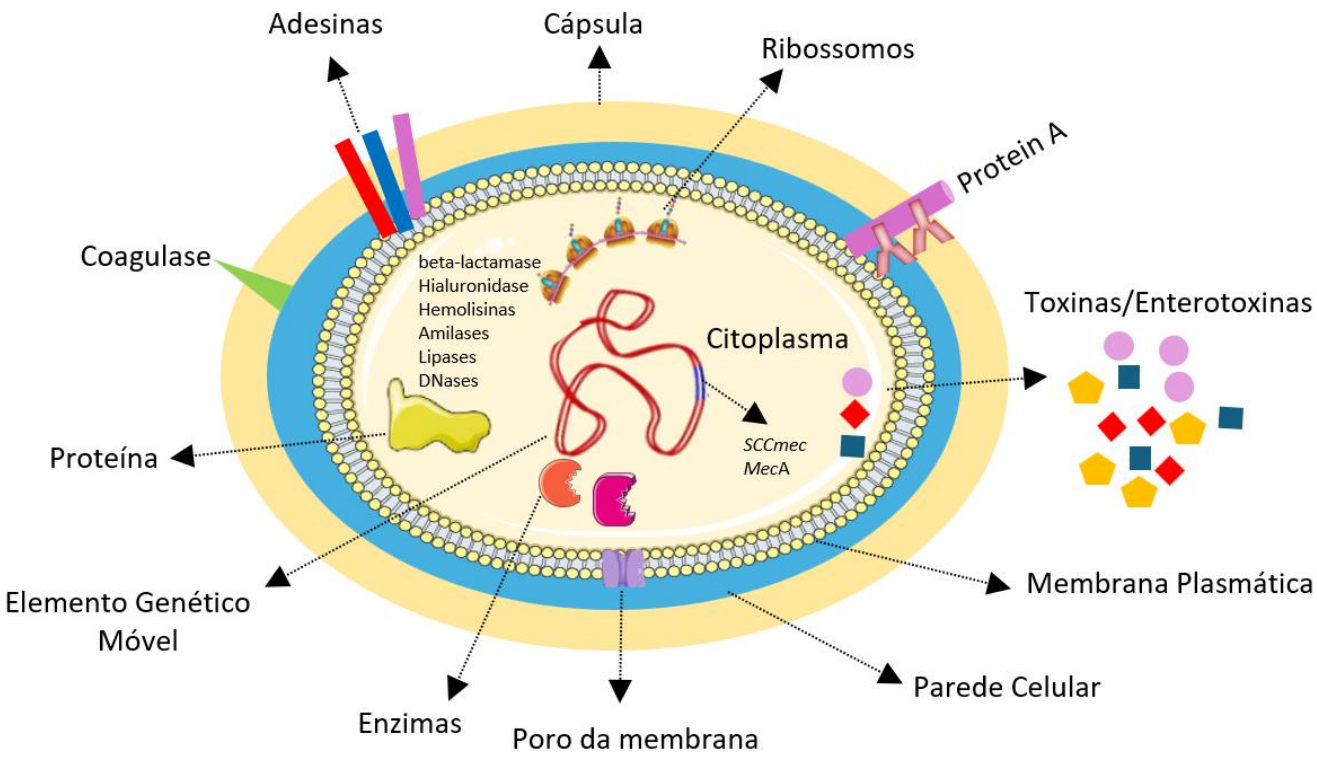
Bardales *et al*. (2024) ressaltam a importância de avaliar parâmetros de qualidade e microbiológicos nos centros de processamento de leite bovino, enfatizando que essas avaliações são cruciais para garantir a saúde pública, a qualidade do produto e impactar positivamente a economia local. A presença de *S. aureus* nos ambientes de produção de laticínios, como fazendas e unidades de processamento, representa uma séria ameaça devido ao seu potencial de contaminação do leite e seus derivados. Os produtores de laticínios devem adotar padrões rigorosos para assegurar uma produção de alta qualidade, destacando que a segurança do leite e seus derivados começa na fazenda (Garcia; Osburn; Cullor, 2019). No entanto, apesar desses padrões, a contaminação pode ocorrer durante a ordenha ou pelo manuseio inadequado do leite cru e dos equipamentos de processamento, o que compromete a qualidade do produto final (Shoaib *et al*., 2023).

A contaminação do leite e dos produtos lácteos por *S. aureus* frequentemente se origina dos próprios animais ou do ambiente da fazenda, que são fontes conhecidas de infecção (Garcia; Osburn; Cullor, 2019). As vacas leiteiras, e outros animais, podem estar contaminados com *S. aureus* especialmente aqueles com mastite, uma infecção das glândulas mamárias. A mastite ocorre quando *S. aureus* entra na glândula mamária através do teto e coloniza o tecido, causando inflamação e infecção. A transmissão do patógeno pode ocorrer durante a ordenha ou devido a práticas inadequadas de higiene, comprometendo a qualidade do leite cru e favorecendo o crescimento bacteriano e a expressão de fatores de virulência (Bencardino; Amagliani; Brandi, 2021; Shoaib *et al*., 2023). Além disso, *S. aureus* pode se disseminar facilmente entre os quartos do úbere e entre diferentes vacas. Equipamentos de ordenha, utensílios de processamento e o ambiente da fazenda podem atuar como vetores dessa contaminação, facilitando a contaminação cruzada e representa riscos à saúde humana (Garcia; Osburn; Cullor, 2019).

A contaminação cruzada é uma preocupação adicional, especialmente porque *S.aureus* pode facilmente contaminar superfícies de equipamentos de ordenha, representando riscos para a qualidade dos produtos lácteos (Bardales *et al*., 2024; Shoaib *et al*., 2023). A higiene inadequada de utensílios e equipamentos, juntamente com superfícies porosas ou danificadas, facilita a contaminação cruzada por *S. aureus* (Bencardino; Amagliani; Brandi, 2021). A implementação de procedimentos eficazes de limpeza e de boas práticas de manuseio são essenciais para mitigar esses riscos

S. aureus é capaz de produzir enzimas extracelulares como proteases, amilases, hialuronidase, hemolisinas, coagulases, lipases, DNases, beta-lactamase e enterotoxinas. Isso ocorre devido a presença de elementos genéticos móveis que codificam essas enzimas, o que facilita a adaptação e sobrevivência do patógeno em diversos ambientes (Figura 1) (Chen *et al*., 2022; Kansaen e*t al*., 2023; Li *et al*., 2022; Shoaib *et al*., 2023).

Figura 1 – Estrutura celular de *Staphylococcus aureus* e seus fatores de virulência



Cápsula: Camada externa que proporciona proteção contra o sistema imunológico do hospedeiro; **Adesinas**: Moléculas na superfície da bactéria que facilitam a aderência a células e tecidos do hospedeiro; **Coagulase**: Enzima que catalisa a coagulação do sangue, auxiliando na formação de coágulos que podem proteger a bactéria de defesas imunológicas; **Proteína A**: Proteína de superfície que se liga a anticorpos F*c*, invertendo sua orientação e interferindo na opsonização e fagocitose. **Enzimas**: Grupo de enzimas que inclui beta-lactamase (resistência a antibióticos), hialuronidase (degrada o ácido hialurônico dos tecidos, facilitando a disseminação), hemolisinas (lisam células do sangue), amilases (degradam amido), lipases (degradam lipídios) e DNases (degradam DNA); ***SCCmec*/*Mec*A**: Elemento genético móvel que confere resistência a antibióticos, como a meticilina; **Toxinas/Enterotoxinas**: Diversas toxinas que podem causar danos teciduais e sintomas de doença, incluindo intoxicações alimentares.

***Fonte**: Adaptado a partir de Choi et al. (2014).*

A diversidade desses fatores de virulência permite ao patógeno aprimorar suas capacidades de aderência, invasão e evasão do sistema imunológico, enquanto também desencadeia potentes respostas inflamatórias (Chen *et al*., 2022). Entre seus fatores de virulência, as toxinas e os superantígenos desempenham um papel fundamental ao danificar as membranas das células hospedeiras, provocando lise e inflamação. Esses elementos também são responsáveis por estimular a produção de citocinas inflamatórias, intensificando os processos inflamatórios no corpo hospedeiro (Abolghait *et al*., 2020; Chen e*t al*., 2022). Além disso, *S. aureus* produz uma variedade de enzimas extracelulares que ajudam a degradar moléculas do hospedeiro, fornecendo nutrientes que promovem a sobrevivência e proliferação da Bactéria (Chen *et al*., 2022).

A contaminação de alimentos por *S. aureus* é uma preocupação crítica de saúde pública devido à sua capacidade de produzir enterotoxinas que causam intoxicações alimentares grave (Berry *et al*., 2022). No Brasil, *S. aureus* foi responsável por cerca de 170 surtos de DVA entre 2013 e 2022, consolidando-se como um dos principais patógenos relacionados à segurança dos alimentos no país (Brasil, 2024).

As enterotoxinas estafilocócicas (*staphylococcal enterotoxin*, SEs) são proteínas de baixo peso molecular, entre 20 e 100 ng, que, quando presentes nos alimentos, podem provocar intoxicação alimentar estafilocócica (*Staphylococcal food poisoning*, SFP). No caso de *S. aureus*, isso ocorre quando sua população ultrapassa 100.000 organismos/g ou mL no alimento. A gravidade da SFP depende das condições do hospedeiro (Cai *et al*., 2023; FDA, 2012). Até o momento, 28 enterotoxinas foram descritas. As enterotoxinas clássicas são designadas como A, B, C, D e E, devido ao seu potencial emético (Berry *et al*., 2022; Bencardino; Amagliani; Brandi, 2021; Chieffi *et al*., 2020; Grispoldi *et al*., 2021; Suzuki *et al*., 2020). Recentemente, outras enterotoxinas foram identificadas, como mostrado na Tabela 2.

Tabela 2 – Caracterização molecular e atividade emética das enterotoxinas estafilocócicas segmentadas por categoria: clássicas, novas e semelhantes

Enterotoxina Estafilocócica	Massa	Tipo	Atividade Emética
SEA	27.1 kDa	Classica	+
SEB	28,4 kDa		+
SEC1	27,5 kDa		+
SEC2	27,6 kDa		+
SEC3	27,6 kDa		+
SED	26,9 kDa		+
SEE	26,4 kDa		+
SEG	27,0 kDa	Nova	+
SHE	25,1 kDa		+
SEI	24,9 kDa		+
Se*l*J	28,5 kDa		nd
Se*l*K	26,0 kDa		+
Se*l*L	26,0 kDa		+
Se*l*M	24,8 kDa		+
Se*l*N	26,1 kDa		+
Se*l*O	26,7 kDa		+
Se*l*P	27,0 kDa		+
Se*l*Q	26,0 kDa		+
Se*l*R	27,0 kDa		+
SES	26,2 kDa		+
SET	27,1 kDa		+
SE*l*U	27,0 kDa	Semelhantes	nd
SE*l*V	27,6 kDa		nd
SE*l*X	19,3 kDa		nd
SE*l*Z	nd		nd
SE*l*W	nd		nd
Se*l*26	nd		nd
SE*l*27	nd		nd
Se*l*01	nd		nd
SE*l*02	nd		nd

Abreviatura: + = potencial emético demostrados em macacos
nd= não potencial emético demostrados em macacos ou não foram testados
Fonte: adaptada de Fisher; Otto; Cheung, (2018)

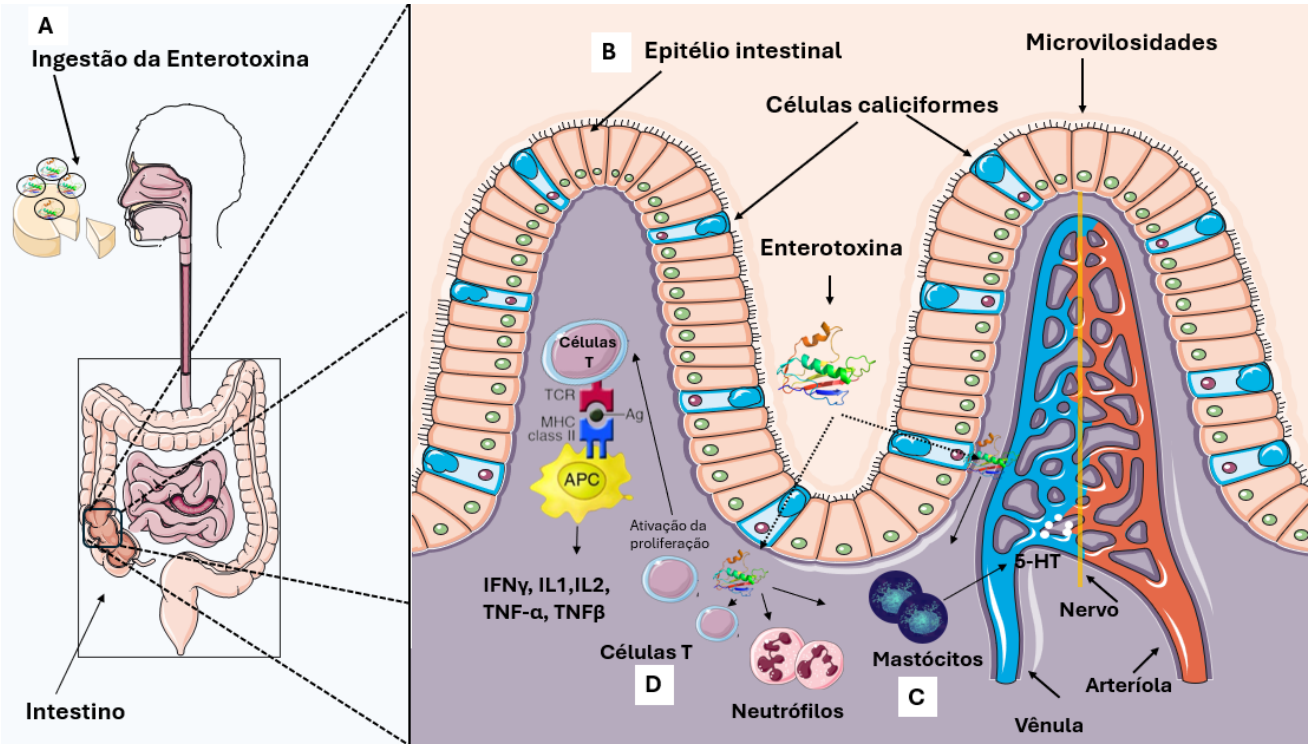
A única exceção é a toxina da síndrome do choque tóxico tipo 1 (TSST-1), originalmente designada SEF, que difere das outras toxinas de *S. aureus* por sua estrutura e mecanismo de ação (Fischer, Otto e Cheung, 2018; Bencardino; Amagliani; Brandi, 2021; Umeda *et al*., 2021; Suzuki *et al*., 2020; Zhang *et al*., 2018). Cerca de 95% dos casos de SFP são causados pelas SEs A, B, C, D e E (Bencardino; Amagliani; Brandi, 2021; Berry *et al*., 2022). As SEs são resistentes ao calor e podem suportar as temperaturas e os tempos de cozimento comumente usados em ambientes domésticos e na indústria (Abolghait *et al*., 2020; Bencardino; Amagliani; Brandi, 2021; Grispoldi *et al*., 2021; Li *et al*., 2022).

A SFP resulta da ingestão de alimentos contaminados com enterotoxinas pré-formadas por cepas patogênicas de *S.aureus*, ou por estafilococos coagulase-negativos que possuem

genes de enterotoxinas que resistem aos processos comuns de cozimento e digestão (Berry *et al*., 2022; Homsombat *et al*., 2021; Suzuki *et al*., 2020). ASFP se manifesta rapidamente com sintomas como gastroenterite acompanhada de diarreia, febre, vômitos e dor abdominal, ocorrendo de 30 minutos a 8 horas após a ingestão de alimentos contaminados (Suzuki *et al*., 2020; Kansaen *et al*., 2023).

Nas células entéricas, as enterotoxinas se ligam às moléculas MHC classe II presentes nas células apresentadoras de antígenos (APCs), como macrófagos e células dendríticas (Berry *et al*., 2022; Fischer, Otto & Cheung, 2018; Suzuki *et al*., 2020). Essas toxinas agem como superantígenos, ativando os linfócitos T, especialmente os T CD4+, de forma não específica. Enquanto os antígenos típicos ativam um pequeno subconjunto de linfócitos T, os superantígenos ativam uma grande proporção dessas células. Essa ativação massiva resulta em uma tempestade de citocinas, levando a uma forte resposta inflamatória. A tempestade de citocinas causa inflamação no trato gastrointestinal, resultando nos sintomas clássicos da doença (Figura 2) (Abolghait *et al*., 2020; Zhang *et al*., 2018).

Figura 2 – Mecanismo de patogenicidade das enterotoxinas estafilocócicas



(A) A exposição inicial começa com a ingestão de alimentos contaminados com enterotoxina estafilocócica pré-formada, que é resistente a altas temperaturas e ácido extremo, permitindo sua sobrevivência até chegar ao intestino, onde inicia seus efeitos prejudiciais**.(B)** No intestino, a toxina se liga às células do epitélio intestinal, através das células caliciformes produtoras de muco e células epiteliais, onde começa seu processo de ação.**(C)** A liberação de serotonina por células mastocitárias ativadas, em resposta à toxina, estimula o nervo vago através da

interação com o precursor 5-HT/serotonina, induzindo a resposta emética**.(D)** Agindo como um superantígeno, a toxina desencadeia a ativação de células T e neutrófilos ao se ligar à molécula MHC II em células apresentadoras de antígenos e ao TCR em células T, levando a uma liberação massiva de citocinas e uma intensa resposta inflamatória.

***Fonte**: Adaptado a partir de Etter et al. (2020).*

No entanto, a gravidade dos sintomas varia com a suscetibilidade do indivíduo, com pessoas que possuem sistemas imunológicos comprometidos, idosos, crianças pequenas ou indivíduos com outras condições médicas subjacentes apresentando sintomas mais graves. Além disso, a quantidade de enterotoxinas ingeridas influencia diretamente a gravidade das reações, tornando o monitoramento e o controle dessas toxinas nos alimentos uma prioridade crítica para a segurança alimentar (Bencardino; Amagliani; Brandi, 2021; Cavaiuolo *et al*., 2023).

A cadeia produtiva de alimentos, especialmente no setor de laticínios, enfrenta desafios significativos no controle das DVA. O processo de controle de qualidade deve começar no campo, abordando aspectos como nutrição, saúde do rebanho, produtividade e sustentabilidade ambiental (Garcia; Osburn; Cullor, 2019; Roy *et al*., 2024). A prevenção de doenças e a adoção de programas de imunização dos animais, juntamente com a redução do uso de antibióticos e outros medicamentos veterinários, são ações vitais para garantir a qualidade final do produto (Roy *et al*., 2024). Este enfoque é crucial para mitigar riscos à saúde pública, manter os produtos em conformidade com as normas sanitárias e prevenir DVA.

3.4.1 *Staphylococcus aureus* resistente a Meticilina: ameaças, resistência e a abordagem de saúde única

Desde a introdução da penicilina na prática clínica, as cepas de *S. aureus* têm desenvolvido resistência antimicrobiana. Em 1944, cepas de *S. aureus* resistentes à penicilina já haviam sido identificadas (Abraham; Chain, 1940), e nos anos seguintes, observou-se o desenvolvimento de resistência a todas as penicilinas naturais. Várias cepas de *S. aureus* resistentes a antibióticos se espalharam globalmente, apresentando sérios riscos à saúde (Titouche *et al*., 2019). *S. aureus* resistente a meticilina (MRSA), destaca-se como um patógeno multidroga resistente na lista de patógenos prioritários da OMS (Ávila-Nova *et al*., 2021; OMS, 2017). Inicialmente reconhecido como agente de infecção nosocomial, o MRSA agora está associado a DVA e foi identificado pela OMS como um patógeno de resistência antimicrobiana de alta prioridade (Abolghait *et al*., 2020; Cavaiuolo *et al*., 2023; Li *et al*., 2022).

O MRSA é classificado em três grupos: associado a cuidados de saúde (HA-MRSA), associado à comunidade (CA-MRSA) e associado à pecuária (LA-MRSA) (Abolghait et al., 2020; Roy *et al*., 2024). As primeiras cepas de MRSA foram relatadas em humanos na década de 1960, inicialmente limitadas a ambientes hospitalares e afetando indivíduos imunocomprometidos. No entanto, infecções adquiridas na comunidade por MRSA surgiram na década de 1990, primeiro na Oceania e depois globalmente (Abolghait *et al*., 2020; Doulgeraki *et al*., 2017).

A resistência à meticilina é mediada pelo gene mecA (ou seu homólogo mecC), que codifica uma nova proteína ligadora de penicilina (*Penicilin Binding Protein*, PBP), chamada PBP2a, com baixa afinidade para antibióticos β-lactâmicos (Silva *et al*., 2020; Titouche *et al*., 2019). As proteínas PBP2a codificadas por mecA e as proteínas PBP2c codificadas por mecC diferem na afinidade aos antibióticos (Silva *et al*., 2020). A baixa afinidade de ligação aos antibióticos concede ao patógeno resistência, protegendo-o da inibição da síntese da parede celular (Aung *et al*., 2017; Roy *et al*., 2024). Isso resulta no espessamento da parede celular, aumento do efluxo de antibióticos, mutações nos alvos citoplasmáticos dos medicamentos e alterações na modificação do antibiótico, que pode envolver a adição de grupos químicos que reduzem a eficácia do antibiótico. Algumas cepas de MRSA também carregam genes para enterotoxinas, o que aumenta a sua virulência (Chen *et al*., 2022).

Outra preocupação é a capacidade do MRSA de formar biofilmes, uma capacidade intrínseca regulada por complexos fatores genéticos (Ávila-Nova *et a*l., 2021). Isso permite que *S. aureus* colonize várias superfícies, aumentando o risco de contaminação cruzada entre superfícies, alimentos e humanos (Silva *et al*., 2020; Doulgeraki *et al*., 2017).

A descoberta de que o MRSA pode colonizar ou infectar animais de produção e humanos expostos a esses animais em vários países destaca a necessidade urgente de entender sua transmissão e impacto na saúde única. O contato direto humano-animal, a exposição a ambientes contaminados e, principalmente, o consumo de alimentos de origem animal contaminados são os principais meios pelos quais o MRSA se propaga para os humanos (Doulgeraki *et al*., 2017). A presença de MRSA em animais produtores de alimentos representa uma ameaça significativa para a agricultura e a saúde pública, com o potencial de causar surtos alimentares de MRSA (Ávila-Nova *et al*., 2021; Titouche *et al*., 2019).

A compreensão da saúde única é crucial para abordar os riscos associados ao MRSA devido à sua natureza multidimensional, que abrange saúde humana, animal e ambiental. A transmissão bidirecional entre humanos e animais, em particular, ressalta a necessidade de estratégias integradas para conter a disseminação desse patógeno resistente (Garcia; Osburn;

Cullor, 2019; Roy *et al*., 2024). A presença de MRSA em animais de produção é uma ameaça significativa, pois o contato direto com animais infectados ou a exposição a alimentos de origem animal contaminados pode introduzir o patógeno em populações humanas.

Nos últimos anos, o termo "saúde única" ganhou visibilidade, mas suas raízes remontam ao século XIX. Naquela época, o médico alemão Rudolf Virchow (1821-1902) já enfatizava a ausência de barreiras entre a medicina animal e humana. No século XX, o veterinário americano Calvin W. Schwabe (1927-2006) reforçou a importância de integrar a saúde humana, animal e ambiental. Em suas pesquisas, ele enfatizou a necessidade de colaboração entre veterinários e médicos para resolver problemas globais de saúde. Em seu livro "Veterinary Medicine and Human Health" (1984), Schwabe introduziu o termo "One Medicine", que mais tarde evoluiu para "One Health" (saúde única) (Garcia; Osburn; Cullor, 2019). O conceito de saúde única enfatiza o equilíbrio entre o bem-estar humano, animal e ambiental, reconhecendo que esses três elementos estão interligados e se influenciam mutuamente. Atualmente, a promoção da saúde única é cada vez mais relevante devido ao aumento de doenças infecciosas.

Os desafios relacionados ao controle de *S.aureus* na cadeia produtiva de laticínios têm implicações que vão além do setor alimentar, pois impactam diretamente na saúde humana e animal, dentro do conceito de saúde única. Nesse contexto, a inovação tecnológica surge como uma ferramenta vital para superar esses desafios. Tecnologias como o US têm mostrado grande potencial para melhorar a segurança e a eficiência na produção de laticínios, contribuindo para práticas mais seguras e saudáveis para todos.

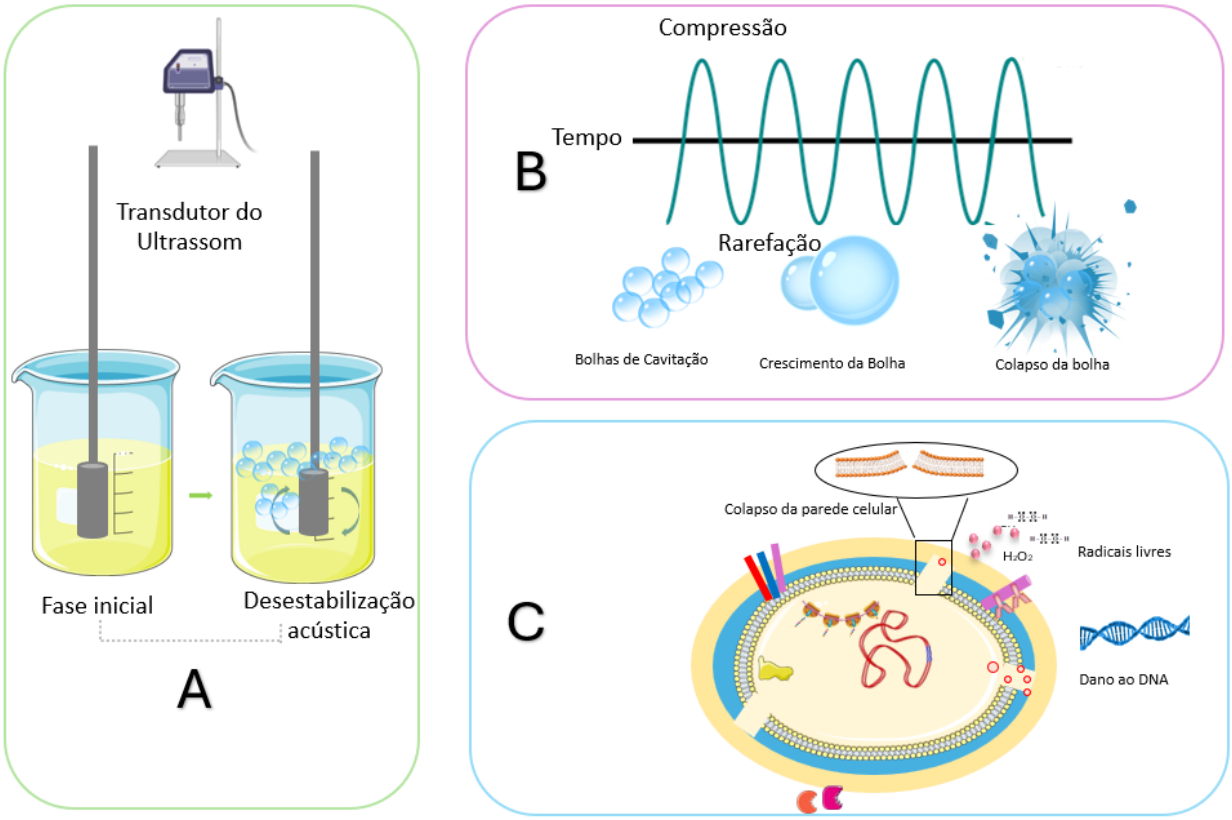
3.5 Uso da tecnologia de Ultrassom na inativação de *S. aureus*

O controle eficaz e a inativação de cepas de *S. aureus* e suas toxinas são essenciais para assegurar a segurança dos alimentos e proteger a saúde pública. A pasteurização, tradicionalmente utilizada na indústria de laticínios, visa reduzir a carga microbiológica nos produtos lácteos (Aaliya *et al*., 2021; Dash *et al*., 2022; Najmitdinova, 2023). Embora eficaz na inativação microbiana, o tratamento térmico convencional tem sido desafiado pela emergência de cepas bacterianas resistentes, como o MRSA, impulsionando a busca por novas tecnologias de processamento (Abolghait *et al*., 2020; Shoaib *et al*., 2023). Neste contexto, a tecnologia de US desponta como uma alternativa promissora, não só pela sua eficácia, mas também por ser ambientalmente amigável (Balthazar *et al*., 2019; Bernardo; Rosário; Conte-Junior, 2021; Bhargava *et al*., 2021; Carrilho-Lopez *et al*., 2021).

O ultrassom é definido como ondas sonoras de alta frequência que ultrapassam o limite de percepção auditiva humana, tipicamente com frequências superiores a 20 kHz (Rathnakumar *et al*., 2023; Scudino *et al*., 2020). As ondas são classificadas por parâmetros como amplitude, comprimento de onda e frequência. Com base na frequência sonora, a aplicação do US pode ser dividida em alta intensidade - baixa frequência (I = 10–1000 Watts por centímetro quadrado W/cm^2) e F = 20 –100 *Kilohertz* (kHz) e baixa intensidade - alta frequência (I < 1 W/cm^2 e F > 1 *Megahertz*, MHz) (Balthazar *et al*., 2019; Bhargava *et al*., 2021; Carrilho-Lopez *et al*., 2021). Assim, o US de alta frequência ou baixa intensidade é empregado para fins diagnósticos não destrutivos, pois não causa modificações agudas na estrutura e nas características do produto. Por outro lado, o US de baixa frequência ou alta intensidade (>1 W/cm²) altera os atributos do produto de maneira favorável e é utilizado em processos destinados a melhorar a qualidade e a segurança dos produtos alimentícios (Balthazar *et al*., 2019; Rathnakumar *et al*., 2023).

O mecanismo de ação do US é fundamentado na cavitação acústica e no *streaming* acústico (Figura 3).

Figura 3 – Mecanismos de ação do ultrassom em meio líquido e seus efeitos microbiológicos.



(A) Fase inicial: Um transdutor de ultrassom é inserido em um recipiente contendo líquido, onde não há atividade aparente. Após a ativação do transdutor, ondas de ultrassom são emitidas, iniciando a formação de microbolhas. **(B)** Comportamento das ondas de ultrassom e cavitação (compressões e rarefações). As Microbolhas de cavitação são formadas durante as rarefações e crescendo em tamanho devido à oscilação das ondas. As bolhas aumentam de tamanho, colapsam, liberando ondas de choque. **(C)** Efeitos biológicos do colapso de bolhas: As ondas de choque causam o rompimento da parede celular da bactéria. A energia liberada forma radicais livres que se transformam em peróxido de hidrogênio (H2O2). O estresse oxidativo resultante pode danificar o DNA das células e causar peroxidação lipídica, que afeta as membranas celulares e pode levar a alterações funcionais e estruturais nas células.

***Fonte**: Adaptado a partir de Carrillo-Lpez et al. (2021).*

Em ambientes geralmente líquidos, as ondas acústicas criam zonas de alta pressão (compressão) e baixa pressão (rarefação). Durante as fases de rarefação, microbolhas de gás podem se formar e crescer. Quando estas bolhas atingem um tamanho crítico, elas colapsam violentamente. Antes do colapso das bolhas, as rápidas variações de pressão induzidas pelas ondas ultrassônicas causam turbulência e deslocamento de volume, conhecido como *streaming* acústico (Guimarães *et al*., 2021; Rathnakumar *et al*., 2023). O processo de geração, crescimento e implosão de bolhas é conhecido como cavitação acústica ou implosão. Cavitação é a formação, crescimento e colapso de microbolhas dentro de uma solução como resultado de flutuações de pressão causadas pelo campo ultrassônico aplicado. Este colapso é a causa da ocorrência de alta turbulência local, gerando o aumento de temperatura e pressão dentro da zona de implosão, capazes de produzir forças de cisalhamento. O colapso violento de uma bolha de cavitação resulta em diversos efeitos físicos e químicos no líquido, tais como microfluxo, agitação, turbulência, jatos de líquido, ondas de choque e formação de Espécies Reativas de Oxigênio (EROs). Esses fenômenos são capazes de induzir efeitos físicos e químicos significativos no material em que são aplicados (Carrilho-Lopez *et al*., 2021; Soltani Firouz; Farahmandi; Hosseinpour, 2019).

3.5.1 Aplicações do Ultrassom na inativação de microrganismos em produtos lácteos

O ultrassom representa uma tecnologia emergente ecologicamente correta, com amplas possibilidades de aplicação para o setor de leite e produtos lácteos (Bernardo; Rosário; Conte-Junior, 2021). Tradicionalmente, os tratamentos térmicos convencionais têm sido amplamente utilizados no setor de laticínios para reduzir a deterioração microbiana e prolongar a vida útil dos alimentos. No entanto, esses métodos frequentemente envolvem o aquecimento do alimento a altas temperaturas por um determinado período, o que pode resultar em perdas

nutricionais e alterações sensoriais significativas (Balthazar *et al*., 2019; Soltani Firouz; Farahmandi; Hosseinpour, 2019; Guimarães *et al*., 2021).

Foi demonstrado que o US de alta intensidade, com níveis de potência variando de 1 a 1.000 W/cm², leva a uma taxa mais rápida de formação de cavidades em comparação ao US de baixa intensidade (<1 W/cm²) (Yu *et al*., 2021). Nesse sentido, estudos recentes têm explorado o uso do US de alta intensidade em laticínios e seus derivados, visando reduzir o tempo de processamento e o consumo de energia, além de melhorar as características físico-químicas dos alimentos (Carrilho-Lopez *et al*., 2021; Soltani Firouz; Farahmandi; Hosseinpour, 2019). Devido à diversidade de produtos lácteos e suas características únicas, o US de alta intensidade tem sido amplamente estudado nesse setor, abrangendo uma variedade de produtos lácteos, como leite cru, leite de ovelha, leite de búfala, iogurte, sorvete, queijo, entre outros (Balthazar *et al*., 2019; Guimarães *et al*., 2021; Jalilzadeh *et al*., 2018; Scudino *et al*., 2020).

Recentemente, diversos estudos têm investigado a aplicação da tecnologia de US na inativação microbiana (Martínez-Moreno *et al*., 2020; Nascimento *et al*., 2023; Yu *et al*., 2021). Essa tecnologia apresenta a vantagem de inativar microrganismos sem causar os efeitos colaterais dos tratamentos térmicos convencionais, que geralmente requerem altos níveis de energia e podem comprometer o valor nutricional dos alimentos (Balthazar *et al*., 2019). O US é uma técnica disruptiva que capaz de alterar as propriedades químicas, bioquímicas, físicas ou mecânicas dos microrganismos, dependendo da intensidade do tratamento aplicado, como frequência, potência, tempo de processamento e volume da amostra (Guimaraes *et al*., 2021).

A inativação de microrganismos pelo US pode ocorrer por meio de diversos mecanismos, conforme ilustrado na Tabela 3.

Tabela 3 – Fatores de processamento e inativação de microrganismos por ultrassom

Fatores de processamento	Unidade de medida	Características	Inativação de microrganismos
Frequência	Hz	-Inversamente proporcional ao tamanho das microbolhas. - No US de Baixa Frequência, as baixas frequências (20–100 kHz) geram grandes microbolhas de cavitação, levando a uma inativação microbiana eficiente. - No US de Alta Frequência, acima de 100 kHz formam pequenas microbolhas, resultando em cavitação insuficiente para inativar bactérias.	-Mais eficaz na faixa de frequência de 20 a 24 kHz.
Intensidade	W, W/L, W/kg, W/cm², J/L	-Quantidade de energia transmitida pelas ondas ultrassônicas.	- Maior intensidade resulta em efeitos mecânicos, térmicos e sonoquímicos mais pronunciados, contribuindo para maior inativação microbiana.
Amplitude da onda	μm, %	- Influencia o tamanho das microbolhas de cavitação.	- Maior amplitude leva a uma intensificação da cavitação, resultando em maior eficiência na inativação microbiana.
Filme de compressão	Cm,mm	-Refere-se à profundidade da sonda dentro do reator	-Otimização crucial para garantir uma distribuição homogênea da intensidade do US e, consequentemente, eficiência uniforme na inativação microbiana

- Hz: Hertz; US: Ultrassom; W: Watts; W/cm²: Watts por centímetro quadrado; J/L: Joules por litro; J/mL: Joules por mililitro; J/cm³: Joules por centímetros cúbicos; μm: Micrômetros; %: Porcentagem.

***Fonte**: Adaptado a partir Beitia et al. (2023).*

Inicialmente, o ultrassom, ao induzir o fenômeno de *streaming* acústico, afeta a parede celular das bactérias, causando-lhes estresse sem danificar a membrana. Tratamentos mais prolongados e intensos, que resultam de rápidas variações de pressão e temperatura, podem levar ao colapso das bolhas e, consequentemente ao rompimento da membrana bacteriana ou da parede celular por meio de cisalhamento. Isso resulta na liberação de material intracelular. Os efeitos mais significativos incluem a ruptura da parede celular causada por microjatos, mudanças na permeabilidade celular, inativação térmica devido a pontos de alta temperatura e a produção de EROs, como os radicais livres de hidroxila. Essas espécies possuem propriedades

oxidativas altamente reativas, capazes de comprometer a membrana celular e danificar o DNA e as enzimas (Balthazar *et al*., 2019; Guimarães *et al*., 2021)

A eficácia letal do ultrassom é influenciada por diversos fatores, incluindo a frequência, a potência ultrassônica, a temperatura da amostra e o tempo de processamento (Guimarães *et al*., 2021; Rathnakumar *et al*., 2023). No entanto, é desafiador comparar os resultados de diferentes estudos, considerando somente esses parâmetros de forma isolada. Isso ocorre porque os efeitos da cavitação dependem desses parâmetros e pequenas variações podem alterar os resultados em diferentes matrizes alimentícias (Aaliya *et al*., 2021). Adicionalmente, o sucesso do tratamento ultrassônico também está relacionado às características físicas e biológicas específicas dos microrganismos tratados. Embora microrganismos como leveduras e bactérias (tanto Gram-positivas quanto Gram-negativas) sejam suscetíveis aos efeitos do US, é necessário considerar as diferenças na estrutura da membrana celular e a possível presença de cápsulas protetoras, que podem reduzir a eficácia do US (Guimarães *et al*., 2021).

Embora a tecnologia de US represente uma abordagem promissora, não destrutiva, não térmica e ecologicamente correta, é importante destacar que sua eficácia pode não ser completamente eficaz em todas as situações. Um dos principais desafios reside na aplicação do US em operações de grande escala. Além disso, o tratamento apenas com US demonstrou possuir atividade antibacteriana e contra biofilmes limitada na inativação de células bacterianas (Martínez-Moreno *et al*., 2020; Yu *et al*., 2021). Diante disso, diversos pesquisadores têm explorado métodos de inativação sinérgica para aprimorar a eficiência dessa técnica.

As limitações inerentes às várias técnicas de inativação microbiana impulsionaram o desenvolvimento e a implementação da tecnologia de obstáculos, também conhecida como tratamento combinado. Essa estratégia incorpora o uso inteligente de combinações de diferentes fatores ou técnicas de preservação (chamados de "obstáculos") para alcançar efeitos de preservação multidirecionais (Leistner; Gorris, 1995). A combinação de tecnologias térmicas e não térmicas desempenha um papel crucial na redução bacteriana. Ao empregar diferentes técnicas conjuntamente, como uma série de obstáculos (químicos, físicos ou ambientais), cria-se um ambiente adverso para os microrganismos nos alimentos, perturbando sua homeostase de maneira temporária ou permanente (Aaliya *et al*., 2021; Bernardo; Rosário; Conte-Junior, 2021).

A sonicação, especialmente quando combinada com calor (termossonicação) ou pressão (manossonicação e manotermossonicação), pode inativar microrganismos patogênicos e deteriorantes presentes nos alimentos. Isso é conseguido através dos efeitos mecânicos e

térmicos da cavitação ultrassônica, que danificam as células bacterianas, levando à sua inativação (Martínez-Moreno *et al*., 2020).

A termossonicação é também destacada como uma estratégia promissora para a inativação microbiana, combinando US e calor, eficaz na redução ou eliminação de microrganismos em alimentos, incluindo bactérias, leveduras e fungos (Martínez-Moreno *et al*., 2020; Nascimento *et al*., 2023; Rathnakumar *et al*., 2023). Esta tecnologia de obstáculo é vantajosa por minimizar o impacto nas características organolépticas e nutricionais dos alimentos, além de requerer menor tempo de processamento e energia, quando combinada com tratamento térmico suave. Entretanto, desafios como custos elevados de equipamento e a definição de parâmetros de processo adequados para a inativação bacteriana sem perda de qualidade sensorial e nutricional são notáveis (Abdulstar; Altemini; Al-Hilphy, 2023).

Neste contexto, nosso grupo de pesquisa, conduziu um estudo sobre a aplicação da termossonicação em produtos lácteos, com um enfoque particular em cremes. Avaliamos a eficácia desta técnica na inativação de MRSA durante processos de pasteurização lenta e rápida, observando uma redução de até 4,72 log UFC/mL em contagens de MRSA em creme. Esses resultados foram comparáveis à redução de 4,82 log UFC/mL obtida por meio da pasteurização convencional, destacando a termossonicação não apenas como uma alternativa energeticamente mais eficiente, mas também eficaz em preservar a segurança microbiológica do creme e reduzir o tempo de processamento (Nascimento *et al*., 2023). Da mesma forma, Balthazar *et al*. (2019) investigaram a aplicação do tratamento ultrassônico em leite de ovelha semidesnatado, tanto fresco quanto congelado, e seus efeitos na qualidade microbiológica. Os tratamentos com US resultaram em uma redução significativa da contaminação bacteriana em comparação com amostras de leite não tratadas, assim como o tratamento de pasteurização em alta temperatura por curto tempo. Especificamente, foi observada a eliminação ou inativação eficaz de coliformes totais e *Staphylococcus* spp.

3.5.2 Desafios para aplicação industrial

A implementação da tecnologia de US em escala industrial enfrenta vários desafios, que incluem os elevados custos de aquisição e instalação dos equipamentos especializados, a necessidade de ajustes na gestão e na linha de produção, e a complexidade operacional e manutenção desses sistemas (Bernardo; Rosário; Conte-Junior, 2021). Contudo, diversos estudos apontam que o US pode se destacar como uma alternativa superior às metodologias tradicionais de processamento, particularmente no setor lácteo, que se destaca como um

seguimento significativo na indústria alimentícia, com uma vasta gama de produtos oriundos de diferentes processos (Guimaraes *et al*., 2021). Os benefícios tecnológicos incluem aprimoramento na homogeneização, emulsificação, redução do tamanho dos glóbulos de gordura e na qualidade nutricional dos produtos (Carrilho-Lopez *et al*., 2021), diminuição do tempo de maturação e fermentação de queijos, bem como na inativação bacteriana (Balthazar *et al*., 2019; Nascimento *et al*., 2023; Rathnakumar *et al*., 2023; Scudino *et al*., 2020). Além disso, o US mostra ser eficaz na inativação de diversas enzimas relacionadas aos produtos lácteos, como fosfatase alcalina, lactoperoxidase e γ-glutamil transpeptidase, preservando os sabores naturais dos alimentos (Rathnakumar *et al*., 2023). Suas aplicações se estendem à estabilização cinética, ruptura celular, liberação de compostos bioativos e melhoria da cristalização de água e lactose, evidenciando sua versatilidade para melhorar a qualidade e segurança dos alimentos, consolidando-se como uma tecnologia eficiente para a conservação de nutrientes, prolongando a vida útil e melhoria na qualidade dos produtos lácteos (Bhargava *et al*., 2021; Guimarães *et al*., 2021).

O ultrassom tem um papel importante na formação firme e estável de gel pela desnaturação das proteínas do soro, fragmentação das micelas de caseína, e recombinação da fração proteica, resultando em um iogurte mais consistente (Carrilho-Lopez *et al*., 2021). Não foram observados efeitos adversos significativos na coesividade ou na elasticidade do queijo branco ultrafiltrado decorrentes do uso do US. A sonicação levou a um aumento da acidez e a uma redução dos valores de pH em comparação com as amostras controle, sem afetar negativamente o conteúdo de gordura ou proteína do queijo. Ademais, o tratamento ultrassônico acelerou os processos de lipólise e proteólise, culminando em uma maior produção de ácidos graxos livres e nitrogênio solúvel em água. Essas alterações contribuem para o aprimoramento do sabor e aroma do queijo, melhorando suas propriedades físico-químicas e sensoriais durante o processo de maturação (Jalilzadeh *et al*., 2018).

As pesquisas realizadas em laboratórios sob condições controladas sugerem que os resultados experimentais alcançados podem não ser facilmente replicáveis em escala industrial, devido às diferenças na quantidade de material processado e à necessidade de sistemas mais robustos para o processamento em larga escala (Soltani Firouz; Farahmandi; Hosseinpour, 2019). Além disso, o uso do US em alta intensidade acarreta algumas desvantagens que exigem estudos mais aprofundados para sua adoção em escala industrial. A aplicação do US em altas intensidades pode gerar calor devido ao aumento da temperatura, o que pode impactar negativamente as características organolépticas e nutricionais dos produtos alimentícios. Pode também levar à peroxidação lipídica, alterar a estrutura física dos alimentos, desintegrar

componentes como proteínas e emulsificantes, modificar o pH e, em casos extremos, gerar subprodutos tóxicos. Os radicais livres, gerados pela cavitação, podem induzir à oxidação lipídica, ocasionando sabores e odores desagradáveis, desnaturação de proteínas, e redução do conteúdo fenólico total devido à degradação do ácido ascórbico. A combinação do US com temperatura e pressão também pode levar à formação de radicais livres que desencadeiam reações danosas à estrutura das proteínas, comprometendo a textura dos alimentos. Assim, a otimização da intensidade e do uso combinado do US antes de sua aplicação se torna essencial (Bhargava *et al*., 2021).

Bernardo, Rosário, Conte-Junior. (2021) ressaltam as principais restrições ao emprego do US em larga escala na indústria de produtos lácteos, como na descontaminação do leite. Essas limitações estão fortemente ligadas à morfologia bacteriana e à composição da parede celular das bactérias. Bactérias Gram-positivas, caracterizadas por uma camada espessa de peptidoglicano, apresentam maior resistência aos efeitos deletérios do US (Balthazar *et al*., 2019; Herceg *et al*., 2020; Shamila-Syuhada *et al*., 2016). A própria composição do leite pode intensificar a resistência bacteriana frente a tratamentos físico-químicos, com a lactose atuando como um agente sonoprotetor ao estabilizar fosfolipídios e proteínas, diminuindo assim a eficácia do US na inativação bacteriana (Gera; Doores, 2011). Esporos bacterianos, devido à sua robustez física, incluindo várias camadas de proteção e a habilidade de se desidratar, mostram maior resistência ao calor úmido em comparação com as células vegetativas (Gao *et al*., 2014).

Estes desafios destacam a falta de consenso sobre a definição e apresentação dos parâmetros de processamento na ultrassonicação, assim como na metodologia para o cálculo da energia acústica real empregada (Guimarães *et al*., 2021; Scudino *et al*., 2020). Esses são aspectos cruciais para permitir comparações efetivas entre diferentes estudos. Além disso, torna-se imprescindível a realização de mais pesquisas para definir parâmetros de processo ajustados a cada objetivo específico de segurança, seja ele tecnológico ou microbiológico, e que sejam adequados ao tipo particular de produto lácteo em análise.

4 MATERIAL E MÉTODOS

4.1 Amostras de Creme de leite e cepas bacterianas

As amostras de creme cru foram adquiridas de uma fábrica de laticínios em São Sebastião do Passé, Bahia, Brasil (coordenadas: 12°30’45,5” S, 38°29’27,2” W), com uma concentração de gordura de 35%. As amostras foram transportadas em caixas isotérmicas, mantendo a temperatura controlada em4 ± 2 ℃) e, posteriormente armazenadas sob refrigeração a 5 ± 2 ℃ até o momento do seu processamento. Este estudo envolveu cinco cepas de *S. aureus* resistentes a β-lactâmicos (MRSA), todas associadas a atividades pecuárias no Brasil, conforme descrito por Silva *et al*., 2020. Destacam-se entre as cepas, a 30PD.1 e 32AD.1, isoladas do leite de vaca; a cepa 3N proveniente da cavidade nasal de um ordenhador; e as cepas 1T.1 e SFT.1, isoladas das tetas das vacas.

4.2 Preparação de suspensões de MRSA

Cada isolado de MRSA foi preservado em caldo tríptico de soja (TSB; HiMedia, Mumbai, Índia) enriquecido com 20% de glicerol e mantido a -80 ℃. No início do experimento, os isolados foram descongelados à temperatura ambiente e cultivados individualmente em 10 ml de caldo *Brain Heart Infusion* (BHI) (Difco, Detroit, EUA) a 36 ℃ durante 24 horas. Para assegurar a pureza das colônias, cada isolado foi semeado em ágar Baird-Parker (Merck, Darmstadt, Alemanha) e incubado a 36 ℃ por 48 horas. Posteriormente, uma única colônia de cada placa foi transferida para 10 ml de caldo BHI e submetida a dois ciclos adicionais de cultivos de 24 horas a 36 ℃. Após a incubação, as culturas foram centrifugadas a 3.500 rpm por 10 minutos a 4 ℃ usando uma centrífuga (Spin Max 80-2B; Medmax, Baueri, Brasil). Os sobrenadantes foram descartados e os sedimentos lavados duas vezes com solução salina tamponada com fosfato (PBS) a pH 7,2. As células foram ressuspensas em PBS utilizando um vórtex. Estas suspensões foram combinadas em proporções iguais em um frasco Erlenmeyer estéril, resultando em uma suspensão mista contendo todas as cinco cepas. A densidade óptica da suspensão foi padronizada para 0,5 (OD600), o que corresponde a 10^8 UFC/mL, confirmada por contagem de colônias em ágar Baird-Parker após um período de incubação de 48 horas a 36 ℃, e medida por um espectrofotômetro (Specord 200plus, Analytik Jena, Alemanha).

4.3 Contaminação artificial de amostras de creme de leite

Para a contaminação artificial das amostras de creme de leite, utilizou-se uma suspensão bacteriana na concentração de 10^6 UFC/mL. Inicialmente, 2 mL do inóculo foram adicionados

a 200 mL de creme de leite. Após a adição, as amostras foram deixadas em repouso por 5 minutos antes de serem submetidas aos tratamentos. Os procedimentos de tratamento foram organizados em duas categorias principais: 1) pasteurização e 2) pasteurização seguida de sonicação, referidas como grupos de termossonicação. Essas categorias foram subdivididas em um total de dez variações de tratamento, conforme detalhado na **Tabela 4**.

Tabela 4 – Parâmetros de processamento utilizados nos tratamentos e contagens de *Staphylococcus* MRSA e microrganismos indicadores (log UFC/mL) em amostras de creme de leite tratadas com pasteurização e termosonicação imediatamente após o processamento (0 d), aos 14 dias e 30 dias de armazenamento refrigerado (5 ± 0,5 °C).

Treatmento	Power (W)	Pulso (s)	Densidade energética (J/cm³)	Temperatura (°C)	Tempo de pasteurização	Tempo de sonicação	Tempo total de processamento
IUNT	-	-	-	-	-	-	-
NIP-72	-	-	-	72	20 sec	-	-
NIP-65	-	-	-	65	40 min	-	-
IP-72	-	-	-	72	20 sec	-	-
IP-65	-	-	-	65	40 min	-	-
US1	31	0	0.39	72	3 sec	3 sec	6 sec
US2	31	0	0.72	72	5 sec	5 sec	10 sec
US3	31	0	1.47	72	10 sec	10 sec	20 sec
US4	35	30	24.44	65	5 min	5 min	10 min
US5	33	30	48.30	65	10 min	10 min	20 min
US6	35	30	119.06	65	15 min	15 min	30 min

UNIT. Amostra inoculado e não tratado; **NIP-72**. Amostra não inoculada e pasteurização rápida; **NIP-65**. Amostra não inoculada e pasteurização lenta; **IP-72**. Amostra inoculada e pasteurização rápida; **IP-65**. Amostra inoculada e pasteurização lenta; **US1**. Amostra inoculada, pasteurização rápida e sonicação por 3s; **US2**. Amostra inoculada, pasteurização rápida e sonicação por 5 s; **US3**. Amostra inoculada, pasteurização rápida e sonicação por 10 s; **US4**. Amostra inoculada, pasteurização lenta e sonicação de 5 min; **US5**. Amostra inoculadoa, pasteurização lenta e sonicação por 10 min; **US6**. Amostra inoculada, pasteurização lenta e sonicação por 15 minutos.
s: segundos; min: minutos; J/cm³: Joules por centímetros cúbicos; °C: graus Celsius

4.4 Armazenamento e tratamento térmico de amostras de creme de leite

Para avaliar o efeito do ultrassom em combinação com o calor (termossonicação) em comparação ao método convencional de pasteurização, volumes iguais de 200mL de creme de leite cru foram tratados de duas maneiras: pasteurização rápida a 72 ℃ por 15 segundos e pasteurização lenta a 65 ℃ por 40 minutos, conforme descrito em nosso estudo anterior (Nascimento *et al*., 2023).

O processo de aquecimento foi realizado em frascos de vidro estéreis e fechados, colocados em um banho-maria com controle térmico e agitação constante, assegurando uma dispersão homogênea do calor nas amostras durante o tratamento. A temperatura do

o banho de aquecimento foi controlado, mantendo-se em 72±1 °C ou a 65±1 °C para facilitar uma transferência de calor eficaz. A temperatura do creme de leite foi monitorada com um termômetro colocados diretamente nos frascos.

Após a conclusão dos tratamentos de pasteurização, as amostras foram resfriadas progressivamente até atingirem aproximadamente 20 ºC, utilizando um banho de gelo. As amostras de creme de leite pasteurizado foram então designadas como amostras controle para todos os experimentos subsequentes.

4.5 Tratamentos Ultrassonicos e Termossônicos de creme de leite

Amostras de creme de leite fresco (200 mL) foram colocadas em béqueres estéreis de 300 mL, utilizados como recipientes para os tratamentos. Os tratamentos ultrassônicos foram efetuados utilizando um processador ultrassônico VC 505 (Vibra-Cell, Sonics & Materials, Connecticut, EUA) que possui potência máxima de entrada 500 W e tensão de 220 V, equipado com uma sonda de 13 mm de diâmetro e frequência de 20 kHz. Detalhes adicionais dos parâmetros de processamento, como potência ultrassônica (P), duração, temperatura (T) e densidade de energia (DE), estão disponíveis na Tabela 4.

A sonda ultrassônica foi imersa na amostra a uma profundidade de 20 mm abaixo da superfície em todos os experimentos. Para a termossonicação, utilizou-se 50% da potência nominal de saída, correspondendo a aproximadamente 31W e 35W para pasteurização lenta e rápida, respectivamente. Os tempos de exposição foram estabelecidos em 5, 6 e 10 segundos para a pasteurização rápida, e 5,10 e 25 minutos para a pasteurização lenta em modo pulsado.

Durante a termossonicação, cada amostra foi aquecida até a temperatura de pasteurização e colocada meticulosamente em um béquer encamisado de 600 mL para manter a temperatura constante. Para assegurar a estabilidade térmica, utilizou-se um sistema de circulação contínua de água no béquer, acoplado a um banho-maria termostático (Tecnal, Modelo: TE2000), permitindo um controle preciso da temperatura, que foi monitorada por um termômetro IR (Raytek - MiniTemp FS, Raytek, Melrose, EUA). Após os tratamentos, todas as amostras foram prontamente resfriadas em banho de água gelada mantido a 2 ± 1 °C subsequentemente armazenadas em refrigerador a 4 ± 0,5 °C até a realização das análises.

4.6 Determinação da densidade energética e variação da temperatura ao longo do tempo

A densidade de energética foi calculada com base na potência nominal (W), tempo (s) de processamento e volume da amostra (mL) (Eq. 1). Além disso, as amostras de creme de leite foram expostas à sonicação sob condições adiabáticas, com a temperatura sendo medida antes e depois do tratamento ultrassonico para estabelecer a relação entre o aumento do tempo de sonicação e o aumento da temperatura (ΔT) (Eq. 2). monitorada no início e ao final do processo.

Equação (1)

$$\text{Densidade energética}\left(\frac{J}{mL}\right) = \frac{\textit{Potência nominal (W) *tempo de processamento (s)}}{\textit{Volume da amostra} \text{ (mL)}}$$

Equação (2)

$$\Delta T = T_{final} - T_{inicial}$$

Onde:

T_{final} é a temperatura ao final do período considerado

$T_{inicial}$ é a temperatura no início desse período

4.7 Análises microbiológicas e avaliação dos tratamentos

A eficácia dos tratamentos foi avaliada por meio das contagens de *S. aureus* imediatamente após o processamento (dia 0) e em intervalos durante o período de estocagem (dias 15 e 30), seguindo as diretrizes estabelecidas pela *American Public Health Association* (APHA).

Além disso, um grupo de controle positivo, composto por amostras inoculadas e não submetidas à sonicação (IP-72 e IP-65), foi incluído para permitir a comparação de reduções logarítmicas. Para a contagem, foram utilizadas três porções de 10mL de creme, as quais foram homogeneizadas e subsequentemente diluídas em série em 90 ml de água peptonada a 0,1% (Merck, Darmstadt, Alemanha), utilizando um Stomacher (Stomacher 80, Seward, Londres, Reino Unido). As diluições resultantes foram então espalhadas em ágar Baird-Parker e incubadas a 36 ± 1 °C por 48 horas. As colônias foram contadas eletronicamente utilizando o contador Flash & Go (IUL Instruments, Barcelona, Espanha), e os resultados expressos em log UFC/mL.

Além de *S. aureus*, a presença de bactérias aeróbias mesófilas e enterobactérias foi avaliada utilizando a técnica pour plate e os meios de cultura *Plate Count Agar* e *Violet Red*

Bile Glucose Agar (VRBGA), respectivamente (PCA, Kasvi®, SP, Brasil), com incubação a 35 °C por 48 horas e 24 horas, respectivamente. A análise de bolores e leveduras foi realizada utilizando a técnica spread plate com o meio de cultura *Dichloran Rose Bengal Chloramphenicol Agar* (DRBC, Difco, Detroit, EUA) e incubação a 25 ºC em BOD por 5 dias. Para a inativação microbiana, o número de reduções logarítmicas (γ) foi calculado e acordo com Pflug (2010), utilizando a seguinte equação:

Equação (3)

$$\gamma = \log_{10}(N_0) - \log_{10}(N_f)$$

Onde:

N_0 representa o número de microrganismos viáveis no produto não processado

N_f é o número de microrganismos viáveis após o tratamento.

4.8 Análise estatística

Todas as análises foram conduzidas em triplicata, tanto na escala analítica quanto experimental. Os resultados foram expressos como média ± desvio padrão (DP). Após a tabulação dos dados, procedeu-se com análises descritivas para explorar as características dos dados coletados. A seleção entre testes estatísticos paramétricos e não paramétricos foi determinada com base no teste de normalidade de Shapiro-Wilk.As comparações entre os tratamentos foram feitas por meio de Análise de Variância para medidas repetidas (One-Way ANOVA), seguida do teste de Tukey para comparações múltiplas. As diferenças entre os diferentes dias de armazenamento foram analisadas por meio de ANOVA seguida de teste de Tukey ou teste t não pareado com correção de Welch, quando apropriado. O nível de significância estabelecido foi de $p<0,05$, com um intervalo de confiança de 95%. O software GraphPad Prism (GraphPad Software, San Diego, EUA) versão 10.2.2 foi utilizado para realizar análises estatísticas e visualizar os dados graficamente.

5 RESULTADOS

Como resultado desta tese foram produzidos dois (02) artigos experimentais e dois (02) de revisão, como reportado abaixo:

- NASCIMENTO, J. C. N.; SALGADO, M. J. G.; ALZATE, K. G.; ALENCAR, J. C. G.; ROSARIO, I. L. S.; SILVA, J. G.; PAULINO, B. N.; COSTA, M. P. Effect of sonication associated with pasteurization on the inactivation of methicillin-resistant *Staphylococcus aureus* in milk cream. *Applied Sciences*, v. 13, n. 22, p. 12093, 2023. DOI: https://doi.org/10.3390/app132212093.

- NASCIMENTO, J. C. N. *et al*. Comparative study of the use of conventional heat treatment and thermosonication for the inactivation of pathogenic microorganisms in milk cream. Em fase de submissão ao periódico *Ultrasonics.*

- NASCIMENTO, J. C. N. *et al. Staphylococcus aureus*, a food pathogen: Current challenges and perspectives in food safety. Submetido ao periódico *Food Science and Nutrition* em 27/06/2024.

- NASCIMENTO, J. C. N. *et al*. Recent advances in ultrasound application in the dairy industry: efficacy and challenges in microorganism inactivation. Submetido ao periódico *Brazilian Journal of Food Technology* em 07/08/2024.

5.1

Artigo: Effect of Sonication Associated with Pasteurization on the Inactivation of Methicillin-Resistant Staphylococcus aureus in Milk Cream

Effect of Sonication Associated with Pasteurization on the Inactivation of Methicillin-Resistant *Staphylococcus aureus* in Milk Cream

Periódico a ser submetido (1ª submissão):	*ISSN: 2076-3417*
Maior percentil (Scopus):	*75%*
Periódico a ser submetido (2ª submissão):	
Maior percentil (Scopus):	

Abstract:

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant challenge to the dairy industry, necessitating robust strategies to ensure food safety. This study focuses on the efficacy of thermosonication, a novel technology combining ultrasound and heat, in reducing MRSA in milk cream. Comparative analysis is conducted with conventional pasteurization, the industry standard. Results indicated that thermosonication effectively reduces MRSA counts by up to 4.72 log CFU/mL, akin to pasteurization's reduction of 4.82 log CFU/mL. This finding highlights the potential of thermosonication as a rapid, energy-efficient alternative to pasteurization in the dairy industry, significantly reducing processing time while maintaining microbial safety. Further exploration and optimization of these techniques promise enhanced food safety and quality control in dairy products, addressing the growing concern of antibiotic-resistant strains like MRSA. This research lays a foundation for innovative approaches and underscores the significance of quantitative data in food safety research.

Keywords: MRSA; ultrasound; thermosonication, bacterial reduction, food safety.

Communication

Effect of Sonication Associated with Pasteurization on the Inactivation of Methicillin-Resistant *Staphylococcus aureus* in Milk Cream

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Citation: Nascimento, J.C.N.; Salgado, M.J.G.; Gutierrez Alzate, K.; de Alencar, J.C.G.; Rosario, I.L.D.S.; da Silva, J.G.; Paulino, B.N.; da Costa, M.P. Effect of Sonication Associated with Pasteurization on the Inactivation of Methicillin-Resistant *Staphylococcus aureus* in Milk Cream. *Appl. Sci.* **2023**, *13*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Received: 22 September 2023
Revised: 17 October 2023
Accepted: date
Published: date

1. Introduction

Milk cream holds a significant place within the realm of dairy products in the diets of Brazilians, primarily due to its versatility. It plays a pivotal role as an essential ingredient in a diverse spectrum of culinary preparations, serving as an integral component in various sweet and savory dishes [1]. It is worth noting that creams available on the Brazilian market exhibit discernible disparities, primarily stemming from variances in their heat treatment processes and fat content. Among the available options, UHT (Ultra-High Temperature) creams, characterized by an average fat content of 20%, offer a unique proposition [2]. These creams undergo processing that imparts an extended shelf life and allows them to be stored at ambient temperatures until opened, enhancing their convenience and

accessibility. In contrast, pasteurized creams, which, on average, contain approximately 35% fat, represent a distinct category [2].

The pasteurization process entails subjecting the cream to a specific temperature for a predetermined duration, thereby ensuring the elimination of harmful microorganisms while preserving the cream's organoleptic attributes. This differentiation in fat content and heat treatment provides consumers with a valuable array of choices, enabling them to select the cream that aligns with their culinary requirements and dietary preferences. However, pasteurized cream is inherently less microbiologically stable due to the lower temperature employed during its production process. The lower temperature range used in pasteurization, while sufficient to eliminate harmful microorganisms, does not provide the same level of microbiological stability as UHT processing. Consequently, pasteurized cream is more susceptible to microbiological variations during storage and possesses a relatively shorter shelf life. In this context, to safeguard the quality and safety of pasteurized cream, it is essential to adhere to stringent microbiological criteria. These evaluations encompass the quantification of total and thermotolerant coliforms, mesophilic aerobic microorganisms, and coagulase-positive *Staphylococcus* [2], which collectively provide valuable insights into the cream's microbiological status and suitability for consumption.

Staphylococcus aureus, particularly its methicillin-resistant strains (MRSAs), raises significant concerns due to its capacity to induce foodborne illnesses and its growing resistance to conventional antibiotics [3]. Notably, recent times have witnessed a substantial upsurge in the exploration of innovative techniques aimed at ensuring the safety and quality of dairy products. This surge is partly attributed to the increasing challenges posed by antibiotic-resistant pathogens, prompting a heightened focus on the development of novel solutions [4–6]. This drive for innovation arises from the need to meet the demands of the global market, as well as consumers' expectations for products that are minimally processed, palatable, healthy, and, above all, safe for consumption [7,8]. Thus, the microbiological safety of dairy products, including creams, stands as a paramount concern, representing a pivotal nexus between the food industry and public health. In response to these imperatives, the application of ultrasound, often referred to as sonication, emerges as a promising alternative technology with the potential to significantly enhance various facets of dairy production processes.

High-intensity ultrasound (HIUS) is a promising approach for enzymatic inactivation, nutritional and sensory quality improvement, and microbiological safety in dairy products [8]. According to Bernardo et al. [9], the integration of ultrasound in milk processing offers advantages like flavor preservation, better homogeneity, and energy efficiency compared with traditional methods. However, it can lead to mild physicochemical changes, like lipid oxidation in raw milk and volatile compound production in pasteurized milk. Some improvements include changes in the freezing point, reduced fat globule size, and dry milk reconstitution. Ultrasound treatment varies in effectiveness based on factors like frequency, amplitude, and duration, and its impact on dairy matrices is influenced by factors like microbiology and energy dissipation from cavitation bubbles [9]. Despite its potential, the implementation of ultrasound technology in the dairy industry requires considerations beyond technical aspects. Factors like cost, profit, consumer perceptions, and financial viability play essential roles in the adoption of this technology [9].

Moreover, thermosonication, an innovative methodology that integrates ultrasound with concurrent heat application, establishes a synergistic process, amplifying the effectiveness of conventional pasteurization techniques. This technique offers compelling advantages in terms of the inactivation of pathogenic microorganisms and the preservation of dairy product quality [10]. Indeed, the existing body of research pertaining to ultrasound treatment in dairy products has predominantly concentrated on milk and dairy beverages. However, it is imperative to extend this investigative framework to encompass a broader array of dairy commodities, including cream [11]. The lipophilic nature of fat renders it an effective sanctuary for microorganisms, shielding them from the rigors of conventional pasteurization procedures. Therefore, it becomes crucial to gain a comprehensive understanding of the effectiveness of ultrasound treatment, especially when combined with pasteurization, as it pertains to milk cream. Such exploration represents a viable strategy for enhancing the safety and quality of this dairy product. Within this context, this communication aims to assess the impact of the pasteurization time (slow and fast) reduction via the association of sonication-pasteurization on the inactivation of MRSA strains in milk cream.

2. Materials and Methods

2.1. Preparation of MRSA Suspensions

Five strains of *mecA*-positive β-lactam-resistant *Staphylococcus aureus* (MRSA) associated with livestock activities in Brazilian territory were used in this study. Specifically, strains 30PD.1 and 32AD.1 were isolated from cow milk, strain 3N originated from a milker's nasal cavity, and strains 1T.1 and SFT.1 were isolated from cow teats [12].

Each isolate was stored in tryptic soy broth (TSB; HiMedia, Mumbai, India) supplemented with 20% glycerol and maintained at −80 °C. To initiate the experiment, the isolates were thawed at room temperature and individually cultured in 10 mL of Brain Heart Infusion (BHI) broth (Difco, Detroit, MI, USA) at 36 °C for 24 h. To ensure the purity of colonies, each isolate was streaked onto Baird-Parker agar (Merck, Darmstadt, Germany) and incubated at 36 °C for 48 h. Subsequently, a single colony from each plate was transferred to 10 mL of BHI broth, followed by two successive 24 h cultivations at 36 °C. After incubation, the contents of the test tubes were centrifuged at 3500 rpm for 10 min at 4 °C using a centrifuge (Spin Max 80-2B; Medmax, Baueri, Brazil). The resulting supernatants were discarded, and the pellets were washed with phosphate-buffered saline (PBS) at pH 7.2, repeating this washing process twice. The cells were then resuspended in PBS using a vortex mixer. These resultant suspensions were combined in equal proportions within a sterile Erlenmeyer flask, resulting in a single pooled suspension containing all five strains. To ensure uniformity, the concentration of the pooled suspension was standardized to an optical density of 0.5 (OD_{600}), corresponding to 10^8 CFU/mL, as confirmed by colony counting on Baird-Parker agar plates following a 48 h incubation at 36 °C, and measured using a spectrophotometer (Specord 200plus, Analytik Jena, Jena,Germany).

2.2. Inoculation, Pasteurization, and Sonication Treatment

Raw cream was acquired from Taquipe Agropecuária (São Sebastião do Passé, Bahia, Brazil; coordinates: 12°30′45.5″ S, 38°29′27.2″ W), with a standardized fat content of 35%. Artificial contamination was performed by introducing a

bacterial suspension at a concentration of 10^6 CFU/mL into 200 mL of milk cream. Specifically, 2 mL of the inoculum was added, followed by a 5 min stand period before proceeding with the treatment. The treatments were divided into two categories, (1) pasteurization and (2) pasteurization and sonication (thermosonication groups), resulting in a total of ten treatment variations (Table 1). Each sample designated for thermosonication was heated to pasteurization temperature and meticulously placed into a 250 mL jacketed beaker to maintain pasteurization temperature.

HIUS technology was employed, utilizing a 13 mm ultrasound probe with a frequency of 20 kHz and a maximum input ultrasonic power of 500 W (VC-505 Ultrasonic Processor; Sonics Materials, Newtown, CT, USA). Within 250 mL glass tubes, 200 mL of each sample underwent the sonication process, with the probe consistently positioned 20 mm below the surface of the sample in all experiments. Detailed processing parameters, including power, duration, temperature, and energy density, can be found in Table 1. To maintain a stable and controlled temperature during the thermosonication process, a jacketed beaker with a continuous water circulation system was employed. This system was linked to a thermostatic water bath (Tecnal, Model: TE2000), ensuring precise temperature control throughout the process, monitored using a calibrated thermometer. Following pasteurization and thermosonication treatments, all samples were promptly cooled in an ice-water bath maintained at a temperature of 2 ± 1 °C and then stored in a refrigerator at 4 ± 0.5 °C until analysis.

Table 1. Processing parameters used in the milk cream treatments.

Treatment	Processing Parameters				
	Power (W)	Pulse (s)	Energy Density (J/cm³)	Time	Temperature (°C)
IUNT	-	-	-	-	-
NIP-72	-	-	-	15 s	72
NIP-65	-	-	-	40 min	65
IP-72	-	-	-	15 s	72
IP-65	-	-	-	40 min	65
US1	31	0	78	3 s	72
US2	31	0	140	5 s	72
US3	31	0	290	10 s	72
US4	35	30	4900	5 min	65
US5	33	30	9700	10 min	65
US6	35	30	23,800	25 min	65

IUNT, inoculated and untreated; NIP-72, not inoculated and rapid pasteurization; NIP-65, not inoculated and slow pasteurization; IP-72, inoculated and rapid pasteurization; IP-65, inoculated and slow pasteurization; US1, inoculated, rapid pasteurization, and 3 s sonication; US2, inoculated, rapid pasteurization, and 5 s sonication; US3, inoculated, rapid pasteurization, and 10 s sonication; US4, inoculated, slow pasteurization, and 5 min sonication; US5, inoculated, slow pasteurization, and 10 min sonication; US6, inoculated, slow pasteurization, and 25 min sonication.

2.3. Bacterial Enumeration

To assess the efficacy of the treatments, microbiological analyses of *S. aureus* counts were conducted immediately after processing (day 0), adhering to the guidelines outlined by the American Public Health Association [13]. Additionally, a positive control group, consisting of an inoculated and untreated sample, was

included to facilitate comparisons of log reductions. For the enumeration process, three cream portions, each weighing 10 g, were homogenized and subsequently serially diluted in 90 mL of 0.1% peptone water (Merck, Darmstadt, Germany) utilizing a stomacher (Stomacher 80, Seward, London, UK). These dilutions were then spread-plated onto Baird-Parker agar and incubated at a controlled temperature of 36 ± 1 °C for 48 h. Finally, to ensure precise quantification, electronic counting was carried out using a Flash & Go electronic counter (IUL Instruments, Barcelona, Spain). The resulting data were expressed as log CFU/mL for comprehensive reporting.

2.4. Statistical Analysis

All analyses were conducted in analytical and experimental triplicate. The results were presented as the mean ± standard deviation (SD). This was carried out using a one-way analysis of variance (ANOVA) with subsequent Tukey post hoc testing, performed at a significance level of 0.05. This statistical evaluation was accomplished using XLSTAT version 2022.1 (Addinsoft, Paris, France). Furthermore, the graphic was plotted using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

The survival levels of MRSA in milk cream are exhibited in Figure 1. The initial analysis revealed that MRSA counts in the treated samples presented a reduction of up to 3.41-fold following pasteurization (4.82 log CFU/mL reduction) and 3.25-fold after thermosonication (4.72 log CFU/mL reduction), in comparison with the untreated group (IUNT). These findings emphasize the significant impact of both pasteurization and thermosonication in reducing MRSA levels, thereby enhancing the safety and quality of the cream. In addition, the treatments subjected to thermosonication, denoted as US1 to US6, exhibited no significant differences ($p > 0.05$) when compared with the inoculated treatments subjected to conventional pasteurization (IP-72 and IP-65) or the thermosonicated treatments themselves. This intriguing observation indicates that thermosonication showcases potential antimicrobial effectiveness on par with conventional pasteurization methods for reducing MRSA levels. Importantly, it achieves this comparable efficacy with significantly reduced processing time during cream treatment, leading to improved productivity and resource utilization.

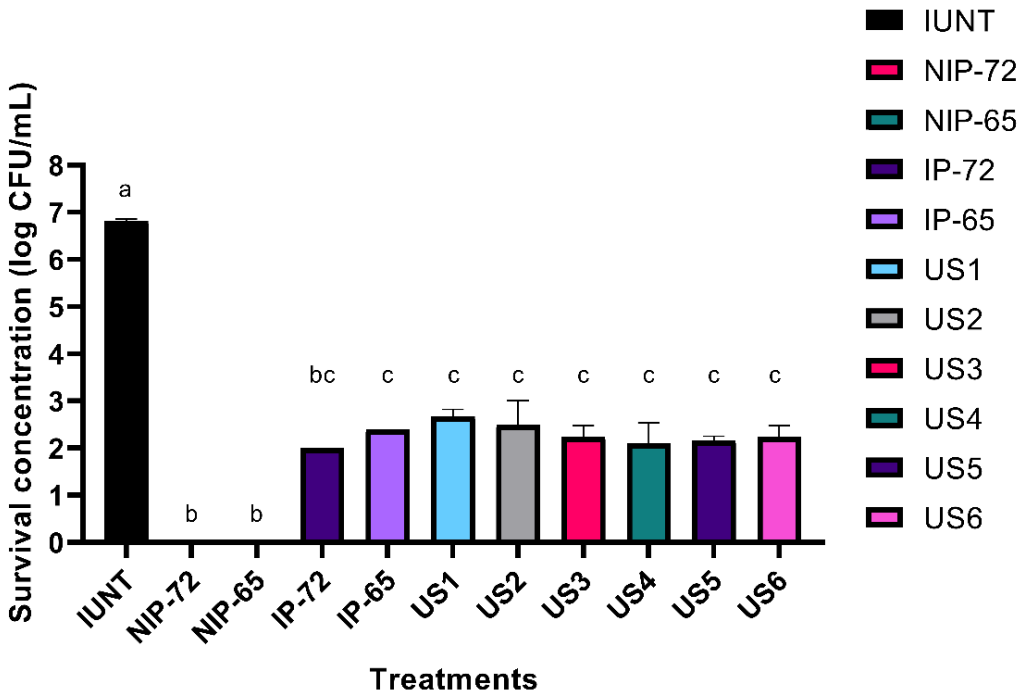

Figure 1. Survival concentrations (log CFU/mL; means ± standard deviation) of *S. aureus* after the application of pasteurization and thermosonication treatments. Different lowercase superscripts indicate significant differences regarding treatments ($p < 0.05$). IUNT, inoculated and untreated; NIP-72, not inoculated and rapid pasteurization; NIP-65, not inoculated and slow pasteurization; IP-72, inoculated and rapid pasteurization; IP-65, inoculated and slow pasteurization; US1, inoculated, rapid pasteurization, and 3 s sonication; US2, inoculated, rapid pasteurization, and 5 s sonication; US3, inoculated, rapid pasteurization, and 10 s sonication; US4, inoculated, slow pasteurization, and 5 min sonication; US5, inoculated, slow pasteurization, and 10 min sonication; US6, inoculated, slow pasteurization, and 25 min sonication.

Further, it is essential to highlight that all treatments subjected to either pasteurization or thermosonication displayed significant differences ($p < 0.05$) when compared with the inoculated and untreated control group (IUNT), underscoring the efficacy of both technological approaches. Thus, the absence of significant differences among these treatments suggests their equal viability as options for enhancing product safety. Furthermore, the clear disparity between these treatments and the untreated control underscores the pivotal role of these processing methods in mitigating bacterial contamination in dairy products, especially when dealing with antibiotic-resistant strains like MRSA. In the dairy industry, the battle against MRSA emerges as a significant concern, and our research demonstrates that both pasteurization and thermosonication yield statistically substantial reductions in MRSA counts in milk cream. The deliberate creation of acoustic cavitation, facilitated by the application of low-amplitude frequencies, has emerged as a pivotal mechanism for cell destruction [9]. This controlled process results in the formation of microbubbles, whose subsequent collapse generates substantial rates of micro-shearing within the cream. It is worth noting that variables such as the frequency, intensity, and duration of ultrasound exposure have surfaced as critical determinants influencing the observed effects on reducing microbial load in milk [14].

Prior investigations have explored the inactivation of *S. aureus* strains in dairy products, shedding light on the pursuit of enhanced food safety. Notably, Li et al.

[11] reported the effects of different hurdle techniques (single ultrasound for 5 min, single heat at 63 °C for 5 min, 5 min ultrasound followed by 5 min heat, 5 min heat followed by 5 min ultrasound, and simultaneous ultrasound and heat for 5 min) at 20 kHz frequency and with a total input power of 600 W, focused on *S. aureus* inactivation in milk. Comparing the diverse treatment techniques to the untreated control, the authors reported up to 1.24-fold cell inactivation when simultaneous ultrasound and heat were applied. This finding not only emphasizes the synergistic potential of thermosonication but also paves the way for the present study, which builds upon this foundation to achieve even higher levels of inactivation.

Moreover, additional investigations have illuminated the substantial potential of these techniques in combating a diverse array of harmful microorganisms, offering a valuable complement to traditional technological methodologies [4]. For instance, Bernardo et al. [15] reported an optimized thermosonication process lasting 1–20 min at temperatures between 62 and 74 °C. This approach resulted in a remarkable reduction of 6.6 log CFU/mL of Shiga toxin-producing *Escherichia coli* in goat milk. In a separate study, Machado et al. [16] noted a reduction of less than 1 log CFU/mL of heat-resistant E. coli isolated from milk. These findings not only underscore the significance and consistency of the techniques employed herein for mitigating microbiological risks in milk cream but also provide further affirmation of ultrasound's efficacy as a valuable tool in combatting staphylococci within the realm of dairy products. The cumulative knowledge derived from these studies reinforces the versatility and potential of these innovative approaches, offering compelling opportunities for enhancing food safety in the dairy industry and beyond. The tailored optimization of factors such as time and temperature showcases the adaptability and nuanced nature of these techniques, encouraging further exploration and refinement for real-world application.

Additionally, it is important to highlight that our analysis did not reveal any statistically significant differences ($p > 0.05$) among the MRSA counts in the inoculated cream subjected to rapid pasteurization (IP-72) and the non-inoculated cream samples that underwent either rapid (NIP-72) or slow (NIP-65) pasteurization, as depicted in Figure 1. This finding is particularly intriguing, as both NIP-72 and NIP-65 demonstrated a notable absence of MRSA growth. Finally, the results conclusively indicate that the synergy of these two technologies significantly reduces the pasteurization time of milk cream by 15 min, all the while maintaining the process's efficiency. Further research and exploration of these methods throughout the storage of cream may provide valuable insights into food safety and quality control.

4. Conclusions

This study highlights the significant impact of ultrasound and heat treatment, emphasizing its viability as a complementary technology to conventional pasteurization. Thermosonication effectively reduced MRSA strains by up to 4.72 log CFU/mL in milk cream, while pasteurization reduced cell counts by 4.82 log CFU/mL. This finding is noteworthy as it demonstrates bacterial inactivation similar to conventional treatment, resulting in a significant reduction in processing time for slow pasteurization—a highly advantageous consideration for the industry. Therefore, thermosonication can be deemed a promising approach for application in cream matrices. This work paves the way for continued exploration and

refinement of these methods, ultimately benefiting both consumers and the food industry by delivering safer and more resilient products. In this context, further research is needed to optimize conditions and assess the impact on the physicochemical composition and stability.

Author Contributions: Conceptualization, J.C.N.N. and M.P.d.C.; methodology, J.C.N.N., M.J.G.S., K.G.A., J.C.G.d.A., J.G.d.S., B.N.P., and M.P.d.C.; formal analysis, J.C.N.N., M.J.G.S., K.G.A., and J.C.G.d.A.; investigation, J.C.N.N., M.J.G.S., K.G.A., J.C.G.d.A., J.G.d.S., B.N.P., and M.P.d.C.; resources, M.P.d.C.; data curation, J.C.N.N. and M.P.d.C.; writing—original draft preparation, J.C.N.N. and M.P.d.C.; writing—review and editing J.C.N.N., M.J.G.S., K.G.A., J.C.G.d.A., I.L.d.S.R., J.G.d.S., B.N.P., and M.P.d.C.; visualization, J.C.N.N., B.N.P., and M.P.d.C.; supervision, J.G.d.S., B.N.P., and M.P.d.C.; project administration, J.G.d.S., B.N.P., and M.P.d.C.; funding acquisition, M.P.d.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Brazil—grant numbers [402430/2018-2], [405728/2018-2], [313119/2020-1], and [303074/2021-3].

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement: The datasets generated for this study are available on request to the corresponding author.

Acknowledgments: The authors are thankful for the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Conflicts of Interest: The authors declare no conflict of interest.

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5.2

Manuscrito: Comparative study of the use of conventional heat treatment and thermosonication for the inactivation of pathogenic microorganisms in milk cream

Comparative study of the use of conventional heat treatment and thermosonication for the inactivation of pathogenic microorganisms in milk cream

Periódico a ser submetido (1ª submissão):	*Ultrasonics - ISSN 0041-624X*
Maior percentil (Scopus):	87%
Periódico a ser submetido (2ª submissão):	
Maior percentil (Scopus):	

Abstract

The present study evaluates the effectiveness of conventional pasteurization compared to thermosonication to the reduction of pathogenic microorganisms, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) and other microorganisms in milk cream. We evalueted the immediate microbial inactivation and the stability of this inactivation over a 30-day of refrigeration period. This study compared traditional pasteurization processes –rapid (72°C for 15 seconds) and slow (65°C for 40 minutes) —with various thermosonication protocols. These protocols involved a combination of rapid pasteurization followed by sonication for durations of 3, 5, and 10 seconds, and slow pasteurization followed by sonication for 5, 10, and 15 minutes, varying ultrasound intensities, duration and temperature. We quantified the populations of MRSA, aerobic mesophilic bacteria, *Enterobacteriaceae*, molds and yeasts immediately post-treatment during throughout the storage time (0, 15 and 30 days). The results demonstrated that both traditional pasteurization and thermosonication effectively reduced MRSA concentrations. Regarding indicator microorganisms, there was variability in the effectiveness of the different treatments applied. However, the combined approach of pasteurization and sonication demonstrated particularly promising results. The synergistic treatment appears to enhance the overall microbial reduction, likely due to the dual action of heat and ultrasonic waves. This integrated technique could potentially offer a more comprehensive and robust solution for controlling microbial populations in dairy products, emphasizing its potential as an advanced strategy in food safety management. Notably, thermosonication showed superior efficacy, particularly in maintaining reduced microbial levels over an extended period, highlighting its potential advantages in enhancing food safety protocols.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, Ultrasound, Microbiological safety, Dairy products, Bioindicators.

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1. Introduction

Milk cream, valued for its rich texture and nutritional benefits, is a versatile ingredient in global cuisine. However, its nutrient-dense composition also makes it susceptible to the growth of pathogenic microorganisms if not properly handled or processed. Ensuring proper storage and processing is essential to maintaining both the safety and quality of milk cream, allowing it to remain a safe and enjoyable component of various dishes worldwide [1]. The presence of *Staphylococcus aureus*, particularly its methicillin-resistant form (MRSA), in dairy products represents a significant food safety risk. *MRSA* is concerning not only for its antibiotic resistance but also for its potential to carry enterotoxin-coding genes, as observed in *mecC*-mediated strains from dairy cattle [2]. These enterotoxins are heat-stable, surviving typical cooking processes, which makes controlling *MRSA* in dairy products crucial. Rigorous hygiene and effective pasteurization are essential to mitigate this risk [3], [4], [5], [6].

Traditionally, pasteurization has served as a critical control measure in the dairy industry to reduce the microbiological load in dairy products. Its thermal processing technique effectively inactivates pathogenic microorganisms and substantially decreases the levels of spoilage bacteria, thereby enhancing the safety and extending the shelf life of dairy products [7]. Pasteurization is effective at reducing microbial load in dairy products, including *Staphylococcus aureus* and its forms MRSA. However, as shown by Aljahani et al. [8], some MRSA strains can survive temperatures up to 90°C for 60 to 90 seconds, with 10% of pasteurized camel milk samples containing heat-resistant MRSA. This indicates that traditional pasteurization may not fully eliminate MRSA in all cases. To address this, new processing technologies are being developed to enhance microbial control while maintaining product quality [8], [9]. One promising approach is thermosonication, which combines heat and ultrasound to improve pasteurization effectiveness by inducing additional cellular destabilization [7].

Thermosonication effectively targets and inactivates heat-resistant bacterial strains like MRSA while improving process efficiency. This method can reduce energy consumption and processing time while preserving the quality attributes of dairy products [7]. Ultrasound, which involves high-frequency sound waves above 20 kHz, plays a crucial role in thermosonication. These sound waves create rapid pressure changes in the liquid, resulting in mechanical stresses that can disrupt microbial cell structures. When combined with heat, ultrasound enhances microbial inactivation, making it a key component of advanced food processing technologies [10], [11]. Thermosonication not

only effectively reduces or eliminates microorganisms such as bacteria, yeasts and fungi, but also helps maintain the organoleptic and nutritional characteristics of food. By minimizing thermal exposure, it preserves natural flavors, textures, and nutritional content, making it an efficient and beneficial method in modern food processing efficient and beneficial method in modern food processing [12]. In addition, ultrasound represents an emerging environmentally friendly technology with wide applications in dairy products [6], [13].

The primary mechanisms driving sonication are acoustic cavitation and acoustic streaming, both of which are mechanical phenomena. Acoustic cavitation involves the formation and subsequent implosion of microscopic bubbles within a liquid. This implosion generates localized increase in temperature and pressure within the implosion zone. Additionally, acoustic streaming, which is the steady flow of the fluid induced by the ultrasound waves, contributes to the process. Together, these phenomena produce significant shear forces capable of disrupting microbial cell walls and membranes, thereby enhancing the microbial inactivation process in foods treated by thermosonication [6], [10], [14], [15], [16], [17]. The effectiveness of ultrasound is influenced by several factors, including frequency, ultrasonic power, sample temperature and processing time. Thus, the higher these settings, the more intense the treatment will be [10], [16].

In light of the growing concerns about food safety, this study aims to evaluate the efficacy of combining traditional pasteurization—both rapid and slow—with thermosonication in reducing MRSA counts in milk cream samples. We seek to determine whether the combined approach of thermosonication and pasteurization offers a more effective and lasting reduction of pathogens compared to conventional pasteurization alone. Our goal is to enhance MRSA inactivation in dairy cream through this integrated method, providing a more robust solution for ensuring the quality and safety of dairy products. By investigating this combination of techniques, we hope to contribute to safer dairy practices and offer greater assurance to consumers regarding the integrity of their food products.

2. Materials and Methods

2.1 Milk cream samples and bacterial strains

Samples of raw milk cream (35% fat) were obtained from a dairy factory located in São Sebastião do Passé, Bahia, Brazil (12°30'45.5" S, 38°29'27.2" W). The samples were transported under controlled refrigeration (4 ± 2 °C) in isothermal boxes and stored

at 5 ± 2 °C until processing. This study used five strains of β-lactam-resistant *Staphylococcus aureus* (MRSA), all of which are associated with livestock farming in Brazil, as reported by Silva et al. [2]. Notable among the strains were 30PD.1 and 32AD.1, isolated from cow's milk; 3N, from the nasal cavity of a milker; and 1T.1 and SFT.1, from the mammary glands of cows.

2.2 Preparation of MRSA inoculum and artificial contamination of milk cream samples

MRSA cultures were preserved in soy triptych (TSB; HiMedia, Mumbai, India) enriched with 20% glycerol at - 80 ℃. For the experiment, the cultures were revitalized in Brain Heart Infusion (BHI; Difco, Detroit, USA) broth at 36℃ for 24 hours and purified on Baird-Parker agar at 36 ℃ for 48 hours. After cultivation, the bacteria were centrifuged (3500 rpm, 10 min, 4 ℃), washed with PBS phosphate-buffered saline (pH 7.2), and resuspended in PBS to form a mixed suspension of all the strains. The optical density was adjusted to 0.5 (OD600), equivalent to 10^8 CFU/mL, and validated by counting on Baird-Parker agar and spectrophotometry (Specord 200plus, Analytik Jena, Germany).

For artificial contamination of the samples, 2 mL of inoculum (10^8 CFU/mL) was added to 200 mL of milk cream. After inoculation, the resulting concentration in the cream was 10^6 CFU/mL. The cream was left to rest for 5 minutes before being subjected to thermal treatments. The treatments consisted of pasteurization and thermosonication, as detailed in Fig.1.

2.3Heat and thermosonic treatments of milk cream samples

For our experiment, we analyzed the effect of pasteurization alone and in combination with thermosonication on milk cream (200 mL) was analyzed. The methods included rapid pasteurization (72°C for 15 seconds) and slow pasteurization (65°C for 40 minutes) using a temperature-controlled water bath with continuous stirring. Additionally, thermosonication was applied after each pasteurization process to evaluate its combined effect. The treatments consisted of pasteurization and thermosonication, as detailed in Table 1.

We utilized a VC 505 ultrasonic processor (Vibra-Cell, Sonics & Materials, Connecticut, USA) that boasts a maximum input power of 500 W and operates at a voltage of 220 V. This processor is equipped with a 13 mm diameter probe and operates at a frequency of 20 kHz. We applied powers of 31W and 35W, with exposure times as

outlined in Fig.1. Thermosonication was performed in a 600 mL jacketed beaker designed to maintain a constant temperature, which was continuously monitored using an IR thermometer (Raytek - MiniTemp FS, Raytek, Melrose, USA). To ensure thermal stability throughout the process, we used a continuous water circulation system within the beaker, linked to a thermostatic water bath (Tecnal, Model: TE2000). This setup allowed for precise temperature control, essential for the effectiveness of the thermosonication process.

Following treatment, the samples were promptly cooled in an ice water bath to halt any further microbial activity and were then stored at a controlled temperature of 4.0 ± 0.5 °C. This meticulous attention to temperature control and sample handling was critical to maintaining the integrity of our results and ensuring the reliability of our study on the inactivation of MRSA in dairy cream.

2.4 Determining energy density and temperature variation over time

The energy density was calculated using Eq. 1, based on the power and volume of the sample. The temperature variation (ΔT) was recorded before and after sonication (Eq. 2).

Equation (1)

$$\text{Energy density}\left(\frac{J}{mL}\right) = \frac{\textit{Rated power (W)* processing time}}{\textit{Sample volume}\ (\text{mL})}$$

Equation (2)

$$\Delta T = T_{\text{final}} - T_{\text{initial}}$$

where:

T_{final} is the temperature at the end of the period considered

T_{initial} is the temperature at the beginning of the period considered

2.5 Microbiological analysis and microbial inactivation efficiency

The effectiveness of the treatments was assessed by *S. aureus* counts immediately after processing (day 0) on days 15 and 30 of storage, following the guidelines established by the American Public Health Association (APHA) [18]. Positive controls (IP-72 and IP-65) were included to compare logarithmic reductions. Counting was carried out using three 10 mL portions of each sample, which were homogenized and subsequently serially diluted in 90 ml of 0.1% peptone water (Merck, Darmstadt, Germany) using a Stomacher

(Stomacher 80, Seward, London, UK) and spread on Baird-Parker agar. The plates were incubated at 36 ± 1 °C for 48 hours, and the colonies counted using a Flash & Go electronic counter (IUL Instruments, Barcelona, Spain). The results were expressed in log CFU/mL.

Analyses of indicator microorganisms were also conducted after processing (day 0) and after 30 days of storage. The presence of mesophilic aerobic bacteria and enterobacteria was determined using pour plate techniques with Plate Count Agar and Violet Red Bile Glucose Agar (VRBGA) media (Kasvi®, SP, Brazil), respectively, with incubation at 35 °C for 48 hours and 24 hours. Analysis of molds and yeasts was carried out using Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Difco, Detroit, USA) by the spread plate technique, incubated at 25 °C for 5 days in a BOD incubator [18].

To determine the microbial inactivation efficiency, the number of logarithmic reductions (γ) was calculated according to the equation proposed by Pflug [19]:

Equation (3)

$$\gamma = \log_{10}(N_0) - \log_{10}(N_f)$$

where:

N_0 represents the number of viable microorganisms in the unprocessed product

N_f is the number of viable microorganisms after treatment.

2.6 Statistical analysis

The data was analyzed in triplicate, both analytically and experimentally, and expressed as mean ± standard deviation. The normality of the data was checked using the Shapiro-Wilk test. Comparisons between treatments were made using Analysis of Variance for repeated measures (One-Way ANOVA), followed by Tukey's test for multiple comparisons. Differences between different storage days were analyzed using ANOVA followed by Tukey's test or unpaired t-test with Welch's correction, when appropriate. GraphPad Prism software (GraphPad Software, San Diego, USA) version 10.2.2 was used to perform statistical analysis and to visualize the data graphically.

3. Results and discussion

3.1. Evaluation of the effectiveness of treatments in reducing MRSA over time

The effectiveness of conventional pasteurization treatments (both rapid and slow) and thermosonication in reducing MRSA counts in cream over 30 days of storage is illustrated in Fig. 2. Initially, the cream samples were inoculated with MRSA and subjected to different treatments, with subsequent evaluations on days 0, 15 and 30 to determine the pathogen's persistence against the treatment methods. The results of day 0 were previously published as a short communication [20].

Fig. 2

According Nascimento et al. [20] a microbiological assessment of raw cream prior to treatment confirmed the absence of *Staphylococcus aureus*, ensuring that any subsequent growth was attributable solely to the strains deliberately inoculated for the study. This initial screening was crucial for establishing a controlled baseline, allowing for precise measurement of the treatment effects on the inoculated MRSA without interference from naturally occurring bacteria. This methodological rigor enhances the validity of our findings, clearly demonstrating the specific impact of the treatments on the introduced pathogenic strains.

As expected, the samples that were not inoculated and subjected to rapid pasteurization (72°C for 15 seconds; NIP-72) and slow pasteurization (65°C for 40 minutes; NIP-65) remained free of viable *S. aureus* cells throughout the study, showing no statistically significant differences ($P > 0.05$). Its confirms the baseline efficacy of these pasteurization methods in maintaining sterility in non-inoculated cream samples.

Conversely, the inoculated samples subjected to rapid pasteurization (IP-72) and slow pasteurization (IP-65) exhibited statistically significant differences ($P < 0.05$) in MRSA counts during the storage period (days 0, 15, and 30). The rapid pasteurization (IP-72) effectively reduced MRSA counts immediately after treatment. However, significant increases in bacterial counts ($P<0.05$) were observed primarily between days 0 and 15 and from days 0 to 30, suggesting a substantial rebound in MRSA levels over time. In contrast, the IP-65 treatment also demonstrated a significant increase in bacterial counts over the storage period ($P < 0.05$), yet the significant growth in MRSA counts primarily occurred after day 15, with no significant differences observed between day 0 and day 15 or between day 15 and day 30.This pattern of recovery in MRSA counts during the storage period suggests that rapid pasteurization, while initially effective, may not provide a long-lasting inhibitory effect on the growth of cells that survive the initial

treatment. This could be due to the relatively brief exposure to heat, which might not sufficiently inactivate all bacterial cells or ensure complete elimination of the microorganisms. These findings highlight the need for further refinement of pasteurization protocols or the integration of additional treatments like thermosonication to achieve more durable microbial control in dairy products. In the groups treated with thermosonication (US1 to US6), all treatments showed significant reduction in the microbial load compared to the untreated control group (IUNT), with statistically significant values ($P < 0.05$) observed throughout the storage period. However, no significant statistical differences were found among the treated groups ($P > 0.05$), suggesting that variations in the duration or the combination of rapid or slow pasteurization with ultrasound did not have immediately distinguishable effects on microbial load reduction. Within the same treatment group, statistically significant differences were observed across the days of treatment ($P < 0.05$), except in the US6 treatment group ($P > 0.05$). This indicates that, while both pasteurization and thermosonication effectively initially reduced bacterial counts to below 3 logs —a safety threshold stipulated by European Union [21] and Brazilian legislation[22]— their effectiveness waned over time. This decrease in effectiveness is evident from the microbial analysis conducted during the refrigerated storage period, as depicted in Fig. 2. These findings underscore the initial efficacy of thermosonication in significantly lowering microbial levels in dairy products. However, the lack of significant differences between the various thermosonication protocols suggests that optimizing the specific parameters of ultrasound application might not be as critical as simply employing the technology in conjunction with pasteurization. The persistence of microorganisms over time, however, points to the need for ongoing assessment of these treatments to ensure long-term food safety and compliance with health standards [23].

The impact of the treatment methods was most pronounced immediately following inoculation, as detailed in our previous study [20]. On day 0, immediately after treatment, all intervention techniques, including slow pasteurization (IP-65), rapid pasteurization (IP-72), and various intensities of thermosonication (US1 to US6), achieved a significant reduction in bacterial counts compared to the untreated control (UNT). From an initial average count of 6.82 log CFU/mL of MRSA, samples subjected to rapid pasteurization (IP-72) exhibited reductions in MRSA counts of 4.82 log CFU/mL, while slow pasteurization (IP-65) showed a reduction of 4.43 log CFU/mL. The thermosonication treatments (US1 to US6) demonstrated a comparable effectiveness to conventional

pasteurization, with an average reduction of 4.72 log CFU/mL in MRSA counts. Although the efficacy of thermosonication aligns closely with that of traditional pasteurization methods, it offers additional advantages, such as reduced processing time and increased energy efficiency. These benefits, documented in the results of our study [20], suggest that thermosonication not only meets the microbial safety standards provided by conventional pasteurization but also enhances operational efficiencies, making it a potentially more sustainable and cost-effective option in dairy processing. Various studies have demonstrated the efficacy of sonication in microbial reduction. For example, Jalilzadeh et al. [24] observed a significant reduction in the *S. aureus* count in feta cheese with the isolated application of sonication (60 kHz, 42 W, 20% intensity, for 20 minutes at 20°C), achieving a reduction of 1.95 log CFU/g after pasteurization of the retentate (78°C, for 1 minute). In addition, the inactivation rates of *S. aureus* at 20 and 40 kHz were 1.10 and 1.03 log UFC/g, respectively. However, Marchesini et al. [25] reported a remarkable resistance of *S. aureus* in raw milk to ultrasound treatments at 24 kHz and temperatures ranging from 15-46 °C for 300 seconds, with log reductions of 0.55 log, which can be attributed to a low-power application and short duration of sonication, which although capable of breaking up or dispersing bacterial clusters, do not have sufficient intensity to cause effective damage to bacterial cells [15].

Similarly, Li et al. [26] found that sonication for 15 minutes resulted in a relatively minor reduction in the *S. aureus* population of only 0.31 ± 0.02 log CFU/mL, suggesting low sublethal cell formation. In contrast, they also reported that the combination of ultrasound and moderate heat (20 kHz, 950 W, 63% input power, for 20 minutes at 55°C) resulted in a significant reduction in the viability of *S. aureus* cells by more than 6 log CFU/mL in 15 minutes, from an initial bacterial population of approximately 8.32 ± 0.08 log CFU/mL, exceeding the results of our most recent study [20]. This highlights the synergistic effectiveness of ultrasound and heat, where immediate mechanical damage combined with progressive heat-induced damage can result in more pronounced lethal effects.

The studies by Herceg et al. [27] and Balthazar et al. [28] corroborate the feasibility of this synergistic approach in dairy products. Herceg et al. [27] demonstrated the effectiveness of combining 20 kHz ultrasound at a temperature of 60°C for 12 minutes on raw milk, with a logarithmic reduction of 1.37 log CFU/mL in the *S. aures* population. Balthazar et al. [28] confirmed the significant inactivation of *S. aureus* (1.6 log CFU/mL after 6 minutes in pulsed mode, reaching temperatures between 59°C and 69°C) in semi-

skimmed sheep's milk. These results reinforce the idea that combined treatments can be especially effective, not only for significant initial inactivation but also for potentially prolonging microbiological safety during storage [29], [30], [31].

In the thermosonication treatments, both rapid (US1 to US3) and slow (US4 to US6), greater variability in bacterial counts was observed. The rapid treatments showed a higher likelihood of significant increases in counts over time, failing to maintain acceptable levels of *S. aureus* below 3 logs [21], [22]. In the slow thermosonication group, the US6 treatment was the only one that did not show significant changes in bacterial counts over time ($P > 0.05$) and managed to maintain acceptable levels immediately after treatment (day 0) and on days 15 and 30 of storage.

The results obtained with the thermosonication treatments (US-1 to US-6) highlight the importance of the operating parameters [16], [29]. Power, energy density, and exposure time were decisive for the long-term success of the treatments. Thermosonication configurations, specifically US1 to US3, which operated at 72°C with exposure times of 3 to 10 seconds and no pulse (total processing time of 6 and 10 seconds, respectively), managed to keep MRSA counts low right after processing (day 0), suggesting that even minimal variations in energy density (0.39 J/cm³, 0.72 J/cm³ and 1.47 J/cm³) have a significant impact on reducing and maintaining bacterial counts. On the other hand, treatments US5 and US6, which applied substantially higher energy densities (48.30 J/cm³ and 119.06 J/cm³, respectively) and for more extended periods (10 and 15 minutes, total processing time of 20 and 30 minutes, respectively), achieved stabilization or continuous reduction of MRSA counts, evidencing the effectiveness of a synergistic approach between ultrasound and prolonged heat treatment in inactivating bacteria.

Notably, the US4 treatment, applying a power of 35 W and a 30-second pulse to reach an energy density of 24.44 J/cm³ for 5 minutes at 65°C (total processing time of 10 minutes), resulted in an immediate and significant reduction in MRSA counts. However, despite the high energy density employed, a notable increase in these counts was observed over the storage period. This phenomenon indicates that the effectiveness of thermosonication can be strongly influenced by the specific configuration of the application parameters, such as time, energy density, and process temperature [29]. This suggests the existence of an optimal configuration of these parameters, which could maximize bacterial inactivation while minimizing the possibility of bacterial adaptation and survival. Determining the optimal model between sonication frequency and bacterial

inactivation rate requires consideration of critical factors such as the power of the ultrasound, the initial microbial load, and the shear strength of the microbial cells [16], [29], [30].

The study by Gao et al. [15] reinforces this perspective, demonstrating that the effectiveness of sonication depends crucially on the balance between the application time and the power employed. Increasing the power of the ultrasound can accelerate the rate of bacterial inactivation, but fine-tuning such parameters is essential to avoid adaptation or resistance of the bacterial cells. Furthermore, the structural and functional differences between Gram-positive and Gram-negative bacteria play a crucial role in the development of effective microbial control strategies [15], [29], [32], [33], [34]. Both Gram-positive and Gram-negative bacteria are affected by the cavitation and thermal effects induced by ultrasound, with their cell wall structures influencing their response to treatment. Therefore, optimizing ultrasound conditions for each bacterial group is crucial for achieving effective microbial control [24].

Acoustic cavitation, which involves the formation, growth, and collapse of microbubbles, can generate sufficient mechanical forces to damage the cellular structures of bacteria [15], [29]. This mechanism was crucial for our study's initial inactivation of *S. aureus* MRSA. However, the efficacy of ultrasonic treatment may vary among different bacterial species, particularly between Gram-positive and Gram-negative bacteria. The study by Shamila-Syuhada et al. [35] highlighted the variability in ultrasound resistance among other species of Gram-positive bacteria, attributing this resistance to differences in cell wall composition and structure. This variability is crucial, as Gram-positive bacteria, like *S. aureus*, have thick, peptidoglycan-rich cell walls, making them more resistant to ultrasound and similar treatments. Balthazar et al. [28] emphasize the need to adjust specific ultrasound regimes to overcome natural bacterial defenses, underscoring the importance of customization when applying such technologies.

The main mechanism of action of ultrasound in bacterial inactivation is structural damage to the cell membranes and walls [29]. Additionally, Li, Ding et al. [31] found that acoustic cavitation can weaken cell walls and increase membrane permeability, facilitating the entry of antimicrobial agents like SAEW and resulting in internal damage. Lentacker et al. [33] explain that sonoporation, characterized by the temporary formation of pores in the cell membrane, can be a temporary that the growth of microorganisms after treatments such as pasteurization and thermosonication. Moreover, the structure of the bacterial capsule plays a crucial role in protecting bacteria against the shear forces

generated by cavitation. The physical properties of the capsule, such as shape, size, thickness, and rigidity, significantly influence the ability of the bacteria to withstand these forces [15]. For instance, a thicker or more rigid capsule can increase the distance between the cavitation and the plasma membrane, reducing the impact of shear stress. This can make certain types of bacteria, such as *Staphylococcus aureus*, more resistant to ultrasound treatments regardless of their Gram-positive status [34]. Therefore, the complexity of the interactions between ultrasound parameters, bacterial characteristics, and the application environment must be considered when designing microbial inactivation protocols. Therefore, the complexity of the interactions between ultrasound parameters, bacterial characteristics and the application environment must be considered when designing microbial inactivation protocols.

After observing significant reductions in MRSA counts, provided by both pasteurization and thermosonication, it becomes essential to expand our analysis to other microorganisms that impact the safety and quality of milk cream in the dairy industry. Typically, in the food industry, microbial contamination is generated not from a single strain but involves multiple microorganisms. Additionally, bacteria are often gathered as a biofilm on the surface of food [29]. MRSA is a critical indicator of health risks due to its antibiotic resistance. However, other hygiene indicator microorganisms, such as aerobic mesophilic bacteria, enterobacteria, molds and yeasts, also compromise the quality and integrity of the final product and therefore require attention (Sert and Mercan, 2020).

3.2 *Evaluation of the efficiency of treatments in reducing mesophilic aerobic bacteria over time*

The effectiveness of conventional pasteurization (rapid and slow) and thermosonication treatments on mesophilic aerobic bacteria counts in cream samples was investigated during a 30-day refrigerated storage period (Table 2). To assess the efficacy of the applied treatments and the evolution of mesophilic aerobic bacteria counts over time, the unpaired Welch's test was employed. This statistical analysis was conducted on untreated raw material during the storage period. In the treatment inoculated with MRSA and untreated (IUNT), before the application of treatments, there was a statistically significant increase in the counts of mesophilic aerobic bacteria ($P<0.05$). Mesophiles showed a variable response depending on the treatment type. Notably, the untreated and inoculated control (IUNT) exhibited a significant increase in mesophilic populations over

the 30-day period. Specifically, the average counts started at 10.40 log CFU/mL on day 0 and rose to 12.48 log CFU/mL on day 30. However, compared to the IUNT, all implemented treatments demonstrated a significant reduction in the quantity of mesophilic aerobic bacteria (P>0.05).

(Table 2).

The inoculated samples subjected to rapid pasteurization (72°C for 15 seconds; IP-72) and slow pasteurization (65°C for 40 minutes; IP-65) showed no statistically significant differences in mesophilic bacteria counts between day 0 and day 30 ($P > 0.05$). For the non-inoculated samples subjected to slow pasteurization (NIP-65), there was also no statistically significant increase in bacterial counts ($p > 0.05$). However, in the uninoculated samples that underwent rapid pasteurization (NIP-72), there was a significant increase ($p < 0.05$) in bacterial counts from 3.47 log CFU/mL to 4.57 log CFU/mL (Table 2). This increase can be attributed to the recovery of microorganisms sublethally affected by the treatments, which indicates that although refrigeration slows down microbial growth, it does not entirely eliminate it [26], [37]. The resilience of bacterial spores, particularly genera such as *Bacillus* and *Paenibacillus*, which are notoriously resistant to extremes of temperature, acids, alkalinity and oxidizing agents, suggests that incomplete initial inactivation may allow these dormant forms to recover, thus compromising the shelf life of dairy products [26], [37].

In the thermosonication-treated groups (US-1 to US-3), which were subjected to rapid pasteurization followed by sonication for 3, 5 and 10 seconds (total processing time of 6, 10 and 20 seconds, respectively), respectively, there were variations in the counts of mesophilic bacteria, between storage times (day 0 and day 30). However, these counts remained within the limits set by regulatory standards [21], [22]. Specifically, the short sonication treatments (3 and 5 seconds) for US1 and US2, which started with lower initial counts of 3.15 and 3.30 log CFU/mL, respectively, showed statistically significant increases ($P < 0.05$) in bacterial counts at the end of the treatment, reaching 4.14 and 4.24 log CFU/mL (Table 2). These results are consistent with those observed by Lim, Benner, and Clark [37], who found that bacterial counts in milk increased after short periods of sonication (20, 30, and 60 seconds) following pasteurization at 72.5°C. These counts exceeded acceptable microbiological limits during refrigerated storage, highlighting the need for careful evaluation. The increase in bacterial counts in US1 and US2 indicates

that brief sonication periods may not sustain reductions in bacterial load, possibly due to a rapid recovery of bacteria after the initial treatment. In addition, the high pasteurization temperatures appear to be the predominant factor in the initial reduction in bacterial load [7],[11]). Although pasteurization alone effectively reduces bacterial counts initially, the subsequent rise in counts suggests a limitation in long-term bacterial control when sonication is applied for very short periods [15], [37]. Group US3, with a 10-second sonication, showed no statistically significant changes ($p > 0.05$) in the counts, suggesting greater stability under this treatment regime.

In groups US4 to US6, which were subjected to slow pasteurization followed by sonication for periods of 5, 10 and 15 minutes, respectively (total processing time of 10, 20, and 30 minutes, respectively), there were no statistically significant changes ($p > 0.05$) in bacterial counts over time, indicating bacterial stability. This result suggests that the combination of slow pasteurization and prolonged sonication can effectively keep bacterial counts within stable limits, without promoting a significant reduction or increase in the microbial load. This finding contrasts with the results of other studies investigating the application of ultrasound and thermosonication to food products, which have shown considerable diversity in their results. For example, Balthazar et al. [28] reported a significant reduction in bacterial load in semi-skimmed sheep's milk using sonication at 20 kHz with powers of 78 W and 104 W. Initial bacterial counts were low, at 1.81 and 2.7 log CFU/mL, respectively, and remained stable at 1.7 and 2.7 log CFU/mL during storage at 4°C ± 0.5. In particular, one of the treatments with 104 W and 936 J/mL for 6 minutes at temperatures of 59°C and 69°C showed no bacterial growth either during processing or after refrigerated storage. On the other hand, Sert and Mercan [36] observed a significant reduction in the counts of aerobic mesophilic bacteria in the butter from cream after treatments at 85°C for periods of 5, 10 and 15 minutes.

The variation in results between different studies and those observed in our research can be attributed to differences in treatment conditions and particular characteristics of the food matrices used. The effectiveness of sonication in microbial inactivation is strongly influenced by the operating conditions, particularly the power and energy density of the ultrasonic equipment [28]. Power, defined as the amount of energy (in joules) transmitted per second to the medium, and energy density, which quantifies the energy per volume of liquid treated (in joules per cubic centimeter), are determining factors for the effectiveness of the treatment [37].

In our study, the power used was 35 W, which may have been insufficient to induce effective acoustic cavitation, which is essential for the violent collapse of microbubbles in the milk cream. This phenomenon generates intense mechanical forces capable of breaking bacterial cell walls [7]. In contrast, Barukčić et al. [38] reported that increasing the power from 480 W to 600 W, with treatments at 55 °C for 8 minutes, resulted in a reduction of one logarithmic cycle in the viable cell count. This suggests that higher powers may be more effective in inducing cavitation and, consequently, bacterial inactivation. Additionally, energy density is a crucial factor. In our study, the energy density was 24.44 J/cm³, considerably lower than 93.6 J/cm³ used by Balthazar et al. [28]. High energy densities intensify the mechanical (sonoporation) and thermal effects generated by sonication, enhancing microbial inactivation [29], [33]. This disparity in operating conditions may be responsible for the lower efficacy observed in our study compared to others that used more intense sonication conditions.

Although most of the treatments did not show statistically significant changes, groups US5 and US6 exhibited particularly remarkable bacterial stability, between storage times (day 0 and day 30).

The precise configuration of the sonication parameters in these groups directly impacted the stability of the bacterial load over the storage period. The US5 treatment carried out with a power of 33 W and an energy density of 48.30 J/cm³, involved 10 minutes of sonication in pulsed mode at 65 ºC. Treatment US6 used a power of 35 W and an energy density of 119.06 J/cm³, with 15 minutes of sonication also in pulsed mode at 65 ºC (total processing time of 30 minutes). These adjustments resulted in minimal variations in bacterial growth, with growth variations of just 0.03 and 0.11 log, respectively, between the beginning and end of the 30 days of refrigerated storage. These results emphasize the effectiveness of these specific treatments in suppressing bacterial proliferation, suggesting that carefully adjusted sonication parameters may be fundamental to maintaining bacterial stability and ensuring food safety during prolonged storage [11]. The stability observed in groups US5 and US6 highlights the importance of precisely adjusting parameters such as power, energy density and sonication duration, particularly in pulsed mode, which includes cooling periods between pulses. In addition, applying a temperature of 65 °C for 10 and 15 minutes, respectively, in the treatments proved to be effective in stabilizing the microbial load. This thermal regime, shorter than the typical 40 minutes of slow pasteurization without sonication (IP-65), minimizes thermal damage to the product and maximizes the physical destabilization of bacterial

cells. This approach balances microbial efficacy and product integrity, with total processing times of 20 and 30 minutes, respectively.

Although our results show a moderate effectiveness in containing the growth of mesophilic bacteria, keeping the counts within acceptable limits after 30 days of refrigerated storage, studies such as those by Barukčić et al. [38] and Balthazar et al. [28] highlight the significant influence of treatment conditions on the viability of microbial cells. For example, Barukčić et al. [38] reported an increase of approximately two logarithmic cycles in the viable cell count after applying thermosonication at 45°C with 600 W for 10 minutes, a phenomenon attributed to the disintegration of microbial cell clusters that released viable cells without exerting complete lethal effects. In contrast, Balthazar et al. [28] observed greater effectiveness in destabilizing and rupturing bacterial cells when applying higher temperatures, 59°C and 69°C, for 6 minutes.

The composition of the food matrix is essential for the effectiveness of ultrasonic energy transmission and thermal distribution, directly influencing the uniformity and effectiveness of the ultrasonic treatment. In this study, focused on raw milk cream - a viscous, high-fat matrix - significant differences are observed compared to semi-skimmed sheep's milk [28] and whey [38], both in nutritional composition and physical properties [39]. The high viscosity of milk cream can reduce the efficiency of ultrasonic energy transmission, resulting in slower and less uniform propagation of ultrasonic waves, requiring precise adjustments to treatment parameters to ensure adequate antimicrobial efficacy [29].

In addition, the high fat concentration in milk cream can serve as a protective barrier for microorganisms, exceptionally those capable of associating with or infiltrating lipid phases, making them less accessible to antimicrobial agents [39], [40]. In emulsions such as cream, fat globules can encapsulate microorganisms, protecting thermal or chemical treatments by isolating bacteria and minimizing their direct exposure to treatment agents [39]. This feature highlights the need to adapt treatment technologies, such as thermosonication, to the particularities of the food matrix to guarantee effective microbial reduction and maintain the quality and safety of processed foods.

The literature on ultrasonic and thermosonic treatments is characterized by a notable lack of standardization, which prevents direct comparisons between studies due to the significant variations in treatment configurations. The absence of a consensus on how to quantify the acoustic energy applied intensifies the difficulties of effectively comparing results [16]. This diversity in treatment parameters highlights the need for in-

depth investigations that explore how these parameters interact with the specific characteristics of food matrices. Furthermore, the development of a standardized experimental protocol for the inactivation of microorganisms in products such as milk cream using ultrasound would be extremely useful, although such a protocol is not yet available [11], [16].

3.3 *Evaluation of the efficiency of treatments in reducing enterobacteria over time*

Our study evaluated the impact of conventional pasteurization (rapid and slow) and thermosonication treatments on *Enterobacteriaceae* counts in milk cream during a 30-day refrigerated storage period. The initial counts of these microorganisms in the raw material were 2.95 log CFU/mL before treatment application (day 0), increasing to 8.15 log CFU/mL by day 30, indicating a statistically significant difference ($P<0.05$) (Table 2). Interestingly, no significant statistical differences ($P> 0,05$) were observed between the pasteurized and thermosonic treatments immediately after processing, as both managed to reduce the microbial load to zero. All pasteurization and sonication treatments effectively reduced *Enterobacteriaceae* to non-detectable levels immediately after processing. Interestingly, there was a varying degree of regrowth in some treatments after 30 days, notably in the IUNT and thermosonication-enhanced treatments like US3 and US6. This suggests that, for this specific microorganism and on this specific day, all treatments were equally effective.

The absence of enterobacteria immediately after the treatments indicates that both pasteurization and thermosonication have the potential for initial sterilization. However, the increase in counts during refrigerated storage highlights the challenges of controlling these bacteria in the long term. Statistical analysis revealed significant differences ($P < 0.05$) between treatments in terms of limiting the growth or absence of growth of enterobacteria, with treatments such as IP-72, US2, US4 and US5 showing no statistically significant differences between them ($P > 0.05$), suggesting that they are equally the best treatments for reducing the count of this microorganism. This suggests the superiority of some thermosonication protocols.

It has been observed that the initial absence of enterobacteria after treatments, followed by an increase in subsequent storage periods, may be due to sublethal damage to the microorganisms [29]. The mechanical effects caused by cavitation cause different types of physical damage to cell walls. Gram-negative bacteria often show a higher sensitivity to these effects compared to Gram-positive bacteria. This is because the

microflows and shock waves induced by cavitation cause mass transfer processes and localized damage to cell walls. Such damage may not always lead to complete cell death, allowing bacteria to repair and recover over time [32]. The differential susceptibility of Gram-negative and Gram-positive bacteria to ultrasonic treatments, as evidenced in this study, illustrates the complexity of the interaction between ultrasound technology and food microbiology. The unique structure of the cell walls of Gram-negative bacteria, which includes an outer membrane rich in lipopolysaccharides (LPS), offers initial protection against hydrophobic agents and reduces mechanical damage [15]. Most Gram-negative bacteria show high resistance to lipophilic natural antibacterial agents due to the impermeable nature of their complex outer membrane structure, where the ultrasound technique can be effective [29]. However, this same structure makes these bacteria particularly vulnerable to ultrasound-induced cavitation. Gram-negative bacteria have been shown to be less resistant to ultrasound than Gram-positive bacteria [13], [29].

As pointed out by Gao et al. [15], large cells are more easily inactivated by ultrasound than small cells, and rod-shaped bacteria are considered more sensitive than cocci. Complementing these observations, Gera and Doores [30] results highlight that *Escherichia coli*, which belongs to the *Enterobacteriaceae* family, shows significantly shorter decimal reduction times than *Listeria monocytogenes*, a Gram-positive bacterium, under ultrasonic treatment. This suggests faster inactivation, possibly due to the lower resistance of their cell walls to cavitation. However, the growth observed after 30 days of refrigerated storage shows that these microorganisms were able to multiply during this time. In addition, the presence of lactose has been identified as a factor that can offer additional protection to bacteria [27]. In milk cream, the presence of lactose exerts a sonoprotective effect during treatment with ultrasonic waves. Gera and Doores [30] observed that the presence of lactose in the solution can protect bacteria against inactivation by ultrasound. The increased stability of cell membranes was attributed to the bond between the polar head group (phosphate group) of the phospholipid layer and the free -OH group of the sugar. They identified lactose as a component that exerts a sonoprotective effect due to its ability to stabilize cellular structures, such as proteins and membranes, and to act as a compatible solute, maintaining osmotic balance and protecting bacteria from the physical damage caused by ultrasound [30]. This finding suggests that the response of bacteria to ultrasound may vary not only depending on the bacterial species, but also on the composition of the environment in which they are found. The study by Herceg et al. [27] confirms that *Escherichia coli* is more susceptible to

ultrasound than *Staphylococcus aureus* in cow's milk, reinforcing the idea that the specific characteristics of both the environment and the microorganism can significantly influence the effectiveness of the treatment.

3.4 *Evaluation of the treatment efficiency in reducing molds and yeasts over time*

Table 2 shows the effectiveness of conventional pasteurization treatments (rapid and slow) and thermosonication in controlling the growth of fungi in milk cream over 30 days. Initial analysis of the raw material indicated a significant increase in fungal counts, from 1.00 log CFU/mL on day 0 to 8.26 log CFU/mL on day 30. This observation is pertinent because creams, typically kept under refrigeration and often produced from thermally treated milk, including some fermented products, pose specific preservation challenges [41]. Interestingly, the US-6 treatment was the least effective on day 0, significantly increasing ($P<0.05$) the microbial count compared to the IUNT treatment. Similar to *Enterobacteriaceae*, most treatments significantly reduced molds and yeasts, with many maintaining low levels throughout the storage period. The IUNT showed a notable increase, suggesting that the lack of initial processing allowed for substantial fungal growth over time.

The uninoculated samples subjected to rapid pasteurization (72°C for 15 seconds; NIP-72) showed a significant increase ($P < 0.05$) in fungal counts between day 0 (with no counts) and day 30 with counts of 3.68 log CFU/mL (Table 2). This indicates that rapid pasteurization was not effective in controlling fungi over the refrigerated storage time. This increase can be attributed to the intrinsic ability of molds and yeasts to enter states of dormancy or resistance, allowing them to survive in sublethal conditions and recover later when conditions become more favorable. In addition, their complex cell structure, which can include thick cell walls and protective substances, contributes to this resilience ([15], [42].

In contrast, slow pasteurization (NIP-65) showed no significant difference ($P > 0.05$) in fungal counts, suggesting greater efficacy in fungal control over storage time. For the inoculated treatments, slow pasteurization (IP-65) and rapid pasteurization (IP-72) showed no significant differences ($P > 0.005$) between day 0 and day 30. This suggests that, the pasteurization treatments maintained stability in the fungal counts.

In the thermosonication-treated groups (US-1 to US-6), there were variations in mold and yeast counts over the storage time. Groups US-1, US-2 and US-3, which were subjected to rapid pasteurization followed by sonication for 3, 5 and 10 seconds (total

processing time of 6 and 10 seconds, respectively), respectively, showed statistically significant differences ($p < 0.05$) between day 0 and day 30. Treatments US1 and US3 showed initial counts of 2.58 log UFC/mL and 2.45log UFC/mL, respectively, on day 0. However, there was no detectable fungal growth on day 30. This suggests that the combination of rapid pasteurization and sonication was effective in controlling fungi over time. However, temperature may have been the determining factor. In short times of exposure to sonication, a very small amount of sonoporation occurs, accompanied by the formation of microcracks and ruptures in the cell wall. On the other hand, at prolonged times, sonoporation occurs in excess, resulting in severe mechanical shear and cell rupture [14].

On the other hand, the US-2 treatment showed an increase in fungal counts, from 2.46 log UFC/mL to 3.55 log UFC/mL between day 0 and day 30, indicating a growth of 1.10 log. This result suggests that sonication for 5 seconds (total processing time of 10 seconds) was not effective in controlling fungi over the storage period. In the group of samples subjected to slow pasteurization followed by sonication in minutes, there were significant differences ($P < 0.05$) in the fungal counts between day 0 and day 30 in groups US4 and US5. The US4 group had an initial count of 2.00 log UFC/mL, which increased to 4.62 log UFC/mL, while the US5 group went from no growth on day 0 to growth of 2 log UFC/mL on day 30. This indicates that, despite the increase in energy density, 10 minutes of slow thermosonication was not enough to control fungal growth. In the US-6 group, the fungal counts showed no significant differences ($P > 0.05$) over time, suggesting that the 15-minute sonication treatment resulted in a total processing time of 30 minutes, which may have been moderately effective in controlling fungi. However, it is worth noting that this treatment already had a high count on day 0, with 4.89 log CFU/mL, compared to other treatments which started with lower initial counts (Table 2). This suggests that, despite the extended sonication time, the high initial fungal load may have affected the effectiveness of the treatment.

In contrast to our studies, Jalilzadeh et al. [24] observed no significant differences between different ultrasound frequencies for the deactivation of *P. chrysogenum* in ultrafiltered Iranian feta cheese stored for 30 days. The reductions on the seventh day of ripening at frequencies of 20, 40 and 60 kHz were 1.11, 1.07 and 1.11 log CFU/mL, respectively. The resistance of fungi to inactivation treatments can be attributed to specific characteristics of certain species, which show resistance to heat, especially due to the presence of ascospores. These ascospores can be activated when heat treatments do

not reach temperatures high enough to inactivate them. In addition, the characteristics of the fungi's cell walls confer greater resistance to sonication [15], [41].

In the present study, which used low-frequency ultrasound (20 kHz), some inactivation was observed in treatments such as US-1 and US-3 over 30 days of storage, but growth was observed in treatments US-4 to US-6. Despite the use of high-frequency ultrasound (850 kHz) by Gao et al. [42], the inactivation of *A. pullulans* suspensions was also less effective. The acoustic cavitation induced by high-frequency ultrasound could have caused mechanical effects and sonochemical reactions, such as the formation of hydroxyl radicals in the aqueous medium. However, low-frequency and high-power ultrasound treatments, such as those used in our studies, showed inactivation that can be attributed to the mechanical effects of acoustic cavitation. The growth observed in fungi over the 30 days of storage under refrigeration can largely be attributed to the differences in the composition of the cell wall of these microorganisms compared to bacteria. This finding was also made by Gao et al. [15], who studied microorganisms of different sizes. They selected the Gram-negative bacterium *Enterobacter aerogenes*, with a peak diameter of 1.1 μm, and the yeast *Aureobasidium pullulans*, with a peak diameter of 7.6 μm. In comparison, the yeast proved to be more resistant to ultrasound, due to its greater ability to withstand shear forces. The predominant composition of the cell wall of *A. pullulans*, composed mainly of mannoproteins and glucans, confers superior resistance to ultrasound when compared to *E. aerogenes*.

3.5 *Evaluation of the temperature variation of thermosonication treatments on milk cream samples*

As shown in Table 3, the temperature variation was significant ($p < 0.05$) between the six treatments subjected to thermosonication. This indicates that sonication affected the final temperatures in different ways. Treatments US1, US2 and US3 were subjected to short sonication periods of 3, 5 and 10 seconds (total treatment time of 6 and 10 seconds, respectively). No change in temperature was observed during these treatments, as indicated by a ΔT equal to 0, probably due to the short time the thermosonication applied. On the other hand, in treatments US4 to US6, which were subjected to longer sonication periods of 5, 10 and 15 minutes, the temperature variation was more noticeable, resulting in total processing times of 10, 20, and 30 minutes, respectively. The US4 treatment (thermosonication for 5 minutes) showed the greatest temperature variation, while US5 (10 minutes of thermosonication) showed the least variation, resulting in total processing

times of 10 and 20 minutes, respectively. On the other hand, in treatments US4, US5 and US6, which were subjected to longer periods of sonication (5, 10 and 15 minutes, respectively), the temperature variation was more noticeable. Treatment US4 (5 minutes of thermosonication) showed the greatest temperature variation, while treatment US5 (10 minutes of thermosonication) showed the least variation.

Table 3

These findings align with observations made by Balthazar et al. [28], who reported significant temperature variations during ultrasound treatments. For instance, their study on semi-skimmed sheep's milk revealed that sonication at 20 kHz with a power of 104 W for 6 minutes led to temperature ranges from 59°C to 69°C. In contrast, a different treatment in their study, US-2, showed temperatures between 53°C and 63°C ± 1. The rise in temperature is a direct consequence of the cavitation generated by ultrasound, which can increase the effectiveness of the treatment. Cavitation leads to the formation of microbubbles that implode, releasing thermal and mechanical energy. This process contributes to the observed temperature rise, which can be useful for microbial inactivation [43].

To avoid overheating the sample in our study, we used a jacketed container connected to a water bath to allow water circulation and temperature control, as in the studies by Sert and Mercan [36] with cream for butter production, and Barukčić et al. [38] who studied thermosonication in sweet whey samples. The use of a jacketed container provides more precise temperature control during heat treatments, which is an important consideration when comparing treatments involving ultrasound, since cavitation and the collapse of gas bubbles also cause rapid temperature changes [43].

The temperature of the milk cream during the application of ultrasound confirms the occurrence of the cavitation phenomenon, characterized by the collapse and implosion of bubbles, with a notable increase at the end of the process. A significant variation in temperature (high ΔT) may have enhanced the thermal effect, thus increasing the effectiveness of inactivating *S. aureus* MRSA and other indicator microorganisms. This increase in temperature may have enhanced the bactericidal effect of ultrasound-induced cavitation. Furthermore, this assumption is supported by comparing the calculated ultrasonic intensities, which were considerably lower in the case of sonication treatments combined with temperature [14].

Finally, the results of this study indicate that combining pasteurization with thermosonication is generally more effective in reducing microbial loads than pasteurization alone. The effectiveness of sonication appears to be both time and intensity-dependent, with longer or more intense sonication periods leading to lower microbial counts initially and throughout the storage period.

4. Conclusion

The study underscores the importance of optimizing milk cream processing techniques to balance microbial safety with quality preservation. The integration of thermosonication with pasteurization offers a promising approach to enhance microbial inactivation, though the specific parameters (duration and intensity of sonication) need careful adjustment to maximize efficacy and prevent microbial regrowth during storage. This study's findings contribute to the broader field of food microbiology by providing insights into the microbial dynamics in processed dairy products, which is crucial for developing safer and higher-quality food products.

Author Contributions:

Conceptualization, J.C.N.N. and M.P.C.; methodology, J.C.N.N. M.J.G.S., K.G.-Á., J.C.G.A., J.G.S., B.N.P. and M.P.C.; formal analysis, J.C.N.N. M.J.G.S., K.G.-Á. and J.C.G.A.; investigation, J.C.N.N. M.J.G.S., K.G.-Á., J.C.G.A., J.G.S., B.N.P. and M.P.C.; resources, M.P.C..; data curation, J.C.N.N. and M.P.C.; writing—original draft preparation, J.C.N.N. and M.P.C.; writing—review and editing J.C.N.N., M.J.G.S., K.G.-Á., J.C.G.A., R.B.S., J.G.S., B.N.P. and M.P.C.; visualization, J.C.N.N., B.N.P. and M.P.C.; supervision, J.G.S., B.N.P. and M.P.C.; project administration, J.G.S., B.N.P. and M.P.C.; funding acquisition, M.P.C. All authors have read and agreed to the published version of the manuscript**.**

Declaration of competing interest

The authors declare that there were no commercial or financial relationships that could be interpreted as a potential conflict of interest in the conduct of the study.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Brazil—grant number [303074/2021-3].

782 **Acknowledgements**

783 The authors are thankful for the financial support provided by Conselho Nacional de
784 Desenvolvimento Científico e Tecnológico (CNPq), Laboratório de Controle de
785 Qualidade de Produtos Farmacêuticos (CQFar) and Laboratório de Inspeção e Tecnologia
786 de Leites e Derivados (LaITLácteos).

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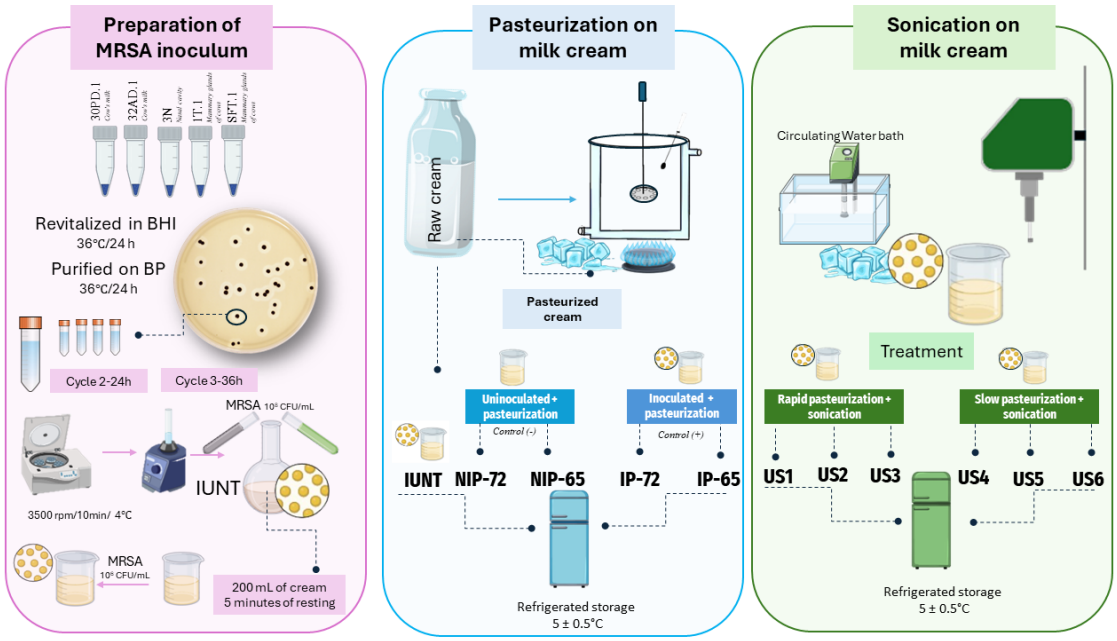
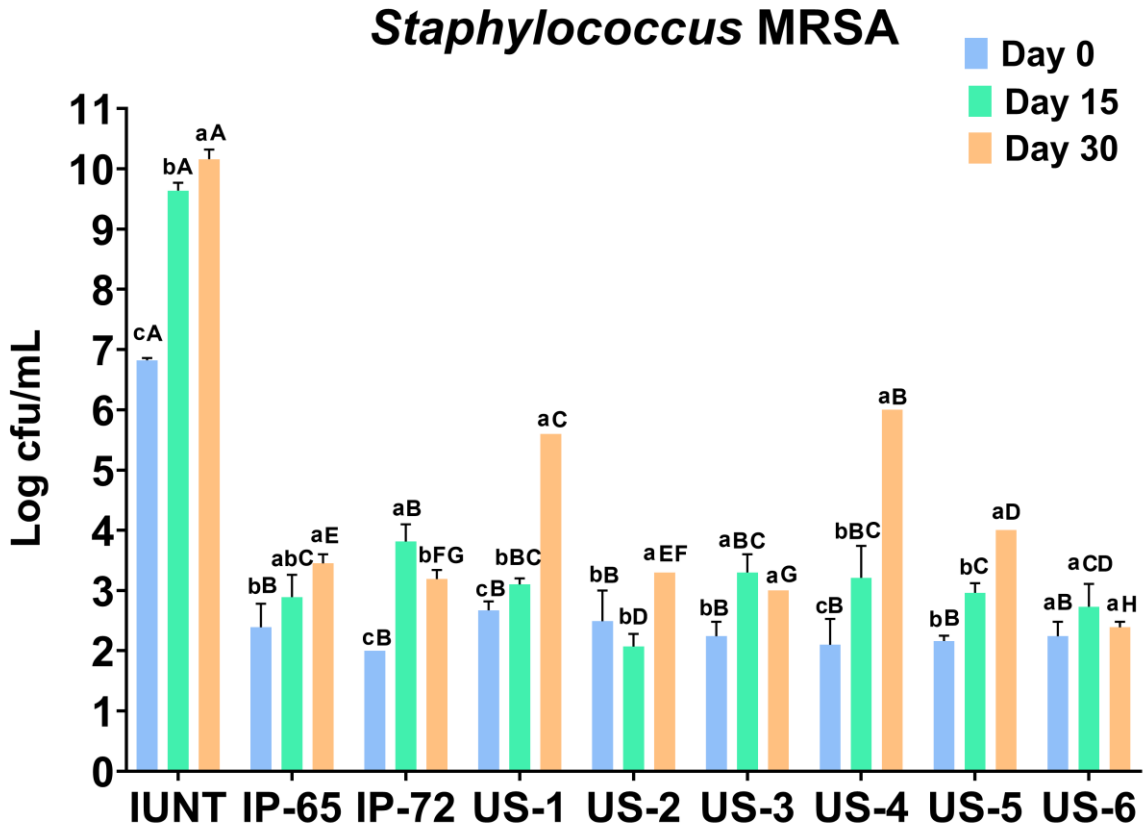
FIGURE

Fig. 1. Processing parameters used in the treatments of cream and *Staphylococcus* MRSA counts (log CFU/mL) in milk cream samples treated with pasteurization and thermosonication immediately after processing.



IUNT, inoculated and untreated; NIP-72, uninoculated and rapid pasteurization; NIP-65, uninoculated and slow pasteurization; IP-72, inoculated and rapid pasteurization; IP-65, inoculated and slow pasteurization; US-1, inoculated, rapid pasteurization and sonication for 3 s; US-2, inoculated, rapid pasteurization and sonication for 5s;US-3, inoculated, rapid pasteurization and sonication for 10s; US-4, inoculated, slow pasteurization and 5 min sonication; US-5, inoculated, slow pasteurization and sonication for 10 min; US-6, inoculated, slow pasteurization and Sonication for 15 minutes.

Fig. 2. *Staphylococcus aureus* MRSA counts (log CFU/mL) of sour cream samples immediately after processing (0 d), 15 days (15 d) and 30 days (30 d) of refrigerated storage at 5 °C (±0. 5).



NIP-72, uninoculated and rapid pasteurization; NIP-65, uninoculated and slow pasteurization; IP-72, inoculated and rapid pasteurization; IP-65, inoculated and slow pasteurization; US-1, inoculated, rapid pasteurization and sonication for 3 s; US-2, inoculated, rapid pasteurization and sonication for 5 s;US-3, inoculated, rapid pasteurization and sonication for 10 s; US-4, inoculated, slow pasteurization and 5 min sonication; US-5, inoculated, slow pasteurization and sonication for 10 min; US-6, inoculated, slow pasteurization and Sonication for 15 minutes.
Staphylococcus MRSA(A); total count of mesophilic aerobic bacteria (B); Enterobacteriaceae count (C); Mold and Yeast Count (D). Different letters mean meaning difference $p < 0.05$.
Means ± standard deviation followed by different lowercase letters within rows indicate statistical difference between treatments ($p < 0.05$). Means ± standard deviation followed by different uppercase letters within columns indicate statistical difference between days ($p < 0.05$).

Table 1. Processing parameters used in the treatments of cream and *Staphylococcus* MRSA counts (log CFU/mL) in milk cream samples treated with pasteurization and thermosonication immediately after processing (0 d). at 14 days and 30 days of refrigerated storage (5 ± 0.5 °C).

Treatment	Power (W)	Pulse (s)	Energy density (J/cm^3)	Temperature (°C)	Pasteurization Time	Sonication Time	Total Processing Time
IUNT	-	-	-	-	-	-	-
NIP-72	-	-	-	72	20 sec	-	-
NIP-65	-	-	-	65	40 min	-	-
IP-72	-	-	-	72	20 sec	-	-
IP-65	-	-	-	65	40 min	-	-
US1	31	0	0.39	72	3 sec	3 sec	6 sec
US2	31	0	0.72	72	5 sec	5 sec	10 sec
US3	31	0	1.47	72	10 sec	10 sec	20 sec
US4	35	30	24.44	65	5 min	5 min	10 min
US5	33	30	48.30	65	10 min	10 min	20 min
US6	35	30	119.06	65	15 min	15 min	30 min

IUNT. inoculated and untreated; **NIP-72**. uninoculated and rapid pasteurization; **NIP-65**. uninoculated and slow pasteurization; **IP-72**. inoculated and rapid pasteurization; **IP-65**. inoculated and slow pasteurization; **US-1**. inoculated. rapid pasteurization and sonication for 3 s; **US-2**. inoculated. rapid pasteurization and sonication for 5 s; **US-3**. inoculated. rapid pasteurization and sonication for 10 s; **US-4**. inoculated. slow pasteurization and 5 min sonication; **US-5**. inoculated. slow pasteurization and sonication for 10 min; **US-6**. inoculated. slow pasteurization and Sonication for 15 minutes.

840 **Table 2.** Survival concentrations (log CFU/mL) of indicator microorganisms in milk cream samples treated with pasteurization and thermosonication
841 immediately after processing (0 d) and during refrigerated storage (30 d) (5 ± 0.5 °C).

Treatments	Mesophiles		*Enterobacteriaceae*		Molds and Yeasts	
	0d	30 d	0d	30 d	0d	30 d
IUNT	10.38 ± 0.40^{bA}	12.48 ± 0.00^{aA}	2.95 ± 0.09^{bA}	8.15 ± 0.04^{aA}	1.00 ± 0.00^{bCD}	8.26 ± 0.11^{aA}
NIP-72	3.47 ± 0.09^{bDE}	4.57 ± 0.26^{aCE}	0.00 ± 0.00^{bB}	2.60 ± 0.00^{aC}	0.00 ± 0.00^{bD}	3.68 ± 0.06^{aC}
NIP-65	4.89 ± 0.70^{aBC}	5.48 ± 0.10^{aB}	0.00 ± 0.00^{bB}	1.73 ± 0.69^{aD}	2.79 ± 0.71^{aB}	3.07 ± 0.16^{aD}
IP-72	4.49 ± 0.43^{aBCD}	5.06 ± 0.22^{aBC}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aE}	2.31 ± 0.21^{aBC}	2.00 ± 0.00^{aE}
IP-65	3.95 ± 0.57^{aDE}	4.81 ± 0.00^{aBE}	0.00 ± 0.00^{bB}	3.13 ± 0.04^{aC}	2.82 ± 0.81^{aB}	2.81 ± 0.10^{aD}
US1	3.15 ± 0.15^{bE}	4.14 ± 0.23^{aE}	0.00 ± 0.00^{bB}	4.53 ± 0.05^{aB}	2.58 ± 0.75^{aB}	0.00 ± 0.00^{bF}
US2	3.30 ± 0.00^{bE}	4.24 ± 0.12^{aDE}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aE}	2.46 ± 0.45^{bBC}	3.52 ± 0.35^{aC}
US3	5.00 ± 0.00^{aB}	4.75 ± 0.28^{aCE}	0.00 ± 0.00^{bB}	3.90 ± 0.50^{aB}	2.45 ± 0.54^{aBC}	0.00 ± 0.00^{bF}
US4	5.02 ± 0.04^{aB}	4.89 ± 0.50^{aBCD}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aE}	2.00 ± 0.00^{bBC}	4.62 ± 0.04^{aB}
US5	4.34 ± 0.38^{aBCD}	4.35 ± 0.04^{aDE}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{bD}	2.00 ± 0.00^{aE}
US6	4.68 ± 0.17^{aBC}	4.79 ± 0.29^{aCE}	0.00 ± 0.00^{bB}	4.51 ± 0.00^{aB}	4.89 ± 0.81^{aA}	4.49 ± 0.02^{aB}

842 **IUNT**. inoculated and untreated; **NIP-72**. uninoculated and rapid pasteurization; **NIP-65**. uninoculated and slow pasteurization; **IP-72**. inoculated and rapid pasteurization; **IP-**
843 **65**. inoculated and slow pasteurization; US-1. inoculated. rapid pasteurization and sonication for 3 s; **US-2**. inoculated. rapid pasteurization and sonication for 5 s; **US-3**.
844 inoculated. rapid pasteurization and sonication for 10 s; US-4. inoculated. slow pasteurization and 5 min sonication; **US-5**. inoculated. slow pasteurization and sonication for 10
845 min; **US-6**. inoculated. slow pasteurization and Sonication for 15 minutes.
846 Values were expressed as a mean±SD (n=2).
847 $^{a–c}$ Different lowercase superscripts indicate significant differences among storage times ($p<0.05$).
848 $^{A–D}$ Different uppercase superscripts indicate significant differences among treatments of milk cream samples ($p<0.05$).
849

850

Table 3. Change in temperature of cream samples during thermosonication treatments

Treatment	Initial T (°C)	Final T (°C)	Δ T
US1	72 ± 0.00^{a}	72 ± 0.00^{a}	0 ± 0.00^{a}
US2	72 ± 0.00^{a}	72 ± 0.00^{a}	0 ± 0.00^{a}
US3	72 ± 0.00^{a}	72 ± 0.00^{a}	0 ± 0.00^{a}
US4	60.83 ± 0.12^{b}	64.53 ± 0.59^{b}	3.7 ± 0.52^{b}
US5	62.70 ± 0.30^{b}	65.23 ± 1.25^{b}	2.5 ± 1.12^{b}
US6	64.00 ± 0.20^{b}	67.17 ± 0.35^{ab}	3.2 ± 0.32^{b}

US-1. inoculated. rapid pasteurization and sonication for 3 s; **US-2**. inoculated. rapid pasteurization and sonication for 5s; **US-3**. inoculated. rapid pasteurization and sonication for 10 s; **US-4**. inoculated. slow pasteurization and 5 min sonication; **US-5**. inoculated. slow pasteurization and sonication for 10 min; **US-6**. inoculated. slow pasteurization and Sonication for 15 minutes.

Different letters mean meaning difference $p < 0.05$.

ΔT Temperature change, defined as the difference between the final and initial temperatures ($\Delta T = T\_final - T\_initial$).

$^{a–b}$ Different lowercase superscripts indicate significant differences among treatments of milk cream samples ($p<0.05$).

5.3

Manuscrito: Staphylococcus aureus, a food pathogen: Current challenges and perspectives in food safety

Staphylococcus aureus, a food pathogen: Current challenges and perspectives in food safety

Periódico a ser submetido (1ª submissão):	*Trends in Food Science & Technology* ISSN: 0924-2244
Maior percentil (Scopus):	99%
Periódico a ser submetido (2ª submissão):	*Critical Reviews in Food Science and Nutrition – ISSN: 1040-8398*
Maior percentil (Scopus):	98%

Abstract

Staphylococcus aureus is is an important zoonotic pathogen causing severe infections in both humans and animals. Food contaminated with staphylococcal enterotoxins can lead to food poisoning, presenting symptoms like nausea, vomiting, and diarrhea. The emergence of antimicrobial resistance, particularly multidrug-resistant strains transmitted through food, is a pressing global issue. Understanding these challenges is crucial for food safety. This review provides a comprehensive look at enterotoxigenic staphylococci and their toxins in various foods, discussing their impact on public health. It specifically addresses methicillin-resistant strains involved in foodborne illnesses. Strategies for prevention and control include hygiene practices, temperature management, and advancements in detection technologies. Innovations in natural antimicrobial agents and thermal/non-thermal technologies are improving food safety measures without compromising quality.Despite advancements, challenges remain in combating *Staphylococcus aureus* contamination. Ongoing research focuses on developing new technologies to deactivate the pathogen and its toxins. In conclusion, addressing these challenges requires sustained efforts to enhance food safety protocols, alongside identifying future research needs in technology development.

Keywords: Enterotoxin; Methicillin-resistant *Staphylococcus aureus*; Antimicrobial resistance; Inactivation technology; Food safety

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1. Introduction

Unsafe food poses a serious threat to human health. Each year, up to 600 million people worldwide become ill after eating contaminated food, and approximately 420,000 die, with 40% of these deaths occurring among children under 5 years of age (WHO 2022). The Center for Disease Control and Prevention (CDC) estimates that 48 million people in the United States (one in six Americans) develop foodborne disease, resulting in 128,000 hospitalizations and 3,000 deaths each year (CDC 2022; FDA 2019). In 2021, the 27 country members of the European Union (EU) reported 4,005 outbreaks of foodborne illness, an increase of 29.8% compared to 2020, with bacteria being the leading causative agents (EFSA 2022a). More broadly, over half of all global bacterial deaths in 2019 were attributed to just five pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Ikuta et al. 2022). The widespread impact of these pathogens highlights the need for global surveillance systems, improved diagnostic methods, and enhanced strategies for infection prevention and control.

Interestingly, the COVID-19 pandemic had an unexpected impact on food safety, with a global decrease in reported outbreaks, likely due to measures taken to combat the virus worldwide (EFSA 2022b). Between 2013 and 2022, an average of 6,523 outbreaks of foodborne illnesses were reported annually in Brazil, with 107,513 patients (16 patients/outbreak), 12,722 hospitalized, and 112 deaths, reflecting significant underreporting and the importance of prevention efforts (Brazil 2023).

In this context, the focus on food safety shifts to microorganisms that significantly impact the global incidence of foodborne diseases. Among these, *Staphylococcus aureus* (*S. aureus*) not only stands out as one of the top five pathogens responsible for global bacterial deaths but also as a notorious source of food contamination. This Gram-positive microorganism, present

in about one-third of the global population, causes a range of infections, from staphylococcal food poisoning to toxic shock syndrome and pneumonia. Its global prevalence and link to foodborne disease outbreaks underscore a persistent challenge to public health, requiring ongoing vigilance and prevention efforts tailored to both regional and global realities (Cavaiuolo et al. 2023; Ikuta et al. 2022; Li et al. 2022a; Zhang et al. 2022a). This bacterium secret multiple toxins, such as staphylococcal enterotoxins (SEs), hemolysins, coagulases, lipases, DNases, lactamases, and capsules, which facilitate the colonization of host tissues and evasion of the immune response (Chen et al. 2022a; Kansaen et al. 2023; Li et al. 2022a; Shoaib et al. 2023).

Staphylococcal enterotoxins (SEs) are significant for their ability to disrupt adaptive immunity by over-stimulating T cells and causing inflammation, with their resistance to common cooking and digestion processes allowing doses as low as 20-100 ng to induce serious symptoms (Cai et al. 2023; Wan et al.2023). Currently, 28 types of SEs have been identified, highlighting the adaptability and danger of these toxins. *S. aureus* poses a continuous threat throughout the food chain, particularly in animal products, leading to potential foodborne outbreaks. The emergence of beta-lactam-resistant strains, including those resistant to methicillin, in various foods, underscores the growing challenge of ensuring food safety and emphasizes the critical need for stringent monitoring and adherence to health standards to prevent infections from contaminated foods (Abolghait et al. 2020; Berry et al. 2022; Fischer, Otto and Cheung 2018; Suzuki et al. 2020; Grispoldi et al. 2021; Umeda et al. 2021; Bencardino, Amagliani, and Brandi 2021; Fanelli et al. 2022; Khairullah et al. 2023; Li et al. 2022b; Zhang et al. 2022a).

A significant gap in the current literature is the absence of a detailed and comprehensive analysis of the current trends related to *S. aureus* as a foodborne pathogen. While there is a significant amount of individual research addressing this topic, most focus on isolated aspects,

such as epidemiology, pathogenicity, or detection methods. However, a holistic analysis that gathers and synthesizes the latest findings on contamination trends, production of new enterotoxins, resistance to antimicrobials, and prevention strategies is notably missing. Based on these aspects, this review aimed to describe an overview of enterotoxigenic staphylococci, methicillin-resistant staphylococci in different foods, and their relationship to pathogenicity mechanisms. This article also briefly discusses new trends in technologies being investigated in the methods of control and inactivation of *S. aureus* and its toxins.

Our review provides a comprehensive overview of enterotoxigenic staphylococci, methicillin-resistant *Staphylococcus aureus* (MRSA) in various foods, and their relationship with pathogenic mechanisms. Additionally, it briefly discusses emerging trends in technologies being investigated for the control and inactivation of *S. aureus* and its toxins. To construct this narrative review, we conducted a thorough examination of both original research and review articles, utilizing reputable databases such as PubMed, Elsevier, Science Direct, Wiley, Taylor & Francis. We employed a strategic search methodology, using keywords such as "Food poisoning," "Prevention and control," "Enterotoxins," "Food safety," in conjunction with "*Staphylococcus aureus*" or "Methicillin-resistant *Staphylococcus aureus* (MRSA)," and "Detection technologies," to gather pertinent data that clarify this intricate relationship. This diligent research process enabled us to compile and analyze critical findings that underscore the significant role of *S. aureus* as an important zoonotic pathogen and strategies to mitigate its impact on food safety.

2. *Staphylococci aureus* and staphylococcal foodborne poisoning

2.1 *Staphylococci and S. aureus – an overview*

Staphylococci constitute a genus of bacteria notable for their widespread presence in the environment and the human body. These bacteria are distinguished by their dual nature: some species act as harmless commensals, while others emerge as opportunistic and pathogenic agents capable of causing a spectrum of diseases (Bencardino, Amagliani, and Brandi 2021). Within this genus, *Staphylococcus* is remarkably diverse, encompassing more than 81 species, with numerous subspecies characterized to date (https://lpsn.dsmz.de/genus/staphylococcus) (Shoaib et al. 2023). Furthermore, *Staphylococcus* spp. is classified into two groups based on their ability to produce coagulase (an enzyme that clots plasma) (Cavaiuolo et al. 2023; Kansaen et al. 2023). This classification is crucial for understanding staphylococcal infection's pathogenicity and treatment strategies.

Coagulase-positive staphylococci (CPS) represent a subgroup within the *Staphylococcus* genus, classified as gram-positive. These bacteria are either aerobic or facultatively anaerobic, highlighting their adaptability to various environmental conditions (Cavaiuolo et al. 2023). The most common CPS species are *S. aureus, S. schleiferi* subsp *coagulans, S. lutrae, S. hyicus; S. intermedius, S. argenteus, S.schweitzeri* and *S. delphini* (Cavaiuolo et al. 2023; Kansaen et al. 2023). CPS, especially *S. aureus*, is distinguished by its ubiquity and virulence. This spherical bacteria naturally colonize the normal microbiota of human skin and respiratory mucosa (main reservoir) and, to a lesser extent, the environment. It is the most pathogenic species of *Staphylococcus* spp (Shoaib et al., 2023).

Humans and food-producing animals are the main reservoirs of *S. aureus* transmission (Oniciuc et al. 2017; Shoaib et al. 2023). In addition, *S. aureus* is responsible for nosocomial and community-acquired infections with a wide geographic distribution (Cavaiuolo et al. 2023). Human contamination by *S. aureus* can occur through direct human contact, nasal carriers in food processing environments, contaminated food, objects, surfaces such as doorknobs, faucets, hospital equipment (fomites), and food processing environments. Animals (dairy cows) and

also the integration of companion animals in households can contribute to this contamination (Bencardino, Amagliani, and Brandi 2021; Chaves et al. 2018). Infection by S. aureus is not limited contact or environmental exposure. It can also occur through ingesting foods that are handled or stored improperly.

However, under certain conditions, *S. aureus* can become an opportunistic pathogen that causes various infections in humans and animals (Cavaiuolo et al. 2023; Li et al. 2022a; Zhang et al. 2022). There is a diversity of conditions caused by the disease, ranging from superficial skin infections of the skin to more severe conditions such as toxic shock syndrome, osteoarticular diseases, bacteremia, pneumonia, endocarditis, and sepsis (Abolghait et al. 2020; Alghizzi and Shami 2023; Bencardino, Amagliani, and Brandi 2021; Chen et al. 2022a; Kansaen et al. 2023; Li et al. 2022a). Furthermore, the resistance of *S. aureus* to various antibiotics, including methicillin and vancomycin, poses an additional challenge to effectively treating infections, making antimicrobial resistance management a public health priority (Zaher et al. 2023).

2.2 *Epidemiology of S. aureus*

S. aureus is recognized as an opportunistic foodborne pathogen, attracting significant attention from scientists and health professionals due to its implications for animal health, human health, and food safety (Cavaiuolo et al. 2023; Li et al. 2022a; Zhang et al. 2022). This interest is evidenced by extensive and recent research that highlights its consequences in various areas, underscoring the need for surveillance and control.

This focus on food safety and public health is directly connected to the recognition of *S. aureus* as a significant global challenge, as demonstrated by the Global Burden of Disease Study 2019. This systematic report not only emphasizes *S. aureus* position as the leading

pathogenic agent in mortality in 135 countries, with over a million deaths attributed to it in 2019 but also stresses the importance of addressing its global impact. This pathogen stands out among 33 evaluated bacteria, particularly for its lethality in individuals over 15 years old, accelerating the demand for international strategies to reduce its prevalence (Ikuta et al. 2022).

Moreover, the influence of *S. aureus* extends to the realm of foodborne diseases, marking its significant presence in different regions. In the United States, *S. aureus* is among the top five pathogens causing foodborne illness (estimated at 241,148 cases). In the European Union, it ranks among the top three, and in Brazil, it is noted as the third most identified etiological agent in 10.8% of waterborne and foodborne disease (DTHA) outbreaks, only surpassed by *Escherichia coli* (32.3%) and *Salmonella* (10.9%) (Brasil 2023; CDC 2022; EFSA 2022a). The prevalence of *S. aureus* as a food contaminant, especially through enterotoxigenic strains that can cause food poisoning, highlights the critical importance of monitoring and controlling this pathogen to protect public health.

In Brazil, specifically, S. aureus was responsible for approximately 170 reported cases of foodborne disease outbreaks between 2013 and 2022, consolidating its position as one of the primary pathogens regarding food safety in the country (Brasil 2023). These data emphasize the urgency of implementing robust surveillance and effective preventive measures globally to mitigate the risks associated with *S. aureus*. Additionally, it highlights the need for coordinated actions among public health bodies, healthcare professionals, and the food industry to ensure food safety and protect collective health.

2.3 *Mechanisms of pathogenicity, virulence factors, and production of enterotoxins*

The ability of *S. aureus* to cause a wide range of infections, from food poisoning to severe clinical conditions such as septicemia and pneumonia, is primarily influenced by its sophisticated mechanisms of pathogenicity (Shoaib et al. 2023).

S. aureus has various virulence factors, underscoring its adaptive and pathogenic capacity across diverse environments. Different classes of mobile genetic elements encode these factors and include a range of extracellular enzymes such as proteases, amylases, hyaluronidase, hemolysins, coagulases, lipases, DNases, lactamase, capsules, and, especially, enterotoxins (Chen et al. 2022a; Freitas et al. 2023; Kansaen et al. 2023; li et al. 2022a; Shoaib et al. 2023). The broad virulence of *S aureus* enables survival in distinct environments. This adaptative capability enhances adhesion, invasion, and immune evasion and triggers inflammatory responses through pathogen-associated molecular patterns (Chen et al. 2022a).

Among the various virulence factors, toxins and superantigens play a crucial role, whose primary function is to disrupt host cell membranes and induce lysis and inflammation of target cells, producing inflammatory cytokines (Abolghait et al. 2020; Chen et al. 2022a). Furthermore, *S. aureus* produces extracellular enzymes that break down host molecules into nutrients, promoting bacterial survival and proliferation (Chen et al. 2022a).

SEs, which act as superantigens, trigger excessive immune responses by activating the major histocompatibility complex class II (MHC-II) receptors on antigen-presenting cells and the variable region of the beta chain (Vβ) of the T cell receptor (TCR) on T lymphocytes (Berry et al. 2022; Fischer, Otto and Cheung 2018; Suzuki et al. 2020). This activation leads to the release of a series of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-2, interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-α), and TNF-β, which are crucial for the induced toxicity, as depicted in Figure 1 (Abolghait et al. 2020; Wan et al. 2023; Zhang et al. 2018).

[Figure 1 near here]

Furthermore, the effects of SEs, including the induction of emesis, are attributed to their superantigenic activity, which promotes an intense inflammatory response in the intestine and degranulation of intestinal mast cells (Bencardino, Amagliani and Brandi 2021; Wan et al. 2023). In this sense, it has been postulated that emesis induced by enterotoxins is due to the translocation of enterotoxins through mucus-producing goblet cells and epithelial cells of the intestinal epithelium. The enterotoxin interacts with mast cells to induce the release of 5-hydroxytryptamine (precursor of 5-HT/serotonin), which interacts with the vagus nerve to cause an emetic response. Additional cellular targets, including T cells and neutrophils, may also play a role in the pathogenesis of staphylococcal food poisoning (Fischer, Otto, and Cheung 2018).

2.4 *Sources and routes of S. aureus contamination in foods*

The food contamination by *S. aureus* is a critical public health issue due to its ability to produce enterotoxins that cause severe intoxications (Berry et al. 2022). The presence of *S. aureus* in various foods is facilitated by its ubiquity in the environment and the flora of humans and animals, underscoring the need to identify sources and pathways of contamination to prevent outbreaks (Umeda et al. 2021).

Although *S. aureus* sp are commonly associated with nosocomial and community-acquired infections, many of these coagulase-positive strains are enterotoxigenic and pose a contamination risk for food, causing Staphylococcal food poisoning (SFP) (Berry et al. 2022; Cavaiuolo et al. 2023; Chen et al. 2022a; Li et al. 2022a; Suzuki et al. 2020; Umeda et al. 2021). The primary sources of contamination include raw meats and meat products, poultry, eggs, and unpasteurized milk, as well as products derived from these foods (Abolghait et al. 2020; Li et al. 2022b). Other risk factors include retail-sold foods, inadequate food handling practices, and

ready-to-eat foods. The latter can provide ideal conditions for the proliferation of *S. aureus*, due to exposure to pratices such as failure in temperature control, cross-contamination, improper storage, and insufficient hygiene during preparation. Such conditions favor bacterial growth, increasing the risk of harmful bacterial activity (Abolghait et al. 2020; Cavaiuolo et al. 2023; Fanelli et al. 2022; Li et al. 2022b; Park et al. 2020; Umeda et al. 2021; Zhang et al. 2022a; Zhang et al. 2022b).

The adaptability of *S. aureus* to complex food environments is notable, especially under conditions of high humidity, broad pH variation (5 to 9.6), and high concentrations of sugar or salt. These factors, coupled with competition with other microorganisms and specific environmental conditions, including temperature ranges conducive to growth (10 to 46 °C), a water activity (aw) of 0.86 to 0.99, and a redox potential ranging from -100 to +200 mV, significantly contribute to its ability to survive and multiply in various food environments (Abolghait et al. 2020;Grispoldi et al. 2021; Kansaen et al. 2023; Li et al. 2022a). The capability of *S. aureus* to secrete enzymes and produce toxins, especially enterotoxins, amplifies its competitive advantage amidst this complexity. These toxins not only facilitate overcoming other microorganisms but also accelerate *S. aureus* ability to establish itself in these niches effectively. Forming biofilm further protects the bacteria against host immune responses, endowing it adaptability in these food contexts (Tuncay and Sancak 2023).

In animal-derived products, such as meats, poultry, eggs, and unpasteurized milk and its derivatives, the risk of foodborne outbreaks linked to *S. aureus* is notably high (Bencardino, Amagliani, and Brandi 2021). This risk is intensified by the ability of *S. aureus* to colonize a wide range of animals, especially cattle, which can be asymptomatic carriers of the pathogen and sometimes develop mastitis. Such a condition in dairy cows can lead to the contamination of raw milk with S. aureus, creating a conducive environment for bacterial growth and the production of enterotoxins (Necidová et al. 2019; Shoaib et al. 2023). This chain of

contamination extends beyond the animal source, intrinsically linked to insufficient hygiene practices at various stages of food processing. From the production phase through handling, shipping, slicing, storage, and the point of sale, animal-derived foods are subject to contamination (Zaher et al. 2023). The complexity of contamination, especially evident in dairy products, encompasses various factors, such as the milking environment, the production process, the personnel involved in processing, production tools and equipment, water quality, and environmental conditions, as depicted in Figure 2 (Tuncay and Sancak 2023). This complex network of potential contamination points highlights the critical need to implement rigorous food safety practices to ensure the quality and safety of animal-derived foods and their dairy derivatives.

[Figure 2 near here]

The growth of community-acquired infections and the urgency to monitor antibiotic-resistant strains of *S. aureus* have motivated studies on its transmission through food handlers. These professionals, often exposed to the pathogen, not only face the risk of contracting infections but also act as conduits for the spread of pathogenic strains during the food processing and distribution stages (Bencardino, Amagliani, and Brandi 2021; Berry et al. 2022). Cross-contamination, where microorganisms are transferred from surfaces or other elements to foods, is identified as a significant cause of food poisoning, necessitating effective control and prevention strategies (Bencardino, Amagliani, and Brandi 2021).

The prevalence of nasal carriers of *S. aureus* spp. in food processing environments highlights the dangers of cross-contamination. The presence of *Staphylococcus* spp. in Brazilian institutions, home kitchens, and cream-based desserts underscores the need for vigilance and effective preventive measures to mitigate food contamination by *S. aureus* spp. (Chaves et al.

2018; Chaves et al. 2022). Furthermore, cases of food poisoning in South Korea associated with the consumption of food at a buffet, evidence of the often food handler-linked origin of contamination, emphasizing the importance of hygiene and sanitary control (Park et al. 2020). These cases underscore the critical importance of surveillance and appropriate preventive measures in mitigating the food contamination attributed to *S. aureus*, ultimately safeguarding public health.

3. Enterotoxigenic Staphylococci aureus and their toxins

Enterotoxigenic staphylococci, predominantly coagulase-positive staphylococci, are known for producing enterotoxins that can cause diseases in humans. Among the various diseases caused by this pathogen, SFP is particularly relevant. SFP is caused by the ingestion of food contaminated with adequate amounts of SEs secreted by certain pathogenic strains of *S. aureus* and, occasionally, coagulase-negative staphylococci carrying enterotoxin genes (Berry et al. 2022; Homsombat et al. 2021; Suzuki et al. 2020).

SFP manifests rapidly with symptoms such as gastroenteritis with diarrhea, fever, vomiting, and abdominal pain, occurring 30 min to 8 hours after consuming contaminated food (Alghizzi and Shami 2023; Kansaen et al. 2023). It results from ingesting toxins produced by strains of *S. aureus* present in contaminated food. The severity of the clinical condition can vary according to the individual's degree of susceptibility, concentration, and the amount consumed in food (Bencardino, Amagliani, and Brandi 2021; Cavaiuolo et al. 2023).

The dose of SEs required to cause SFP varies considerably among individuals, depending on their susceptibility and other factors (Abolghait et al., 2020). According to the FDA, the toxic dose of SE is less than 1.0 ng, achievable when the count of *S. aureus* is reached when its population exceeds 100,000 organisms/g or mL in food (FDA 2012). However, some authors

reported that doses around 20–100 ng caused food poisoning, reinforcing the need for strict control measures to limit the presence of this pathogen in foods and ensure consumer safety (Cai et al. 2023; Wan et al.2023).

Often, cases of SFP are underreported and can be confused with diseases caused by other foodborne pathogens due to the similarity of symptoms (FDA, 2012). The enterotoxigenic capacity has also been identified in S. argenteus, a new species of CPS, highlighting its role as a foodborne pathogen in Europe and Brazil (Cavaiuolo et al. 2023; Rossi et al. 2020). Moreover, coagulase-negative staphylococci, such as *S. sciuri, S. lentus* and S. *cohnii* ssp. *cohnii*, have also been reported as producers of enterotoxins, despite not producing coagulase, expressing virulence factors that qualify them as opportunistic pathogens. These microorganisms can form biofilms, facilitating the colonization of surfaces and contributing to their pathogenicity (Chaves et al. 2018; Kansaen et al. 2023). This diversity of staphylococci capable of producing enterotoxins underscores the need for surveillance and strict control measures to prevent food contamination and protect public health.

Research conducted in the East Java region of Indonesia highlighted the widespread dissemination of *Staphylococcus* genera, both coagulase-positive and harmful strains, in agricultural environments. The presence of these pathogens in cow's milk and in the hands of farmers underscores the widespread dissemination of these microorganisms in agriculture-related environments. The discovery emphasizes the importance of rigorous hygiene practices in preventing food contamination and ensuring food safety (Khairullah et al. 2023).

The global relevance of *S. aureus* as a causative agent of food poisoning is evidenced by documented outbreaks in various countries (Table 1). Notable incidents include an outbreak in a nursing home in Osaka, Japan, in May 2016, an event at a Korean festival in 2018, and a significant outbreak in a school in the Ninh Binh province, Vietnam, in 2018 (Umeda et al. 2021; Park et al. 2020; Le et al. 2021). The analysis of the mentioned outbreaks demonstrates

the risk of enterotoxigenic *S. aureus* in food environments and the need for ongoing surveillance and strict control measures. These incidents underline the challenges in managing foodborne outbreaks and stress the necessity of hygiene practices throughout the food production and distribution. Consequently, it is crucial to implement strategies against *S. aureus*, involving better agricultural and food processing methods to reduce pathogen exposure and developing public health policies for effective surveillance and quick outbreak responses. Integrating scientific research with policy and food safety practices is critical to mitigating these pathogens' risks and ensuring global food safety.

[Table 1 near here]

SEs demonstrate remarkable resistance to extreme conditions, including high temperatures, proteolytic enzymes, and varied pH levels. Furthermore, they are relative to the general sterilization method (100 °C, 30 min), making heat treatment ineffective in destroying these toxins once produced (Abolghait et al. 2020; Bencardino, Amagliani, and Brandi 2021; Grispoldi et al. 2021; Kansaen et al. 2023; Li et al. 2022a; Wan et al. 2023).

A detailed understanding of the relationship between the growth of *S. aureus* and its ability to produce enterotoxins is essential for assessing and mitigating food safety risks. Table 2 summarizes critical findings from recent studies, highlighting the impact of environmental variables such as temperature, pH, water activity (aw), and salinity, including the presence of sodium chloride (NaCl), on the regulation of these biological processes.

[Table 2 near here]

It is evident that temperature plays a crucial role in toxin production dynamics, with SEA production significantly higher at 25°C compared to 8°C after 16 hours. Moreover, optimal environmental conditions, characterized by a pH above 5.0, a water activity (aw) above 0.86,

and temperatures above 15°C, promote increased expression of the SEA and SEB toxins (Homsombat et al. 2021; Ding et al. 2016). The remarkable ability of these toxins to resist high temperatures illustrates the importance of thermal stability and refolding capability under certain pH conditions (Necidová et al. 2019).

The variable sensitivity of enterotoxins to enzymatic digestion and thermal treatment adds another layer of complexity to the challenge of neutralizing them. For example, SEB (SE02) demonstrates significant reduction and complete inhibition when exposed to heating (100 °C) and digestion by pepsin, respectively (Suzuki et al. 2020). The analysis of the thermal stability of SEA, SEB, and SEH using Differential Scanning Calorimetry (DSC) revealed the critical influence of pH and ionic strength of foods in designing effective thermal inactivation methods (Berry et al. 2022). Predictive models play a crucial role in this context, employing environmental variables such as pH, aw, and temperature to estimate the toxigenic expression of *S. aureus*. The logistic regression model exemplifies this approach, while innovative techniques, including artificial neural networks, enhance the accuracy of predictions for various food products, thus contributing to food safety (Cai et al. 2023; Ding et al. 2016; Gunvig et al. 2018).

In summary, effective prevention of *S. aureus* contamination and ensuring food safety require the implementation of strict hygiene practices at all stages of food production and processing, as well as control strategies adapted to the resistance and variability of staphylococcal enterotoxins.

3.1 *Characterization of Staphylococcal Enterotoxins*

SEs are classified within the family of pyrogenic toxins and are known for their potent superantigenic activity, which can induce immunosuppression and promote nonspecific proliferation of T cells (Abolghait et al. 2020).

The nomenclature rules established by the International Committee on the Nomenclature of Staphylococcal Superantigens (INCSSN) differentiate classic SEs from staphylococcal enterotoxin-like toxins (SEls), based on their emetic potential (Lina et al. 2004). Theretofore, twenty-eight SEs and SEls have been categorized into toxins with confirmed emetic activity (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SElK, SElL, SElM, SElN, SElO, SElP, SElQ, SER, SES, and SET) and those without confirmed emetic activity or that have not yet been approved or tested (SElJ, SElU, SElV, SElX, SElW, SElZ, SEl26, SEl27, SEl01, and SEl02) (Umeda et al. 2021; Suzuki et al. 2020). Toxins with recognized emetic capability include SEA, SEB, SEC, SED, and SEE (classic enterotoxins), responsible for approximately 95% of all cases of staphylococcal food poisoning (Bencardino, Amagliani, and Brandi 2021; Berry et al. 2022). The only exception to this rule is the Toxic Shock Syndrome Toxin-1 (TSST-1), named initially SEF (Bencardino, Amagliani, and Brandi 2021; Fischer, Otto, and Cheung 2018; Suzuki et al. 2020; Umeda et al. 2021; Zhang et al. 2018). Notably, SEs are heat-stable, resisting temperatures and cooking times typical in domestic and industrial environments.

These toxins are part of a superfamily that shares structural and functional similarities, consisting of non-glycosylated globular proteins of low molecular weight (19 to 29 kDa) and superantigen activity (Abolghait et al. 2020; Fischer, Otto, and Cheung 2018; Wan et al. 2023). The genes encoding SEs and SEls are found in various mobile genetic elements, such as genomic islands, prophages, plasmids, and transposons, facilitating their horizontal transfer among bacterial strains (Bencardino, Amagliani, and Brandi 2021; Grispoldi et al. 2021). These characteristics combined make SEs category B bioterrorism agents by several US federal agencies (Berry et al. 2022; Fischer, Otto, and Cheung 2018). The main characteristics of

399 staphylococcal enterotoxins, including the locations of the SE/SEl genes and their biological
400 properties, are detailed in Table 3.
401
402 [Table 3 near here]
403
404 In addition to structural diversity, SEs are classified into different variants, such as SEs
405 C, which are subdivided into SEC1, SEC2, and SEC3, based on their sequence identity
406 similarity (Berry et al. 2022; Chaves et al. 2018). The enterotoxin SE02 was recently identified
407 and isolated from two strains of *S. aureus* (Tokyo12480 and Tokyo12482) related to an
408 outbreak in Tokyo (Suzuki et al. 2020). The genes responsible for encoding the enterotoxins
409 are predominantly found in various mobile genetic elements, contributing to the significant
410 variability of SEs among isolates of *S. aureus* (Abolghait et al. 2020; Fischer, Otto, and Cheung
411 2018). Recent research has also revealed that new toxins, similar to SEs, including SElK, SElL,
412 SElM, SElN, SElO, SElP, and SElQ, can induce emetic reactions in animal models, such as
413 monkeys, playing a potential role in staphylococcal food poisonings. However, higher doses,
414 about 100 μg/kg, are required to trigger such effects (Zhang et al. 2018).
415 Recently, enterotoxin-like toxins, specifically SEl26 and SEl27, have been identified in
416 *Staphylococcus argenteus* and *Staphylococcus schweitzeri*, marking the discovery of new
417 species closely related to *S. aureus* (Zhang et al. 2018). The genetic diversity and the emergence
418 of new toxins in *S. aureus* isolates expand understanding of the genetic diversity within the
419 *Staphylococcus* genus and highlight the constant evolution of these microorganisms.
420 Heretofore, worldwide records of the prevalence of SEs in food contaminated by
421 *Staphylococcus* spp. are not available. Several studies on the subject, including reports of
422 possible outbreaks involving these bacteria, have indicated the predominance of food outbreaks
423 by classic SEs (Abolghait et al. 2020; Le et al. 2021; Li et al. 2022b; Park et al.2020; Pereira et

al. 2022). Freitas et al. (2018) highlighted the significant detection of Staphylococcal Enterotoxin D (SED) on the hands of workers in institutional and home kitchens and in their nostrils, reinforcing the need for rigorous hygiene practices. Moreover, recent investigations have revealed that new SEs and their associated genes have been identified in strains of *S. aureus* involved in food poisonings, suggesting an increasing role of these emerging toxins in outbreaks (Li et al. 2022a; Park et al. 2020; Umeda et al. 2021).

A study conducted by Li et al. (2022a) in Jilin, China, analyzed samples from retail foods and foodborne outbreaks, isolating *S. aureus* strains that carried enterotoxin genes, including both classic and new SEs. This study revealed a notable distribution of SE genes among the isolates, with a significant percentage carrying genes for SEA (40/125, 32%), SEE (36/125, 28.8%), SEC (29/125, 23.2%), SElL (29/125, 23.2%), SEB (25/125, 20%), SEH (22/125, 17.6%), SED (6/125, 4.8%), SElQ (6/125, 4.8%), and SElK (6/125, 4.8%). These findings point to the importance of considering both classic and new SEs as potential causes of food poisoning outbreaks (Table 1), highlighting the ongoing need for surveillance and prevention measures adapted to the evolving toxigenicity profile of *S. aureus*.

3.2 *Challenges and advances in the detection of S. aureus and its enterotoxins*

The detection of *S. aureus* and its SEs is crucial for diagnosing SFP. Criteria for confirming the diagnosis of SFP include the detection of SEs in food debris, the isolation of the same strain of *S. aureus* from clinical patient specimens (vomit and stool) and food debris, and the recovery of greater than 10^5 CFU/g of CPS, and the identification of *S. aureus* from food remains (Cai et al.2023; FDA 2012).

Despite the critical importance of this detection for public health, identifying SEs in foods presents challenges and is commonly performed in reference laboratories or specialized

research institutes (Freitas et al. 2023). Currently, only the first discovered enterotoxins (SEA to SEE) are routinely detectable by commercial kits (Cavaiuolo et al. 2023). There is a growing demand for advanced diagnostic methods that identify *S. aureus* and its SEs, including new variants, to ensure food safety and public health (Chen et al. 2022b; Zaher et al. 2023).

To ensure food safety and protect consumer health, minimizing the risk of SFP in the European Union, Regulation (EC) No 2073/2005, issued by the European Commission in 2005, establishes specific criteria for the detection of SE and the enumeration of coagulase-positive staphylococci in products like cheeses, powdered milk, and whey powder. In the Brazilian context, ANVISA set requirements for SE identification through the Collegiate Board Resolution (RDC) No. 724/2022 and the Normative Instruction (IN) No. 161/2022, specifying that the detection limit for SEs must be no more than 1 ng/g in food categories such as milk and derivatives, dairy supplements, and ready-to-eat foods (Brazil 2022a; Brazil 2022b). This measure reinforces compliance with international food safety standards and highlights Brazil's commitment to ensuring the integrity of foods available to consumers.

Current challenges in food safety related to *S. aureus* include difficult diagnosis, underreporting, and antimicrobial resistance (Freitas et al. 2023; Mestrovic et al. 2022; Oniciuc et al. 2017). These issues were highlighted in a recent systematic review focusing on the prevalence and types of SEs in foods. The review indicated significant gaps in surveillance protocols and medical, epidemiological, and laboratory standards adherence. Often, the brief duration of illnesses caused by these toxins leads to reduced seeking of medical care, exacerbating the problem of underreporting (Freitas et al. 2023). This reality underscores the urgent need to improve communication between the medical, veterinary, and agri-food industries and consumers, adopting the integrated "One Health" approach that considers all aspects of the food chain (Oniciuc et al. 2017). The precise and rapid detection of *S. aureus* and its enterotoxins is essential to ensure safe food and preserve public health (Chen et al. 2022b).

3.2.1 *Traditional and advanced methods for S. aureus detection*

The detection and identification of *S. aureus* have traditionally relied on conventional laboratory techniques. Among these, bacterial culture stands out as the gold standard method, complemented by the coagulase test and biochemical assays for accurate identification (Cavaiuolo et al. 2023; Petrucci et al. 2021; Hameed, Xie and Lee 2018; Shrivastava, Lee, and Lee 2018). Innovations in culture procedures, such as the development of chromogenic agars, have improved sensitivity and specificity, simplifying detection by eliminating the need for screening additives (Petrucci et al. 2021). However, these traditional approaches are notably slow and laborious, yet more accurate and reliable than modern detection techniques, encouraging research into faster and more efficient methods. By doing so, scientists can achieve better outcomes (Hameed, Xie, and Lee 2018).

In recent years, advanced and promising techniques have emerged that operate independently of microbiological cultures, aimed at the rapid and automated detection of *S. aureus*. The Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) are particularly notable for allowing the specific detection of *S. aureus* antigens and bacterial DNA, respectively (Cavaiuolo et al. 2023; Chen et al. 2022b; Grispoldi et al. 2021; Hameed, Xie, and Lee 2018). Furthermore, methods such as immunochromatographic assays and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) offer advantages in speed and specificity. However, they face challenges in terms of complexity, costs, and the requirement for technical specialization (Chen et al. 2022b; Wu et al. 2023).

Flow cytometry and fluorescent assays, especially those employing nanomaterials, are significant advancements, offering high sensitivity and reduced cost. However, complex samples can challenge these techniques' specificity, raising concerns about potential false

positives (Hameed, xie, and Lee 2018; Wu et al. 2023). The exploration of in situ technologies, including smartphones and portable microscopy, indicates a promising path for rapid and accessible detections. Still, the reliability of these innovative approaches can be compromised by the interference of substances present in complex samples (Shrivastava, Lee and Lee 2018).

The evolution in detecting *S. aureus* reflects a transition from traditional methods to faster and technologically advanced strategies. Selecting the most suitable method requires a balance between accuracy, speed, cost, and the capability for in situ detection. While progressive methods promise significant advancements, the barriers of complexity and cost remain substantial. The future trend in food pathogen detection will likely focus on developing techniques that combine sensitivity, specificity, rapidity, and portability, aiming for innovative solutions in food safety. Advances, especially in advanced methods, stand out for their ability to provide rapid, accurate, and quantitative detections, crucial for preventing outbreaks of foodborne diseases and protecting public health.

3.2.2 *Traditional and advanced methods for the detection of SEs*

The conclusive identification of SFP requires the detection of SEs in food or the recovery of, at a minimum, 105 CFU per gram or milliliter of *S. aureus* in food samples (FDA 2012). Initially, biological assays were implemented as pioneering methods for identifying SEs. However, variability in the sensitivity of the tested organisms, the lack of specificity, limited practicality, and ethical concerns associated with the use of animal models have rendered these techniques inadequate for the effective confirmation of SFP (Wu et al. 2016).

With technological advances in diagnostic methods, the implementation of serological tests, including latex agglutination and double immunodiffusion assays in gel, marked a significant advancement, based on antigen-antibody interactions (Féraudet Tarisse et al. 2021).

However, the contemporary era has witnessed the advent of advanced molecular biology techniques, with PCR standing out for its high sensitivity and precision, even in samples with low concentrations of microorganisms. Despite these benefits, PCR faces limitations, such as the need for prior isolation of staphylococcal strains from foods and the focus solely on DNA analysis, without providing information on the expression of enterotoxins in consumed foods. Additionally, routine implementation of PCR can be costly and complex, requiring highly specialized operators to conduct it (Féraudet Tarisse et al. 2021; Shalaby et al. 2023; Wu et al. 2016).

Instrumental methods, such as high-performance liquid chromatography and mass spectrometry, offer detailed analyses of analytes, based on molecular weights or primary amino acid sequences, with notable advantages including the elimination of preliminary toxin isolation steps and the capability for quantification with lower detection limits (Féraudet Tarisse et al. 2021; Shalaby et al. 2023; Wu et al. 2016). However, despite their promise, these techniques face challenges such as interferences in complex matrices, high costs, limited sample processing capacity, and the need for highly specialized personnel (Wu et al. 2016).

Notably, the ELISA method stands out as one of the most effective and widely used techniques for the detection of SEs, due to its speed, sensitivity, and ability to process numerous samples simultaneously. This method, based on the use of specific antibodies, whether polyclonal or monoclonal, for toxin identification, has established itself as the dominant approach in the field, also benefiting from the availability of specialized commercial kits (Féraudet Tarisse et al. 2021; Grispoldi et al. 2021; Li et al. 2022; Zhang et al. 2018; Umeda et al. 2021). The detection of "non-classical" SE variants in *S. aureus* isolates derived from food poisoning underscores the need for new detection methods, as these kits do not cover all SE variants (Li et al. 2022a; Zhang et al. 2018; Umeda et al. 2021). Consequently, the demand for highly sensitive, specific, and rapid immunological assays has driven the enhancement of

immunoassay techniques, including monoplex and multiplex lateral flow immunoassays based on the sandwich ELISA, which stand out for their robustness, ease of use, superior performance, cost-effectiveness, and speed, eliminating the need for specialized technical infrastructure for their operation (Féraudet Tarisse et al. 2021; Umeda et al. 2021).

Although immunoassays represent a reliable platform for the detection of SEs, featuring high sensitivity, a broad linear detection range, and practicality, the quality of the employed antibodies plays a critical role. Generating antibodies through animal immunization procedures can be an extensive and costly process. Moreover, the produced antibodies may exhibit instability and susceptibility to post-translational modifications, which can limit their applicability in certain applications (Wu et al. 2016). Concurrently, aptamers emerge as viable alternatives in the detection of staphylococcal enterotoxins, surpassing traditional antibodies in terms of affinity and specificity. Selected through in vitro techniques, such as SELEX, these single-stranded oligonucleotides (DNA or RNA) offer advantages such as ease of synthesis, modification, and enhanced stability, enabling in situ applications (Wu et al. 2016).

This advancement in the detection of SEs reflects ongoing progress in the field of food science and microbiology, where precision, speed, and specificity are essential for ensuring food safety and public health. As new methods are developed and refined, more efficient and comprehensive detection of SEs is expected, thereby contributing to the prevention of food poisoning outbreaks and the improvement of food control and surveillance strategies.

4. Antimicrobial-resistant *S. aureus*: focus on Methicillin Resistance in Foods

MRSA is an infectious agent of interest to global public health, initially described as an agent of nosocomial infections. However, MRSA is currently associated with foodborne illness (FBD) and has been identified by the WHO as one of the high-priority antimicrobial resistance pathogens (Abolghait et al. 2020; Cavaiuolo et al. 2023; Li et al. 2022a).

According to the literature, MRSA has been classified into three groups: healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA) (Abolghait et al. 2020; Li et al. 2022b; Wu et al. 2022). Clinical manifestations of MRSA infection include light to several symptoms, since skin infections until septicemia and toxic shock (Shoaib et al. 2023), and CA-MRSA causes less severe infections in animals and humans as compared to HA-MRSA (David and Daum 2010). However, not necessarily a MRSA strain causes more serious illnesses than a regular *S. aureus* (Shoaib et al. 2023); some factors in the hostesses contribute to the illness gravity, like age, health status, body condition score, hygiene conditions and long-term antibiotic therapy (Shoaib et al. 2020). Besides, the crescent resistance of MRSA to a lot of antibiotics raises concerns among the scientific community.

Methicillin resistance is conferred by the presence of the mecA gene (or its homolog mecC) that encodes a penicillin-binding protein (PBP), PBP2a, with low activity for β-lactam antibiotics and regulatory genes (Alghizzi and Shame 2023; Kansaen et al. 2023; Shoaib et al. 2023; Silva et al. 2020). PBP2a proteins encoded by mecA and PBP2c proteins encoded by mecC have functional differences in drug affinity (Silva et al. 2020).

In 2019, MRSA alone accounted for over 100,000 deaths. This same research predicts that globally, 204 nations will witness a total of 4.95 million deaths associated with antibiotic resistance and 1.27 million attributed deaths in the same year (Mestrovic et al. 2022). Furthermore, the number of deaths from MRSA across all age groups, attributable to antibiotic resistance, was analyzed in super-regions by the Global Burden of Disease (GBD), led by the Institute for Health Metrics and Evaluation (IHME) in 2019. These MRSA-related deaths, associated with antibiotic resistance, were higher in the super-region of Southeast Asia, East Asia, and Oceania, while fewer were observed in the super-region of Central Europe, Eastern

598 Europe, and Central Asia, as illustrated in Figure 3. These data underscore the urgency of
599 addressing antibiotic resistance on a global scale (Ikuta et al. 2022).

600 [Figure 3 near here]

601 The urgency of addressing antibiotic resistance on a global scale is further underscored
602 by the ability of MRSA to form biofilm. Like other staphylococcal strains, this intrinsic ability
603 (an important virulence factor) on biotic and abiotic surfaces is highly regulated by complex
604 genetic factors (Kansaen et al. 2023); this allows *S. aureus* to colonize different surfaces,
605 enabling its spread, while increase the risk of cross-contamination between surfaces, food, and
606 humans (Silva et al. 2020). MRSA strains have recently been isolated in a lot of animals
607 involved in food production processes, including cattle and sheep carcasses (Zaher et al. 2023),
608 chicken meat and offal (Abolghait et al. 2020), meat and associated food products (Li et al.
609 2022b), cow milk (Khairullah et al. 2023; Lodhi et al. 2021; Tuncay and Sancak 2023), as well
610 as in farmers and slaughterhouse workers (Khairullah et al. 2023; Zaher et al. 2023). Tracing
611 strain origins is challenging due to the high prevalence of *S. aureus* in different environments
612 and food.

613 Direct contact between humans and animals, exposure to environmental sources
614 contaminated with livestock production residues, and, especially, the consumption of animal-
615 derived foods are considered potential sources of MRSA transmission to humans originating
616 through the food chain (Ávila-Nova et al. 2021). A recent study revealed a notable prevalence
617 of MRSA, with a contamination rate of manual swab samples from slaughterhouse workers in
618 the provinces of Egypt reaching 29.4%. Additionally, the study identified MRSA prevalences
619 in human feces (40%), followed by beef (35%) and sheep meat (26.6%) (Zaher et al. 2023).
620 The spread of MRSA infections in food animals was initially thought to be slower, but now it's
621 becoming a serious problem for food industries too (Shoaib et al. 2023); when you add high
622 transmissibility to a resistant, biofilm-forming pathogen, capable of being transmitted on a large

scale through food, a serious safety problem arises, and that must be solved through the application of safety process standards.

In their investigation, Tuncay and Sancak (2023) characterized strains of *S. aureus* circulating in raw milk obtained from the Van province, Turkey. It was determined that only 2 out of the isolates harbored the mecA gene. Notably, all obtained isolates (100%) exhibited resistance to both penicillin G and cefoxitin. Similarly, another study investigated the prevalence of *S. aureus* and MRSA in dairy products. Of the 141 samples of dairy products collected, 26% were positive for *S. aureus* and 14% of the *S. aureus* isolates were MRSA (Ávila-Nova et al. 2021). Another study investigated the prevalence and enterotoxicity of MRSA in broiler meat and offal. Most MRSA isolates (75%, 6/8) contained the SEB gene (seb) (Abolghait et al. 2020).

A four-year follow-up study investigated the antimicrobial resistance profile and molecular characteristics of LA-MRSA from animal farms and pork markets in China. A total of 1667 samples were collected from different farms from 2013 to 2016. *S. aureus* was detected in a proportion of 3.7% (n = 62) and 18 isolates were identified as MRSA with the presence of mecA and mecC genes. All *S. aureus* isolates resisted penicillin and ampicillin, but it's crucial to highlight that cefoxitin is the reference antimicrobial for detecting phenotypic resistance in MRSA strains (Li et al. 2022b).

Another strain of MRSA was isolated in China in 2015 from a sample of frozen dumplings resistant to more than 18 antibiotics: florfenicol, chloramphenicol, clindamycin, tiamulin, erythromycin, ampicillin, cefepime, ceftazidime, kanamycin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole and linezolid (Zhang et al. 2022b). Similarly, 12 genetically diverse strains of *S. aureus* isolated from ready-to-eat foods in Algiers, Algeria, have been confirmed to be resistant to antibiotics such as ofloxacin, erythromycin, lincomycin, tetracycline and kanamycin (Fanelli et al. 2022). On the American continent, the first report of

LA-MRSA containing mecC variants from several sources (milk, milker hands, and milking utensils), was reported by Silva et al. (2020). In this study, the authors reported a genomic trait of β-lactam resistance. In silico analysis identified homologous mecC genes associated with different PBP. Toxicity analysis revealed that this strain contained the gene encoding SEA. The authors conclude that detecting the same MRSA mecC clone from different sources suggests that new multidrug-resistant substances may circulate within herd environments and among people in close contact with animals. The ability to acquire antimicrobial resistance mechanisms is concerning (Silva, Rodrigues, and Silva 2019).

These studies provide insight into the role of antibiotic overuse in animal production, and the presence of LA-MRSA in raw milk and dairy products underscores the importance of addressing potential transmission routes to humans. Contamination during food handling and individuals carrying the pathogen further highlights the significance of hygiene practices.

The first case of food-borne MRSA infection occurred in the Netherlands in 1995, resulting from a hospital outbreak of MRSA due to the ingestion of contaminated food by patients admitted to the Erasmus Medical Center in Rotterdam. One patient developed severe sepsis and died. In the study, *S. aureus* was isolated from the oropharynx of food handlers and served foods (Kluytmans et al. 1995). Therefore, MRSA is no longer just a nosocomial pathogen. In addition to the MRSA outbreak in the Netherlands in 1995, there have been other outbreaks of foodborne MRSA in different parts of the world. This suggests that the epidemiology of MRSA has changed with the emergence of CA-MRSA strains worldwide.

The first reported community-based outbreak of CA-MRSA gastrointestinal illness happened in Tennessee (USA), affecting three members of the same family, and caused by eating barbecue pulled pork and cabbage salad. Contaminated cabbage salad from an asymptomatic food handler was the source of MRSA. All family members who ate the food within 30 minutes of purchase from a deli developed gastrointestinal symptoms. Stool samples

673 from sick family members and S. aureus isolates obtained from cabbage salad and food preparer
674 nasal swabs were identical. Strains implicated in the outbreak produced SEC and were
675 identified as MRSA (Jones et al. 2002). In 2007, 178 CPS isolates were recovered from 31 SFP
676 outbreaks in France, of which two strains of S. aureus were methicillin-resistant (Kérouanton
677 et al. 2007).

678 Several methods for phenotypic or genotypic identification of MRSA strains are usually
679 combined. Using the standard Kirby-Bauer disk diffusion method to determine susceptibility to
680 antimicrobials (Silva, Rodrigues, and Silva 2019). Furthermore, proteomic techniques like as
681 MALDI-TOF-MS and PCR are widely used to screen for MRSA (Chen et al. 2022b).

682 The aforementioned studies show evidences that although the prevalence of antimicrobial
683 resistance and MRSA in food is low, MRSA may also be involved in SFP and represent a
684 potential risk to human health, and deserves attention. In this way, follow sanitary laws and
685 biosecurity protocols is essential to every food service, aiming to reduce potential sources and
686 food contamination.

687 **5. Emerging Trends in the Control and Inactivation of *S. aureus* in Food**

688

689 5.1 *Challenges in controlling S. aureus using conventional and unconventional approaches*

690

691 *S. aureus* continues to be an important foodborne pathogen that poses challenges to food
692 safety. Its ability to acquire resistance makes it even more difficult to combat. Factors that
693 influence the control and successful inactivation of *S. aureus* and its toxins include the presence
694 of complex regulatory networks, structural differences in the cell envelope, the existence of
695 gene expression and the ability to secrete a variety of virulence factors and invasive enzymes
696 (Abolghait et al. 2020; Chen et al. 2022a; Chen et al. 2022b; Freitas et al. 2023; Kansaen et al.
697 2023; Li et al. 2022a; Shoaib et al. 2023). These factors may contribute to its high pathogenicity

and are one of the reasons why antimicrobial resistance poses major challenges for clinical management and infection control (Zhao et al. 2022). Furthermore, its ability to form biofilms is a major issue in the food industry. Cleaning with chemical disinfectants and high-pressure washing is commonly used for control. However, the effectiveness of conventional cleaning is relatively low, mainly attributed to strong biofilm adhesion on food contact surfaces and low penetration of disinfectants into the structure of the biofilm (Yu et al. 2021).

Effective control and inactivation of S. aureus strains and their toxins are essential to ensure food safety and protect public health. To prevent food contamination by *S. aureus*, it is imperative to implement comprehensive surveillance throughout the food chain, incorporating continuous monitoring and the adoption of Good Production and Handling Practices. It is crucial to develop guidelines and programs that prevent the extended use of antibiotics, avoiding antimicrobial resistance (Zaher et al. 2023).

5.2. *Conventional Heat Treatment (CHT)*

Thermal processing is widely used in the food industry to inactivate microorganisms, representing one of the most effective to control *S. aureus* (Shao et al. 2021). Although heat is efficient in inactivating the pathogen, it's crucial to acknowledge the heat resistance of the enterotoxins produced by *S. aureus*. Effective toxin inactivation, without compromising food quality, requires heating the food to a specific temperature for a certain period (Pereira et al. 2022). However, the heat resistance of the enterotoxins suggests the need for complementary approaches beyond heating.

For thermal treatments, two critical parameters are applied in food processing, including decimal reduction time (DT) and the thermal coefficient (z). The DT is the in minutes or equivalent necessary for a logarithmic reduction in the microbial population at a given temperature (T), and z represents the temperature difference necessary for reducing a

723 logarithmic unit in the value of DT (Stumbo 1973). These parameters help optimize the thermal
724 process, ensuring pathogen inactivation while seeking to preserve the desirable characteristics
725 of the food.

726 Although conventional heat treatment is the most used in the food industry and shows
727 efficacy on microbial inactivation, it can result in the loss of heat-sensitive nutritional
728 components and sensory quality of foods and produce chemical residues (Allai et al. 2023; Dai
729 et al. 2020; Rocha-Pimienta et al. 2020). Furthermore, conventional hot air, hot water, and
730 steam have disadvantages such as long treatment times for large volume samples, and samples
731 treated by hot water or steam may have to be dried again before storage (Gao et al. 2023).

732

733 5.3. *Alternatives approaches to CHT*

734

735 5.3.1. *Natural Antimicrobials*

736

737 An alternative to thermal treatments, natural antimicrobials can be effective in inhibiting
738 and controlling *S. aureus*. Several products of natural origin have been described as alternative
739 antimicrobials for the control of foodborne pathogen including bacteriocins, monoterpene rich
740 essential oils, curcumin, phenolics and protein extracts, and other others (Nisa et al. 2023;
741 Bezerra et al, 2022; Ramón-Sierra et al, 2020; Li et al, 2020).

742 In this context, Li et al. (2022c) described the antimicrobial activity of clove essential oil
743 against *S. aureus* in pork. The results showed a morphological damage and intracellular
744 components leakage in *S. aureus* cells yielded by essential oils. At the metabolic level, clove
745 essential oil significantly inhibited the respiratory metabolism of *S. aureus* and SEA expression
746 decreased by approximately 82.97%. The antimicrobial activity of eugenol against the
747 tetracycline-resistant strain IS-58 of *S. aureus* and its toxicity in *Drosophila melanogaster* were

examined in the study conducted by Bezerra et al. (2022). The researchers observed that eugenol inhibits the function of the TetK efflux pump, which is one of the major contributors to antimicrobial resistance in bacteria. Furthermore, toxicity tests in *Drosophila melanogaster* suggest that eugenol may exhibit safety for human use. Many other studies demonstrate that essential oils and volatile compounds are interesting for controlling *S. aureus*, but practical application or in food encounters limitations related to physicochemical, toxicological and stability properties (Rout et al. 2022).

On the other hand, the antibacterial activity of the bacteriocin LFX01 produced by *Lactobacillus plantarum* LQ80 was evaluated against *S. aureus* and *E. coli* in both planktonic and biofilm states, as well as its efficacy in preventing contamination of these bacteria in pork. The results demonstrated that LFX01 effectively inhibited bacteria growth as well as the biofilm formation, even at low concentrations. Furthermore, LFX01 successfully prevented the contamination of *S. aureus* and E. coli in pork. The study suggests that LFX01 could be a promising alternative to chemical preservatives in pork products (Xin et al. 2023). Previously, a bacteriocin (XJS01) from *L. salivarius* CGMCC2070 was able to inhibit both planktonic and biofilm states of *S. aureus* strain 2612:1606BL1486 isolated from chicken meat at MIC value of 9.85 μg/mL (Li et al. 2021). In addition, this bacteriocin exhibited good resistance to heat treatment and antibiofilm properties, where the biofilm formation was significantly inhibited by XJS01 bacteriocin at concentrations of 18, 36, and 54 μg/mL.

The antibiofilm activity of bacteriocins produced by lactic acid bacteria makes these natural compounds promising antimicrobial agents for application in food industry as an alternative against resistance of biofilm cells, including *S. aureus*, to conventional sanitizers. Regarding the antibiofilm properties, it is hypothesized that bacteriocins can be reduce the planktonic cell population and bacterial attachment on surfaces and/or contribute to biofilm eradication by inactivating the cells in preformed biofilms (Pang et al. 2022).

Just like essential oils, the application of bacteriocins as antimicrobials presents certain important limitations, mainly related to the high cost related to culture media and purification methods employed and low yields achieved through the processes of production. Moreover, other factors as stability of these compounds in the food matrix and during processing must also be considered. Regarding the use for inhibition of *S. aureus*, more studies are needed to establish a standardization in terms of concentration to be used and the effectiveness of different bacteriocins against conventional and resistant strains of *S. aureus*.

5.3.2. *Emerging technologies*

Innovative technologies have provided promising solutions for effectively inactivating *S. aureus* and its toxins in food. Despite the challenges posed by this foodborne pathogen and its resistance control, technological advancements hold promise for better control and containment. Currently, efforts have been made to develop new, more efficient, and sustainable which maintain foods, organoleptic and nutritional properties, have a low environmental impact, and are safe and economical (Allai et al. 2023).

Emerging technologies can be classified as non-thermal and thermal. The main emerging non-thermal technologies are: high hydrostatic pressure (Rocha-Pimienta et al. 2020), cold atmospheric pressure plasma (Lee et al. 2021), pulsed electric field (Qi et al. 2021; Freire et al. 2021), ozone (Sarron et al. 2021), supercritical fluids (Cristianini et al. 2023), photodynamic techniques (Li et al. 2020; Sen and Nyokong 2021), ultrasound (Yu et al. 2021; Martínez-Moreno et al. 2020) and ultraviolet radiation (Cristianini et al. 2023). The emerging thermal technologies are ohmic heating (Shao et al. 2021) and dielectric heating (microwave heating and radio frequency heating) (Leong and Oey 2022). Regarding microbial inactivation, the processing conditions differ among emerging technologies in the food industry. Nevertheless,

798 promising results have been observed across all these studies, indicating their potential
799 application in food processing. The benefits of these technologies are discussed in this section,
800 and a summary of the results is provided in Table 4. Apart from the positive aspects of the
801 technologies, the table also addressed the parameters of processes, the disadvantages of each
802 technique used, and the degree of microbial reduction in each cited study.

803 [Table 4 near here]

804 These innovative approaches offer significant advantages over the commonly employed
805 traditional pasteurization techniques in the food industry (Freire et al. 2021; Li et al. 2020;
806 Martínez-Moreno et al. 2020; Sem and Nyokong 2021; Sun et al. 2022; Urrutia et al. 2022; Yu
807 et al. 2021). Non-thermal processing techniques are widely used to enhance food safety while
808 minimizing the impact on nutritional quality compared to conventional heat treatment methods.
809 The innovative technologies aim to overcome the limitations of conventional thermal treatment
810 and preserve the original benefits of treated food products (Allai et al. 2023). The primary
811 thermal and non-thermal technologies, along with their mechanisms of action, are highlighted
812 in Figure 4.

813 [Figure 4 near here]

814

815 5.3.2.1. *Emerging non-thermal technologies - advantages and challenges*

816

817 These technologies offer promising prospects as alternatives to conventional thermal
818 treatment methods, efficiently inactivating *S. aureus* with a low impact on food quality. They
819 offer potential advantages in terms of microbial inactivation with minimal impact on food
820 quality. Together, these techniques can enhance the efficacy of microbial inactivation and
821 provide a synergistic approach to ensure food safety.

Photodynamic technology using photosensitizers is an innovative non-thermal treatment approach widely employed to enhance food safety. In this technique, photosensitizers are applied to the food and activated by light, leading to the generation of reactive oxygen species that are highly effective in microbial inactivation (Urrutia et al. 2022). A study conducted by Li et al. (2020) demonstrated the efficacy of using curcumin as a photosensitizer in combination with chitosan spray and photodynamic treatment to inactivate *S. aureus* and its biofilms. The results showed that the combination of curcumin and photodynamic treatment could effectively target bacterial cells and *S. aureus* biofilms. Furthermore, chitosan sprays with curcumin were shown to be effective in inactivating microorganisms on food equipment surfaces and fresh products. Another study investigating the enhancement of photodynamic inactivation of *S. aureus* using zinc phthalocyanines substituted with Schiff base-conjugated nanoparticles demonstrated high efficacy against *S. aureus*. The nanocomposites exhibited excellent photodynamic antibacterial activity upon light exposure, reducing bacterial growth by 100% (Sem and Nyokong 2021).

A challenge in using photodynamic technology is that light is generally the limiting factor given its low penetration depth. This makes inactivating microbial cells hidden below the top surface a challenge. The physicochemical properties of certain photosensitizers may also reduce the effectiveness of treatment. Thus, the choice becomes of utmost importance for the success of the process (Ghate et al. 2019).

Pulsed Electric Field involve the application of short pulses of high-voltage electric fields aimed at disrupting cell membranes and thus inactivating microorganisms (Freire et al. 2021). Electric fields have amplitudes in the range of 100–300 V/cm to 20–80 kV/cm and are applied to foods for short durations (from milliseconds to microseconds). It is widely used in the processing of liquid foods such as dairy products, eggs, and juices. Furthermore, the treatment practically has no effects on the sensory and nutritional properties of the foods. To date, the

exact details about how the pulsed electric field inactivates microorganisms have not yet been completely elucidated, however, the indications are that there is an increase in permeability and subsequent rupture of microbial membranes. The main disadvantage of the technology in question is the initial costs for its installation (Ghoshal 2023).

Plasma can be described as an ionized gas containing reactive oxygen or nitrogen species, ultraviolet radiation, free radicals and charged particles. When ionized gas is produced through low energy (1–10 eV) and electron density of up to 1010 cm-3, it is called cold plasma (Laroque et al. 2022). Foods are exposed to ionized gases at room temperature and atmospheric pressure. Cold atmospheric pressure plasma generates reactive species that can enter microbial cells and cause oxidative stress, leading to cell death (Lee et al. 2021). In general, the cold plasma process has high efficiency at low pH due to partial denaturation of proteins and cell leakage. However, depending on the composition of the gas used and the length of the process, the treatment can negatively influence the food components, potentially degrading them. Therefore, more studies are needed to improve the efficiency of microbial inactivation and minimize adverse effects (Laroque et al. 2022).

The use of high-pressure in food processing and preservation involves several effects, including the microbial inactivation through protein denaturation and cell structure rupture (Rocha-Pimienta et al. 2020). It is considered a cold pasteurization technique, in which pre-packaged foods are placed in a container and subjected to high levels of isostatic pressure, in the range of 300 to 600 MPa (Houška et al. 2022). The effect of high hydrostatic pressure (HHP) on inactivation of *S. aureus* sub. *aureus* and *Bacillus cereus* in human milk was recently reported (Rocha-Pimienta et al. 2020). Aiming for maximum bacterial inactivation, processing should be carried out at pressure intensity of 593.96 MPa for 233 s, that lead to decrease of 5.81 and 6.93 log CFU.mL−1 in *S. aureus* sub. *aureus* and vegetative *Bacillus cereus*, respectively.

These results suggested that this non-thermal technology could be a useful tool to increase milk safety in human milk banks.

The pressurized techniques can be used both individually and in combination with heat to increase the rate of microbial inactivation. The main advantages of high-pressure processing including rapid and uniform pressure distribution in the food matrix, processing at ambient or lower temperatures, minimal impact in nutritional or sensory properties of the food. On the other hand, the main limitations of this technique are related to the high cost of operating the pressurized system, compatibility of the technique with the food matrix and definition of parameters that allow microbial inactivation while ensuring the preservation of food quality (Gokul Nath, Pandiselvam, and Sunil 2023).

Ozonation is a promising technology that has its food disinfection mechanism based on the redox potential (2.08 eV) and the increase of intracellular reactive oxygen species, causing metabolic alterations, damage to nucleic acids, and lysis of bacterial cells (Xue et al. 2023). It is a GRAS (Generally Recognized As Safe) technology, approved by the US Food and Drug Administration (FDA) as an antimicrobial additive for food contact (Sarron et al. 2021).

The use of ozone Gas, O3(g), has been reported as effectively for disinfection and sterilization in different fields, including environmental, medical and food sectors (Ofori et al. 2018; Britton et al. 2020; Xue et al. 2023). Recently, a study performed by Taba et al. (2022) evaluated the inactivation of *S. aureus* in experimentally contaminated surgical needles, after submitting them to low pressure, ozone gas, and the combination of these techniques. Among the results obtained, the authors highlight that an ideal protocol for inactivating *S. aureus* would be using low pressure for 30 min and to ozone gas for 30 min with a 60 mgO3/cm2 dosage.

Some advantages of this technique comprises the environmental compatibility due to low waste generation, short disinfection contact time compared to other methods, no generation of heat during treatment (which means an advantage for use in heat-sensitive foods). However,

there are some limitations: Ozone is corrosive at ppm greater than 4 ppm, its storage is not possible as it is an unstable gas and decomposes quickly (it must be produced in situ at the place of use), it requires monitoring to check for possible leaks. When inhaled, it can cause health problems (Prabha et al. 2015).

Microbial inactivation by supercritical fluids generally occurs by supercritical CO2, which can modify the intracellular pH of microorganisms and induce protein denaturation and enzymatic inactivation by altering the conformation of these structures. The diffusion time of CO_2 is crucial for the treatment. The microbial inactivation process can be carried out in both continuous and batch mode, and minimizes the loss of heat-labile compounds, especially in fruit and vegetable juices, is easy to handle, safe and has a low cost of acquiring critical CO_2, in addition to the possibility of work in coupled mode with other technologies (Cristianini et al. 2023). Something important for implementing supercritical fluid technology is checking possible chemical changes in food due to the process and identifying the nutritional content of the food, in order to validate the process (Braga, Gaspar, and Sousa. 2023).

Ultraviolet radiation causes microbial inactivation through the absorption of UV light incident on the DNA of microorganisms, leading to injuries that impact the replication of their genetic material. It is generally applied to liquid foods and beverages, in addition to fresh products. Shortwave radiation (200-280 nm) is the most lethal and could inactivate several pathogenic bacteria, such as *Staphylococcus*. UV radiation is energy efficient and cost-effective when compared to other methods, and minimally affects nutritional and sensory compounds (Cristianini et al. 2023). However, the method has some restrictions, as shortwave radiation has limited penetration and therefore it is necessary to ensure that the entire surface of the food is exposed through rotation or fluidization. Another aspect is the lack of globalized regulatory approval for broad implementation on an industrial scale (Cristianini et al. 2023).

Recently, many studies have examined the application of ultrasound technology in microbial inactivation (Martínez-Moreno et al. 2020; Nascimento et al. 2023; Sun et al. 2021; Yu et al. 2021). Ultrasound is a form of vibrational energy characterized by acoustic waves with frequencies above 20 kHz, exhibiting directionality, penetrability, and reflectivity. In the food industry, ultrasound is often used for food processing, with ultrasonic transducers producing frequencies ranging from 20 kHz to 10 MHz. This technology is considered environmentally friendly, highly efficient, non-toxic, and cost-effective (Zhang et al. 2022). It has been demonstrated that high-intensity ultrasound, with power levels ranging from 1 to 1,000 W/cm^2, leads to a faster rate of cavity formation compared to low-intensity ultrasound (<1 W/cm^2) (Yu et al. 2021).

Ultrasound technology is a non-destructive, non-thermal, and environmentally friendly approach widely used in the food industry. However, a major challenge in using ultrasound is its use in large-scale equipment operations, and treatment with ultrasound alone has shown limited antibacterial and antibiofilm activity in inactivating bacterial cells (Martínez-Moreno et al. 2020; Sun et al. 2021; Yu et al. 2021). Therefore, many researchers have been investigating synergistic inactivation methods to enhance the technique efficiency. Ultrasound can, for example, be combined with vacuum and heat, with the resulting technique called vacuum thermosonication (Martínez-Moreno et al. 2020). Moreover, He et al. (2021) described that combined treatment with ultrasound and photodynamic techniques significantly reduced *S. aureus* populations by 3.21-4.51 log CFU/mL. However, after 3, 6, and 9 minutes of sonication, the *S. aureus* population decreased from 0.03 to 0.36 log CFU/mL. It is believed that sonication facilitates the entry of larger photosensitizing agents into the bacterial cell, enhancing their intrinsic biocidal effect and ultimately leading to cell death. Additionally, it is important to note that the frequency and power of ultrasound are crucial factors during the treatment.

5.3.2.2. *Emerging thermal technologies - advantages and challenges*

Emerging thermal technologies have positive characteristics in providing more uniform, rapid, and sustainable heating, all with lower costs and environmental impacts. They rely on the generation of heat directly within the food, the complexity, and inherent properties of the food in question (Leong and Oey 2022).

Non-ionizing radiation heating (radiofrequency and microwaves) occurs through the interaction of electromagnetic waves in their frequency ranges with dielectric materials, providing volumetric heating within the food (Cristianini et al. 2023). Radiofrequency generates heat inside the samples through the rotation of polar molecules and ionic movements, resulting in volumetric heating effects. It is capable of penetrating through non-metallic packaging and allowing contactless heating (Gao et al. 2023). Microwave heating of food results from the electrical transfer of the electromagnetic field in the device's chamber. Its use increases production efficiency in the food industry due to reduced heat treatment time and energy consumption (Guzik et al. 2021). However, due to its longer wavelength, which means greater depth of penetration, radiofrequency is more suitable for treating large volume samples than by microwaves and is a technique with the potential to replace conventional heating methods (Gao et al. 2023).

Thermosonication can also be cited as an interesting approach to microbial inactivation by combining ultrasound and heat (Martínez-Moreno et al. 2020; Nascimento et al. 2023). It is an effective method to reduce/eliminate the microbial load in general from foods, including bacteria, viruses, yeasts, and fungi, for example. Furthermore, it has little impact on the organoleptic and nutritional characteristics of foods and is a process that requires less processing time and energy expenditure. Some disadvantages of this approach including high costs of related to equipment and establishment of process parameters that are suitable for

bacterial inactivation and that do not cause loss of sensorial and nutritional quality of the food matrix (Abdulstar, Altemini, and Al-Hilphy 2023). In this sense, Nascimento et al. (2023) conducted an investigation into the application of thermosonication in dairy products, with a specific focus on cream, assessing the efficacy of this technique in inactivating MRSA during slow and fast pasteurization processes. The results revealed that thermosonication was able to reduce MRSA counts by up to 4.72 log CFU/mL in cream, a result comparable to the 4.82 log CFU/mL reduction achieved through conventional pasteurization. This study highlighted thermosonication not only as an energy-efficient alternative but also as adepted of preserving the microbiological safety of cream with a significant reduction in processing time.

Ohmic heating, also known as Joule heating, is a technology that converts electrical energy into thermal energy without creating temperature gradients within the product, resulting in a more uniform heat dissipation throughout the food matrix. This approach prevents overheating of the food surface, preserving nutrients and sensory attributes (Shao et al. 2021). Ohmic heating can inactivate bacteria not only through its heating effects, but also through the non-thermal (physical chemical) effects of the electric current. The chemical effect occurs through the formation of free radicals, oxygen, hydrogen, hydroxyl, and mineral ions, which cause the death of microbial cells. The mechanical effect occurs through the rupture of the cell membrane (Alkanan et al. 2021).

It is a quick and homogeneous treatment, ideal for products sensitive to shear due to the low flow speed, and which causes minimal nutritional loss. Disadvantages of the method include the initial cost of ohmic heating systems, higher than that of conventional heating methods, and the non-effective heating of foods containing fat granules due to non-conduction due to lack of water and salts. Consequently, pathogenic bacteria present inside the fat granules are less exposed to heat treatment than bacteria outside the fat particles (Alkanan et al. 2021).

Although traditional thermal methods are effective in microbial inactivation, they often adversely affect the nutritional and sensory quality of food products, in addition to presenting limitations against biofilm structures. Conversely, non-thermal alternatives, including the use of natural antimicrobials and emerging technologies that do not apply heat, stand out for their potential to preserve the organoleptic and nutritional characteristics of foods intact. The selection of the optimal technology must take into account the type of food, the desired qualities for the final product, operational costs, as well as effectiveness against specific pathogens. High-value and heat-sensitive foods are ideal candidates for the adoption of these non-thermal technological innovations. Therefore, an efficient strategy may involve the synergistic combination of methods, exploiting the complementary advantages of multiple technologies to achieve effective inactivation of pathogens such as *S. aureus*, while preserving or even enhancing the quality and safety of foods.

6. Concluding remarks

This review underscores the significant food safety and public health challenges posed by *S. aureus* and its enterotoxins, highlighting the severity of food poisoning from consuming contaminated foods. The emergence of antimicrobial-resistant strains of *S. aureus* further complicates the control and prevention of these infections. Although proper hygiene practices and temperature control are fundamental measures to minimize the risk of contamination, technological advancements have provided promising solutions for the effective inactivation of *S. aureus* and its toxins in food.

However, despite these advancements, significant challenges remain. The gaps identified in this review point to the need for ongoing development and enhancement of innovative techniques to improve the inactivation of *S. aureus* and its toxins. Specifically,

the current literature lacks studies detailing the action mechanisms of new technologies on different strains of *S. aureus*, particularly those resistant to multiple antimicrobials. Moreover, there is a dearth of research integrating contamination prevention strategies at various points in the food production chain, from production to the end consumer.

Therefore, this review emphasizes the importance of continuous surveillance and the adoption of innovative technologies to control and contain *S. aureus* and its toxins, stressing that to advance food safety and protect public health against this persistent foodborne pathogen, it is crucial to address the identified knowledge gaps and fully explore the potential of technological advances. Multidisciplinary collaboration among microbiologists, food technologists, public health professionals, and the food industry will be key to developing comprehensive control and prevention strategies, aligned with the complexities of this global health challenge.

Acknowledgments

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico(CNPq) Brazil—grant numbers [402430/2018-2], [405728/2018-2], [313119/2020-1], [303074/2021-3], [311863/2022-1] and [404304/2022-2] .

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Table 1**.** Occurrence of foodborne transmission and outbreaks related to *Staphylococcus aureus* published worldwide.

Country/ Year	**Cases /Clinical symptoms**	**Vehicle**	**SE detected**	**Infectious dose**	**Method of detection**	**Reference**
China (2011 – 2016)	N/A	4300 (retail foods)	*SEA and SEH*	N/A	Microbiological Analysis Polymerase Chain Reaction PCR Whole-genome sequence	Zhang et al. 2022
France (2016-2017)	Vomiting, diarrhea, abdominal pain, nausea	Salad with eggplant Grilled cucumber, Smoked chicken	SEB, SEG and SEI	N/A	Microbiological Analysis, Whole-genome sequence analysis	Cavaiuolo et al. 2023
Vietnã (2018)	352 children nausea, vomiting, abdominal pain, and headache	Deep-fried shrimp chicken floss	SEA and SEC	≥0.211 ng	Microbiological and Molecular Analysis	Thi Le et al. 2021
Korea (2018)	86 participants/ Vomiting, diarrhea, nausea, abdominal pain and chills	Rice cakes and Food handler	SEA, SEG, SEI, SEM, SEN, SEO, SEU, SAK and SE*l*W	N/A	Microbiological na Phylogenetic analysis Whole-genome sequencing *In silico* molecular typing	Park et al. 2020
Egypt (2018)	N/A	144 chilled or frozen broiler chicken meat	SEB	N/A	Microbiological na Phylogenetic analysis DNA extraction	Abolghait et al. 2020
Brazil (2018)	N/A	Institutional and domestic kitchens hands and nostrils of the	SEA and SED	6 ng	Microbiological Analysis PCR	Freitas et al. 2018

		kitchen personnel				
China (2021)	N/A	4262 (retail food), 61 (food outbreaks)	SEA, SEE, SEC, SE*l*L, SEB, SEH, SED , SE*l*Q and SE*l*K	N/A	Microbiological na Phylogenetic analysis Whole-genome sequencing	Li et al. 2022
Brazil (2018)	N/A	Food handlers	SEA, SEB, SEC1, SEC5 and SED	N/A	Microbiological na Phylogenetic analysis PCR	Pereira et al. 2021

Abbreviations: N/A= not available.

Table 2. Comparative analysis of production capacity, resistance conditions, and predictive models of Staphylococcal Enterotoxins

Enterotoxin	Production capacity	Resistance conditions	In vivo effects [a]	In vitro effects[b]	Prediction models[c]	References
SEA	- Higher expression at room T (25°C) compared to 8°C after 16 hours [1] - Significant expression at 37°C for 24 hours [2] - Production more likely at pH > 5.0, aw > 0.86, and T > 15°C [4]	-Thermal stability with reduced growth rates: -100°C/3min. (36.8%) -110°C/3min. (34.2%) -121°C/3min. (31.6%) [2] Refolding capacity at pH 4.5 in the presence of zinc. No refolding at pH 4.5 with irreversible loss of structure [3]	N/A	- UHT milk [1] - Pasteurized milk with 1.5% fat content [2] - (DSC) and Biological activity assays [3]	Linear logistic regression model [4]	Homsombat et al. 2021 [1]; Necidová et al. 2019[2] Berry et al. 2022[3] Ding et al. 2016 [4]
SEB	- Higher expression at 24°C when MRSA reached 7.3 × 10^3 ± 1.2 × 10^3 CFU/g [5]	- Refolding capacity at pH 4.5 - Higher residual biological activity at pH 4.5. - Affected by ionic strength and elevated pH (pH 5.6) [3]	N/A	- DSC and biological activity assays [3] - Raw chicken liver [5]	N/A	Berry et al. 2022[3] Abolghait et al. 2020[5]
SEB (SE02)	- Higher expression observed between 20°C and 25°C.	- Reduction after heat treatment: reduction after 1 hour and inhibition after 6 hours at 100°C. - Resistance to enzymatic digestion: rapidly degraded by pepsin in acidic pH (acetic acid, pH 4.5), quickly degraded by trypsin.	Animal models	N/A	N/A	Suzuki et al. 2020
SEC	Higher expression at room T (25°C) compared to 8°C after 16 hours [1]	N/A	N/A	-UHT milk [1]	N/A	Homsombat et al. 2021[1]

SED	- Notable production of SE (47,300 ng/mL and 49,200 ng/mL) at 37°C for 24/48 hours [6]	N/A	N/A	-UHT milk - Genomic analysis	N/A	Chieffi et al. 2020
SEH	N/A	- Refolding capacity at pH 6.8. - No refolding at pH 4.5 with irreversible loss of structure.	N/A	- DSC and biological activity assays	N/A	Berry et al. 2022
SElX	N/A	- Resists high T up to 100°C. - Resistance to enzymatic digestion: pepsin at different pH conditions (4.0 and 4.5)	Animal models	- Biological activity assays	N/A	Wan et al. 2023
SEA a SED	- T > 30°C, pH 4.6 – 6.0 - NaCl (2.2–5.6%) and $NaNO_2$ (0–150 ppm) influence growth and production	- T: 10–40°C, optimum >30°C. - pH: Inhibition at pH 2.5. - Effect of temperature and pH: Between T of 35°C and 40°C with pH 5.8 and 2.5% WPS + 80 ppm sodium nitrite reduce production. - Intensification of inhibition: Increase to 150 ppm of sodium nitrite intensifies inhibition.	N/A	- Meat products	Predictive model using neural network based on logistic regression	Gunvig et al. 2018
General	- T (32°C and 44°C) and Aw (0.938) affect growth rate and latency time. - Minimum inoculum (3.22 log CFU/g) for enterotoxin production.	N/A	N/A	- Kazakh cheese	Predictive model using artificial neural network	Cai et al. 2023

Legend: DSC: Differential Scanning Calorimetry; aw: Water Activity; T: Temperature

(a) In Vivo: Specific conditions found in studies involving animal models; (b) In Vitro: Specific conditions found in studies involving experimentally contaminated food categories, in controlled culture media, and biological assays; (c) Prediction Model: Results from simulations or predictive models regarding the stability or resistance of enterotoxins.Notes: Resistance conditions may vary significantly between different types of foods. This table provides a generalized summary; Some values, such as temperatures and pH ranges, are presented as intervals or general conditions to reflect variations found in the literature.Abbreviations: N/A= not available.

Table 3**.** Major characteristics of staphylococcal enterotoxins .

SE	Sequence Length	Mass (kDa)	Genetic Element	Type	Emetic activity
SEA	233	27.1	Prophage	Classical	+
SEB	238	28,4	Chromosome, Plasmid	Classical	+
SEC1	nd	27,5	SaPI	Classical	+
SEC2	239	27,6	SaPI	Classical	+
SEC3	239	27,6	SaPI	Classical	+
SED	nd	26,9	Plasmid	Classical	+
SEE	206	26,4	Prophage	Classical	+
SEG	233	27,0	SaPI	New	+
SEH	214	25,1	Transposon	New	+
SEI	nd	24,9	SaPI	New	+
SE*l*J	nd	28,5	Plasmid	New	nd
SE*l*K	nd	26,0	Prophage, SaPI	New	+
SE*l*L	nd	26,0	Prophage, SaPI	New	+
SE*l*M	nd	24,8	SaPI	New	+
SE*l*N	227	26,1	SaPI	New	+
SE*l*O	nd	26,7	Transposon	New	+
SE*l*P	nd	27,0	Prophage	New	+
SE*l*Q	nd	26,0	Prophage, SaPI	New	+
SE*l*R	nd	27,0	Plasmid	New	+
SES	nd	26,2	Plasmid	New	+
SET	nd	27,1	Plasmid	New	+
SE*l*U	nd	27,0	SaPI	New	nd
SE*l*V	nd	27,6	Chromosome	New	nd
SE*l*W	nd	nd	SaPI	New	nd
SE*l*X	nd	19,3	Chromosome	New	nd
SE*l*Z	nd	nd	nd	New	nd

Abbreviations: SE = staphylococcal enterotoxins; SaPI = *S. aureus* pathogenicity island; nd= not demonstrated or unavailable; + = presence of emetic activity.

Source: adapted from Fisher, Otto, Cheung, (2018)

Table 4. Effect of new technologies (thermal and non-thermal) on the inactivation of *S. aureus* in biofilm, bacterial suspension, and food products, as well as advantages and disadvantages

Technology	Study Matrix	*S. aureus* strains	Treatment Parameters	Reduction	Advantages	Disadvantages	Reference
Ultrasound	Biofilm (*S. aureus)*	ATCC 6538	20 kHz,240 W, >20ºC, 10 min, Acoustic pressure: 1.38×10^{5} Pa	4.21 ± 0.10 log 10 CFU/cm^2	-Nonthermal technology -Change in cell structure -Effective biofilm detachment	-The technique needs to be combined to be effective. -HIU alone may not be a promising approach -Bactericidal effect is very limited even at high potency	YU et al. 2021
Ultrasound and chlorogenic acid	- Mutton was - Biofilms (*S. aureus)*	*S. aureus*	50 kHz, 800 W	1.14 log CFU/g/ 60 min	-Nonthermal technology -The synergistic bactericidal effect had produced by US and CA - Changed the morphology of *S. aureus* cells - Led the leakage of internal constituents - Reduced the concentration of polysaccharide in biofilms	-US treatment alone exhibits weak antibacterial and antibiofilm activities for the inactivation of cells *S. aureus*	Sun et al. 2021
Ultrasound and thyme essential oil	Bacterial suspension	ATCC 25923	3.33, 5.00, and 6.67 W/mL for 3, 6, and 9 min on a pulsed mode (2s on: 2s off)	3.21–4.51 log CFU/mL	-Nonthermal technology	-US alone did not produce a clear decrease in the number of S. aureus cells	He et al. 2021

					-The combined treatment significantly reduced *S. aureus* -Synergistic effect causing physical damage on cell morphology -Synergic effect facilitated the entry of the essential oil into the bactéria -Reduction of bacterial membrane hydrophobicity		
Curcumin photodynamic technology	Biofilm (*S. aureus)*	ATCC 25923	25 or 50 μmol/L curcumin-loaded chitosan photosensitizer	5.0 log CFU/mL	-Nonthermal technology -Avoidance of the development of resistance -Absence of undesirable chemical residues -Suitable for heat-sensitive foods and materials	-Low water-solubility of curcumin -Inactivation effects depends of curcumin concentration, co-incubation time and illumination time	Li et al. 2020
High voltage electrostatic field	Medium plates and the surface of salmon, griskin,	NCTC 8325-4	Salmon -98.3 ± 0.2% Griskin -98.6 ± 0.3% Cheese -99.8 ± 0.1% Sausage - 99.6 ± 0.1% (13 kV for 30 min)	2.29 logs in the first 11 min, and treatments for an additional 10 min	-Nonthermal technology -Great antibacterial effects against *S. aureus* on solid surfaces -Maintains the organoleptic and nutritional properties roughly unchanged -Low energy consumption	-HVEF and HVAEF were not effective in the sterilization on *S. aureus* in all the supporting environments.	Qi et al. 2021

	cheese and sausage		three kinds of electric fields – HVEF, HVAEF and HVPEF	only caused a reduction of 0.46 logs	-High environmental protection -Economic feasibility		
High hydrostatic pressure	human milk	CECT 976	593.96 Mpa/233 s	6–7 log cfu mL^{-1}	-Nonthermal technology -Causes critical damages in microorganisms from 100MPa onwards -Prevents nutritional loss -Effective alternative to holder pasteurization	-However, the agri-food industry is limited to applying treatments of 600 MPa since it uses water as a pressure transmitting fluid	Rocha-Pimienta et al. 2020
Cold Atmospheric Pressure Plasma	Bacterial suspension	SA1, SA2 and SA3	0.05 A, 65.0V and operating power of 50mW Plasma jet operating in argon/ 5 to 120 s	1-2 log cycles	-Nonthermal technology -Shorter times required for treatment -No handling of chemicals required -No need to use water -Can be used at products sensitive to thermal process or surfaces of equipment in food industry	-No significant impact on membrane permeabilization, RNA and DNA damage, or esterase activity - But longer treatment times are necessary	Lee et al. 2021

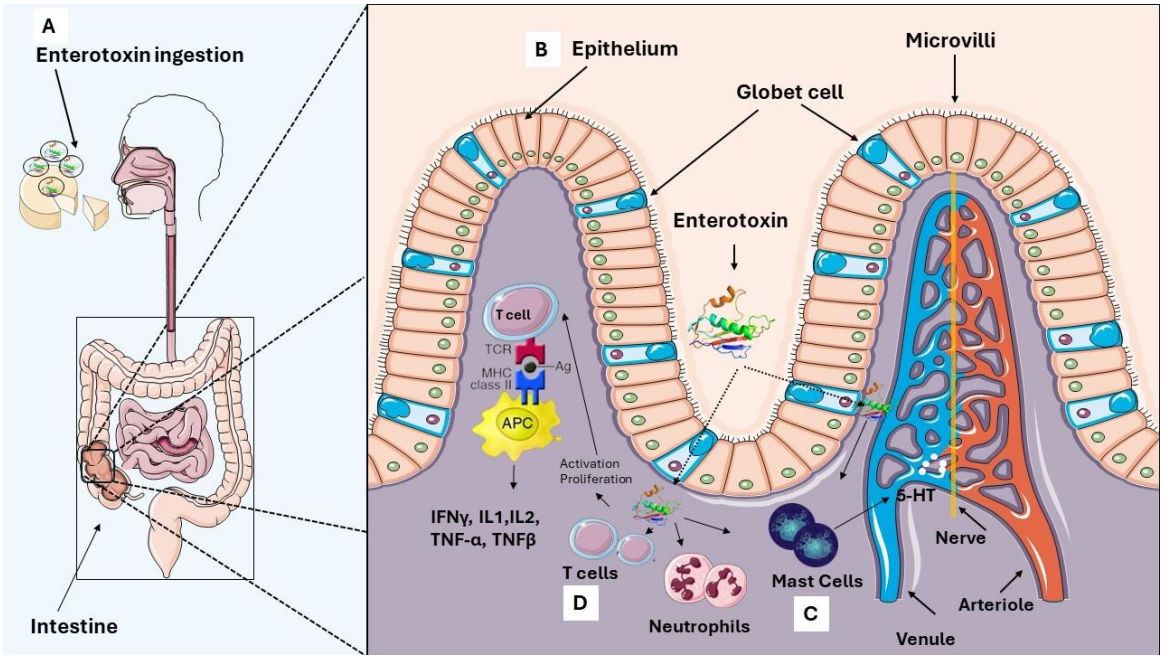
Pulse Electric Fields	Bacterial suspension	CECT 4459	18 and 25 kV/cm 20 to 400 μs Frequency of 0.5 Hz to minimize sample heating	Generation of pores with a radius higher that 0.7 nm	-Nonthermal technology -Strong antibacterial effect - Release of cellular components -Maintains food organoleptic and nutritional properties roughly unchanged -Low energy consumption -High environmental protection -Economic feasibility	-Reversely permeabilized cells might be able to reseal their envelopes and outgrow, given the appropriate recovery conditions are provided	Freire et al. 2021
Photodynamic inactivation / of Phthalocyanines	Bacterial suspension	ATCC 25923	Various irradiation - 0.5 W cm^{-2} Irradiance energy doses 0.9 kJ cm^{-2} to 3.6 kJ cm^{-2}/ 20 min - 80 min	9.67 log	-Nonthermal technology -Stability, durable and broad-spectrum antimicrobial properties of Silver - Efficient delivery of drug to bacteria site through enhanced permeability - Efficient inactivation of resistant strains	-Low solubility or high aggregation tendency prevents ((Phthalocyanines) them from showing a good therapeutic antimicrobial effect	Sen and Nyokon 2021
Vacuum-thermosonication	Sourso p puree	ATCC 33862	Vacum - 16.93 kPa heat - 50 °C 1–3 intermittent vacum pulses (24 kHz/0.34 W/g/10 min)	≥7 log CFU	-Effective against *S. aureus* cells -Great potential to be applied in viscous food such as fruit purees or similar, a limiting of ultrasound alone	-Thermal technology -Additional researches are needed to generate more knowledge on the effect of the VTS technology on different food matrix	Martínez-Moreno et al. 2020

			Acoustic energy density of 85 W/cm ^{2}		-Can be scalable to industrial applications because it is cheaper than MTS -Can be recognized as a "green" and sustainable alternative due to the reduction of energy consumption, elimination of waste-water, decrease in processing time and costs, insurance of safety		
Ohmic heating	Milk, apple juice and broth	ATCC 6538	10 V/cm e 50 Hz 24.8–26.3 °C	1.93 and 1.69 log CFU/ mL Broth - 1.87 and 1.15 log CFU/mL	- Inactivation of *S. aureus* in the food system, especially those with lower pH	-Requires specialized equipment and can be expensive to integrate -Food quality had no significant difference with conventional heat treatment.	Shao et al. 2021

Abbreviations: HIU = High-Intensity Ultrasound; CFU - Colony Forming Units; AP = Acoustic Pressure; US = Ultrasound; CA = Chlorogenic Acid; HVEF = High Voltage Electrostatic Field; HVAEF = High Voltage Alternating Electric Filed; HVPEF = Prick Electrostatic Field

Figure caption

Figure 1. Pathogenicity mechanism of staphylococcal enterotoxins.



(A) The initial exposure begins with the ingestion of foods contaminated with preformed staphylococcal enterotoxin, which is resistant to extreme heat and acid, allowing it to survive until reaching the intestine where it starts its harmful effects. **(B)** In the intestine, the toxin binds to the cells of the intestinal epithelium, entering the body through mucous-producing goblet cells and epithelial cells, where it begins its action process. **(C)** The release of serotonin by activated mast cells, in response to the toxin, stimulates the vagus nerve through interaction with the 5-HT/serotonin precursor, inducing the emetic response. **(D)** Acting as a superantigen, the toxin triggers the activation of T cells and neutrophils by binding to the MHC II molecule on antigen-presenting cells and to the TCR on T cells, leading to a massive release of cytokines and an intense inflammatory response.

Figure 2. Potential *S.aureus* contamination routes in Dairy Products in Milking, Production and Processing Environments

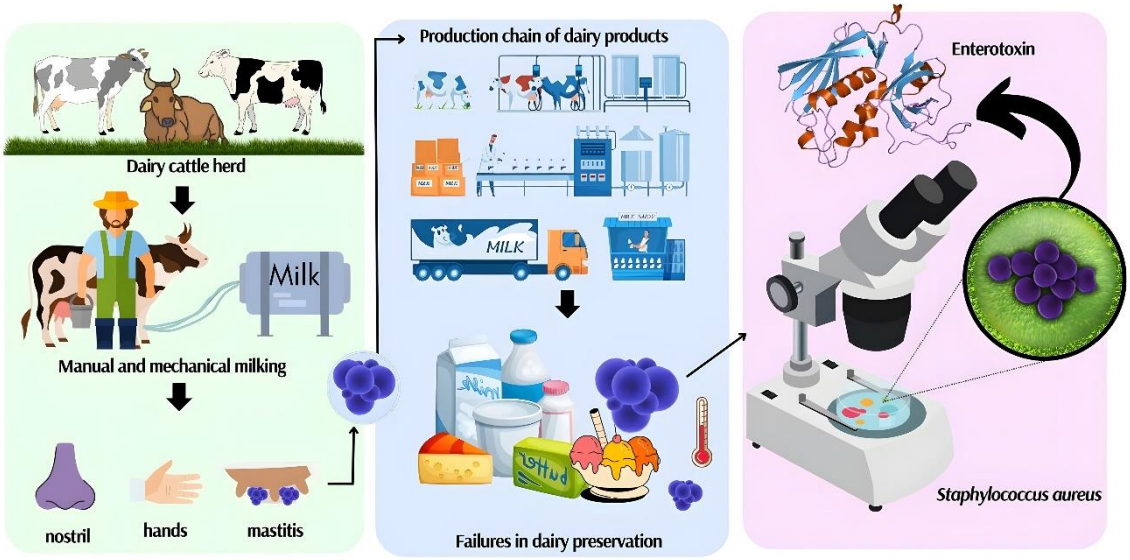


Figure 3. Number of all-age MRSA deaths attributable to antimivrobial resistance. For the 7 GBD super-regions, 2019

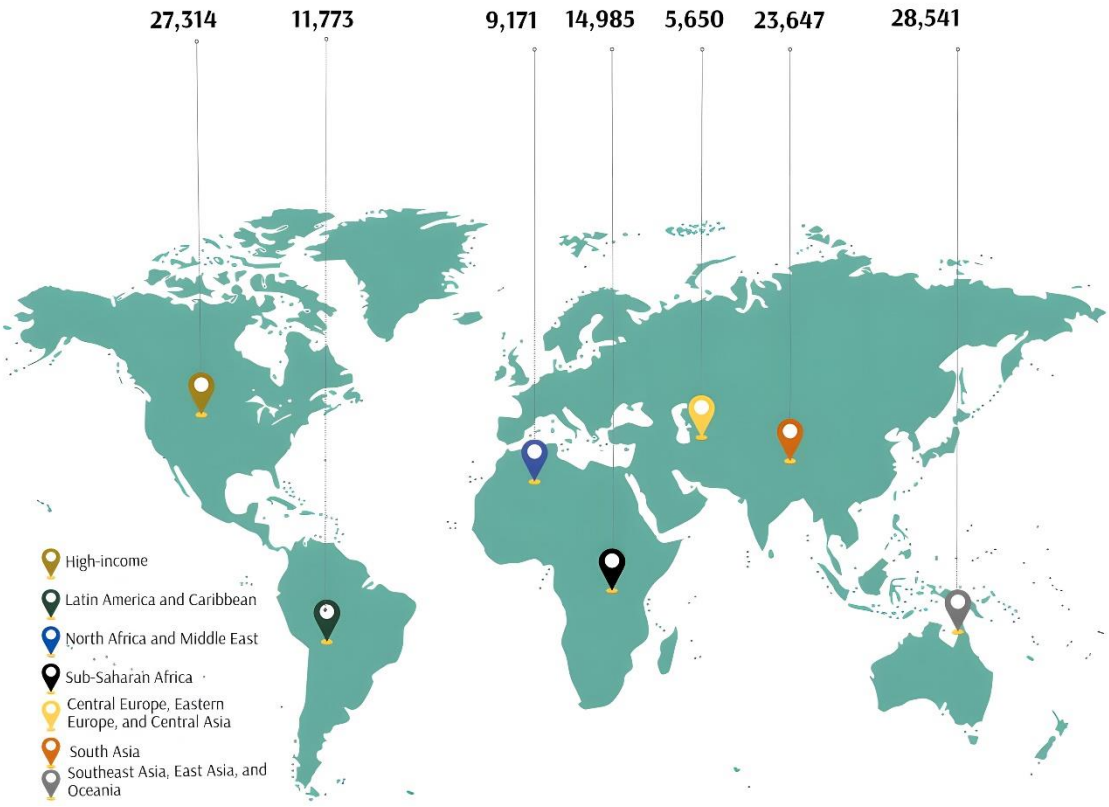
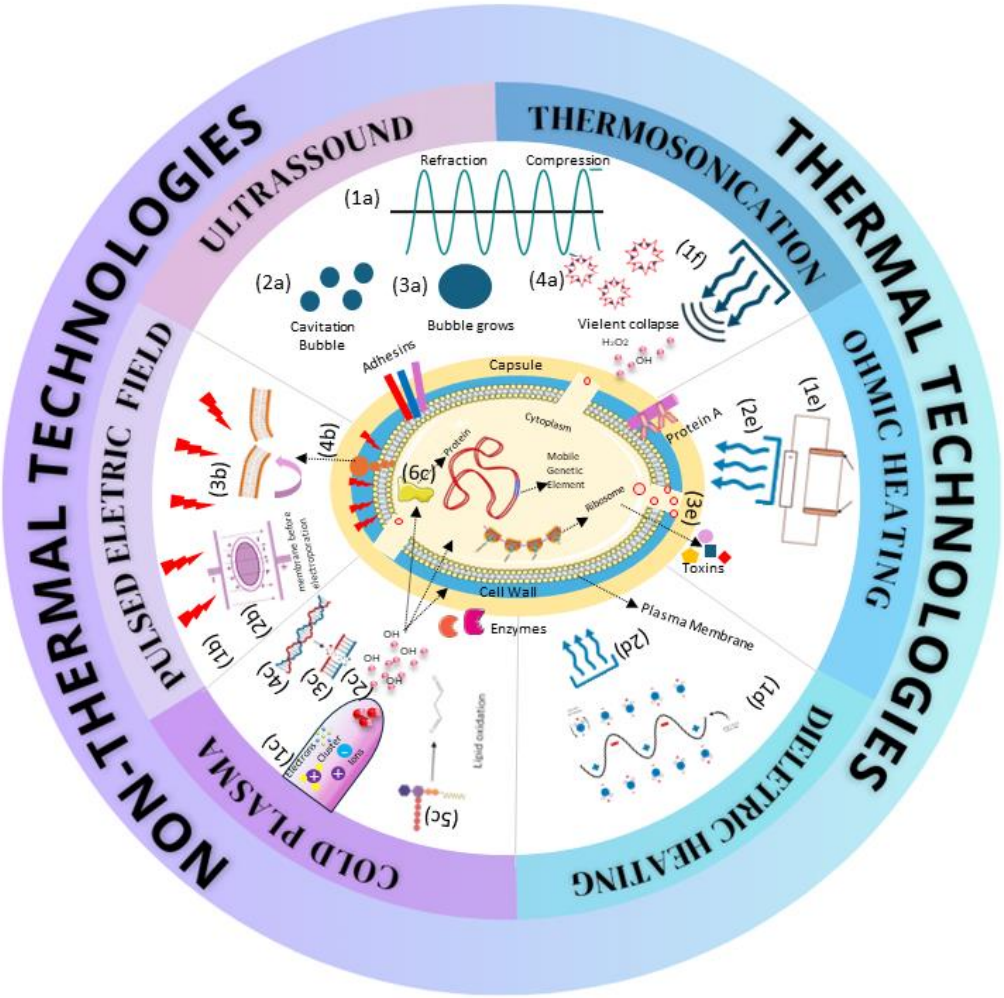


Chart: IHME Source: IHME Get the data Created with Datawrapp

Source: adapted from Ikuta et al., 2022

Figure 4. Mechanism of microbial inactivation using the main non-thermal and thermal Technologies



Microbial inactivation (non-thermal Technologies) - Ultrasound- (1) Generation of high-frequency sound waves by ultrasonic equipment (acoustic cavitation); **(2)** Bubble formation by negative pressure; **(3)** Growth and collapse of bubbles under compression and expansion of waves; **(4)** Violent collapse of bubbles produces free radicals and hydrogen peroxide, damaging the cell walls. **Pulsed electric field: (6)** Electric field applied externally for microseconds; **(7)** Creation of intense electric field across the cell membrane; **(8)** Electroporation and disintegration of the cell membrane; **(9)** Electric field generates pores in the membrane, damaging the cell wall. **Cold plasma: (1c)** Gas energized by high voltage becomes plasma; **(2c)** Formation of reactive oxygen, nitrogen, and other species by electrostatic forces; **(3c)** Reactive species induce oxidative stress in cells, damaging the cell membrane; **(4c)** Destruction of genetic material; **(5c)** Lipid oxidation; **(6c)** Protein denaturation leading to cell destruction.

Microbial inactivation (Thermal Technologies) - Dielectric heating: (1d) Oscillating electric field applied to the dielectric material induces periodic changes in polarity; **(2d)** Generating heat and damaging cellular structures. **Ohmic heating: (1e)** Electric current heats the conductive material (Joule effect); **(2e)** Efficient conversion of electricity into heat, distributing it quickly and uniformly; **(3e)** Damage to cellular structures due to the increase in temperature. **Thermosonication: (1f**) combines ultrasound and heat.

5.4

Manuscrito: Recent advances in ultrasound application in the dairy industry: efficacy and challenges in microorganism inactivation

Recent advances in ultrasound application in the dairy industry: efficacy and challenges in microorganism inactivation

ADVANCES IN ULTRASOUND FOR THE DAIRY INDUSTRY

Periódico a ser submetido (1ª submissão):	*Brazilian Journal of Food Technology ISSN: 1516-7275*
Maior percentil (Scopus):	*62%*
Periódico a ser submetido (2ª submissão):	
Maior percentil (Scopus):	

Abstract

This literature review explores the application of ultrasound technology to inactivate microorganisms in dairy products. By examining recent studies from the past decade obtained from databases such as Google Scholar, SciELO, Scopus, and ScienceDirect, this review evaluates the effectiveness and potential of ultrasound as an alternative to traditional thermal methods. The main focus is ultrasound mechanisms, including cavitation, and its impact on dairy products' sensory and nutritional properties. Key findings highlight the advantages of ultrasound, such as its non-thermal nature, which helps preserve the quality of dairy products, and its effectiveness in reducing microbial loads. Additionally, the review discusses the technical, economic, and regulatory challenges that must be addressed for widespread industrial adoption. Future research directions are suggested to enhance the application of ultrasound in the dairy industry and improve food safety and quality.

Highlights

-Ultrasound effectively reduces microorganisms in dairy products with energy efficiency.
-The integration of ultrasound and heat enhances microbial inactivation and preserves quality.
- Combined ultrasound and thermal treatment are effective for microbial inactivation in some milk products.
-Challenges in industrial applications include costs and the need for parameter optimization.

- The integration of ultrasound with heat treatment can be a viable alternative to traditional thermal processing methods.

Keywords: High-Intensity Ultrasound; Microbial Control; Dairy Products; Thermosonication; Food Safety; Energy Efficiency

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1 Introduction

Milk and dairy products are often recommended as part of the daily diet due to their rich composition of essential nutrients. However, these nutrients can also promote the growth of pathogens, leading to outbreaks of foodborne illnesses (Qi et al., 2024). The high demand for dairy products underscores the importance of ensuring their quality and safety. Due to their composition, these products can provide an ideal environment for the proliferation of pathogenic microorganisms, which compromise both sensory and nutritional quality and food safety. Therefore, ensuring the microbiological safety of dairy products is crucial for protecting consumer health and maintaining product integrity. (Aaliya et al., 2021; Dash et al., 2022).

Traditionally, the dairy industry employs thermal methods, such as pasteurization and sterilization, for microorganism inactivation. Pasteurization, for example, involves heating milk to specific temperatures for a determined period to eliminate pathogenic microorganisms and reduce the total microbial load. Although effective, these methods can negatively affect the sensory and nutritional properties of the products, resulting in nutrient loss and changes in flavor and texture (Shoaib et al., 2023). Inadequate thermal treatments can also contribute to product recalls and outbreaks of foodborne illnesses. Dairy products, especially raw milk, have frequently been associated with outbreaks of foodborne diseases (Table 1).

Table 1

Given the limitations of traditional thermal methods, ultrasound technology emerges as a promising alternative for microorganism inactivation in dairy products (Balthazar et al., 2019). Ultrasound uses high-frequency sound waves to generate cavitation, a phenomenon that results in the formation and collapse of bubbles in a liquid, producing intense mechanical forces capable of inactivating microorganisms. In addition to being a non-thermal technique, ultrasound can be applied controlled to minimize negative impacts on the sensory and nutritional properties of dairy products (Rathnakumar et al., 2023; Scudino et al., 2020).

This review aims to explore the application of ultrasound technology for microorganism inactivation in dairy products, highlighting its effectiveness and the challenges associated with its industrial implementation. The review will discuss the mechanisms of ultrasound action, its advantages and limitations compared to traditional methods, and the technical and economic obstacles that need to be overcome for

widespread adoption of this technology in the dairy industry. Finally, future perspectives and emerging research areas will be presented, which may contribute to advancing the application of ultrasound in microorganism inactivation in dairy products.

2 Methodology

This literature review focused on applying ultrasound for microorganism inactivation in dairy products. A detailed search was conducted on various sites and databases such as Google Scholar, SciELO, Scopus, and ScienceDirect prioritizing studies published in the last ten years. The main search terms included "ultrasound," "microorganism inactivation," "dairy products," "ultrasound technology," "cavitation," and "microbiological quality."

3 Fundamentals and applications of ultrasound in microorganism inactivation

Effective control and inactivation of microorganisms are essential to ensure food safety and protect public health. Pasteurization, traditionally used in the dairy industry, aims to reduce the microbial load in dairy products (Aaliya et al., 2021; Dash et al., 2022; Najmitdinova, 2023). Although effective in microbial inactivation, conventional thermal treatment has been challenged by the emergence of resistant bacterial strains, driving the search for new processing technologies (Shoaib et al., 2023). In this context, ultrasound technology emerges as a promising alternative, not only for its effectiveness but also for being environmentally friendly (Balthazar et al., 2019; Bernardo, Rosário, Conte-Junior, 2021; Bhargava et al., 2021; Carrilho-Lopez et al., 2021).

Ultrasound uses high-frequency sound waves that exceed the human hearing range, typically with frequencies above 20 kHz (Alvarenga et al., 2021; Rathnakumar et al., 2023; Scudino et al., 2020). The waves are classified by amplitude, wavelength, and frequency. Based on sound frequency, ultrasound applications can be divided into high intensity - low frequency (I = 10–1000 W/cm² and F = 20–100 kHz) and low intensity - high frequency (I < 1 W/cm² and F > 1 MHz) (Balthazar et al., 2019; Bhargava et al., 2021; Carrilho-Lopez et al., 2021).

High-frequency or low-intensity ultrasound is used for non-destructive diagnostic purposes, as it does not cause acute modifications in the structure and characteristics of the product. On the other hand, low-frequency or high-intensity ultrasound (>1 W/cm²) favorably alters product attributes and is used in processes aimed at improving the quality and safety of food products (Balthazar et al., 2019; Rathnakumar et al., 2023). The

mechanism of ultrasound action is based on acoustic cavitation and acoustic streaming (Figure 1).

Figure 1

In typically liquid environments, acoustic waves create high pressure (compression) and low pressure (rarefaction) zones. During rarefaction phases, gas microbubbles can form and grow. When these bubbles reach a critical size, they collapse violently. Before the bubbles collapse, the rapid pressure fluctuations induced by ultrasonic waves cause turbulence and volume displacement, known as acoustic streaming (Guimarães et al., 2021; Rathnakumar et al., 2023). The bubble generation, growth, and implosion process is known as acoustic cavitation or implosion (Alvarenga et al., 2021). Cavitation is the formation, growth, and collapse of microbubbles within a solution due to pressure fluctuations caused by the applied ultrasonic field. This collapse leads to high local turbulence, resulting in increased temperature and pressure within the implosion zone, capable of producing shear forces. The violent collapse of a cavitation bubble results in various physical and chemical effects on the liquid, such as microflow, agitation, turbulence, liquid jets, shock waves, and the formation of reactive oxygen species. These phenomena can induce significant physical and chemical effects on the material they are applied to (Carrilho-Lopez et al., 2021; Soltani Firouz et al., 2019).

Microorganism inactivation by ultrasound can occur through various mechanisms, as illustrated in Table 2.

Table 2

Initially, ultrasound, by inducing the phenomenon of acoustic streaming, affects the bacterial cell wall, causing stress without damaging the membrane. Prolonged and intense treatments, resulting from rapid pressure and temperature fluctuations, can lead to bubble collapse and, consequently, rupture of the bacterial membrane or cell wall through shear forces. This results in the release of intracellular material. The most significant effects include cell wall rupture caused by microjets, changes in cell permeability, thermal inactivation due to high-temperature points, and the production of reactive oxygen species, such as hydroxyl radicals. These species have highly reactive oxidative properties, capable of compromising the cell membrane and damaging DNA and enzymes (Alvarenga et al., 2021; Balthazar et al., 2019; Guimarães et al., 2021).

3.1 Inactivation of microorganisms in dairy products

The inactivation of microorganisms in dairy products by ultrasound represents an innovative and effective approach to ensuring food safety and preserving quality (Bernardo, Rosário, Conte-Junior, 2021). This technology offers a promising alternative to traditional thermal methods, better preserving dairy products' sensory and nutritional properties . (Balthazar et al., 2019; Soltani Firouz et al., 2019; Guimarães et al., 2021).

With the growing interest in applying less invasive and more sustainable techniques, ultrasound has garnered significant attention in the scientific literature. Several recent studies have analyzed the application of ultrasound in microbial inactivation (Martínez-Moreno et al., 2020; Nascimento et al., 2023; Yu et al., 2021). Table 3 summarizes the treatment parameters and the effectiveness of ultrasound in inactivating various microorganisms in dairy products, highlighting variations in effectiveness based on the different parameters used.

Table 3

The data presented in Table 3 illustrate the variability in the effectiveness of ultrasound for microorganism inactivation in dairy products, highlighting how different treatment parameters, such as frequency, intensity, and exposure time, influence the results. For example, Jalilzadeh et al. (2018) observed a more pronounced reduction in *E. coli* with higher frequencies (60 kHz), while *P. chrysogenum* showed a lower inactivation rate regardless of the frequency used. Guimarães et al. (2019) revealed that higher ultrasound powers (600 W) resulted in greater bacterial inactivation. Additionally, Balthazar et al. (2019) demonstrated that variations in power and treatment duration directly affect effectiveness, with a power of 104 W for 6 minutes being the most efficient for semi-skimmed sheep milk. These discrepancies reinforce the need to optimize ultrasound parameters for each type of product and specific microorganism, suggesting that a one-size-fits-all approach may not be ideal for all cases. Therefore, careful selection of ultrasound parameters is essential to maximize effectiveness in microbial inactivation, especially in industrial contexts.

3.1.1 Advantages of Ultrasound in Microbial Inactivation

The main advantage of ultrasound is its ability to inactivate microorganisms without the side effects of conventional thermal treatments, which often require high energy levels and can compromise the nutritional value of foods (Balthazar et al., 2019; Soltani Firouz et al., 2019; Guimarães et al., 2021). Ultrasound is a disruptive technique that can alter the chemical, biochemical, physical, or mechanical properties of microorganisms, depending on the treatment intensity, such as frequency, power, processing time, and the volume and temperature of the sample (Guimaraes et al., 2021; Rathnakumar et al., 2023).

High-intensity ultrasound, with power ranging from 1 to 1,000 W/cm², has shown a faster rate of cavitation formation compared to low-intensity ultrasound (<1 W/cm²) (Yu et al., 2021). Recent studies have explored the use of high-intensity ultrasound in various dairy products to reduce processing time and energy consumption, as well as to improve the physicochemical characteristics of foods (Carrilho-Lopez et al., 2021; Soltani Firouz et al., 2019). The diversity of dairy products and their unique characteristics have led to extensive research on high-intensity ultrasound, covering raw milk, sheep milk, buffalo milk, yogurt, ice cream, cheese, among others (Balthazar et al., 2019; Guimarães et al., 2021; Jalilzadeh et al., 2018; Scudino et al., 2020).

However, it is challenging to compare results from different studies considering these parameters in isolation, as the effects of cavitation depend on these parameters and slight variations can alter results in different food matrices (Aaliya et al., 2021). Additionally, the success of ultrasonic treatment is also related to the physical and biological characteristics of the microorganisms being treated. While both yeasts and bacteria (Gram-positive and Gram-negative) are susceptible to ultrasound, differences in cell membrane structure and the presence of protective capsules can reduce treatment effectiveness (Alvarenga et al., 2021; Guimarães et al., 2021).

3.1.2 Challenges and Limitations of Ultrasound

Although ultrasound technology represents a promising, non-destructive, non-thermal, and environmentally friendly approach, it is important to note that its effectiveness may not be fully realized in all situations (Jalilzadeh et al., 2018; Guimarães et al., 2019). Furthermore, treatment with ultrasound alone has shown limited antibacterial activity and efficacy against bacterial biofilms (Martínez-Moreno et al., 2020; Yu et al., 2021).

Additionally, the effect of ultrasound can be influenced by the product matrix, as the presence of solids, fats, and viscosity can interfere with the propagation of acoustic waves and, consequently, the efficiency of the inactivation process. Heterogeneity in the

distribution of ultrasonic waves can lead to areas with varying levels of effectiveness, making it challenging to ensure uniform treatment in large volumes of product (Nascimento et al., 2023).

The application of high-intensity ultrasound can generate heat due to the increase in temperature, negatively impacting the organoleptic and nutritional characteristics of food products. Additionally, high-power ultrasound can cause adverse physical and chemical effects on foods. Free radicals generated by cavitation can lead to lipid oxidation, resulting in undesirable flavors and odors, protein denaturation, and a reduction in total phenolic content due to ascorbic acid degradation. The combination of ultrasound with temperature and pressure can also form free radicals that trigger damaging reactions to protein structure, compromising food texture. Therefore, optimizing ultrasound intensity and combined use before its application becomes essential (Bhargava et al., 2021).

Another challenge is the cost associated with ultrasound technology, which can be relatively high compared to traditional thermal treatment methods. Investment in equipment and maintenance can limit some producers' economic feasibility, especially in smaller-scale operations (Bernardo, Rosário, Conte-Junior, 2021; Yu et al., 2021). Continued research is necessary to address these limitations and improve the effectiveness and applicability of ultrasound in the dairy industry.

3.1.3 Combined strategies and recent advances

In recent years, the application of ultrasound for microorganism inactivation in dairy products has advanced significantly, reflecting the growing interest in more efficient and sustainable processing technologies (Table 3). A major advancement is the implementation of high-intensity ultrasound, which has shown enhanced potential for the inactivation of a wide range of pathogenic microorganisms (Guimarães et al.2021; Martínez-Moreno *et al*., 2020).

The combination of ultrasound with other thermal and non-thermal technologies plays a crucial role in bacterial reduction. Aaliya et al. (2021) discuss the application of hurdle technology, which utilizes a synergistic combination of thermal and non-thermal techniques to enhance microorganism elimination effectiveness. This approach allows for more robust microbial load control by leveraging the advantages of different processing methods to ensure food safety without compromising its sensory qualities (Aaliya et al., 2021).

Sonication, when combined with pressure (manosonication), can inactivate pathogenic and spoilage microorganisms present in food. This is achieved through the mechanical effects of ultrasonic cavitation, which damage bacterial cells, leading to their inactivation (Alvarenga et al., 2021; Martínez-Moreno et al., 2020). When combined with heat (thermosonication), it is also highlighted as a promising strategy for microbial inactivation, effective in reducing or eliminating microorganisms in food, including bacteria, yeasts, and molds (Martínez-Moreno et al., 2020; Nascimento et al., 2023; Rathnakumar et al., 2023). Research conducted with creams observed a reduction of up to 4.72 log CFU/mL in MRSA counts, comparable to the reduction achieved with conventional pasteurization, highlighting thermosonication as an efficient solution for the microbiological safety of dairy products (Nascimento et al., 2023). Similarly, ultrasonic treatment of semi-skimmed sheep milk showed a significant reduction in bacterial contamination, comparable to high-temperature short-time pasteurization treatment (Balthazar et al., 2019).

Additionally, recent research has investigated the use of more sophisticated ultrasound devices, such as adjustable frequency transducers and controlled cavitation systems, which offer more precise and efficient control over the microorganism inactivation process (Yu et al., 2021). Further studies have also explored the combination of ultrasound with hydrogen peroxide and the active lactoperoxidase system, demonstrating the effectiveness of these integrated approaches in eliminating pathogenic and spoilage bacteria in dairy products (Shamila-Syuhada et al., 2016).

These advancements highlight the importance of integrating ultrasound with other technologies to overcome specific limitations and optimize microorganism inactivation in dairy products. Ongoing development and innovative combinations of processing methods are expanding the potential of ultrasound as an effective solution for food safety.

3.2 Challenges for industrial application

The implementation of ultrasound technology on an industrial scale faces several challenges, including the high costs of acquiring and installing specialized equipment, the need to implement changes in management and production lines, as well as the operational complexity and maintenance of these systems (Bernardo, Rosário, Conte-Junior, 2021). However, various studies suggest that ultrasound may stand out as a superior alternative to traditional processing methodologies, particularly in the dairy sector, which is a significant segment of the food industry with a wide range of products derived from different processes. (Guimaraes *et al*., 2021).

Technological benefits include improvements in homogenization, emulsification, reduction in fat globule size, and nutritional quality of products (Carrilho-Lopez et al., 2021), as well as a decrease in cheese maturation and fermentation time, and bacterial inactivation (Balthazar et al., 2019; Nascimento et al., 2023; Rathnakumar et al., 2023; Scudino et al., 2020). Moreover, ultrasound proves effective in the inactivation of various enzymes related to dairy products, such as alkaline phosphatase, lactoperoxidase, and γ-glutamyl transpeptidase, while preserving the natural flavors of foods (Rathnakumar et al., 2023). Its applications extend to kinetic stabilization, cell rupture, release of bioactive compounds, and improvement of water and lactose crystallization, highlighting its versatility in enhancing food quality and safety. Ultrasound thus establishes itself as an efficient technology for nutrient preservation, extending shelf life, and improving the quality of dairy products (Bhargava *et al*., 2021; Guimarães *et al*., 2021).

Ultrasound plays a significant role in the firm and stable gel formation by denaturing whey proteins, fragmenting casein micelles, and recombining the protein fraction, resulting in a more consistent yogurt (Carrilho-Lopez et al., 2021). No significant adverse effects were observed on the cohesiveness or elasticity of ultrafiltered white cheese due to the use of ultrasound. Sonication led to an increase in acidity and a reduction in pH values compared to control samples, without negatively affecting the fat or protein content of the cheese. Additionally, ultrasonic treatment accelerated lipolysis and proteolysis processes, resulting in higher production of free fatty acids and water-soluble nitrogen. These changes contribute to flavor and aroma enhancement in cheese, improving its physicochemical and sensory properties during the maturation process (Jalilzadeh *et al*., 2018).

Research conducted in laboratories under controlled conditions suggests that experimental results achieved may not be easily replicable on an industrial scale due to differences in the amount of material processed and the need for more robust systems for large-scale processing (Soltani Firouz *et al*., 2019)..

Bernardo et al. (2021) highlight the main constraints of using ultrasound on a large scale in the dairy industry, such as in milk decontamination. These limitations are strongly related to bacterial morphology and cell wall composition. Gram-positive bacteria, characterized by a thick peptidoglycan layer, exhibit greater resistance to the deleterious effects of ultrasound (Balthazar et al., 2019; Herceg et al., 2020; Shamila-Syuhada et al., 2016).

These challenges emphasize the lack of consensus on defining and presenting processing parameters in ultrasonic treatment, as well as the methodology for calculating the actual acoustic energy used (Guimarães et al., 2021; Scudino et al., 2020). These are

crucial aspects for enabling effective comparisons between different studies. Furthermore, conducting more research to establish process parameters tailored to specific safety objectives, whether technological or microbiological, and appropriate for the particular type of dairy product under analysis is essential.

4 Final considerations

The use of ultrasound for microorganism inactivation in dairy products has proven effective, enhancingsafety and product quality. This technology reduces processing time and energy consumption while preserving the nutritional and sensory properties of foods, making it appealing for commercial applications due to its eco-friendly and efficient approach. However, variability in microorganism response and differing treatment conditions necessitate a tailored approach for each product and specific context. Despite these advantages, the industrial implementation of ultrasound faces challenges, requiring significant investment and effort to advance research, large-scale application, and commercialization.

Acknowledgements

The authors are thankful for the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant number [303074/2021-3].

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Table 1. Data on Dairy Product Outbreaks and Recalls

Year	Product	Identified Issue	Consequences
2016	Raw milk[(1)]	*Listeria monocytogenes* contamination	Foodborne illness, 2 death and 8 hospitalizations
2017	Raw milk cheese [(2)]	*Listeria monocytogenes* contamination	Foodborne illness, 1 death and 2 hospitalizations
2022	Whole Nutrition Infant Formula, Milk Based Powder with Iron [(3)]	Potential for cross-contamination with *Cronobacter sakazakii*	Product withdrawa
2022	Various cheeses[(3)]	Potential to be contaminated with *Listeria monocytogenes*	Product withdrawa
2023	Pepper Jack Raw Milk Cheese [(3)]	Presence of *Listeria monocytogenes*	Product withdrawa
2023	Tome De Brebis Sheep Milk Cheese[(3)]	Potential *Foodborne illness*	Product withdrawa
2023	Tome Corse Sheep Milk Cheese [(3)]	Potential *Foodborne illness*	Product withdrawa
2023	Sophelise, Tobasi, and Berkshire Bloom Cheeses [(3)]	Potential contamination with *Listeria monocytogenes*	Product withdrawa
2024	Ice Cream Products[(3)]	*Listeria monocytogenes* Contamination	Product withdrawa
2024	Powdered Goat Milk Infant Formula [(3)]	*Cronobacter* spp. contamination	Product withdrawa
2024	Aged Cojita Mexican Grating Cheese [(3)]	Potential *Listeria monocytogenes* contamination	Product withdrawa
2024	Nutramigen Powder infant fórmula [(3)]	Potential *Cronobacter sakazakii* contamination	Product withdrawa
2024	Queso Fresco and Cotija Cheese [(4)]	Potential *Listeria monocytogenes* contamination	Outbreak and Product withdrawa
2024	Raw Cheddar Cheese [(4)]	*Escherichia coli*	Outbreak, 2 death and 11 hospitalizations

Source: CDC (Centers for Disease Control and Prevention), 2016 [(1)], 2017[(2)], 2024[(4)]; FDA (Food and Drug Administration), 2024[(3)]

Table 2. Key Processing factors and their impact on microorganism inactivation by ultrasound

Processing Factors	Unit of Measurement	Description	Impact on Microorganism Inactivation
Frequency	Hz	Determines bubble size and cavitation intensity.	Lower frequencies (20–100 kHz) generate larger bubbles, enhancing microbial inactivation. Higher frequencies (>100 kHz) produce smaller bubbles, often resulting in less effective inactivation.
Intensity	W, W/L, W/kg, W/cm², J/L	Energy transmitted by ultrasonic waves.	Higher intensities increase mechanical, thermal, and sonochemical effects, leading to more effective microbial inactivation.
Amplitude	µm, %	Affects the size and distribution of cavitation bubbles.	Greater amplitude intensifies cavitation, improving microbial inactivation efficiency.
Exposure Time	s (seconds)	Duration of ultrasonic treatment.	Longer exposure times can enhance microbial inactivation but may also affect product quality.
Temperature	°C (Celsius)	Temperature of the product during treatment.	Higher temperatures can increase the efficiency of cavitation and microbial inactivation, though excessive heat may affect the product's quality.
Medium Viscosity	mPa·s (millipascal-seconds)	Thickness of the product.	Higher viscosity can hinder bubble formation and cavitation, reducing the efficiency of microbial inactivation.

Note: Hz: Hertz; W: Watts; W/cm²: Watts per square centimeter; J/L: Joules per liter; µm: Micrometers; %: Percentage; s: Seconds; °C: Celsius; mPa·s: Millipascal-seconds.

Source: Adapted from Beitia et al. (2023).

528 **Table 3**. Efficacy of Ultrasound in the Inactivation of Target Microorganisms in Dairy Products
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Dairy Product	Target Microorganisms	Treatment Conditions	Efficacy (Log Reduction, CFU/NMP Reduction, D-value)	Challenges Observations	Reference
Raw milk	*Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.aureus*)	Ultrasound probe, 12.0 mm, 600 W, 20 kHz, 60°C	*S. aureus*: D-value of 4.80 min *E. coli*: D-value of 2.78 min	*E. coli* is more susceptible to ultrasonic treatment than *S. aureus*.	Herceg et al. (2012)
Reconstituted skim milk (≤1.25% fat)	*Enterobacter aerogenes*	Ultrasound probe, 12.7 mm, 20 kHz and 850 kHz, 20 min	20 kHz: Water: 3.64 after 60 min - 5%, 10% and 15% skim milk: 2.73, 2.31 and 2.21 log, respectively 850 kHz: no significant reduction	Low-frequency ultrasound effective; high-frequency ineffective	Gao et al. (2014a)
Raw Milk	*Listeria monocytogenes*, *E. coli, Salmonella Typhimurium*, Pseudomonas *fluorescens*	Ultrasound probe, 14 mm, 24 kHz, 62.5 - 125mm	*Listeria monocytogenes, E. coli and Salmonella Typhimurium*: ~3 log reduction with H_2O_2 (0.05%) *Pseudomonas fluorescens*: < 1 log	High effectiveness with H_2O_2.	Shamila-Syuhada et al. (2016)
Retentate of UF milk (16% fat) for Iranian UF white cheese	*E. coli*, *S. aureus*, *Penicillium chrysogenum, Clostridium sporogenes*	Ultrasound probe, 20, 40, 60 kHz, 80% intensity, 20 min	*E. coli* O157:H7: - 20/40/60 kHz: 4.08, 4.17 and 4.28 log, respectively *S. aureus*: - 20/40/60 kHz: 1.10, 1.03 and 1.95 log, respectively *Penicillium chrysogenum*: - 20/40/60 kHz: 1.11, 1.07 and 1.11 log, respectively *Clostridium sporogenes*: - 20/40/60 kHz: 2.11, 2.03 and 2.17 log, respectively	Efficacy increases with frequency for *E. coli*; less effective for other.	Jalilzadeh et al. (2018)
Chocolate milk beverage	Aerobic mesophilic bacteria, Total coliforms and Thermotolerant	Ultrasound probe, 13 mm, 900 W, 3.0 kJ/cm³, 19 kHz	Aerobic mesophilic: 3.56 log Coliforms: < 3 MPN/mL	Effective for improving microbiological quality.	Monteiro et al. (2018)

Raw Milk	Aerobic mesophilic bacteria, *Enterobacteriaceae*	Ultrasonic processor, 1500 W, 20 kHz, 10 -15 min, 95% intensity	Aerobic Mesophilic Bacteria: 2.45- 4.8 CFU/mL *Enterobacteriaceae*: - 3.41 log or lower	Longer treatment times and higher amplitudes improve microbial reduction.	Hernández-Falcón et al. (2018)
Low- and High-Fat Milk	*Listeria monocytogenes*, *Salmonella Typhimurium*	Ultrasound probe,24 kHz, 400 W power at 124 µm (100%) wave amplitude for 15 min	*Listeria monocytogenes*: - 3.0 (CEO), 0.6 (US), 4.5 (CEO + US) *Salmonella Typhimurium*: - Low-Fat Milk: 2.3 (CEO), 1.6 (US), 3.1 (CEO + US) - High-Fat Milk: 2.2 (CEO), 1.1 (US), 3.8 (CEO + US)	CEO + US resulted in the greatest reduction.	Mortazavi & Aliakbarlu (2019)
Prebiotic soursop whey beverage	*Enterobacter aerogenes*	Ultrasound probe, 13 mm, 19 kHz, 200–600 W, energy density 8, 16, and 24 W/mL for 60 min	- Water: 3.64 log - 5%, 10% and 15% milk: 2.73, 2.31 and 2.21, respectively	Effectiveness decreases with higher milk concentrations.	Guimarães et al. (2019)
Iranian UF white cheese	*E. coli*, *S. aureus*, *Clostridium sporogenes*, *Penicillium chrysogenum*	Ultrasound probe, 22 mm, 24 kHz, 85 W/cm²	*E. coli* and *S. aureus*: ~7 log *Clostridium sporogenes*: 2.11 – 2.17 log *Penicillium chrysogenum*: 1.07 - 1.11 log	High efficacy for *E. coli* and *S. aureus; l*ess effective for *Penicillium chrysogenum*.	Martínez-Moreno et al. (2020)
Semi-skimmed sheep milk (1.6% fat) fresh and frozen	Aerobic mesophilic bacteria, Total coliform count, *Staphylococcus spp.*,	Ultrasound probe, 78 -104 W, 6 - 8 min	Aerobic Bacteria: 1.6 - 2.7 log Coliforms: Absence *Staphylococcus* spp.: 1.6 - 2.7 log	US4 is most effective for reducing microbial contamination.	Balthazar et al. (2019)
Raw milk	Aerobic mesophilic heterotrophic bacteria, Total coliforms, Thermotolerant coliforms	Ultrasound probe, 13 mm, 100 - 475 W, 1 - 7 kJ/mL	Aerobic mesophilic: 3.92 log Coliforms: < 3 MPN/mL	Variable effectiveness: higher energy densities improve reduction.	Scudino et al. (2020)

Raw camel milk	*E. coli*, *Salmonella Typhimurium*	Ultrasound probe, 13 mm, 900 W, 20 kHz, 15 min	*E. coli*: Complete inactivation *Salmonella Typhimurium*: 4.4 log	Effective at complete inactivation of *E. coli*; significant reduction of *Salmonella*.	Dhahir et al. (2020)
Milk Cream	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Ultrasound probe, 13 mm, 20 kHz, 500 W	MRSA: 4.82 log (after pasteurization), 4.72 log (after thermosonication)	Efficacy for MRSA	Nascimento et al. (2023)

530 Legend: UF – Ultrafiltrated; CEO – Cinnamon Essential Oil; US – Ultrasound; CEO + US – Cinnamon Essential Oil combined with Ultrasound

531 **FIGURE**

532

533 **Figure 1.** Mechanisms of Ultrasound Action in Microorganism Inactivation.
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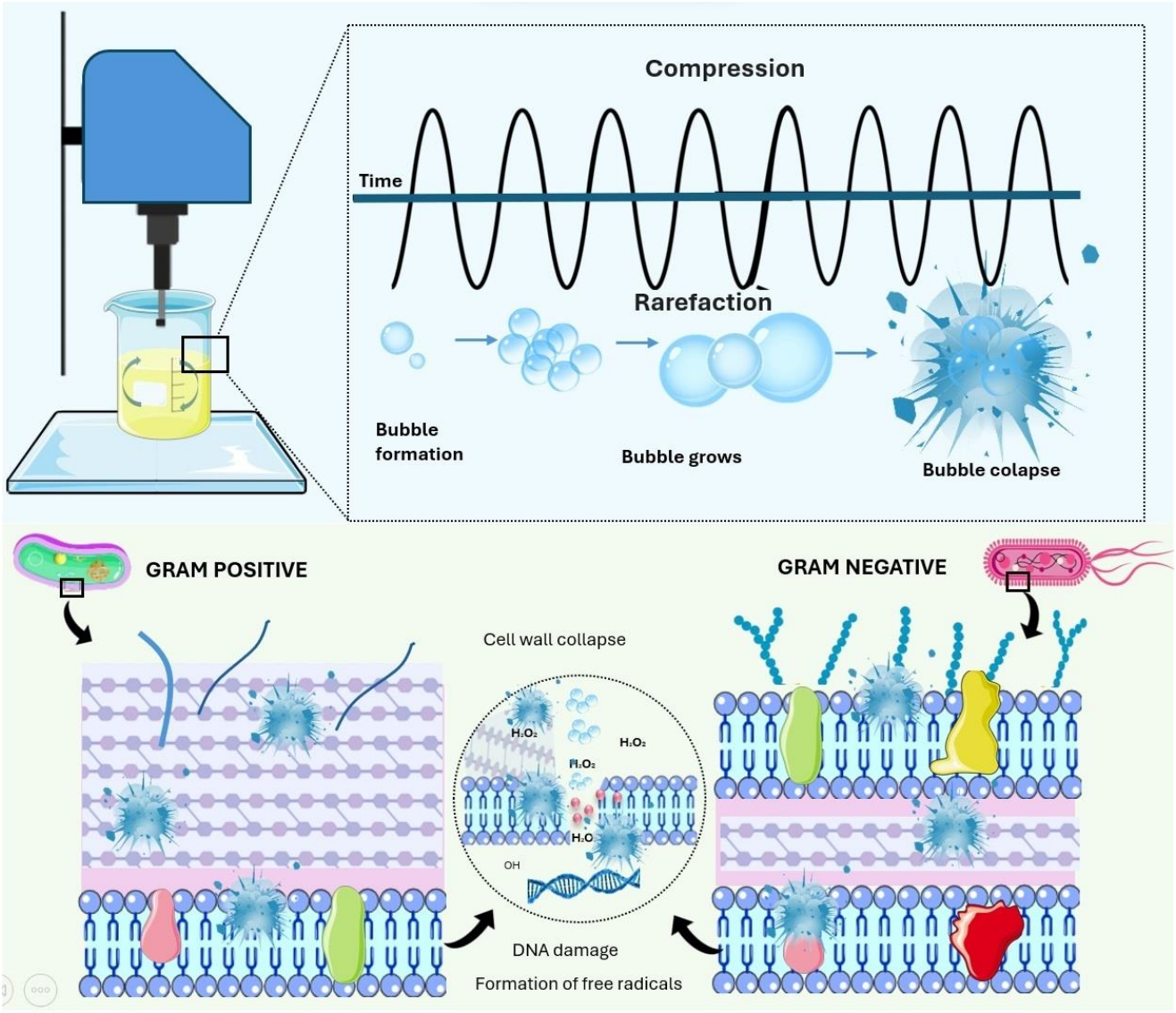

6 CONCLUSÃO GERAL

A presente tese investigou a eficácia da combinação entre técnicas de pasteurização, nas suas modalidades rápida e lenta, com a termossonicação na inativação de *Staphylococcus aureus* resistente à meticilina (MRSA) e outros microrganismos indicadores presentes em creme de leite. Os resultados obtidos demonstraram que a aplicação conjunta de termossonicação e pasteurização convencional revelou-se uma estratégia eficaz e promissora para aprimorar a redução microbiana em comparação ao uso isolado da pasteurização.

A sinergia observada entre as técnicas mostrou que o efeito térmico da pasteurização é amplificado pela ação mecânica das ondas ultrassônicas, resultando em uma inativação mais eficiente de MRSA e demais microrganismos. Essa combinação não apenas resultou em uma redução significativa das contagens microbianas imediatamente após o tratamento, mas também promoveu a manutenção prolongada desses níveis reduzidos ao longo do período de armazenamento, especialmente quando comparada às técnicas tradicionais.

As revisões realizadas no âmbito deste estudo reforçam que *Staphylococcus aureus*, particularmente as cepas resistentes a antimicrobianos como o MRSA, continua a representar um desafio significativo para a segurança dos alimentos. Embora as práticas convencionais de processamento, como a pasteurização, sejam fundamentais, sua eficácia pode ser substancialmente aumentada pela combinação com tecnologias emergentes, como a termossonicação. Adicionalmente, a revisão sobre a aplicação de ultrassom na indústria de laticínios destaca as vantagens dessa tecnologia, como sua capacidade de promover uma redução microbiana eficaz. No entanto, a variabilidade na resposta microbiana ao tratamento e a necessidade de otimizações específicas para cada tipo de produto indicam que a implementação em escala industrial ainda enfrenta desafios significativos.

Em síntese, esta tese contribui para o entendimento e aplicação prática da termossonicação combinada com a pasteurização como uma estratégia inovadora para a inativação de microrganismos patogênicos e indicadores em creme de leite. Os resultados ressaltam que, com a combinação adequada de parâmetros, essa abordagem pode oferecer uma solução mais robusta para a segurança microbiológica de produtos lácteos, superando as limitações das técnicas convencionais. Pesquisas futuras devem continuar a explorar essa combinação, visando a otimização dos parâmetros para diferentes produtos e escalas de produção, com o objetivo final de aprimorar a segurança dos alimentos de forma sustentável e eficiente.

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PRODUÇÃO TÉCNICO CIENTÍFICA

(2020-2024)

Atuação Profissional

Laboratório de Microbiologia de Alimentos da Faculdade de Farmácia da Universidade Federal da Bahia.
Cargo: Farmacêutica Bioquímica de Alimentos
Vínculo: Celetista (2004-atual) pela Fundação de Apoio à Pesquisa e Extensão

Uniceusa- Centro Universitário Salvador
Cargo: Professor Adjunto III
Vínculo: Celetista (2020-atual)
Disciplinas ministradas: Microbiologia de Alimentos, Análises de Alimentos, Controle de Qualidade Microbiológico de Medicamentos, Produção Técnico Científico Interdisciplinar (TCC), Bromatologia e Análises de Alimentos, Farmácia Integrada, Microbiologia e Micologia Clínica, Estágio em Alimentos, Fitoterapia Clínica, Práticas Educativas em Saúde e Fisiologia Humana.

Participação em Projetos de Pesquisa:

Tema: Emprego da Modelagem Matemática e Microbiologia Preditiva no Estudo de Leites e Derivados Lácteos
Local de desenvolvimento: Laboratório de Inspeção e Tecnologia de Leite e Derivados (LaITLácteos)
Instituição: Escola de Medicina Veterinária e Zootecnia - UFBA
Orientadora: Profa. Marion Pereira da Costa

Participação em Projetos de Extensão:

Tema: Projeto de Desenvolvimento Institucional Visando Ações na Quialidade e Segurança Microbiológica de Alimentos e Águas Comercializados na Messoregião Metropolitana da Bahia
Cadastro SIPAC: 387/2022
Local de desenvolvimento: Laboratório de Microbiologia de Alimentos (2023-atual)
Instituição: Faculdade de Farmácia - UFBA
Coordenação: Profa. Mariana Nougalli Roselino

Tema: Programa de Apoio ao Desenvolvimento de Ações Educacionais, Científicas e Extensionistas em Análises e Segurança de Alimentos no Estado da Bahia
Cadastro SIPAC: 113/2019
Local de desenvolvimento: Laboratório de Microbiologia de Alimentos (2019-2024)
Instituição: Faculdade de Farmácia - UFBA
Coordenação: Profa. Clícia Capibaribe Leide e Profa. Mariana Nougalli Roselino

Artigo publicado

ANDRADE, I. H. P.; **NASCIMENTO, J.C.N**.; JESUS ASSIS, D.; ANDRADE, R. C.; CAMILLOTO, G. P.; CRUZ, R. S. Innovation waves: Analysis and modeling of patents on

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NASCIMENTO, J. C. N.; SILVA, J. G.; COSTA, M. P. *Staphylococcus aureus* em produtos lácteos: a ameaça invisível que você precisa conhecer. 2023; Tema: Blog. Milkpoint. (Site). https://www.milkpoint.com.br/artigos/industria-de-laticinios/staphylococcus-aureus-em-produtos-lacteos-a-ameaca-invisivel-que-voce-precisa-conhecer-234742/

Trabalhos apresentados em Congressos Nacionais e Internacionais

Resumos Expandidos

MATOS, J. R.; **NASCIMENTO, J. C. N.**; VILAS BOAS, D. M.; PAZ, F. G.; LEITE, C. C. Qualidade Microbiológica de Sorvetes e Picolés Comercializados na Cidade de Salvador -

BA. In: Congresso Brasileiro de Inovação e Tecnologia na Gastronomia e Ciência de Alimentos, 2020, Fortaleza.

MATOS, J. R.; **NASCIMENTO, J. C. N**.; VILAS BOAS, D. M.; PAZ, F. G.; LEITE, C. C. Avaliação Microbiológica dos Utensílios Utilizados em Cozinhas e Restaurantes de Resorts localizados no Litoral Norte Baiano. In: Congresso Brasileiro de Inovação e Tecnologia na Gastronomia e Ciência de Alimentos, 2020, Fortaleza.

VILAS BOAS, D. M.; LEITE, C. C.; MATOS, J. R.; PAZ, F. G.; **NASCIMENTO, J. C. N.**; SANTANA, A. S. Ecologia Microbiana da Água Mineral em Poço de Extração e como Produto Final. In: Congresso Brasileiro de Inovação e Tecnologia na Gastronomia e Ciência de Alimentos, 2020, Fortaleza.

VILAS BOAS, D. M.; MATOS, J. R.; PAZ, F. G.; **NASCIMENTO, J. C. N.**; LEITE, C. C. Ocorrência de Microrganismos Patogênicos em Alimentos Prontos para o Consumo. In: Congresso Brasileiro de Inovação e Tecnologia na Gastronomia e Ciência de Alimentos, 2020, Fortaleza.

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PAZ, F. G.; **NASCIMENTO, J. C. N.**; VILAS BOAS, D. M.; MATOS, J. R.; LEITE, C. C. Qualidade Microbiológia da Água de Coco Industrializada. In: Congresso Virtual UFBA 2021 - Universidade em Movimento, 2021, Salvador.

PAZ, F. G.; NASCIMENTO, J. C. N.; VILAS BOAS, D. M.; MATOS, J. R.; LEITE, C. C. Qualidade microbiológica de saladas cruas provenientes de restaurantes institucionais e comerciais localizados na mesorregião metropolitana da cidade de Salvador-BA. In: Congresso Virtual UFBA 2021 - Universidade em Movimento, 2021, Salvador.

MATOS, J. R.; NASCIMENTO, J. C. N.; SILVA, R. A. I.; SANTOS, S. S.; SOUTO, R. B. Avaliação da qualidade de antimicrobianos de uso veterinário. In: Congresso Virtual UFBA 2021 - Universidade em Movimento, 2021, Salvador.

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MATOS, J. R.; VILAS BOAS, D. M.; PAZ, F. G.; NASCIMENTO, J. C. N.; LEITE, C. C. Qualidade microbiológica de manteigas produzidas em diferentes mesorregiões do estado da Bahia. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020. Qualidade microbiológica de manteigas produzidas em diferentes mesorregiões do estado da Bahia, 2020.

MATOS, J. R.; NASCIMENTO, J. C. N.; PAZ, F. G.; VILAS BOAS, D. M.; LEITE, C. C. Avaliação dos parâmetros de qualidade microbiológica aplicados a diferentes variações de queijos de búfala. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020. Avaliação dos parâmetros de qualidade microbiológica aplicados a diferentes variações de queijos de búfala, 2020.

PAZ, F. G.; VILAS BOAS, D. M.; NASCIMENTO, J. C. N.; MATOS, J. R.; LEITE, C. C. Contagem de bactérias aeróbias mesófilas em dietas enterais provenientes de hospitais da cidade de Salvador - BA. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020.
PAZ, F. G.; VILAS BOAS, D. M.; NASCIMENTO, J. C. N.; MATOS, J. R.; LEITE, C. C. Microbiota superficial de carcaças resfriadas de frango. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020.

VILAS BOAS, D. M.; MATOS, J. R.; PAZ, F. G.; NASCIMENTO, J. C. N.; LEITE, C. C. Ocorrência de *Pseudomonas aeruginosa* em amostras de água potável submetidas à filtração, destinadas à ingestão humana. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020.

VILAS BOAS, D. M.; NASCIMENTO, J. C. N.; PAZ, F. G.; MATOS, J. R.; LEITE, C. C. Qualidade microbiológica do gelo utilizado para consumo e para refrigeração de alimentos na cidade de Salvador- BA. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020.

MATOS, J. R.; NASCIMENTO, J. C. N.; VILAS BOAS, D. M.; PAZ, F. G.; LEITE, C. C. Contaminação microbiológica em alimentos prontos para o consumo - risco de veicular doenças de origem alimentar. In: Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2020.

MATOS, J. R.; **NASCIMENTO, J. C. N.**; SANTOS, S.; SOUTO, R. B. Controle de qualidade de antimicrobianos de uso veterinário: Estudo normativo e análise da literatura. In: I Simpósio de Farmácia Veterinária do Programa de Pós-graduação da Universidade Federal do Rio de Janeiro, 2020.

Participação em eventos científicos

II Simpósio Integrado dos Programas Pós-Graduação da Faculdade de Farmácia (SIPPFar). Universidade Federal da Bahia. 2023. (Simpósio).

2º International Symposium of Food Science SInCA.2º International Symposium of Food Science SInCA. 2022. (Simpósio).

II Colóquio Virtual de Ciência de Alimentos: Tópicos Contemporâneos: Alimentos Emergentes, Cacau e Chocolate Alergias e Intolerâncias Alimentares. Universidade Federal da Bahia. 2021. (Simpósio).

II Congresso Virtual UFBA 2021. Universidade Federal da Bahia (Congresso).

Seminário de Avaliação da Chamada CNPq/Sescoop.Seminário de Avaliação da Chamada CNPq/Sescoop. 2021. (Seminário).

Colóquio Virtual: Impactos da presença de contaminantes em alimentos e ações sustentáveis para produção de alimentos seguros. Universidade Federal da Bahia. 2020. (Simpósio).

Congresso Virtual UFBA 2020. Simpósio. 2020. (Congresso).

I COICTA - International Online Congress of Food Science and Technology - "Food Security: Strategies in the Pandemic Period". 2020. (Congresso).

I Simpósio de Ciência e Tecnologia de Leite e Derivados. Núcleo de Estudos em Processamento, Qualidade e Segurança de Leite e Derivados (NUPROLAC). 2020. (Simpósio).

I Simpósio Internacional em Ciência e Tecnologia de Alimentos- V Seminário em Inovação em e Tecnologia na Área de Alimentos. 2020. (Simpósio).

Microbiologia dos Alimentos - Produtos de Origem Animal produzido pelo Grupo de Estudos de Tecnologia e Inspeção de Produtos de Origem Animal (UNESP-Araçatuba).. 2020. (Outra).

Tutoria de alunos de iniciação científica

Aluno: Jessica Borges dos Santos Silva.
Trabalho: Impactos da fraude por adição de água ao leite nas características microbiológicas de iogurte tradicional adoçado. EDITAL PROPCI/UFBA 01/2021 PIBIC (vigência 01/09/2021 à 31/08/2022). 2021. Iniciação Científica. (Graduando em Medicina Veterinária)
Órgão de fomento: Conselho Nacional de Desenvolvimento Científico e Tecnológico. **Orientadora**: Profa. Marion Pereira da Costa.

Aluno: Rafael Alves Castor de Cerqueira.
Trabalho: Emprego da Modelagem Matemática e Microbiologia Preditiva no Estudo de Leite e Derivados Lácteos. 2020. Iniciação Científica. (Graduando em Medicina Veterinária)
Órgão de fomento: Conselho Nacional de Desenvolvimento Científico e Tecnológico. **Orientadora**: Profa. Marion Pereira da Costa.

Aluno: Arlen Carvalho de Oliveira Almeida.

Trabalho: Análises físico-químicas de sorvete de búfala artesanal com baixo teor de gordura e açúcar. EDITAL_03_2020_PIBITI (vigência 01/09/2020 até 30/08/2021). 2020. Iniciação Científica. (Graduando em Medicina Veterinária)
Órgão de fomento: Conselho Nacional de Desenvolvimento Científico e Tecnológico.
Orientadora: Profa. Marion Pereira da Costa.

Coorientação de aluno de graduação

Aluna: Juliana Ranzan Matos.
Trabalho: Desenvolvimento e validação de método para doseamento do antibiótico de uso veterinário Enrofloxacino. 2023. Trabalho de Conclusão de Curso. (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientador: Prof. Ricardo Bizogne Souto.

Banca de Trabalho de Conclusão de Curso

Aluna: Flora Aline Davino Gomes.
Trabalho: Caracterização molecular in silico da família gênica preniltransferase na mamona: uma análise genômica comparativa. 2023. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientador: Prof. Paulo Roberto Ribeiro de Jesus.

Aluno: Leonel Barbosa Barros.
Trabalho: Câncer de mama entre os gêneros: compreensão das peculiaridades para auxiliar na conduta e tratamento dos homens. 2023. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientadora: Profa. Júnia Raquel Dutra Ferreira.

Aluno: Paulo Daniel de Freitas Machado.
Trabalho: Explorando a evolução da pesquisa científica com cloroquina e hidroxicloroquina para COVID-19: uma análise bibliométrica de dados da *web of science* (2018-2022). 2023. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientadora: Profa. Thaís Rodrigues Penaforte.

Aluna: Flora Aline Davino Gomes.
Trabalho: Detecção de Fumonisina em Milho pelo Método de Espectroscopia no Infravermelho Próximo: Revisão Integrativa da Literatura. 2023. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientadora: Profa. Carolina Oliveira Souza.
Aluno: Karim Fawzi Sayah.
Trabalho: Composição química preliminar da amêndoa de monguba (*Pachira aquatica Aubl*.). 2023. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientador: Prof. Ederlan de Souza Ferreira.

Aluna: Claudia Miranda Barreto.
Trabalho: Mecanismos de ação do resveratrol na síntese de fatores vasoativos do endotélio. 2023. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.

Orientador: Prof. Ederlan de Souza Ferreira.

Aluna: Bárbara de Castro dos Santos Silva.
Trabalho: Certezas e Incertezas dos Impactos a Saúde do Consumo de Alimentos Geneticamente Modificados. 2022. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientador: Prof. Ederlan de Souza Ferreira.

Aluna: Rhaissa Coelho Andrade.
Trabalho: Desenvolvimento e caracterização de bebida bubble juice com microrganismos probióticos encapsulados. 2022. Trabalho de Conclusão de Curso (Graduação em Nutrição) - Universidade Federal da Bahia.
Orientadora: Profa. Mariana Nougalli Roselino.

Aluno: Jonathan Victor Ramos Sanches.
Trabalho: Métodos Analíticos utilizados para a determinação da Lactose em Produtos Lácteos: Uma Revisão Bibliográfica. 2022. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientadora: Profa. Maria Eugênia Oliveira Mamede.

Aluno: Helio M. A. do Sacramento Júnior.
Trabalho: Condição microbiológica de saladas cruas consumidas em restaurantes institucionais na cidade de Salvador e municípios vizinhos - Bahia. 2021. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia. **Orientadora**: Profa. Clícia Capibaribe Leite.

Aluno: Clecio Rodrigues Oliveira.
Trabalho: Abordagem teórica sobre os compostos antioxidantes presentes na cerveja e sua relação com a saúde humana.2021. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientadora: Profa. Karina Guedes

Aluna: Maiane Silva das Neves."
Trabalho: Alimentos sem química? controvérsias entre alimentos orgânicos *versus* alimentos convencionais. 2021. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientador: Prof. Renan Geovanny Oliveira Araujo.

Aluna: Uiara Moreira Paim.
Trabalho: Avaliação da presença de microrganismos indicadores de qualidade no ambiente da ordenha e no leite cru em propriedade rural leiteira. 2021. Trabalho de Conclusão de Curso (Graduação em Medicina Veterinária) - Universidade Federal da Bahia.
Orientadora: Profa. Marion Pereira da Costa.

Aluna: Laís Duarte Soares.
Trabalho: Influência da contaminação microbiana em produtos de maquiagem de uso coletivo: controle sobre o processo de utilização. 2020. Trabalho de Conclusão de Curso (Graduação em Farmácia) - Universidade Federal da Bahia.
Orientador: Prof. Mileno Oliveira.

Banca de Trabalho de Conclusão de Curso de Especialização

Aluno: Jonatas Silva dos Santos.
Monografia: Perfil epidemiológico das bactérias causadoras de doenças veiculadas por alimentos no Brasil. 2021. Monografia (Aperfeiçoamento/Especialização em Curso de Especialização em Microbiologia) - Universidade Federal da Bahia.
Orientadora: Profa. Clícia Capibaribe Leite.

Prêmios e Reconhecimentos

2024 - Professora Homenageada da Turma 2023.2 do curso de Farmácia da UNICEUSA, UNICEUSA - Centro Universitário Salvador.

2022 - Menção Honrosa em reconhecimento á valorosa contribuição e dedicação, nas atividades desenvolvidas junto à Faculdade de Farmácia da Universidade Federal da Bahia. FACFAR/UFBA,

2022 - Professora Homenageada da Turma 2022.1 do curso de Farmácia da UNICEUSA - Centro Universitário Salvador.

2021 - Professora Homenageada da Turma 2021.2 do curso de Farmácia da UNICEUSA - Centro Universitário Salvador.

Outras Atividades Acadêmicas em parceria com IES

Orientação de 12 alunos em trabalhos de conclusão de curso de Farmácia da UNICEUSA - Centro Universitário Salvador (2020-2023).
Publicação de 5 artigos em periódicos *Qualis* B e C derivados dos trabalhos de conclusão de curso dos alunos orientados (2020-atual)

Participação em alguns cursos de aperfeiçoamento