UNIVERSIDADE FEDERAL DA BAHIA

FACULDADE DE FARMÁCIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS

KATHERINE GUTIERREZ ALZATE

APROVEITAMENTO DE SUBPRODUTOS INDUSTRIAIS E ENCAPSULAMENTO DE PROBIÓTICOS PARA APLICAÇÃO EM PRODUTOS LÁCTEOS

SALVADOR

2023

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência de Alimentos (PGAli) da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de Mestre/Doutor em Ciência de Alimentos.

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SALVADOR
2023

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

Gutierrez Alzate, Katherine.
Aproveitamento de subprodutos industriais e encapsulamento de probióticos para aplicação em produtos lácteos / Katherine Gutierrez Alzate. - 2023.
117 f.: il.

Orientadora: Profa. Dra. Marion Pereira da Costa.
Dissertação (mestrado) - Universidade Federal da Bahia, Faculdade de Farmácia, Salvador, 2023.

1. Alimentos funcionais. 2. Probióticos. 3. Probióticos - Aspectos da saúde. 4. Microencapsulação. 5. Cupuaçu. I. Costa, Marion Pereira da. II. Universidade Federal da Bahia. Faculdade de Farmácia. III. Título.

CDD - 664
CDU - 664

UNIVERSIDADE FEDERAL DA BAHIA
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TERMO DE APROVAÇÃO

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Aprovada em 28 de fevereiro de 2023.

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Dedico este trabalho,

À Deus, à minha mãe, ao meu pai a toda minha família, que sempre acreditou em mim e me apoia, também aos meus amigos e as pessoas que fizeram parte dessa caminhada.

Meus agradecimentos,

À Deus pai todo poderoso, e à virgem Maria, por seu amor infinito, pela força, sabedoria e por cada um dos anjos que eles colocaram para fazer parte dessa caminhada.

Aos meus pais, Clara e Dario, à minha irmã Jennifer e ao meu adorado sobrinho Juan Pablo, por esse amor incondicional, pela força que vocês significaram para o meu crescimento e, acima de tudo, porque vocês me permitiram realizar meus sonhos, porque mais do que ninguém, vocês conhecem minhas capacidades. Obrigada por ser minha vida;

Á Luis Alfonso, por me acompanhar neste projeto de vida, pelo esforço e compromisso assumidos. Por destacar minhas capacidades, por sempre me lembrar aquilo que alcancei e o que ainda tenho que alcançar. Obrigada pelo respeito e a admiração, é mútua;

À minha orientadora professora Dra. Marion Pereira da Costa, e ao meu coorientador professor Dr. Carlos Cavalheiro por me orientar neste projeto, pela confiança depositada e pelos ensinamentos;

À equipe Laitlácteos por me permitir realizar as análises para este trabalho, por todo o apoio ao Sr. Davino que estava sempre disposto a responder as dúvidas do laboratório, e ao Professor José Givaldino, pela qualidade humana que você proporciona;

Aos Doutores Carolina Oliveira - Labpesca, Leonardo Fonseca -LAPAAC, Stephane - MEVZ-LANA, por me permitir realizar parte da minhas análise em seu laboratório. Muito obrigada também especialmente a Gisele de MEVZ-LANA, pela disposição e boa atitude com o qual ela me recebeu em seu laboratório.

Aos meus amigos na Colômbia que me animaram a seguir este caminho. Também à Lívia, Tau, Wilton e demais amigos, que me acompanharam ao longo desse período aqui no Brasil;

Aos professores, técnicos e colegas do Programa em Ciência de Alimentos pela dedicação e compartilhamento de conhecimentos;

À Coordenação Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa de estudos concedida (nº do processo: 88887.645028/2021-00), sem a qual não seria possível a realização deste trabalho.

RESUMO

Os produtos lácteos têm sido considerados um dos principais alimentos nutricionais da dieta humana. Neste cenário, o aumento da exigência dos consumidores tem pressionado as indústrias lácteas no desenvolvimento de novos produtos sustentáveis, e que ofereçam benefícios à saúde, além das funções nutricionais básicas. Isto tem levado ao crescimento de novas pesquisas, como a adição de produtos ricos em proteínas, amidos, pectina e compostos antioxidantes, através do aproveitamento de subprodutos agroindustriais (sementes e cascas em pó), assim como a adição de micro e nanocápsulas probióticas. Consequentemente, foi desenvolvida uma bebida láctea fermentada com adição de diferentes concentrações de farinha (1,5%; 3%) e polpa (5%; 7,5% e 10%) de cupuaçu, avaliando os parâmetros nutricionais, físico-químicos, microbiológicos e sensoriais. Além disso, foi realizada uma revisão do uso de micro e nanoencapsulação de probióticos em produtos de origem animal, e com ênfase em leite e derivados, considerando as técnicas e materiais mais comumente utilizados neste processo. Foi constatado que a adição da farinha de cupuaçu melhorou a qualidade nutricional, reduziu a sinergia, aumentou a capacidade de retenção de água e melhorou os atributos sensoriais como cor, consistência e firmeza. A polpa melhorou os parâmetros reológicos, especialmente o índice de consistência e a viscosidade aparente na bebida fermentada. Portanto, o desenvolvimento de uma bebida láctea com potencial funcional utilizando polpa e farinha de cupuaçu como ingredientes é promissor em termos de qualidade físico-química, nutricional e microbiana. Por outro lado, no encapsulamento dos probióticos, foi constatado que o nanoencapsulamento tem limitações significativas em comparação ao microencapsulamento devido ao tamanho dos probióticos. Também foi encontrado que o encapsulamento dos probióticos melhora os parâmetros físico-químicos, sensoriais e microbiológicos, o que ajudou a prolongar a vida útil do produto devido à viabilidade probiótica.

Palavras-chave: Cupuaçu. Soro de leite. Microencapsulação. Nanoencapsulação. Probiótico encapsulado.

ABSTRACT

Dairy products have been considered one of the main nutritional foods in the human diet. In this scenario, the increase in consumer demand has put pressure on dairy industries to develop new sustainable products that offer health benefits in addition to basic nutritional functions. This has led to the growth of new research, such as the addition of products rich in protein, starch, pectin, and antioxidant compounds, through the use of agro-industrial by-products (seeds and powdered peels), as well as the addition of probiotic micro and nanocapsules. Consequently, a fermented milk beverage was developed with the addition of different concentrations of cupuassu flour (1.5%; 3%) and pulp (5%; 7.5%, and 10%), evaluating the nutritional, physicochemical, microbiological, and sensorial parameters. In addition, a review of the use of micro- and nanoencapsulation of probiotics in products of animal origin, and with emphasis on milk and dairy products, considering the techniques and materials most commonly used in this process. It was found that the addition of cupuassu flour improved nutritional quality, reduced synereses, increased water holding capacity, and improved sensory attributes such as color, consistency, and firmness. The pulp improved the rheological parameters, especially the consistency index and apparent viscosity in the fermented beverage. Therefore, the development of a dairy beverage with functional potential using cupuassu pulp and flour as ingredients is promising in terms of physicochemical, nutritional, and microbial quality. On the other hand, in the encapsulation of probiotics, it was found that nanoencapsulation has significant limitations compared to microencapsulation due to the size of probiotics. It was also found that encapsulation of probiotics improves the physicochemical, sensory, and microbiological parameters, which helped to extend the shelf life of the product due to probiotic viability.

Keywords*: Cupuassu. Whey. Microencapsulation. Nanoencapsulation. Encapsulated probiotic.*

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

Capítulo I

Aproveitamento de subprodutos industriais e encapsulamento de probióticos para aplicação em produtos lácteos

1 INTRODUÇÃO

Atualmente a indústria alimentícia busca inovar em produtos que proporcionem benefícios ao consumidor e que sejam sensorialmente aceitos, como é o caso dos produtos funcionais, onde as bebidas lácteas fermentadas são uma das matrizes alimentares mais estudadas e utilizadas para desenvolver novos produtos enriquecidos com ingredientes alimentares funcionais (ANDRADE *et al*, 2019; GUNESER *et al*., 2019), essas bebidas podem ser encontradas como bebidas à base de soro de leite e bebidas lácteas enriquecidas (probióticos, prebióticos, peptídeos, esteróis, vitaminas, minerais, fibras dietéticas e polifenóis) (HATI; MANDAL *et al*., 2019). Estes não só favorecem o bem-estar do corpo humano, pois também permitem o aproveitamento de subprodutos industriais que são fontes de vários componentes bioativos, incluindo aminoácidos, compostos fenólicos, fibras dietéticas, ácidos graxos, probióticos e outros produtos bioquímicos, como minerais, vitaminas e carotenóides (ROUTRAY; ORSAT, 2019).

As bebidas lácteas são geralmente feitas de soro de leite doce fresco, que é um subproduto obtido da fabricação de queijo por separação do coágulo do leite (ARGENTA; SCHEER, 2020). A composição e as características deste subproduto dependem da tecnologia de produção e da qualidade do leite utilizado como matéria-prima na fabricação do queijo, do qual provém a maioria dos nutrientes (HATI; MANDAL *et al*., 2019), tais como proteínas, lactose e minerais (GUO; WANG, 2019). Estes nutrientes favorecem o crescimento de bactérias lácticas (BAL) (RAMA *et al*., 2019). Esta característica principal permite o uso de grandes produções de soro de leite, que ocorre nas indústrias leiteiras, onde uma grande parte destes resíduos é utilizada de forma inadequada e descartada como efluente causando um grave problema ambiental, pois afeta física e quimicamente o solo (HATI; MANDAL *et al*., 2019; PIRES *et al*., 2021).

Outras alternativas também foram implementadas para aumentar a qualidade nutricional, físico-química e sensorial das bebidas lácteas, através da adição de ingredientes de origem vegetal, tais como polpas e pós ou farinhas de frutas obtidas de resíduos industriais, que podem conter quantidades significativas de compostos bioativos (ácidos graxos e compostos fenólicos) que permitiriam a prevenção de algumas doenças cardiovasculares e metabólicas (PENG *et al*., 2020). O uso deste tipo de subprodutos industriais também pode acrescentar o valor das bebidas lácteas, devido ao baixo valor econômico que representam na indústria e às grandes quantidades que são geradas (VODNAR *et al*., 2017). Portanto, o uso de frutas como o cupuaçu (*Theobroma grandiflorum*), que é uma fruta rica em amido, polissacarídeos de

pectina e fibra dietética, poderia melhorar os parâmetros de textura dos produtos lácteos (COSTA *et al*., 2015; PEREIRA *et al*., 2017).

As bebidas lácteas fermentadas devido ao seu alto valor nutricional, pelo teor de proteínas, minerais e vitaminas, proporcionam um meio ideal para o desenvolvimento e crescimento de microrganismos como as bactérias lácticas (BAL), que além de desempenharem um papel muito importante nos produtos lácteos fermentados (prolongar a vida útil dos alimentos, baixar o pH, intensificar o sabor e o aroma), têm efeitos favoráveis sobre o corpo humano, como a construção da microflora intestinal, a redução do colesterol no sangue, o equilíbrio da flora intestinal e o aumento da resistência a patógenos (ZOUMPMPOPOULOU *et al*., 2017; FREIRE *et al*., 2021). Entretanto, esses microrganismos apresentam problemas quando incorporados a qualquer alimento, devido a sua baixa resistência a processos térmicos e diferentes condições ambientais, tais como pH, oxigênio, peróxido de hidrogênio, temperatura e armazenamento (MARTÍN *et al*., 2015; FRAKOLAKI *et al*., 2021).

É aqui que entra a micro e nano-encapsulação, que é um método que fornece uma solução para proteger bactérias probióticas de fatores como calor, umidade e acidez presentes nas matrizes alimentares, incluindo produtos de origem animal (laticínios e carne), onde tais microrganismos podem ser incorporados para garantir a entrega segura e eficaz ao trato gastrointestinal (TGI) (DODOO *et al*., 2017; YAO *et al*., 2020). Este método de acordo com Frakolaki *et al*. (2020) deve ser realizado sob condições de pH moderado, temperaturas relativamente baixas e baixos níveis de oxigênio, para os quais vários materiais de encapsulamento têm sido utilizados através do tempo, incluindo carboidratos (alginato, quitosano, carragena, goma arábica, pectina, etilcelulose e maltodextrina) e proteínas (soro de leite, soja e grãos), que devem ser cuidadosamente escolhidas, assim como técnicas de microencapsulação (secagem por spray, resfriamento por spray, extrusão, emulsificação e electrospinning) para garantir a melhor proteção possível das bactérias sem comprometer as características do produto final (RODRIGUES *et al*., 2020; KOWALSKA *et al*., 2022).

Levando em conta o alto valor nutricional do soro e do cupuaçu, a pouca informação que existe sobre o uso de subprodutos industriais (resíduos de frutas) em bebidas lácteas fermentadas e a importância que o encapsulamento tem gerado para a viabilidade dos probióticos em produtos de origem animal, faz-se necessária o estudo da qualidade nutricional, físico-química, microbiológica e sensorial de bebidas lácteas fermentadas desenvolvidas com polpa e da farinha de cupuaçu. Deve-se também ampliar a pesquisa sobre o uso de nano e microencapsulação de probióticos em produtos lácteos e cárneos.

2 OBJETIVOS

2.1 Objetivo geral

- ✓ Avaliar o efeito do uso de subprodutos industriais sobre os parâmetros de qualidade das bebidas lácteas fermentadas, além de realizar uma revisão de literatura sobre técnicas, materiais e aplicação de micro e nanoencapsulação de probióticos em produtos de origem animal.

2.2 Objetivos específicos

- ✓ Desenvolver uma bebida láctea fermentada com a adição de polpa e farinha de cupuaçu (*Theobroma grandiflorum*) em diferentes concentrações;
- ✓ Avaliar a qualidade nutricional de bebidas lácteas fermentadas;
- ✓ Determinar os parâmetros físico-químicas (pH, sinérese, capacidade de retenção de água, cor e reologia) da bebida láctea fermentada durante o armazenamento;
- ✓ Analisar os parâmetros microbiológicos das bebidas lácteas fermentadas durante o armazenamento;
- ✓ Avaliar o efeito da combinação de polpa e farinha de cupuaçu sobre as propriedades sensoriais do produto;
- ✓ Descrever as diferentes técnicas e materiais mais comumente utilizados na micro e nanocapsulação de probióticos;
- ✓ Identificar as principais aplicações do micro e nanocapsulação em produtos lácteos e cárneos.

3 FUNDAMENTAÇÃO TEÓRICA

3.1 **Bebidas Lácteas Fermentadas**

As bebidas lácteas fermentadas são produtos lácteos feitos de leite, soro de leite ou outros derivados da mesma origem, constituindo uma quantidade majoritária de ingredientes lácteos. Estes podem conter outras substâncias alimentares, mas a porcentagem da base láctea deve ser de pelo menos 51% (m/m) do total de ingredientes do produto, diferentemente de outros produtos alimentares que podem ter uma porcentagem menor de ingredientes lácteos. A mistura de ingredientes junto com a base láctea é fermentada pela ação de micro-organismos específicos e/ou leite fermentado adicionado, sem passar por tratamento térmico após a fermentação, garantindo um mínimo de 10^6 UFC/mL no produto acabado (BRASIL, 2005, GOMES *et al*., 2013).

O processamento de bebidas lácteas fermentadas começa a partir da formulação do produto a ser desenvolvido, o processo de fermentação, o manuseio e a pós-fermentação. Este método de produção pode variar de acordo com a escala de produtividade, porque em uma escala maior (nível industrial) uma quantidade maior de produto normalmente é processada, onde culturas iniciais definidas são comumente usadas, e pode incluir culturas probióticas ou secundárias, de acordo com o tipo de bebida. Enquanto, quando são produzidas artesanal ou em quantidades menores, são utilizadas bactérias ácido lácticas (BAL) nativos e outros micro-organismos (RAMA *et al*., 2019). Durante o processo de fermentação, a lactose é transformada em ácido láctico resultando na redução do pH e na coagulação do leite e outros metabólitos que contribuem para o sabor e o aroma dos produtos e proporcionam benefícios à saúde (PEROTTI *et al*., 2019).

As bebidas lácteas fermentadas têm gerado uma grande demanda no mercado, devido ao aumento da produção que se apresenta mundialmente no mercado de alimentos funcionais, à grande aceitação dos consumidores, à simples tecnologia de produção e à busca por parte de indústrias e pesquisadores de desenvolver formulações cada vez melhores e de melhor qualidade nutricional, com benefícios para a saúde, melhores valores em seus parâmetros físico-químicos, maior economia na produção e aproveitamento de recursos (GUNESER *et al*., 2019).

3.2 Soro de leite

O soro de leite é um subproduto líquido industrial obtido da fabricação de queijo, após coagulação do leite, que pode representar de 85 a 95% de seu volume e conservar cerca de 55%

dos nutrientes (RAMA *et al*., 2019; PIRES *et al*., 2021). Este subproduto é caracterizado por uma cor amarelo-esverdeada devido ao seu teor de riboflavina (vitamina B2), um alto teor de lactose ($C_{12}H_{22}O_{11}$, 70-72 % dos sólidos totais) e proteínas (8-10 % dos sólidos totais) como α-lactalbumina e β-lactoglobulina, albumina bovina sérica, glicomacropeptídeo, imunoglobulinas, lactoperoxidase e lactoferrina. Também é composto de vitaminas e minerais (12-15 % do total de sólidos), este último constituído principalmente por sais de cálcio, potássio, sódio e magnésio (RYAN; WALSH, 2016; ARGENTA; SCHEER, 2020). Além disso, tem a presença de peptídeos bioativos potenciais, que podem ser encontrados pertencentes às caseínas de soro, de modo que os benefícios potenciais à saúde poderiam ser atribuídos a eles, devido à ampla gama de propriedades que possuem, tais como anti-hipertensivo, antioxidante, antimicrobiano, antitrombótico, imunomodulador e opióide (SOMMELLA *et al*., 2016).

O soro de leite é classificado de acordo com o método utilizado para sua produção, entre os quais podemos encontrar soro ácido obtido da coagulação por fermentação (conversão da lactose em ácido láctico) gerado por BAL, pela adição de minerais ou ácidos orgânicos (produção de caseína ácida). O soro de leite resultante tem pH 4,5 - 5,8 (GUO; WANG, 2019; PIRES *et al*., 2021). Outro tipo é o soro doce com um pH entre 6,0 - 6,8, que tem um pH ideal para a coagulação da caseína gerada pela ação enzimática (ARGENTA; SCHEER, 2020). O soro doce difere do soro ácido por seu alto teor de lactose (46,0 - 52,0 g/L) e proteína (6,0 - 10,0 g/L). Além disso, pode ser encontrada uma quantidade até 4 vezes maior de aminoácidos livres em comparação ao soro ácido (BOŽANIĆ *et al*., 2014).

Devido a seu alto teor de matéria orgânica, alta demanda bioquímica de oxigênio e alta demanda química de oxigênio, o soro de leite gera grandes problemas de descarte, representando uma ameaça ambiental se for descartado de forma inadequada (jogado no solo, em corpos d'água ou em águas residuais de laticínios), pois seu potencial poluidor pode exceder o das águas residuais domésticas, levando a uma redução do oxigênio dissolvido nas fontes de água e um risco significativo para a vida aquática, bem como para a saúde humana, para o meio ambiente e o solo devido aos níveis de lactose e minerais (ARGENTA; SCHEER, 2020; PIRES *et al*., 2021). Portanto, algumas pesquisas se focalizaram em aproveitar os nutrientes e contribuir para o desenvolvimento de ingredientes ou produtos de alto valor agregado, alguns dos quais já foram comercializados como produtos minerais lácteos, produtos de fração proteica do soro de leite, concentrado proteico, isolado proteico do soro de leite, soro em pó e lactose. Este último como um dos componentes mais abundantes no soro de leite, é amplamente utilizado em fórmulas infantis, padaria, confeitaria e produtos farmacêuticos (GUO; WANG,

2019). A lactose também foi hidrolisada a fim de desenvolver produtos que possam ser consumidos por pessoas intolerantes a este carboidrato. De acordo com RAMA *et al*. (2019), o soro de leite também pode ser usado como substrato para a produção de outros metabólitos como enzimas ou ácido láctico que favoreceriam o crescimento do BAL.

3.3 Probióticos

Os probióticos são considerados organismos vivos que proporcionam um efeito benéfico à saúde, quando ingeridos em certas quantidades além da própria contribuição nutricional, dado que alguns estudos estabeleceram que estruturas celulares isoladas podem gerar efeitos positivos onde não é necessário que o microrganismo seja viável (FAO/OMS, 2002). Estas são bactérias Gram-positivas, onde o BAL tem sido a espécie mais conhecida, sendo as cepas dos gêneros *Bifidobacterium* e *Lactobacillus* as mais amplamente utilizadas. Outros gêneros como *Enterococcus*, *Lactococcus*, *Pediococcus*, *Streptococcus* e *Leuconostoc* foram atribuídos a propriedades probióticas, assim como outras bactérias não-lácteas ou leveduras como as cepas dos gêneros *Bacillus* e *Saccharomyces* (FRAKOLAKI *et al*., 2020). A viabilidade dos micro-organismos tem mostrado que o consumo gera efeitos benéficos à saúde, tais como melhor absorções de cálcio, síntese de vitaminas e proteínas, estimulação e melhoria do sistema imunológico, sendo um critério permanente na maioria das definições propostas para probióticos, o que se tornou uma nova alternativa para a prevenção de transtornos intestinais e como imunomoduladores gerando efeitos na saúde (ZHANG *et al*, 2015; BAHARI, 2017; MALDONADO *et al*, 2018). São utilizados para tratamento da doença de Crohn, tratamento de úlceras pépticas, gastrite tipo B, artrite reumatoide, câncer gástrico e sintomas de diarreia aguda em bebês e crianças causada por rotavírus. Também como agentes anticancerígenos, antimicrobianos contra patógenos como *Salmonella* e *Helicobacter pylori*, antimutagênios e anti-hipertensivos, bem como ser capaz de reduzir sintomas alérgicos, colesterol sérico, aliviar a intolerância à lactose e estimular o sistema imunológico (BOLTIN, 2016; EVIVIE *et al*., 2017; AL-HINDI; ABD, 2020).

Os produtos lácteos têm sido usados como um meio natural para o transporte de probióticos. Entretanto, a viabilidade do probiótico tende a ser afetada ao ser exposto a fluidos gástricos, o que levou vários estudos a buscar formulações probióticas alternativas para garantir resistência no trato gastrointestinal (TGI) e que estas bactérias possam colonizar o intestino para gerar benefícios à saúde (EVIVIE *et al*., 2017; DODOO *et al*., 2017). As técnicas mais favoráveis são a incorporação de outras substâncias, tais como micronutrientes ou prebióticos, ou o microencapsulamento de probióticos, que também tem sido usado em outros tipos de

matrizes alimentares (produtos não-lácteos) para gerar novas tendências na incorporação de cepas probióticas (FRAKOLAKI *et al*., 2020).

3.4 Bactérias ácido lácticas (BAL)

As bactérias ácido lácticas (BAL) são microrganismos gram positivos capazes de produzir ácido láctico durante o metabolismo homofermentativo ou heterofermentativo, crescendo principalmente em subcondições microaerófilas ou estritamente anaerobic, pertencentes aos gêneros *Enterococcus*, *Carnobacterium*, *Lactobacillus*, *Tetragenococcus*, *Lactococcus*, *Leuconostoc*, *Bifidobacterium*, *Pediococcus*, *Weissela* e *Streptococcus*, entre outros. (BURGAIN *et al*., 2014). Estas bactérias podem estar naturalmente presentes em meios ricos em nutrientes como o trato gastrointestinal urogenital (TGI) ou em alguns alimentos, como laticínios, carnes e grãos. Na indústria alimentícia, esses microrganismos têm sido utilizados para participação em processos de fermentação, como fermentadores (geram rápida .acidificação do meio acelerando e dirigindo o processo de fermentação) ou não fermentadores (fornecem compostos aromáticos), onde é importante levar em conta propriedades como a produção de compostos voláteis e peptidase, a produção de ácidos em diferentes meios e a diferentes temperaturas, atividades de autólise da proteinase, produção de compostos inibidores e resistência a bacteriófagos, para o uso do BAL como bactéria iniciadora (MOTTA; GOMES, 2015).

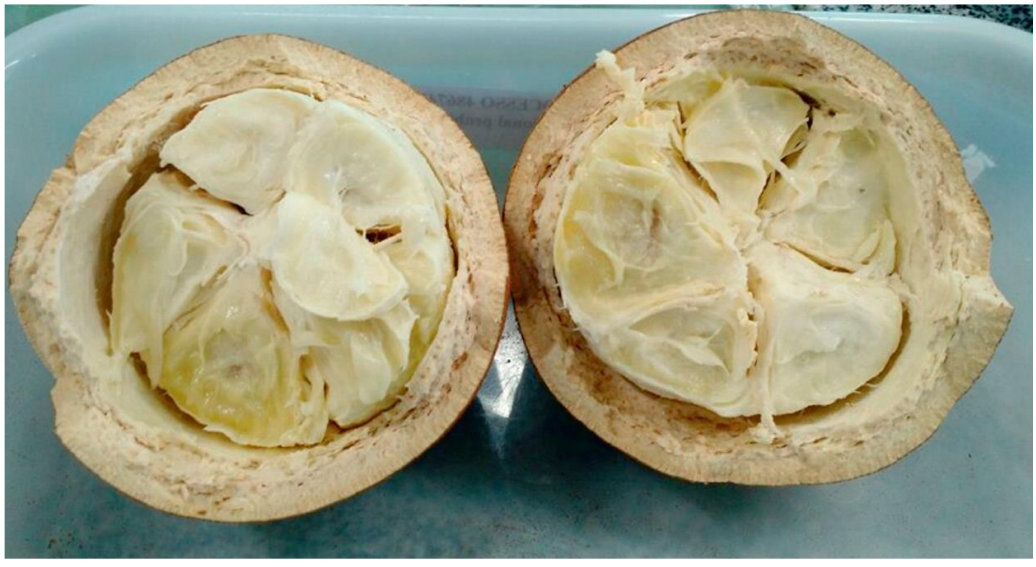
As BAL são também microrganismos que desempenham um papel importante em muitos processos utilizados para transformar e prolongar a vida útil dos alimentos, já que os produtos obtidos de seu metabolismo tendem a ter um pH baixo, ácidos orgânicos, diacetila e acetaldeído e peptídeos antagônicos a outras bactérias (bacteriocinas), que proporcionam segurança higiênica e, ao mesmo tempo, permitem maior estabilidade durante o período de armazenamento. (FREIRE *et al*., 2021). Da mesma forma, estas bactérias têm sido utilizadas em produtos como leite fermentado, queijo, manteiga e iogurte, e no processamento de bebidas alcoólicas, carnes fermentadas, cereais fermentados nos quais contribuem para melhorar o valor nutricional e as propriedades sensoriais, interferindo na intensidade da cor, sabor, aroma e textura dos produtos (EVIVIE *et al*., 2017). As BAL pode proporcionar benefícios à saúde do consumidor atribuídos aos metabólitos bioativos presentes nos fermentos, que poderiam melhorar a digestibilidade, a composição da microbiota intestinal, através da ingestão de produtos fermentados com BAL que poderiam gerar efeitos antialérgicos, antioxidantes, imunomoduladores e efeitos antiobesidade, bem como a redução da dor muscular, reduzindo a

probabilidade de desenvolvimento de doenças cardiovasculares, diabetes mellitus tipo 2, propriedades hipocholesterolemicas e anticarcinogênicas, este impacto na saúde faz que as LAB e os produtos fermentados estejam sendo cada vez mais estudados (MALDONADO *et al*, 2018; MATHUR; BERESFORD; COTTER, 2020)

3.5 Cupuaçu

O cupuaçu (*Theobroma grandiflorum*) é uma fruta tropical sul-americana produzida na região amazônica, pode ser encontrada em países como Bolívia, Peru, Equador, Colômbia, Venezuela, Suriname e principalmente no sul e sudeste do Brasil e nos estados do Maranhão e Pará (CARVALHO; GARCIA; AMAYA-FARFÁN, 2006, RAMOS *et al*., 2019), é uma espécie diplóide, pertencente à família *Sterculiaceae* e ao gênero *Theobroma*, de onde se origina a relação e a familiaridade com o cacau. Os frutos são produzidos entre novembro e junho, com maior incidência em fevereiro e março. Os frutos (Figura 1) são de cor marrom, vêm em diferentes formas de drupa, obovóides, alongados, elípticos, ovais ou redondos, podem medir 1225 cm de comprimento, 1012 cm de diâmetro e pesar entre 200 - 4000 g, contêm uma casca dura de 13 cm de espessura, coberta com um pó marrom, 15 a 50 sementes, envolvidas por uma polpa mucilaginosa, que representa de 38 a 43% dos frutos (SALGADO *et al*., 2011; PEREIRA; ABREU; RODRIGUES, 2018),

Figura 1 cupuaçu (*Theobroma grandiflorum*) – casca, sementes e polpa



Fonte: PEREIRA; ABREU; RODRIGUES, 2018.

O cupuaçu é caracterizado por ter um sabor ácido, aroma e textura fortes (FABER; YUYAMA, 2015), devido à presença de compostos voláteis como ésteres (acetato de etilo, butanoato de etilo, hexanoato de etilo, propanoato de etilo), que dão um alto potencial econômico à fruta cupuaçu, utilizada como ingrediente na produção de produtos lácteos (sorvetes, iogurtes e bebidas fermentadas), sucos, licores, vinhos, geleias, doces, sobremesas e outros produtos como o cupulate (farinha obtido a partir das sementes fermentadas), sendo pouco usado para ser consumido in natura (COHEN; DE SOUSA; JACKIX, 2009; COSTA *et al*., 2015; RAMOS *et al*.,2019).

A polpa de cupuaçu é de cor branca amarelada, tem um pH ácido, tem despertado interesse comercial devido aos múltiplos usos que podem ser feitos devido a sua grande aceitação sensorial, sua composição química particular rica em fibras, o alto teor de amido e polissacarídeos de pectina, e o alto valor nutricional, por isso tem sido atribuído propriedades benéficas para a saúde, devido a sua capacidade antioxidante, potencial antidiabético, inibição da atividade da α-amilase (PINENT *et al*., 2015; PEREIRA *et al*., 2017) atribuídos ao teor de macro e micronutrientes (tabela 1), eles também estão contidos nos subprodutos (casca, semente) obtidos da transformação industrial da polpa, que foram aproveitados pelo processamento das sementes e dando-lhes um uso semelhante ao dado às sementes de cacau, o processo começa com a fermentação do grão, torrefação, moagem e prensagem para obter a gordura e a farinha de cupuaçu, este último é obtido a partir da massa desengordurada (LANNES, 2003)

Tabela 1 – Valores de componentes relacionados de sementes, polpa e farinha de cupuaçu

Composição	Sementes	Polpa	Farinha de cupuaçu
Proteína	7,81	0,48	17,2
Umidade	5,30	81,3 - 89,0	3,05
Fibra	5,56	0,50- 2,12	-
Carboidratos	23,09	34,6	-
Lípidos	60	1,92	28
Antioxidantes (μMTrolox/g)	33,64	1,7 - 2,0	13,0
pH	6,35	3,5	5,68-5,80

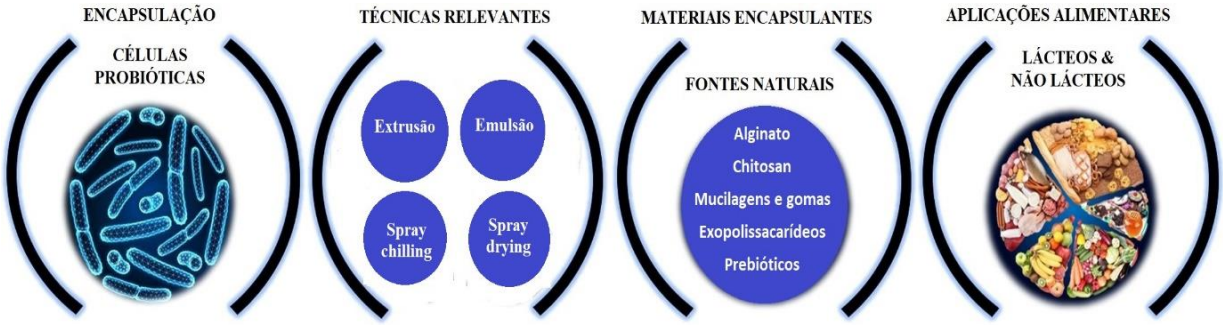
Fonte: *Adaptado a partir de Genovese, Lannes (2008); Pereira, Abreu, Rodrigues (2018) e Ferreira; Jannette (2020).*

Devido às propriedades composicionais do cupuaçu apresenta um perfil de aminoácidos mais equilibrado (leucina, isoleucina) do que o cacau, o que levou ao aumento do uso de sementes de cupuaçu para a produção de chocolates e em produtos de panificação no Brasil, um dos principais países produtores da espécie *Theobroma*, o que tem significado uma boa alternativa para o uso deste subproduto e, ao mesmo tempo, uma excelente matéria-prima para uso na indústria alimentícia (GENOVESE; LANNES, 2008; SALGADO *et al*., 2011; DA COSTA *et al*., 2022).

3.6 Encapsulação

O encapsulamento é uma técnica que tem sido amplamente utilizada na indústria alimentícia para projetar novos produtos funcionais, especialmente aqueles que são enriquecidos com microrganismos probiótico (TIMILSENA; HAQUE; ADHIKARI 2020). O processo consiste em envolver um material do núcleo funcionalmente ativo (material a ser encapsulado) que pode ser líquido ou sólido de tamanho micro ou nano em uma matriz de material inerte (parede, material de revestimento, cápsula, cobertura, membrana ou material portador), que tem a função de isolar e proteger o material do núcleo do ambiente externo, quando usado em probióticos, as propriedades do material de encapsulamento podem reduzir ou inibir os danos ou perdas celulares dos microrganismos encapsulados (MARTÍN *et al*., 2015; WU; ZHANG, 2018). Tudo isso levou a que atualmente se estude a aplicação desta técnica com diferentes tipos de materiais nos quais o objetivo principal é melhorar a resistência dos microrganismos probióticos em condições adversas (processamento, armazenamento, distribuição de alimentos e passagem pelo trato gastrointestinal) garantindo a entrega intacta na fase intestinal (ETCHEPARE *et al*., 2020). As características e desempenho das cápsulas dependem principalmente da técnica e do material de parede utilizado (Figura 2), que deve ser compatível com o material do núcleo das cápsulas, bem como apresentar uma boa estabilidade mecânica, de acordo com a aplicabilidade das cápsulas, para obter uma maior eficiência do processo de encapsulamento (ETCHEPARE *et al*., 2020; RODRIGUES *et al*., 2020).

Figura 2 – Principais técnicas e polissacarídeos para o encapsulamento de células probióticas



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

4 CONSIDERAÇÕES FINAIS

O uso de subprodutos industriais em bebidas lácteas fermentadas é uma boa alternativa para o aproveitamento dos recursos naturais, pois a farinha de cupuaçu aumentou o valor nutricional da bebida. Além a adição da farinha de cupuaçu reduziram a sinéreses e aumentaram a capacidade de retenção de água e a polpa em uma concentração de 10% melhorou os parâmetros reológicos, especialmente o índice de consistência e a viscosidade aparente. Com relação aos parâmetros sensoriais a polpa forneceu um excesso de aroma alcoólico e sabor cupuaçu, a combinação da polpa com uma maior concentração de farinha melhorou a aparência sensorial, a cor, a consistência e a firmeza em formulações combinadas, especialmente quando contendo 10% de polpa. Em geral, a elaboração de uma bebida láctea com potencial funcional utilizando polpa ou semente de cupuaçu como ingredientes é promissora em termos de qualidade físico-química, nutricional e microbiológica.

Por outro lado, considera-se que a nanoencapsulação de probióticos tem grandes limitações em relação à microencapsulação, devido à influência do tamanho das bactérias. Onde o uso de nanocápsulas probióticas em produtos cárneos por electrospinning e microcápsulas em alimentos lácteos por spray drying aumentou, usando o alginato como material de parede, sozinho ou em companhia de outros materiais para melhorar a estrutura da cápsula. Da mesma forma, a micro e nanoencapsulação de bactérias probióticas mostrou ser um caminho extenso, dado que ainda faltam estudos nos quais o comportamento probiótico e a interação de diferentes materiais poliméricos sejam avaliados em produtos cárneos, levando em conta a ampla gama atualmente disponível no mercado.

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Capítulo II

Manuscrito: Cupuassu (Theobroma grandiflorum) pulp and flour improve physicochemical, rheological, and nutritional quality of fermented milk beverage

Cupuassu (*Theobroma grandiflorum*) pulp and flour improve physicochemical, rheological, and nutritional quality of fermented milk beverage.

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Periódico a ser submetido (1ª submissão):	*Foods; ISSN: 2304-8158*
Maior percentil (Scopus):	*95%; 2/34; Health Professions*

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Article

Cupuassu (*Theobroma grandiflorum*) pulp and flour improve physicochemical, rheological, and nutritional quality of arti-sanal fermented milk beverages

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Abstract: The use of fruits and their by-products in food has dramatically impacted the food area due to the nutritional benefits and the technological and sensory effects of food matrices. Therefore, this research aimed to evaluate the effects of adding cupuassu (*Theobroma grandiflorum*) pulp and flour on fermented milk beverages' physicochemical, microbial, and sensory properties during refrigerated storage (0, 7, 14, 21, and 28 days). Twelve formulations were realized with different percentages of cupuassu pulp (0, 5, 7.5, and 10 % w/vol) and flour (0, 1.5, and 3 % w/vol). The treatments with 3% cupuassu flour presented the highest percentages of protein, fat, fiber and carbohydrates, compared to the samples containing pulp. On the other hand, the addition of pulp increased water retention capacity and color parameters (L^*, a^*, b^*, and c^*), decreased pH and syneresis on day 0 of storage. During storage, the samples with pulp showed an increase in pH values, consistency index, and apparent viscosity. In comparison, the cupuassu flour addition decreased syneresis values and increased L^* and b^* during storage, as did the pulp. In addition, the sample HPHF (10% pulp and 3% cupuassu flour) based on just-about-right, penalty, and check-all-that-apply analysis improved some sensory attributes of the fermented milk beverage, such as brown color, acid taste, bitter taste, cupuassu flavor, and firm texture. It can be concluded that the cupuassu pulp and flour addition improves the physicochemical and sensory quality of fermented milk beverages and can provide nutritional value to the product.

Keywords: cupuassu residue; by-products; color analysis; rheological behavior; sensory acceptance

1. Introduction

Artisanal fermented milk constitutes a diverse category of foods created by introducing fermenting bacterial or yeast stains into the milk of different species resulting in products with different sensory properties, comprising acidic, sour, and tangy flavors and fluid, viscous, and thick texture [1]. Some of the most popular artisanal fermented milk include fermented milk beverages, yogurt, kefir, koumiss, and buttermilk [2]. Additionally, many artisanal fermented milk products can be made using traditional methods and locally-sourced ingredients, making them a sustainable and eco-friendly choice for consumers who are also looking to support small-scale farmers and producers. For instance, fermented milk beverages are a well-consumed group of dairy foods in Brazil that must contain a minimum milk base concentration of 51 % vol/vol [3]. In this context, the addition of cheese whey, a by-product of the cheese industry that is generally discarded, as an ingredient in artisanal fermented milk beverages can be an alternative to reduce wasting. Indeed, whey represents up to 95% of the volume of milk and has high nutritional value, containing proteins and water-soluble vitamins, minerals, lactose, lipids, and peptides [4-7]. Therefore, whey has the potential to be used as a raw material in developing new artisanal milk beverages that are not only distictive in flavor and texture but also sustainable and health-promoting.

Moreover, fruit preparations, starches, pectins, xylooligosaccharides, and flours are reported to improve the sensory acceptability, and the physicochemical and rheological properties of milk beverages [8–10]. For instance, the cupuassu (*Theobroma grandiflorum*) has been used in the last years for this purpose [11–13]. Its pulp has an acidic flavor and intense fragrance that gives distinctive flavor over other fruits that are more commonly used for yogurt production, such as strawberry and cantaloupe [14–16]. Likewise, cupuassu can improve some physicochemical properties of milk beverages, such as viscosity and water retention capacity (by decreasing syneresis) [11, 13] due to its carbohydrate content (starch, pectin, and polysaccharides, among others), which gives a high nutritional value to cupuassu pulp as well as fatty acids (palmitic, linoleic, and α-linolenic acids), ascorbic acid (96 - 111 mg/100 g), and phenolic compounds (20.5 mg/100 g). Still, it has a biofunctional potential due to soluble fiber content [15, 17] and antioxidant capacity (1.7–2.0 μM Trolox/g) [18]. Therefore, cupuassu can add nutritional and bio-functional value to dairy products.

Cupuassu flour is a by-product of the fruit obtained from the fermentation, roasting, and grinding of seeds (up to 17% of the fruit) [18]. This flour is mainly constituted of proteins (14.2% - 17.2%), lipids (28.2% - 36.3%), minerals (3.80%), and fibers (22.2%). Additionally, it has phenolic compounds (16.9 mg_{GAE}/g_{DM}), flavonoids (5.92 mg_{CA}/g_{DM}), epicatechin (20.74 $mg/100g_{DM}$), and antioxidant activity in ABTS and DPPH (151 mg $_{TEAC}$/100g $_{CE}$, 85.4 mM Trolox eq/L, respectively) [12, 19]. Thus, cupuassu flour can be an interesting by-product to add value to fermented milk beverages.

The use of cupuassu pulp in different food products has been used to improve the sensory properties of some dairy products such as goat milk yogurt [11, 20], and probiotic beverages[21], being associated with various health benefits, mainly attributed to phenolic compounds, such as flavones, flavonols, catechins, epicatechin, and protoanthocyanidin, which favor the consumer well-being through antioxidant activity, reducing oxidative damage in lymphocyte DNA, presenting anticancer, antimicrobial, anti-inflammatory, and digestive stimulation effects [22-23], allowing to prevent cardiovascular, circulatory, cancer, diabetes, Alzheimer and Parkinson diseases [24, 26].

Besides their potential nutritional, sensory, and techno-functional benefits, cupuassu pulp and flour are not widely used in developing new products. The primary hypothesis of this work is that cupuassu pulp and flour addition enhances the nutritional, sensory, and techno-functional quality of fermented milk beverages. Therefore, this work aimed to evaluate the effect of cupuassu pulp and flour on the physicochemical, microbial, and sensory properties of a fermented milk beverage during 28 days at 4 °C.

2. Materials and Methods

2.1. Materials

Raw cow's milk has been obtained at the Experimental Farm of Entre Rios of Federal University of Bahia (UFBA). The whey was acquired from manufacturing fresh cheese made for other research. The cupuassu pulp and flour were purchased from local markets in São Paulo and Salvador (Brazil).

2.2. Preparation of Artisanal fermented milk beverages

Artisanal fermented milk beverages were prepared as described by Costa et al. [11] with modifications. In all treatments, pasteurized whey (49%) and milk (51%) were used as the liquid base, sugar (3%), and lactic culture (0.1%; Fegurte 3®, Fego Alimentos LTDA, Goiânia, Brazil) composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* were added. The mixture of ingredients was fermented in a water bath (model N1030, Centauro, Atuba, Pinhais, PR, Brazil) at a constant temperature (42 ± 1°C for 5 h) until it reached a pH of 4.6. After interrupting the fermentation process, cupuassu pulp (water 30% and pulp 70%) and/or flour were added to the fermented milk beverages in different concentrations according to Costa et al. [20] and Jovanović et al. [27], depending on the treatment. Therefore, twelve treatments were performed: 1) beverage without added pulp and flour was considered as the control (NPNF), 2) beverage with 1.5% flour (NPLF), 3) beverage with 3% flour (NPHF), 4) beverage with 5% pulp (LPNF), 5) beverage with 7.5% pulp (MPNF), and 6) beverage with 10% pulp (HPNF). In addition, for a low concentration of flour combined with low, middle, and high concentrations of pulp, the treatments were as follows: 7) beverage with 10% pulp and 1.5% flour (HPLF), 8) beverage with 7.5% pulp and 1.5% flour (MPLF), 9) beverage with 5% pulp and 1.5% flour (LPLF). Finally, for a high concentration of flour combined with low, middle, and high concentrations of pulp: 10) beverage with 10% pulp and 3% flour (HPHF), 11) beverage with 7.5% pulp and 3% flour (MPHF), 12) beverage with 5% pulp and 3% flour (LPHF). Lastly, the products were stored at 4±1 °C for 28 days.

2.3. Proximate composition

The proximate composition of the fermented milk beverages was performed on day 0. Moisture content was evaluated through the oven drying method; the ash content by ashing at 550 °C; the protein content by the Kjeldahl method using 6.38 as a factor; and fat content by soxhlet AOAC [28], being the results expressed as %. Neutral Detergent Fiber (NDF) was analyzed in 0.500 g of sample with a Tecnal EQ LCC 08 fiber analyzer using the Ankom system with filter bags [29]. The carbohydrate content was determined by difference, subtracting from 100% the sum of the values obtained previously [27].

2.4. pH, syneresis, water holding capacity (WHC), and instrumental color

The pH, syneresis, water-holding capacity, and instrumental color were evaluated every seven days during the storage period (0, 7, 14, 21, and 28).

The pH was determined by a bench pH meter for aqueous solutions (Model Mpa-210, Tecnopon, Piracicaba, São Paulo, Brazil). Before use, the electrode was calibrated with buffer solutions 4.0, 7.0, and 10.0.

The syneresis and water holding capacity were performed using an Eppendorf laboratory centrifuge model 5702R (Eppendorf Ltd, Stevenage, UK) according to Ladjvardi [30] with slight modifications. Aliquots of 5 g of fermented milk beverages were centrifuged at 3,000 x g for 30 minutes at 10°C, and the whey was separated. Syneresis (%) was calculated as the weight of generated supernatant (whey) per weight of fermented milk beverage multiplied by 100, whereas WHC was given as the percent weight of drained gel (precipitate) relative to the original weight of fermented milk beverage [31].

The values of lightness (L^*, 100 = white, 0 = black), redness (a^*, + red, - green), and yellowness (b^*, + yellow, - blue) of the fermented milk beverages were recorded with a Chroma Meter CR-5 colorimeter (Konica Minolta Business Technologies Inc, Tokyo, Japan) according to Costa et al. [11]. Transmittance with a D-65 light source, a 10° standard observer, and a 26 mm measuring area with a rectangular optical glass cell (lengths of 2 mm, 10 mm, and 20 mm) for the precise measurement of liquid transmittance were employed.

Chroma (c^*), hue angle ($h°$), and total color difference (ΔE^*) were calculated based on the analyzed color coordinates (equations 1 to 3). In addition, the ΔE was calculated matching the spectrum of the freshly prepared fermented milk beverage (day 0) and its relative spectrum at the subsequent storage days according to Lucas et al. [32] as it follows.

$$h° = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$C* = \left(a^{*2} + b^{*2}\right)^{\frac{1}{2}} \quad (2)$$

$$\Delta E = \sqrt{(L_n^* - L_0^*)^2 + (a_n^* - a_0^*)^2 + (b_n^* - b_0^*)^2} \quad (3)$$

2.5. Rheological behavior

Rheological measurements were performed using a rheometer (Haake Rheotest, Mod. 2.1, Medingen, Germany), with concentric cylinders coupled to a water bath for temperature control (25 °C). The flow curves of samples were determined at speeds between 0.56 and 243 rpm; their corresponding shear rates (γ) and shear stresses (σ) were computed from relations given by the instrument manufacturer and then recorded. The experimental data were fitted to the Ostwald-de-Waele model [33] as in equation (4).

$$\sigma = K\gamma^{(n)}, \quad (4)$$

Where σ is the shear stress (Pa), K is the consistency index (Pa.s^n), γ is the shear rate (s-1), and n flow behavior index (dimensionless). This model was used to evaluate the pseudoplastic behavior of each dairy beverage sample.

The rheological data were adapted to the Ostwald-de-Waele model [34] to obtain viscosity values in the upward viscosity/shear rate curves at a shear rate between 25 and 1000 s^{-1} were reported as the apparent viscosity of samples as in equation (5):

$$\mu = K\gamma^{(n-1)}, \quad (5)$$

Where μ is the apparent viscosity, K is the consistency index, γ is the shear rate, and n is the flow behavior index. The viscosity results were expressed in mPa.

2.6. Microbiological analysis

All samples were analyzed for coagulase-positive *Staphylococcus*, molds and yeasts, *Salmonella* spp., total and thermotolerant coliforms, and *Escherichia coli* on days 1 and 28 of storage according to the American Public Health Association [35]. Briefly, 10 mL of each fermented milk beverage was homogenized in 90 mL of 0.1% peptone water. The samples were then subjected to serial dilutions and inoculated on Petri dishes using a Drigalsky loop. Enumeration of coagulase-positive *Staphylococcus* was performed on Baird Parker agar (Neogen, Lansing, MI, USA) after incubation at 35 – 37 ± 1 °C for 48 h. Mold and yeast counts were determined by growth on Dichloran Rose Bengal Chloramphenicol Base (DRBC) agar and incubated aerobically at 25 ±1 °C for 3 - 5 days. The colonies were expressed as log colony forming units (CFU) per mL. For the analysis of *Salmonella* spp., samples were pre-enriched with 1% buffered peptone water (Merck KGaA, Darmstadt, Germany) and incubated at 35 ± 2 °C for 24 h, followed by selective enrichment in Tetrathionate broth (Neogen, Lansing, MI, USA) and Rappaport Vassiliadis broth (RV-Micromed Isofar) at 42 ± 2°C for 24 h. After, aliquots were streaked on Xylose Lysine Deoxycholate agar (Neogen, Lansing, MI, USA), Hektoen enteric agar (Ionlab, Araucária, PR, Brazil), and Bright Green agar (Neogen, Lansing, MI, USA), and incubated at 35 ± 2°C for 24 h.

Finally, the analyses of total and thermotolerant coliforms and *Escherichia coli* were performed following the most probable number (NMP) technique with Lauryl Sulfate Tryptose (LST) broth (Neogen, Lansing, MI, USA), followed by streaking on Eosine Methylene Blue Agar (EMB) for *E. coli* identification.

2.7. Sensory analysis

For the sensory analysis, six treatments (HPHF, MPHF, LPHF, HPLF, MPLF, and LPLF) were used. These Artisanal treatments were selected aiming to measure the sensory effect generated by the cupuassu flour in combination with the pulp in the fermented beverage, based on previous studies reported by Costa et al. [11, 20] who evaluated the same pulp concentrations, where its acceptability in fermented dairy products was evidenced.

The sensory analysis was conducted by the ethical norms for research on humans, the National Health Council, Resolution No. 196/1996, and after approval by the Ethics Committee of the School of Nursing of the Federal University of Bahia under CAAE 60414022.7.0000.5531. The adherence of the individuals to work was through the signing of the Term of Free and Informed Consent. The sensory panel consisted of 61 untrained participants (38 women and 23 men), aged 18 to 56 years, and regular consumers of dairy products. Persons with allergy or latcose intolerance were not recruited. All participants signed the informed consent form. All participants were recruited from the Escola de Medicina Veterinária e Zootecnia (Universidade Federal da Bahia, Brazil). They evaluated the product through a quantitative acceptance test, using a 9-point hedonic scale, where 1 means “I dislike it very much” and 9 means “I like it very much”. The session was conducted in individual booths. Fermented milk beverages were evaluated on day 0 of storage, presented in three-digit blind codes, and 20 mL samples were served one at a time at 7°C, simultaneously with a glass of water for mouth rinsing among samples. The sensory attributes were appearance, color, aroma, flavor, consistency, firmness, and overall impression [36].

The just-about-right (JAR) scale was performed to identify the optimal intensity of each attribute Li et al. [37]. The scale consisted of 5 points from 1- much too little to 5- much too much, with a central point of 3 being "Just About Right". Aroma (acid, alcoholic, cupuassu, and milk), taste (sweet, acid, bitter), color (white, brown, and beige), flavor (cupuassu, caramel), and texture (sandy, consistency, firmness, viscosity, mouthfeel) were evaluated [20, 37].

Participants also performed CATA, using terms referenced in other studies [38-40] to identify product characteristics; flavor (whey, milk, sweetened, cocoa, fermented), taste (acid, bitter), color (artificial, beige, white, dark brown, light brown), aroma (milk, sweet, acid, buttermilk, cupuassu, cocoa, alcoholic), and appearance (homogeneous, sandy, firm, consistent, viscous, liquid, fibrous). Finally, purchase intention was evaluated using a 5-point structured scale (1-certainly would not buy at 5-certainly would buy).

2.8. Estimative of Harrell's optimism on regression models using bootstrap method

The performance of a predictive model is overestimated (optimism) when determined only on the sample used to construct the model. Therefore, internal validation methods aim to estimate model performance accurately in new samples [41]. The estimation of optimism was calculated according to equation (5) [42].

$$o = \frac{\sum_{m=1}^{M} o^{(m)}}{M}, \quad (5)$$

Where, for each bootstrap sample with replacement (m = 1, …, M), $R_{boot}^{2(m)}$= bootstrap coefficient of determination obtained from the fitted model to the bootstrap dataset; $R_{orig}^{2(m)}$= original coefficient of determination obtained by applying the fitted model from the bootstrap dataset to the original dataset; o = optimism of the original model; $o^{(m)} = R_{boot}^{2(m)} - R_{orig}^{2(m)}$; M = number of bootstrap datasets.

The equation (6) was used to calculate the coefficient of determination of the original model after validation.

$$R_V^2 = R_{app}^2 - o, \quad (6)$$

R_V^2= coefficient of determination of the original model
R_{app}^2= apparent coefficient of determination obtained from fitted model to original data.
o = optimism of the original model.

2.9. Statistical analysis

All analyses were performed in analytical and experimental triplicate, and the data obtained were presented as mean ± standard deviation (SD). Comparison of multiple samples was performed by one-way analysis of variance (ANOVA) for centesimal results and two-way ANOVA for physicochemical (pH, syneresis, water holding capacity, instrumental color, and rheological) and microbial results, using GraphPad Prism 8 (San Diego, California, USA). ANOVA was followed by Tukey's multiple comparison tests (two-side, $p < 0.05$). Also, Pearson's correlation test with a 0.05 significance level was performed to evaluate the correlation between variables. The internal validation of the regression models was performed by the bootstrap method (number of bootstrap samples = 200; number of simulations = 1000; bootstrap sample size = original sample size; 95% confidence interval) [42]. In CATA, Cochran's Q test was performed to identify significant differences in the frequency of terms used to describe the samples ($p < 0.05$). When significant

values were found, a Sheskin's multiple pairwise comparison test was employed at a 0.05 significance level to identify significant differences between samples for each sensory term. Statistical analyses were performed with a commercially available statistical package XLSTAT version 2022.3.2.1353 (Addinsoft SARL, New York, USA.

3. Results and Discussion

3.1. Proximate composition

The moisture, ash, protein, fat, fiber, and carbohydrate contents of the Artisanal fermented beverage are presented in Table 1. HPNF presented the highest moisture value and LPHF the lowest ($p < 0.05$), indicating that the cupuassu pulp and the flour addition directly interfered with this parameter. This fact can be attributed to the high moisture content of the pulp [15] and the low moisture content in the flour [19], attributed to the technological process difference that exists between these two products.

Table 1. Proximate composition of the freshly prepared artisanal fermented milk beverages.

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	CHOS (%)
NPNF	88.76 ± 0.19^{c}	0.60 ± 0.01^{i}	1.58 ± 0.02^{f}	2.28 ± 0.20^{b}	0.07 ± 0.03^{d}	6.71 ± 0.03^{c}
NPLF	88.22 ± 0.10^{cd}	0.73 ± 0.01^{f}	1.92 ± 0.03^{c}	1.27 ± 0.06^{de}	0.29 ± 0.17^{abcd}	7.56 ± 0.11^{b}
NPHF	84.64 ± 0.14^{h}	0.78 ± 0.01^{de}	4.42 ± 0.04^{a}	2.38 ± 0.13^{b}	0.39 ± 0.20^{abc}	7.39 ± 0.23^{bc}
LPNF	91.63 ± 0.38^{b}	0.68 ± 0.01^{g}	1.33 ± 0.01^{g}	1.08 ± 0.02^{ef}	0.11 ± 0.01^{cd}	5.18 ± 0.39^{de}
MPNF	92.16 ± 0.17^{b}	0.64 ± 0.01^{h}	0.84 ± 0.01^{i}	0.69 ± 0.01^{g}	0.14 ± 0.02^{bcd}	5.53 ± 0.18^{d}
HPNF	93.02 ± 0.20^{a}	0.63 ± 0.01^{h}	0.94 ± 0.01^{h}	0.80 ± 0.01^{fg}	0.12 ± 0.01^{cd}	4.48 ± 0.18^{e}
HPLF	87.16 ± 0.07^{e}	0.80 ± 0.02^{d}	1.72 ± 0.05^{de}	1.87 ± 0.22^{c}	0.42 ± 0.04^{ab}	8.03 ± 0.11^{b}
MPLF	86.46 ± 0.28^{f}	0.82 ± 0.01^{c}	1.71 ± 0.03^{de}	1.28 ± 0.01^{de}	0.43 ± 0.06^{ab}	9.30 ± 0.31^{a}
LPLF	86.03 ± 0.47^{fg}	0.76 ± 0.01^{e}	1.64 ± 0.03^{ef}	1.59 ± 0.09^{cd}	0.31 ± 0.11^{abcd}	9.67 ± 0.48^{a}
HPHF	87.74 ± 0.08^{de}	0.90 ± 0.01^{a}	1.86 ± 0.01^{c}	1.59 ± 0.01^{cd}	0.56 ± 0.12^{a}	7.35 ± 0.22^{bc}
MPHF	85.71 ± 0.10^{g}	0.86 ± 0.02^{b}	1.77 ± 0.04^{d}	1.77 ± 0.17^{c}	0.52 ± 0.14^{a}	9.37 ± 0.14^{a}
LPHF	83.88 ± 0.13^{i}	0.85 ± 0.01 ^{b}	2.38 ± 0.03^{b}	2.96 ± 0.01^{a}	0.58 ± 0.03^{a}	9.35 ± 0.10^{a}

Results express the mean ± SD of three independent experiments.$^{a–i}$ Different lowercase superscripts in the same column indicate significant differences among treatments of fermented milk beverage ($p < 0.05$).NPNF, control; NPLF, beverage (1.5% flour); NPHF, beverage (3% flour); LPNF, beverage (5% pulp); MPNF, beverage (7.5% pulp); HPNF, beverage (10% pulp); HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour); CHOS, carbohydrates.

The highest values ($p < 0.05$) in protein, fat, fiber, and carbohydrates were found in the samples containing cupuassu flour. This pattern happened due to the concentration of nutrients resulting from the technological transformation to which the cupuassu seeds were subjected [18, 19].

3.2. physicochemical behavior

3.2.1. pH behavior

The variations in pH values presented during the storage period of the artisanal fermented milk beverages are shown in Table 2. The addition of pulp significantly reduced the pH value ($p < 0.05$), which was evident when comparing HPNF (10% pulp, 0% flour) with NPNF (control sample). In this case, pulp addition was a predictor of pH values ($R_v = 0.742$; $p < 0.0001$; Table S1) that can be linked to the acid pH of the cupuassu pulp preparation (3.09 ± 0.01) used.

Similar behaviors were presented by Costa et al. [11], who added 10% of pasteurized cupuassu pulp to goat milk yogurt, presenting decreased pH after pulp addition. However, the pH values were higher (4.57 − 4.43) than those herein, which can be attributed to the matrices and pulp conditions.

Table 2. pH of artisanal fermented milk beverage with cupuassu pulp and flour during 28 days of storage at 4°C.

Parameters	Sample	Storage time (days)				
		0	7	14	21	28
pH	NPNF	4.40 ± 0.02^{cA}	4.37 ± 0.01^{cB}	4.38 ± 0.01^{dAB}	4.30 ± 0.01^{dC}	4.37 ± 0.01^{eB}
	NPLF	4.49 ± 0.03^{bA}	4.48 ± 0.01^{abA}	4.50 ± 0.01^{bA}	4.48 ± 0.02^{bA}	4.47 ± 0.01^{aC}
	NPHF	4.67 ± 0.02^{aA}	4.53 ± 0.01^{aD}	4.63 ± 0.01^{aAB}	4.59 ± 0.01^{aBC}	4.58 ± 0.03^{aC}
	LPNF	4.18 ± 0.03^{gB}	4.13 ± 0.01^{fC}	4.19 ± 0.01^{hAB}	4.14 ± 0.02^{gC}	4.23 ± 0.01^{gA}
	MPNF	4.11 ± 0.02^{hBC}	4.19 ± 0.04^{eA}	4.10 ± 0.01^{jBC}	4.09 ± 0.01^{hC}	4.14 ± 0.01^{hAB}
	HPNF	3.99 ± 0.02^{iC}	4.11 ± 0.03^{fAB}	4.14 ± 0.010^{iA}	3.98 ± 0.01^{iC}	4.08 ± 0.01^{iB}
	HPLF	4.21 ± 0.02^{fgA}	4.21 ± 0.01^{eA}	4.24 ± 0.01^{gA}	4.10 ± 0.01^{hB}	4.12 ± 0.03^{hiB}
	MPLF	4.23 ± 0.01^{efgAB}	4.23 ± 0.02^{eAB}	4.22 ± 0.01^{gAB}	4.25 ± 0.02^{eA}	4.21 ± 0.02^{gB}
	LPLF	4.28 ± 0.01^{deBC}	4.29 ± 0.01^{dAB}	4.30 ± 0.01^{fAB}	4.26 ± 0.01^{eC}	4.31 ± 0.01^{fA}
	HPHF	4.30 ± 0.04^{dA}	4.30 ± 0.02^{dA}	4.30 ± 0.01^{fA}	4.19 ± 0.01^{fC}	4.34 ± 0.01^{efA}
	MPHF	4.25 ± 0.01^{defC}	4.34 ± 0.01^{cdB}	4.35 ± 0.01^{eB}	4.25 ± 0.01^{eC}	4.42 ± 0.01^{dA}
	LPHF	4.44 ± 0.01^{bcB}	4.43 ± 0.03^{bB}	4.45 ± 0.01^{cB}	4.38 ± 0.01^{cC}	4.52 ± 0.01^{bA}

Results express the mean ± SD of three independent experiments. $^{a–i}$ Different lowercase superscripts in the same column indicate significant differences among treatments of artisanal fermented milk beverage ($p < 0.05$). $^{A-D}$ Different uppercase superscripts in the same row indicate significant differences among storage times ($p < 0.05$). NPNF, control; NPLF, beverage (1.5% flour); NPHF, beverage (3% flour); LPNF, beverage (5% pulp); MPNF, beverage (7.5% pulp); HPNF, beverage (10% pulp); HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour).

In addition, it was observed that the pH of the cupuassu flour (5.70 ± 0.02) presented a correlation ($p < 0.05$) with the percentage of added flour, the model had a poor predictive power ($R_v = 0.599$), given that the higher the percentage of cupuassu flour, the higher the pH value, with NPHF (0% pulp, 3% flour) being the treatment with the highest value ($p < 0.05$). This behavior was also present in the samples that had a combination of pulp and flour, whose pH values were intermediate, compared to the samples that contained only pulp or only flour. According to Yadav et al. [43], the decrease or increase in pH values can vary due to the acidity of the flour used.

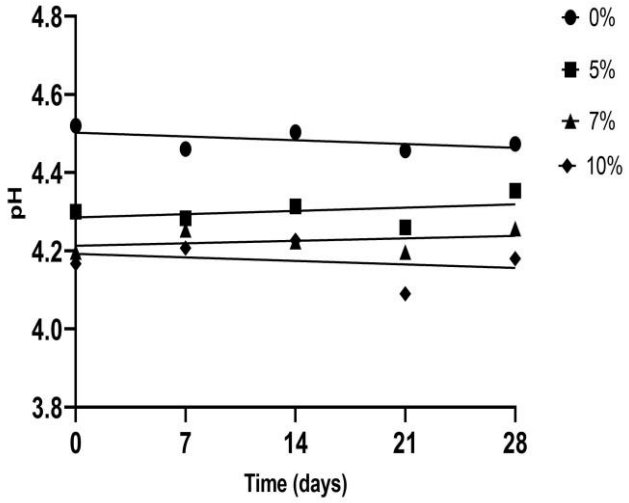


Figure 1. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of pH in relation to cupuassu pulp of artisanal fermented milk beverages stored at 4 °C.

During the storage period, NPNF and the samples with the addition of cupuassu flour showed a decrease in pH values, except for the samples MPLF and LPLF combined with pulp and 1.5% cupuassu flour. This reduction can be attributed to the post-acidification generated in the dairy beverage by transforming lactose into lactic acid by lactic acid bacteria (LAB), allowing to maintain the metabolic activity and acidification during the storage time under refrigeration [44]. The pH decrease in the sample with flour alone during storage could be due to a possible synergistic effect towards starter cultures, attributed to the fiber and protein content of cupuassu flour, in addition to post acidification, concomitant protein/fiber catabolism which caused the pH to increase slightly (although significantly) compared to the control. This behavior was similar to those found by Zare et al. [45] and Kaur et al. [46] with the addition of lentil and soybean flour in fermented milk and chickpea flour in yogurts, respectively. It enhanced the acidification rate of probiotic cultures.

Furthermore, when comparing storage days 0 and 28, adding cupuassu pulp increased pH values ($p < 0.05$), except in the HPLF and MPLF treatments. On the other hand, it was observed that NPNF (control sample) had a decrease in pH values on days 0 and 21 of storage. While this is true, the treatments with combination of cupuassu pulp and flour only showed this decrease on day 21, which indicates that the combination of pulp and flour in the beverage generated a slower post-acidification process, which would help to prolong the shelf life of the product [44], which could be due to the presence of the cupuassu yeasts (*Pichia* genus) that metabolize acid, causing the increase in pH [45]. Despite the observed alkalinization during storage, it was observed that the combination of cupuassu pulp and flour maintained pH values between 4.0 and 4.6, which is known to allow the development of LAB and prevent the growth of pathogenic microorganisms [19].

3.2.2. Syneresis and Water Holding Capacity (WHC)

The results of syneresis presented in Table 3 show that NPNF (control sample), in general, presented the highest water loss regardless of the storage day ($p < 0.05$). The contraction of the casein network formed during fermentation

justifies this behavior [48, 49]. The percentage of cupuassu pulp and flour were inversely correlated, with syneresis values the greater the percentage of pulp or flour, the lower the syneresis values, where HPHF (3% flour, 10% pulp) presented the lowest value ($p < 0.05$). Since the syneresis is an indicator of quality, using these ingredients can mitigate this technological defect, which commonly affects the sensorial parameters of this type of product. The reduced syneresis can be attributed to the pectin polysaccharides contained in cupuassu, which strengthen the casein network formed by interacting with the positive charges on the surface of the micelles [50, 51].

Table 3. Syneresis and water holding capacity of artisanal fermented milk beverage with cupuassu pulp and flour during 28 days of storage at 4°C.

Parameters	Sample	Storage time (days)				
		0	7	14	21	28
Syneresis	NPNF	36.95 ± 1.0^{aA}	35.81 ± 2.15^{aA}	37.73 ± 0.87^{aA}	35.58 ± 0.72^{abA}	36.04 ± 0.80^{aA}
	NPLF	34.77 ± 0.33^{abcAB}	34.07 ± 0.21^{abcdB}	35.76 ± 0.79^{abA}	35.77 ± 0.12^{aA}	34.29 ± 0.88^{abAB}
	NPHF	33.79 ± 0.02^{bcdA}	32.00 ± 0.71^{defAB}	31.20 ± 1.04^{fgB}	32.54 ± 0.55^{deAB}	31.40 ± 1.21^{cdB}
	LPNF	33.13 ± 0.46^{bcdeC}	34.97 ± 0.36^{abAB}	35.48 ± 0.12^{bcA}	33.85 ± 1.03^{bcdBC}	34.17 ± 0.26^{bABC}
	MPNF	34.67 ± 0.16^{abcA}	34.42 ± 0.39^{abcA}	33.99 ± 1.26^{bcdA}	34.68 ± 0.58^{abcA}	34.96 ± 0.03^{abA}
	HPNF	31.49 ± 0.20^{defC}	31.99 ± 0.88^{defBC}	32.24 ± 0.50^{defBC}	33.40 ± 0.01^{cdAB}	34.29 ± 0.57^{abA}
	HPLF	32.36 ± 0.05^{cdefB}	32.74 ± 0.05^{bcdeB}	32.20 ± 0.10^{defB}	32.92 ± 0.65^{dB}	33.81 ± 0.16^{bA}
	MPLF	33.11 ± 1.0^{bcdeAB}	32.34 ± 0.20^{cdefB}	33.55 ± 0.06^{cdeAB}	33.87 ± 0.54^{bcdA}	34.53 ± 0.45^{abA}
	LPLF	35.09 ± 2.34^{abA}	34.94 ± 0.34^{abA}	33.72 ± 0.74^{bcdeBC}	30.89 ± 1.01^{eB}	33.16 ± 0.04^{bcBC}
	HPHF	29.97 ± 0.16^{fA}	30.32 ± 0.38^{fA}	30.03 ± 0.29^{gA}	30.83 ± 0.50^{eA}	30.25 ± 0.74^{dA}
	MPHF	30.97 ± 0.65^{efA}	30.48 ± 0.93^{efA}	31.01 ± 0.99^{fgA}	32.25 ± 0.01^{deA}	31.67 ± 0.45^{cdA}
	LPHF	30.28 ± 0.52^{fC}	31.59 ± 0.76^{efBC}	31.77 ± 0.21^{efgB}	33.35 ± 0.04^{cdA}	31.22 ± 0.61^{dBC}
WHC	NPNF	63.05 ± 1.00^{fA}	64.18 ± 2.15^{eA}	62.26 ± 0.87^{gA}	64.42 ± 0.72^{efA}	63.97 ± 0.80^{dA}
	NPLF	65.23 ± 0.33^{defAB}	65.93 ± 0.21^{bcdeA}	64.24 ± 0.79^{fB}	64.23 ± 0.12^{fB}	65.71 ± 0.88^{cdAB}
	NPHF	66.21 ± 0.02^{cdeB}	68.00 ± 0.71^{abAB}	68.80 ± 1.04^{abA}	67.46 ± 0.55^{abcAB}	68.60 ± 1.21^{abA}
	LPNF	66.87 ± 0.46^{bcdeA}	65.03 ± 0.36^{deBC}	64.52 ± 0.12^{efC}	66.14 ± 1.03^{cdeAB}	65.83 ± 0.26^{cABC}
	MPNF	65.33 ± 0.16^{defA}	65.58 ± 0.39^{cdeA}	66.00 ± 1.26^{defA}	65.32 ± 0.58^{defA}	65.04 ± 0.03^{cdA}
	HPNF	68.51 ± 0.20^{abcA}	68.01 ± 0.88^{abAB}	67.76 ± 0.50^{bcdAB}	66.60 ± 0.01^{bcdBC}	65.71 ± 0.57^{cdC}
	HPLF	67.64 ± 0.05^{abcdA}	67.26 ± 0.06^{bcdA}	67.80 ± 0.10^{bcdA}	67.08 ± 0.65^{bcdA}	66.19 ± 0.16^{cB}
	MPLF	66.89 ± 1.01^{bcdeAB}	67.66 ± 0.20^{abcA}	66.45 ± 0.06^{cdeAB}	66.13 ± 0.54^{cdeB}	65.47 ± 0.45^{cdB}

LPLF	64.88 ± 2.34^{efB}	65.06 ± 0.34^{deB}	66.28 ± 0.74^{deAB}	69.11 ± 1.01^{aA}	66.84 ± 0.04^{bcAB}
HPHF	70.03 ± 0.16^{aA}	69.68 ± 0.38^{aA}	69.97 ± 0.29^{aA}	69.17 ± 0.50^{aA}	69.75 ± 0.74^{aA}
MPHF	69.03 ± 0.65^{abAB}	69.88 ± 0.69^{aA}	68.54 ± 0.61^{abAB}	68.08 ± 0.58^{abB}	68.33 ± 0.45^{abAB}
LPHF	69.71 ± 0.56^{aA}	67.99 ± 0.26^{abB}	68.34 ± 0.07^{abcB}	66.65 ± 0.04^{bcdC}	68.78 ± 0.61^{aAB}

Results express the mean ± SD of three independent experiments. $^{a–i}$ Different lowercase superscripts in the same column indicate significant differences among treatments of artisanal fermented milk beverage ($p < 0.05$). $^{A-D}$ Different uppercase superscripts in the same row indicate significant differences among storage times ($p < 0.05$).NPNF, control; NPLF, beverage (1.5% flour); NPHF, beverage (3% flour); LPNF, beverage (5% pulp); MPNF, beverage (7.5% pulp); HPNF, beverage (10% pulp); HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour);WHC, water holding capacity.

During storage time, the control sample did not show any alterations ($p > 0.05$). However, the addition of HPNF pulp produced a slight increase, contrary to the addition of 3% flour alone, which decreased syneresis values ($p < 0.05$), presenting as a predictor ($R_v = 0.689$; $p < 0.0001$; Table S1) between flour percentage and syneresis (Figure 2). On the other hand, HPLF increased during storage, while LPLF and LPHF decreased. In general, syneresis was not affected by storage time, and when there were significant differences, they were slight. Thus, the addition of cupuassu did not appear to be a relevant parameter on syneresis in relation to storage days.

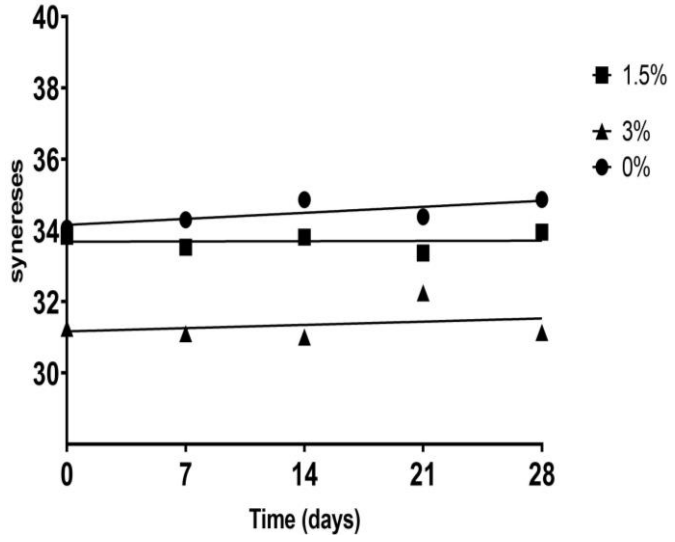
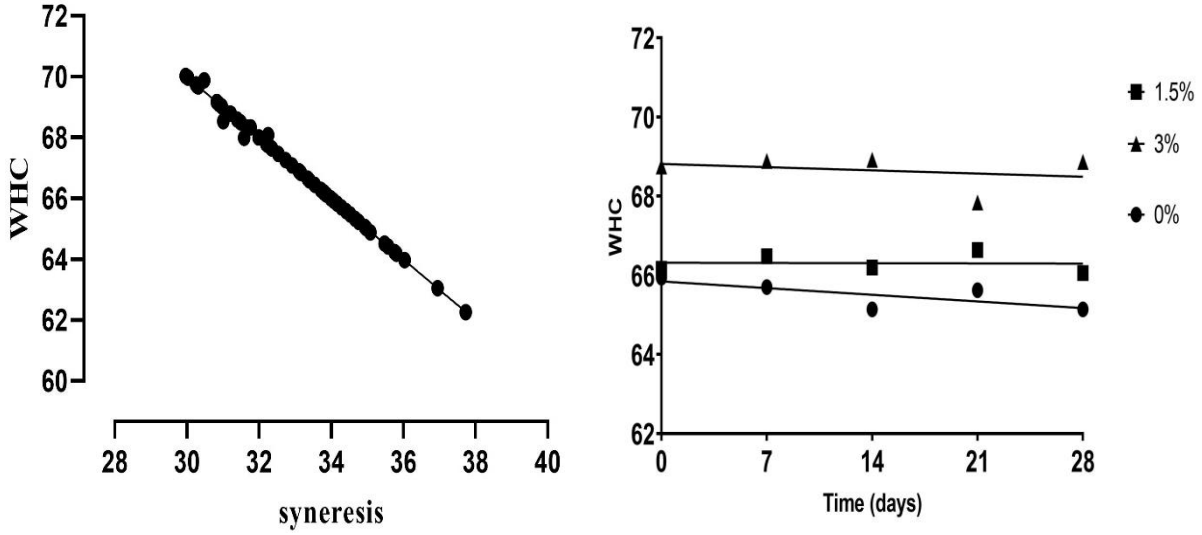


Figure 2. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of syneresis in relation to cupuassu flour of artisanal fermented milk beverages stored at 4 °C.

The results obtained in WHC confirmed the syneresis values and showed the inverse correlation between them, where the syneresis was a strong predictor of the WHC ($R_v = 0.998$; $p < 0.0001$; Table S1; Figure 3a), since the low water-holding capacity of proteins increases whey separation in fermented milk beverages [52]. In addition, the percentages of pulp and flour used were found to be positively correlated with the WHC (Figure 3b). Also, similar to syneresis,

WHC was slightly altered, if at all, by storage time. This behavior is related to the different pH values that occurred during the storage period since low pH values are one of the causes that generate the release of whey in the product [53].



(**a**) (**b**)

Figure 3. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of WHC in relation to: (**a**) syneresis; (**b**) Flour of artisanal fermented milk beverages stored at 4 °C.

3.2.3. Instrumental color

The instrumental color parameters L^* (lightness), a^* (greenness-redness), b^* (blueness-yellowness), C^* (chroma), and $h°$ (hue angle) of artisanal fermented milk drinks during the 28 days of storage are presented in Table 4. It is shown that adding pulp, in general, significantly reduced the L^* parameter ($p < 0.05$), except on day 0, where there was no significant difference compared to the control. This fact may be attributed to the pigments present in the pulp, which makes the beverage darker [13]. Likewise, the L^* values decreased more sharply in treatments with flour than with pulp. Thus, the flour intensified the darkening when combined with pulp, as evidenced in LPHF (5% pulp, 3% flour), which exhibited the lowest value for this parameter ($p < 0.05$). Therefore, flour content was a strong predictor of L^* ($R_v = 0.867$; Figure 4) resulting from the dark pigmentation of the

dust due to the oxidation of phenolic compounds in the cupuassu seeds during technological processing [18].

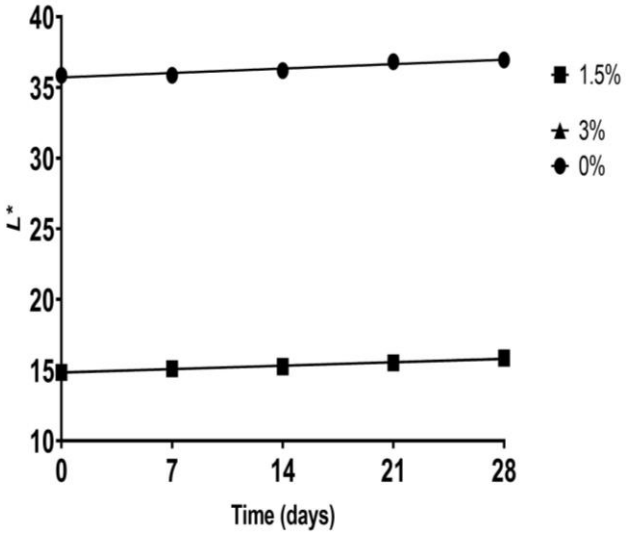


Figure 4. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of L^* in relation to cupuassu flour of artisanal fermented milk beverages stored at 4 °C.

During the storage time, L^* values increased progressively in all samples during the 28 days, except for NPNF, which only increased until day 7 ($p < 0.05$). The reduction of the size of the casein micelles due to proteolysis during storage can increase L^* values due to increased scattering light [54]. Still, considering the treatments with the addition of cupuassu pulp, the degradation of pigments during storage may also have contributed to this behavior [47].

Table 4. Color parameters values of artisanal fermented milk beverage with cupuassu pulp and flour during refrigerated storage.

Parameters	Sample	Storage time (days)				
		0	7	14	21	28
*L**	NPNF	35.95 ± 0.88^{abB}	37.41 ± 0.09^{aA}	37.27 ± 0.01^{aA}	37.74 ± 0.05^{aA}	37.49 ± 0.10^{bA}
	NPLF	14.41 ± 0.06^{deD}	14.26 ± 0.01^{hE}	14.74 ± 0.05^{dC}	14.89 ± 0.03^{hB}	15.34 ± 0.01^{fA}
	NPHF	14.88 ± 0.50^{cdeAB}	14.28 ± 0.03^{hC}	14.68 ± 0.01^{dBC}	14.89 ± 0.02^{hAB}	15.31 ± 0.02^{fA}
	LPNF	35.13 ± 0.06^{bE}	35.29 ± 0.04^{cD}	35.69 ± 0.09^{bC}	35.94 ± 0.01^{dB}	36.29 ± 0.02^{cA}
	MPNF	36.80 ± 0.02^{aAB}	35.58 ± 0.08^{bB}	36.19 ± 1.04^{bB}	37.53 ± 0.02^{bA}	37.79 ± 0.05^{aA}
	HPNF	35.56 ± 0.07^{bC}	35.17 ± 0.03^{dD}	35.64 ± 0.06^{bC}	36.05 ± 0.04^{cB}	36.23 ± 0.02^{cA}
	HPLF	15.02 ± 0.01^{cdeD}	15.87 ± 0.02^{eC}	16.32 ± 0.02^{cA}	16.20 ± 0.04^{eB}	16.34 ± 0.01^{eA}
	MPLF	15.32 ± 0.02^{cD}	15.45 ± 0.01^{fC}	14.94 ± 0.01^{dE}	15.87 ± 0.01^{fB}	16.72 ± 0.03^{dA}
	LPLF	14.56 ± 0.25^{cdeC}	14.77 ± 0.02^{gBC}	15.02 ± 0.03^{dAB}	15.10 ± 0.03^{gA}	15.03 ± 0.01^{gAB}
	HPHF	15.05 ± 0.01^{cdeD}	15.86 ± 0.01^{eC}	16.28 ± 0.01^{cA}	16.21 ± 0.04^{eB}	16.31 ± 0.01^{eA}
	MPHF	15.28 ± 0.01^{cdD}	15.44 ± 0.01^{fC}	14.94 ± 0.01^{dE}	15.86 ± 0.01^{fB}	16.67 ± 0.01^{dA}
	LPHF	14.26 ± 0.02^{eE}	14.79 ± 0.02^{gD}	14.98 ± 0.01^{dC}	15.10 ± 0.02^{gA}	15.02 ± 0.01^{gB}
*a**	NPNF	1.140 ± 0.12^{fA}	0.530 ± 0.01^{iC}	0.6567 ± 0.08^{gC}	0.570 ± 0.02^{iC}	0.880 ± 0.05^{iB}
	NPLF	1.897 ± 0.01^{dC}	1.970 ± 0.02^{dB}	2.130 ± 0.02^{dA}	1.817 ± 0.01^{dD}	1.915 ± 0.60^{dC}
	NPHF	1.857 ± 0.01^{dA}	1.877 ± 0.02^{eA}	1.757 ± 0.03^{eB}	1.777 ± 0.05^{deB}	1.915 ± 0.01^{dA}
	LPNF	2.177 ± 0.03^{cE}	2.447 ± 0.01^{cA}	2.407 ± 0.02^{cB}	2.320 ± 0.02^{cC}	2.220 ± 0.01^{cD}
	MPNF	2.590 ± 0.03^{bC}	2.757 ± 0.06^{bB}	2.867 ± 0.01^{bA}	2.600 ± 0.01^{bC}	2.595 ± 0.04^{bC}
	HPNF	3.347 ± 0.08^{aC}	3.590 ± 0.03^{aB}	3.647 ± 0.02^{aAB}	3.710 ± 0.01^{aA}	3.360 ± 0.01^{aC}

	HPLF	1.560 ± 0.03^{eC}	1.730 ± 0.04^{ghB}	1.620 ± 0.01^{fC}	1.717 ± 0.01^{efB}	1.815 ± 0.01^{eA}
	MPLF	1.847 ± 0.03^{dB}	1.977 ± 0.02^{dA}	1.630 ± 0.01^{fD}	1.697 ± 0.04^{fgC}	1.645 ± 0.02^{fCD}
	LPLF	1.817 ± 0.01^{dA}	1.867 ± 0.02^{efA}	1.830 ± 0.04^{eA}	1.590 ± 0.01^{hB}	1.560 ± 0.01^{ghB}
	HPHF	1.577 ± 0.02^{eD}	1.690 ± 0.02^{gB}	1.630 ± 0.03^{fC}	1.670 ± 0.01^{fgBC}	1.760 ± 0.01^{eA}
	MPHF	1.877 ± 0.01^{dA}	1.917 ± 0.03^{deA}	1.580 ± 0.01^{fC}	1.730 ± 0.03^{efB}	1.620 ± 0.02^{fgC}
	LPHF	1.770 ± 0.02^{dA}	1.800 ± 0.01^{fgA}	1.787 ± 0.01^{eA}	1.637 ± 0.04^{ghB}	1.500 ± 0.05^{hC}
	NPNF	26.290 ± 1.26^{aA}	26.997 ± 0.14^{aA}	26.677 ± 0.19^{bA}	26.810 ± 0.18^{aA}	26.455 ± 0.03^{aA}
	NPLF	-2.340 ± 0.02^{bC}	-2.020 ± 0.01^{eA}	-2.090 ± 0.02^{eA}	-2.290 ± 0.01^{defgC}	-2.215 ± 0.06^{fB}
	NPHF	-2.210 ± 0.08^{bBC}	-2.000 ± 0.02^{eA}	-2.310 ± 0.04^{fgC}	-2.270 ± 0.04^{defC}	-2.145 ± 0.01^{eB}
	LPNF	26.300 ± 0.15^{aA}	26.327 ± 0.13^{bA}	26.180 ± 0.07^{cAB}	25.970 ± 0.03^{cB}	26.175 ± 0.02^{bAB}
	MPNF	26.820 ± 0.01^{aB}	25.950 ± 0.04^{cD}	27.650 ± 0.02^{aA}	26.397 ± 0.01^{bC}	25.495 ± 0.02^{cE}
	HPNF	26.557 ± 0.12^{aA}	25.270 ± 0.08^{aC}	25.267 ± 0.06^{dC}	25.897 ± 0.01^{cB}	25.195 ± 0.03^{dC}
b*	HPLF	-2.537 ± 0.04^{bC}	-2.380 ± 0.05^{fgB}	-2.447 ± 0.03^{ghB}	-2.247 ± 0.01^{deA}	-2.260 ± 0.01^{fA}
	MPLF	-2.327 ± 0.02^{bB}	-2.167 ± 0.02^{efA}	-2.590 ± 0.01^{hD}	-2.300 ± 0.01^{defgB}	-2.440 ± 0.01^{gC}
	LPLF	-2.177 ± 0.02^{bA}	-2.190 ± 0.03^{efA}	-2.227 ± 0.08^{efA}	-2.450 ± 0.02^{gB}	-2.725 ± 0.01^{hC}
	HPHF	-2.600 ± 0.01^{bC}	-2.410 ± 0.02^{fgB}	-2.400 ± 0.02^{fghB}	-2.237 ± 0.015^{dA}	-2.250 ± 0.01^{fA}
	MPHF	-2.410 ± 0.19^{bB}	-2.070 ± 0.01^{eA}	-2.537 ± 0.01^{hB}	-2.417 ± 0.01^{fgB}	-2.420 ± 0.02^{gB}
	LPHF	-2.167 ± 0.02^{bA}	-2.507 ± 0.23^{gBC}	-2.230 ± 0.01^{efAB}	-2.410 ± 0.04^{efgABC}	-2.685 ± 0.03^{hC}
	NPNF	26.317 ± 1.27^{aA}	27.007 ± 0.14^{aA}	26.687 ± 0.19^{bA}	26.817 ± 0.19^{aA}	26.470 ± 0.02^{aA}
	NPLF	3.010 ± 0.010^{bA}	2.850 ± 0.04^{eB}	2.980 ± 0.01^{efA}	2.930 ± 0.01^{deAB}	2.930 ± 0.07^{fgAB}
	NPHF	2.887 ± 0.07^{bA}	2.757 ± 0.03^{eB}	2.900 ± 0.02^{efA}	2.887 ± 0.06^{deA}	2.875 ± 0.01^{fgA}
	LPNF	26.387 ± 0.15^{aA}	26.440 ± 0.12^{bA}	26.297 ± 0.07^{cAB}	26.077 ± 0.04^{cB}	26.275 ± 0.02^{bAB}
	MPNF	26.947 ± 0.01^{aB}	26.097 ± 0.04^{cD}	27.800 ± 0.02^{aA}	26.520 ± 0.01^{bC}	25.625 ± 0.02^{cE}
c*	HPNF	26.760 ± 0.12^{aA}	25.527 ± 0.09^{dC}	25.527 ± 0.06^{dC}	26.167 ± 0.01^{cB}	25.415 ± 0.03^{dC}
	HPLF	2.977 ± 0.05^{bA}	2.947 ± 0.07^{eA}	2.930 ± 0.02^{efA}	2.827 ± 0.01^{deB}	2.900 ± 0.01^{fgAB}
	MPLF	2.967 ± 0.01^{bB}	2.930 ± 0.02^{eC}	3.060 ± 0.01^{eA}	2.857 ± 0.02^{deD}	2.945 ± 0.01^{fBC}
	LPLF	2.830 ± 0.01^{bC}	2.877 ± 0.04^{eBC}	2.880 ± 0.03^{efBC}	2.920 ± 0.02^{deB}	3.135 ± 0.01^{eA}
	HPHF	3.047 ± 0.01^{bA}	2.947 ± 0.01^{eB}	2.907 ± 0.04^{efB}	2.790 ± 0.02^{eD}	2.855 ± 0.01^{gC}
	MPHF	2.920 ± 0.01^{bB}	2.820 ± 0.01^{eC}	2.987 ± 0.01^{efA}	2.970 ± 0.01^{dA}	2.910 ± 0.03^{fgB}
	LPHF	2.807 ± 0.03^{bC}	2.827 ± 0.05^{eBC}	2.857 ± 0.02^{fBC}	2.917 ± 0.06^{deB}	3.075 ± 0.05^{eA}
	NPNF	87.530 ± 0.15^{dC}	88.870 ± 0.01^{fA}	88.610 ± 0.14^{fA}	88.777 ± 0.04^{fA}	88.090 ± 0.11^{gB}
	NPLF	308.987 ± 0.34^{abD}	313.780 ± 0.24^{aB}	315.610 ± 0.53^{aA}	308.417 ± 0.08^{aD}	310.865 ± 0.19^{bC}
	NPHF	309.980 ± 0.95^{aAB}	311.520 ± 1.44^{bcA}	307.080 ± 0.94^{cC}	308.027 ± 0.21^{aBC}	311.760 ± 0.01^{aA}
	LPNF	85.270 ± 0.03^{eA}	84.690 ± 0.01^{gD}	84.750 ± 0.04^{gD}	84.887 ± 0.04^{gC}	85.155 ± 0.01^{hB}
	MPNF	84.477 ± 0.07^{eA}	83.947 ± 0.13^{gD}	84.090 ± 0.01^{gCD}	84.370 ± 0.02^{gAB}	84.190 ± 0.07^{iBC}
h°	HPNF	82.820 ± 0.12^{fA}	81.917 ± 0.05^{hC}	81.790 ± 0.02^{hC}	81.850 ± 0.01^{hC}	82.410 ± 0.01^{jB}
	HPLF	301.647 ± 0.17^{cE}	306.017 ± 0.07^{eC}	303.520 ± 0.14^{deD}	307.310 ± 0.05^{bB}	308.775 ± 0.05^{cA}
	MPLF	308.407 ± 0.57^{bB}	312.410 ± 0.02^{abA}	302.137 ± 0.05^{eE}	306.420 ± 0.72^{cC}	304.020 ± 0.38^{eD}
	LPLF	309.817 ± 0.21^{aA}	310.450 ± 0.08^{cdA}	309.457 ± 1.64^{bA}	302.970 ± 0.05^{eB}	299.800 ± 0.02^{fC}

	HPHF	301.257 ± 0.40^{cE}	305.097 ± 0.52^{eC}	304.150 ± 0.23^{dD}	306.817 ± 0.02^{bcB}	307.985 ± 0.24^{dA}
	MPHF	309.907 ± 0.35^{aB}	312.787 ± 0.49^{abA}	301.950 ± 0.10^{eE}	306.207 ± 0.05^{cC}	303.860 ± 0.12^{eD}
	LPHF	309.267 ± 0.11^{abA}	309.540 ± 0.54^{dA}	308.657 ± 0.02^{bcA}	304.080 ± 0.11^{dB}	299.235 ± 0.53^{fB}
ΔE*	NPNF	NA	2.03 ± 1.10^{aAB}	1.89 ± 0.86^{aAB}	2.26 ± 1.00^{aA}	1.89 ± 0.72^{aAB}
	NPLF	NA	0.37 ± 0.01^{cdB}	0.47 ± 0.01^{bcB}	0.48 ± 0.09^{bB}	0.93 ±0.06^{bcdA}
	NPHF	NA	0.68 ±0.38^{bcdA}	0.42 ± 0.27^{cA}	0.36 ± 0.23^{bA}	0.50 ± 0.43^{dA}
	LPNF	NA	0.32 ± 0.01^{dD}	0.62 ± 0.04^{bcC}	0.90 ± 0.02^{bB}	1.18 ± 0.06^{abcdA}
	MPNF	NA	1.51 ± 0.08^{abA}	1.29 ± 0.50^{abB}	0.84 ± 0.03^{bB}	1.66 ± 0.01^{abA}
	HPNF	NA	1.37 ± 0.17^{abcA}	1.33 ± 0.08^{abA}	0.91 ± 0.02^{bB}	1.52 ± 0.08^{abA}
	HPLF	NA	0.89 ± 0.01^{bcdD}	1.30 ± 0.02^{abB}	1.22 ± 0.03^{bC}	1.37 ± 0.01^{abcA}
	MPLF	NA	0.24 ± 0.02^{dAD}	0.51 ± 0.01^{bcC}	0.57 ± 0.01^{bB}	1.42 ± 0.01^{abcA}
	LPLF	NA	0.23 ± 0.21^{dBC}	0.46 ± 0.24^{bcABC}	0.66 ± 0.22^{bAB}	0.79 ± 0.14^{cdA}
	HPHF	NA	0.84 ± 0.01^{bcdC}	1.25 ± 0.01^{abB}	1.22 ± 0.04^{bB}	1.33 ± 0.01^{abcA}
	MPHF	NA	0.39 ± 0.18^{cdC}	0.49 ± 0.06^{bcBC}	0.62 ± 0.02^{bB}	1.42 ± 0.02^{abcA}
	LPHF	NA	0.66 ± 0,18^{bcdC}	0.72 ± 0.02^{bcBC}	0.89 ± 0.01^{bAB}	0.96 ± 0.01^{bcdA}

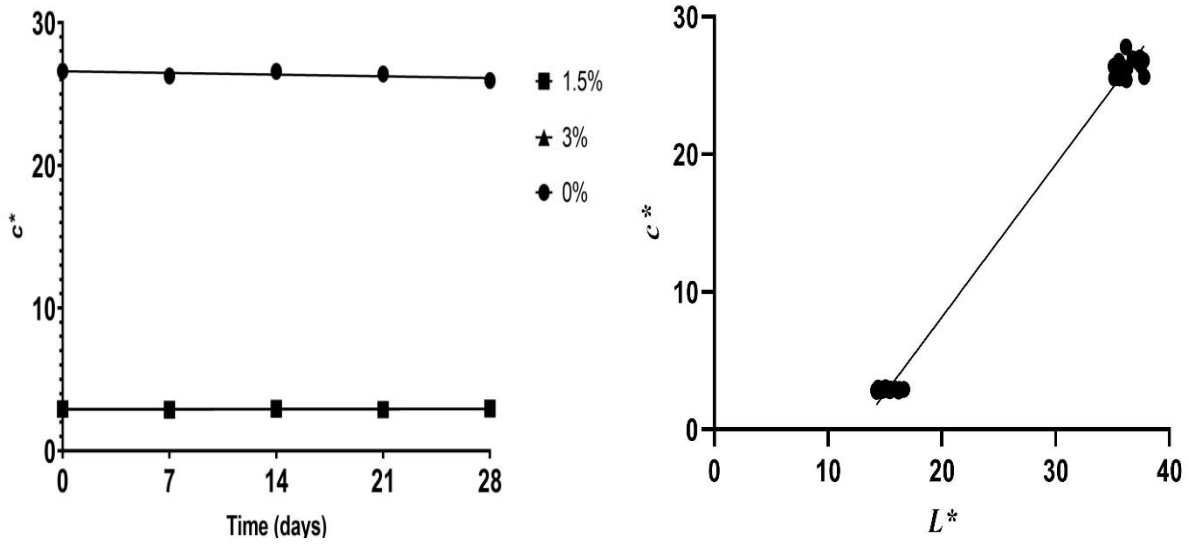
$^{a–i}$ Different lowercase superscripts in the same column indicate significant differences among treatments of artisanal fermented milk beverage ($p < 0.05$). $^{A-D}$ Different uppercase superscripts in the same row indicate significant differences among storage times ($p < 0.05$). NPNF, control; NPLF, beverage (1.5% flour); NPHF, beverage (3% flour); LPNF, beverage (5% pulp); MPNF, beverage (7.5% pulp); HPNF, beverage (10% pulp); HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour); L^*, Lightness; a^*, redness; b^*, yellowness; c^*, Chroma; and $h°$, hue angle; ΔE^*, total color difference.

The addition of cupuassu increased the values of a^* compared to the control. However, the values obtained with pulp were higher than those with flour due to the reddish color developed by the cupuassu catechins by oxidation, a product of fermentation [22, 24]. Coherently, there was a positive correlation between a^* and pulp concentration, while a negative correlation between a^* and flour was observed in the beverages (Table S1). Thus, treatments combined with pulp and flour presented a^* values higher than control, but lower than those for pulp alone. Regarding storage time, NPNF showed a noticeable decrease during this period ($p < 0.05$), which could be associated with the pH behavior of the samples during storage being a low predictor of the a^* parameter ($p < 0.0001$). On the other hand, for samples containing cupuassu, the values of a^* remained unchanged, increased or decreased. This can be attributed to the influence of pH as well as the stability of catechin in each treatment.

The b^* values were significantly higher in the control ($p < 0.05$) and in the treatments with pulp alone, regardless of storage day. It is attributed to the yellow-green color of the whey due to the presence of riboflavin and the yellowish-white of cupuassu pulp [18, 55]. The control had a stable behavior during the 28 days of storage. In contrast, treatments with flour or pulp alone had reduced b^* values or unchanged. An increase of b^* values is typical of non-enzymatic browning (Maillard) reactions [56]. Thus, flour and pulp apparently mitigated the Maillard reaction. Phenolic compounds, which are present in cupuassu, suppress the development of Maillard browning compounds in foods [25]. On the other hand, flour and pulp had the opposite effect when combined, raising b^* values. It is possible that the carbohydrate and protein content added

from the 2 ingredients (Table 1) superimposed the suppressive effect of the phenolic compounds.

The chroma (c*) results indicated that treatments with pulp alone presented c^* values similar or slightly lower than the control. In contrast, samples with flour alone sharply decreased chroma, including when combined with pulp (Figure 5a). Therefore, the lowest color saturation in samples containing flour can be attributed to its effect in decreasing L^* and b^* compared to the control, which flour content was a strong predictor of c^* values(R_v = 0. 869), as reported in Table S1 and Figure 5b. Comparing the samples in end of storage with those freshly prepared, control and flour treatment remained stable regarding chroma. In general, samples with pulp alone had color saturation reduced, while combined treatments were similar or higher than control, such as LPLF and LPHF. The chroma behavior during storage was similar that b^*, which can be explained by significant correlation among them ($p < 0.001$), as shown in Table S1.



(**a**) (**b**)

Figure 5. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of c^* in relation to: (a) cupuassu flour; (b) L^* of artisanal fermented milk beverages stored at 4 °C.

The hue angle ($h°$) values identify the relative orientation of the color with respect to the origin as 0° (red-purple), 90° (yellow), 180° (blue-green), and 270° (blue) to specify the color. The $h°$ values obtained indicated that the pulp-only samples were significantly lower than the control, presenting a yellow color perception ($p < 0.05$). On the other hand, the samples containing flour showed an increase in the value of $h°$ (Figure 6a) associated with the magenta color, which can be attributed to the pigmentation of the flour and the reduction of syneresis, allowing a better perception of the hue, the latter generated by the increase in pH and WHC, positively influencing the decrease in L^* (Figure 6b), b^* and c^* compared to the control, which explains the significant correlation with the values of $h°$ (R_v = 868; $p < 0.0001$; Table S1).

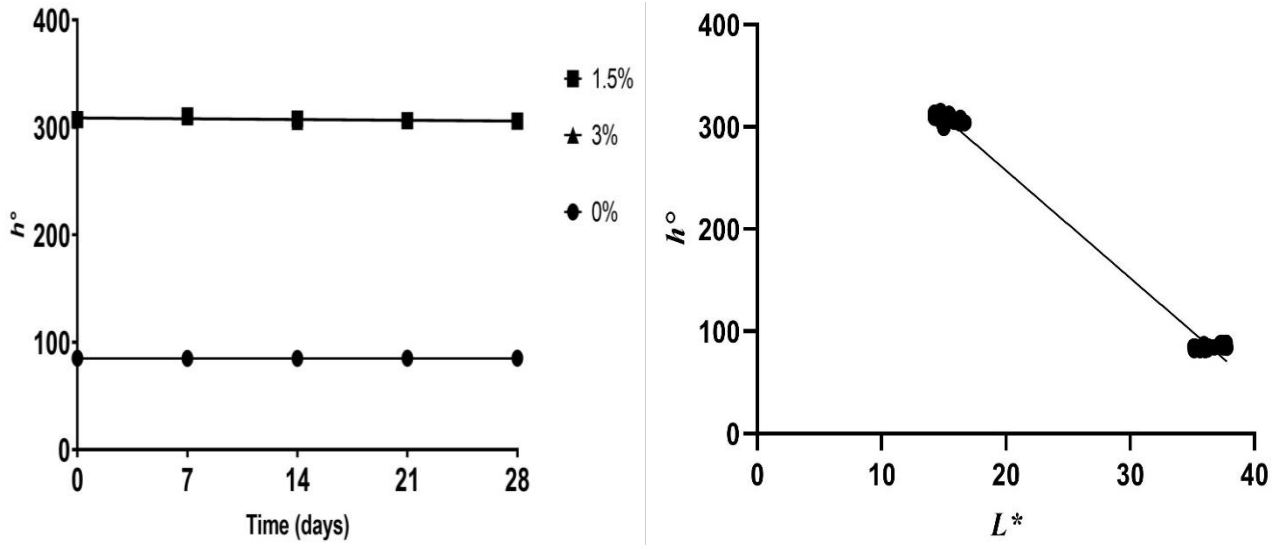


Figure 6. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of $h°$ in relation to: (a) cupuassu flour; (b) L^* of artisanal fermented milk beverages stored at 4 °C.

On day 28 of storage, the samples containing only flour and the control showed an increase in $h°$ values compared to day 0. On the contrary, the addition of pulp caused a decrease in $h°$. The same behavior was observed in the combined treatments with pulp and flour, which decreased, except for HPLF and HPHF, which increased at the end of storage. The behavior of h° during storage was similar to that of a^*, indicating a significant correlation between them ($p = 0{,}001$; Table S1).

ΔE indicates the color stability present among the samples. Samples LPNF, NPLF, and NPHF showed significantly lower values ($p < 0.05$) compared to the control. The same behavior was presented in the combined samples of pulp and flour, where MPLF and LPLF showed significantly lower values ($p < 0.05$), indicating that the higher percentages of pulp and flour resulted in a more stable color during storage, which is excellent from the consumer's point of view. During the remaining days of storage (14, 21, and 28), ΔE values increased in all samples except NPHF and NPNF, which showed no significant difference during storage ($p > 0.05$).

3.3. Rheological behavior

The behavior of the fermented dairy beverage with adding of cupuassu pulp and flour was described using the Ostwald-De-Waele model, as reported in Table 5. This model relates the shear stress to the shear rate of the fluid [33]. According to Moreira et al. [57], it provides a better adjustment to the experimental data for whey-based products, similar to that reported herein. The values obtained for the correlation coefficient in the different samples were greater than 0.910 ($R^2 \geq 0.910$), which indicates that the power law model provided suitable adjustment parameters. The consistency index (K) gives an idea of the fluid viscosity; the K values were generally unaffected by flour alone compared to the control, regardless of added concentration ($p > 0.05$). On the other hand, medium and high pulp concentrations elevated K values, while a low concentration was insignificant. Similarly, 10 % and 7.5 % of pulp for combined treatments provided higher consistency indexes than the control, regardless of flour concentration. In contrast, 5 % pulp generally did not affect

K, regardless of flour concentration combined. The addition of pulp presented a positive correlation concerning K (data no shown). The pulp acidified the pH and reduced syneresis, mainly when at a higher concentration. Inline, these factors contributed to increased K, as reported by the inverse correlation between them (Table S1). Still, increased WHC by adding pulp also favored a higher consistency (p = 0.03; Table S1).

During storage time, despite fluctuations, control, treatments with flour alone and with pulp alone at 10% finished storage with K similar to the freshly prepared product. In contrast, pulp alone at the middle and low concentrations progressively increased the consistency index. The behavior was varied for combined treatments with 3% flour; HPLF and MPLF finished lower and higher than the control, respectively, while LPLF was similar to the control at 28 days. For combined treatments with 1.5 % flour, middle and low pulp concentrations elevated K over storage, while high concentration finished similar to the control. These differences in behavior can be attributed to the different effects of treatments on pH, syneresis and WHC during storage (Table S1).

Table 5. Rheological parameters obtained by Ostwald de Waele model for artisanal fermented milk beverage.

Parameters	Sample	Storage time (days)				
		0	**7**	**14**	**21**	**28**
K (mPa.s^n)	NPNF	230.59 ± 41.49^{efC}	208.88 ± 35.18^{gC}	351.11 ± 17.01^{eAB}	402.64 ± 14.38^{dA}	283.49 ± 65.70^{efBC}
	NPLF	161.89 ± 1.94^{fC}	198.50 ± 47.44^{gBC}	385.46 ± 39.57^{eA}	290.75 ± 49.20^{eAB}	235.03 ± 2.51^{fBC}
	NPHF	413.84 ± 47.67^{cdA}	166.83 ± 15.96^{gC}	306.27 ± 65.92^{eB}	388.41 ± 20.70^{dAB}	357.08 ± 24.35^{eAB}
	LPNF	174.29 ± 44.94^{efD}	252.67 ± 46.45^{fgCD}	365.38 ± 5.42^{eBC}	449.35 ± 39.63^{dAB}	530.60 ± 61.26^{dA}
	MPNF	406.37 ± 19.27^{cdD}	368.59 ± 30.99^{deD}	543.88 ± 11.13^{cdC}	774.28 ± 1.07^{bB}	1284.53 ± 57.90^{aA}
	HPNF	556.90 ± 1.95^{cA}	574.04 ± 54.25^{abA}	650.88 ± 38.77^{bA}	640.47 ± 22.88^{cA}	563.23 ± 44.25^{dA}
	HPLF	1699.10 ± 146.40^{aA}	502.12 ± 23.98^{bcC}	663.73 ± 13.83^{bC}	944.51 ± 50.64^{aB}	530.26 ± 23.16^{dC}
	MPLF	390.43 ± 63.37^{dC}	625.36 ± 43.18^{aB}	581.54 ± 15.67^{bcB}	625.30 ± 52.86^{cB}	949.46 ± 7.44^{bA}
	LPLF	176.59 ± 2.52^{efC}	213.04 ± 50.82^{gC}	331.69 ± 27.51^{eAB}	407.97 ± 14.13^{dA}	253.21 ± 32.37^{efBC}
	HPHF	843.50 ± 5.47^{bA}	473.90 ± 38.64^{bcdC}	769.92 ± 26.69^{aB}	849.56 ± 16.26^{abA}	797.68 ± 27.82^{cAB}
	MPHF	477.13 ± 34.40^{cdC}	453.05 ± 35.28^{cdeC}	490.73 ± 3.52^{dC}	664.53 ± 16.36^{cB}	926.42 ± 19.85^{bA}
	LPHF	331.82 ± 36.22^{deB}	356.20 ± 16.87^{efB}	313.91 ± 13.26^{eB}	449.45 ± 37.20^{dA}	496.54 ± 4.63^{dA}
n	NPNF	0.438 ± 0.03^{bcdA}	0.461 ± 0.07^{abA}	0.398 ± 0.03^{bcA}	0.382 ± 0.02^{abA}	0.426 ± 0.05^{bcA}
	NPLF	0.458 ± 0.02^{bcA}	0.454 ± 0.04^{abA}	0.333 ± 0.01^{deB}	0.405 ± 0.03^{aA}	0.444 ± 0.01^{abA}
	NPHF	0.385 ± 0.02^{deB}	0.450 ± 0.02^{abA}	0.398 ± 0.04^{bcAB}	0.349 ± 0.02^{bcdB}	0.379 ± 0.05^{cdB}
	LPNF	0.488 ± 0.05^{abA}	0.479 ± 0.03^{aA}	0.417 ± 0.01^{abAB}	0.351 ± 0.02^{bcdB}	0.363 ± 0.02^{deB}
	MPNF	0.350 ± 0.01^{efB}	0.416 ± 0.02^{abA}	0.359 ± 0.01^{cdB}	0.309 ± 1E-3^{dC}	0.229 ± 2E-3^{gD}

	HPNF	0.348 ± 0.01^{efB}	0.383 ± 0.01^{bcA}	0.378 ± 2E-3^{bcdA}	0.375 ± 3E-3^{abA}	0.383 ± 0.01^{cdA}
	HPLF	0.297 ± 0.01^{fC}	0.401 ± 0.01^{abA}	0.349 ± 2E-3^{deB}	0.312 ± 0.01^{cdC}	0.401 ± 0.02^{bcdA}
	MPLF	0.397 ± 0.30^{cdeA}	0.310 ± 0.01^{cB}	0.307 ± 0.01^{eB}	0.342 ± 0.01^{bcdB}	0.308 ± 0.01^{fB}
	LPLF	0.534 ± 0.01^{aA}	0.458 ± 0.04^{bcA}	0.413 ± 0.02^{abCD}	0.384 ± 0.01^{abD}	0.482 ± 0.02^{aAB}
	HPHF	0.359 ± 0.03^{efB}	0.434 ± 0.02^{abA}	0.343 ± 3E-3^{deB}	0.348 ± 2E-3^{bcdB}	0.360 ± 0.01^{defB}
	MPHF	0.375 ± 0.01^{deB}	0.427 ± 1E-3^{abA}	0.371 ± 3E-3^{bcdB}	0.358 ± 0.01^{abcC}	0.322 ± 1E-3^{efD}
	LPHF	0.402 ± 0.01^{cdeBC}	0.412 ± 0.02^{abAB}	0.457 ± 0.01^{aA}	0.360 ± 0.03^{abcC}	0.382 ± 0.01^{cdBC}
Apparent viscosity (mPa·s)	NPNF	57.12 ± 15.78^{efB}	49.50 ± 19.00^{eB}	98.06 ± 12.77^{fgAB}	118.04 ± 12.10^{efA}	74.05 ± 27.57^{fAB}
	NPLF	37.10 ± 3.04^{deC}	47.34 ± 17.20^{eBC}	132.32 ± 19.19^{efA}	80.17 ± 20.10^{fB}	56.35 ± 1.49^{fBC}
	NPHF	119.56 ± 6.17^{efA}	39.39 ± 5.72^{eB}	87.07 ± 29.14^{gA}	126.60 ± 14.28^{efA}	105.60 ± 12.57^{efA}
	LPNF	37.45 ± 15.04^{eD}	54.82 ± 14.61^{deCD}	95.50 ± 2.54^{fgBC}	146.00 ± 22.78^{deAB}	165.7 ± 31.10^{dA}
	MPNF	131.90 ± 8.56^{deCD}	97.25 ± 15.15^{cdD}	171.07 ± 5.73^{cdC}	286.56 ± 2.23^{bB}	614.21 ± 32.71^{aA}
	HPNF	181.54 ± 3.04^{cdA}	167.47 ± 18.40^{bA}	191.70 ± 13.36^{bcA}	191.42 ± 7.67^{cdA}	164.28 ± 17.93^{dA}
	HPLF	655.25 ± 77.48^{aA}	138.27 ± 11.04^{bcC}	216.09 ± 7.25^{bC}	345.76 ± 24.14^{aB}	146.11 ± 12.68^{deC}
	MPLF	110.35 ± 28.32^{bcC}	230.93 ± 21.90^{aB}	216.52 ± 9.52^{bB}	208.23 ± 24.42^{cB}	352.67 ± 5.87^{bA}
	LPLF	31.63 ± 1.27^{fC}	49.95 ± 17.31^{eC}	88.01 ± 12.18^{gB}	118.46 ± 3.33^{efA}	54.01 ± 10.27^{fC}
	HPHF	266.20 ± 19.98^{bA}	117.42 ± 15.169^{cB}	255.02 ± 5.60^{aA}	277.42 ± 7.27^{bA}	250.37 ± 10.14^{cA}
	MPHF	142.52 ± 8.35^{deC}	114.67 ± 8.48^{cD}	148.76 ± 3.13^{deC}	209.97 ± 5.37^{cB}	328.24 ± 8.78^{bA}
	LPHF	91.18 ± 12.57^{efB}	94.87 ± 10.05^{cdB}	72.34 ± 6.04^{gB}	142.43 ± 25.40^{eA}	145.34 ± 3.42^{deA}

$^{a–f}$ Different lowercase superscripts in the same column indicate significant differences among treatments of artisanal fermented milk beverage ($p < 0.05$). $^{A-D}$ Different uppercase superscripts in the same row indicate significant differences among storage times ($p < 0.05$).NPNF, control; NPLF, beverage (1.5% flour); NPHF, beverage (3% flour); LPNF, beverage (5% pulp); MPNF, beverage (7.5% pulp); HPNF, beverage (10% pulp); HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour); K (mPa s^n), consistency index; n, flow behavior index; R^2, linear correlation coefficient; apparent viscosity (measured at 25 °C with a 25 s^{-1} shear rate).

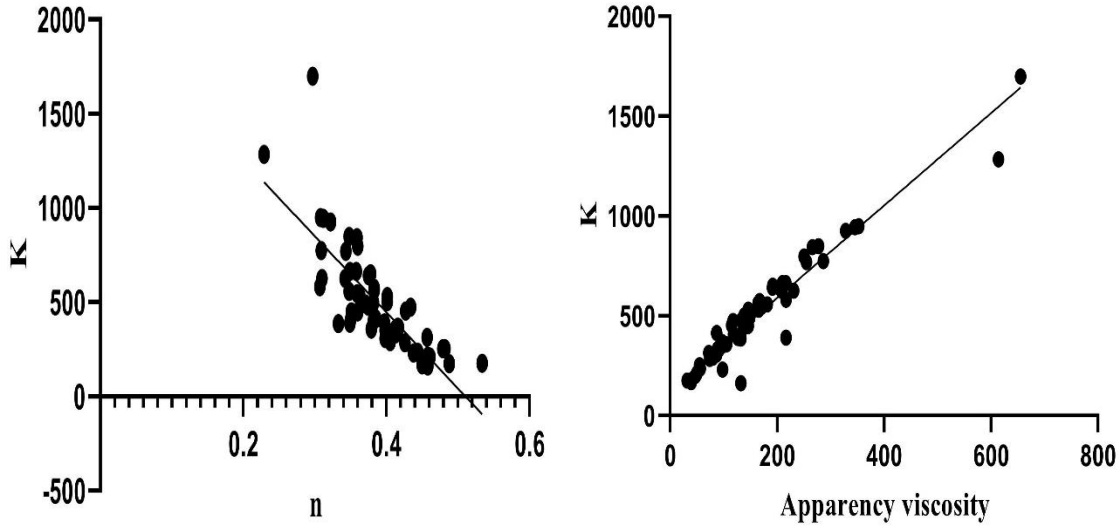
The index n indicates the degree of deviation from the Newtonian flow (n = 1). As n values were ≤ 0.534 (Table 5), all samples presented a non-Newtonian behavior. It indicates that the beverages have a typical performance of pseudoplastic fluids, which is characteristic of this type of dairy product [58, 59]. This behavior is corroborated by Figure S1, where the shear stress increased as a function of shear rate in all samples, regardless of storage time. The values of n were not affected by the addition of pulp alone, at low concentration compared to the control ($p > 0.05$), while medium and high concentrations of pulp decreased the values of n ($p < 0.05$). On the other hand, the addition of flour, in general, did not affect the values of n, regardless of the concentration added ($p > 0.05$). Differently combined samples with high pulp concentrations provided lower consistency indices than the control, regardless of flour concentration,

while samples with medium pulp concentration showed no significant difference, regardless of flour concentration, as did LPHF containing 5% pulp and 3% flour, in contrast to LPLF containing the same concentration of pulp but less flour which was significantly higher ($p < 0.05$).

During storage time, the control showed no significant difference ($p > 0.05$), in contrast to the samples only with flour and only with pulp LPNF and MPNF decreased n values, while HPNF increased concerning time, indicating that storage time, pulp addition, and pH favored n (Table S1). The combined samples HPLF and MPHF showed an increase in n on day 7 of storage and a subsequent decrease until days 21 and 28, respectively. MPLF decreased during storage, while LPLF only after day 7. HPHF and LPHF presented the highest values on days 7 and 14, respectively. Consistently, K was a strong predictor of n (R_v = 0.786; $p < 0.001$; Table S1): As K values increased, n values decreased (Figure 7a).

Apparent viscosity describes the flow behavior, an important parameter that influences sensory attributes and quality in dairy beverages since the addition of whey generates low viscosity in this type of product [59]. Table 5 presents the values obtained for viscosity, where it was observed that the addition of pulp at the highest concentration increases the apparent viscosity compared to the control ($p < 0.05$), while the flour did not generate a significant effect regardless of the concentration ($p > 0.05$). On the other hand, the combination of flour and pulp ingredients in the LPLF, MPHF, and LPHF samples did not show significant changes compared to the control ($p > 0.05$), in contrast to HPLF, MPLF, and HPHF, which showed a significant increase in apparent viscosity values ($p > 0.05$).

During storage, the control sample showed an increase in viscosity on days 21 and 28, this behavior was also present in the samples with 1.5% flour, while the sample with 3% showed a significant variation only on day 7, obtaining the lowest value ($p < 0.05$). Samples with only pulp addition increased viscosity with respect to storage time, which may be attributed to the reduction in pH due to the addition of pulp favoring a higher apparent viscosity (p = 0,04; Table S1). Samples combined with the lower pulp concentration (LPLF and LPHF) increased apparent viscosity values on days 21 and 28, respectively ($p < 0.05$). However, LPLF showed no significant difference from the control on day 28 ($p > 0.05$). Concerning the samples combined with the mean pulp concentration (MPHF and MPLF), they showed a decrease at day 7, where MPHF subsequently increased until day 28 ($p < 0.05$), showing a higher apparent viscosity value than the control. In general, HPLF and HPHF samples had the highest apparent viscosity at the beginning of storage ($p < 0.05$). However, these had the lowest values on day 7, which can be attributed to the decrease in n during storage and the increase in K caused by the addition of pulp, which positively affected the viscosity (Figure 7b; $p < 0.001$; Table S1).



(**a**) (**b**)

Figure 7. Significant correlations ($p < 0.05$) and internally validated by the Bootstrap method of K in relation to: (a) n; (b) Apparency viscosity of artisanal fermented milk beverages stored at 4 °C.

3.4. Microbiological Analysis

In the count of molds and yeasts at day 0 of storage, the lowest values were found in the LPLF and NPNF samples, with counts ≤ 4 log CFU/mL, while HPLF had the highest value with 5.83 ± 0.08 log CFU/mL ($p < 0.05$). On day 28, samples MPNF, LPHF, HPLF, NPHF, and NPLF obtained the highest values (between 6.11 ± 0.12 and 6.20 ± 0.32), while NPNF had the lowest value, with 5.57 ± 0.13 ($p > 0.05$). Therefore, treatments without or with lower cupuassu content had less fungal growth than treatments with higher levels. It can be attributed to the yeast (*Pichia* and *Hanseniaspora*) content of the cupuassu fruit microbiota and organic compounds present in the cupuassu pulp and seeds [47]. Regarding time, all counts increased significantly until the end of storage ($p < 0.05$), except HPHF and LPLF, which remained stable (data not shown). The relatively high pH and water activity of the artisanal fermented milk beverage favors the growth of this type of microorganism.

Total and thermotolerant coliforms, *Escherichia coli*, coagulase-positive *Staphylococcus*, and *Salmonella* spp. were absent in the artisanal fermented milk beverage during storage, indicating good hygienic and sanitary practices during the production and storage of the beverage (data not shown).

3.5. Sensory analysis

The results of the acceptability test and purchase intention of the artisanal fermented milk beverages with cupuassu flour and pulp are shown in Table 6 and Figure S2. Formulations with 1.5 % flour tended to present lower sensory scores than formulations with 3 % regarding appearance, color, consistency, and firmness. Thus, MPLF and LPLF were lower than HPHF, while only HPLF was lower than MPHF and LPHF for appearance. For color, HPHF was superior to all treatments with 1.5 % flour, while MPHF and LPHF were higher than MPLF and LPLF. MPLF was lower than all treatments with 3% flour for consistency, and it was lower than HPHF and LPHF for firmness. The high flour concentration positively affected the appearance ($R_v = 0.825$; $p = 0.032$, Table S2), color ($R_v = 0.936$; $p = 0.005$, Table S2), and flavor ($R_v = 0.803$; $p = 0.039$) of the

artisanal fermented milk beverages, which were attributed to the decrease in syneresis caused by the increase in WHC, which increased the score in the appearance and color attributes (Figure S3A, B and Figure S3C, D), as well as the color and carbohydrate content typical of cupuassu flour [19]. On the other hand, flour concentration was not relevant regarding flavor, taste, overall acceptability, and purchase intention.

Table 6. Sensory acceptance of artisanal fermented milk beverage with cupuassu pulp and flour.

Sample	Attribute[1)]							
	Appearance	Color	Flavor	Taste	Consistency	Firmness	Overall Acceptability	Purchas Intentio
HPLF	6.03 ± 2.02^{abc}	5.79 ± 1.99^{bc}	6.30 ± 1.57 ^{a}	5.59 ± 2.09^{a}	6.07 ± 1.93^{a}	6.03 ± 1.91^{ab}	5.49 ± 1.82^{a}	2.71 ± 1.1
MPLF	5.15 ± 2.15^{c}	5.16 ± 2.06^{c}	5.77 ± 1.87^{a}	4.39 ± 2.13^{b}	5.00 ± 1.97^{b}	5.08 ±1.88^{b}	4.62 ± 1.99^{a}	2.13 ± 1.0
LPLF	5.43 ± 2.11^{bc}	5.48 ± 2.12^{c}	5.92 ± 1.70^{a}	5.23 ± 1.97^{ab}	5.98 ± 1.81^{a}	5.70 ± 1.80^{ab}	5.51 ± 1.77^{a}	2.59 ± 1.1
HPHF	6.89 ± 1.72^{a}	6.93 ± 1.63^{a}	6.48 ± 1.54^{a}	4.67 ± 2.50^{ab}	6.18 ± 1.74^{a}	6.31 ±1.81^{a}	5.26 ± 2.26^{a}	2.33 ± 1.1
MPHF	6.39 ± 1.80^{ab}	6.56 ± 1.74^{ab}	6.41 ± 1.90^{a}	4.59 ± 2.28^{ab}	5.97 ± 1.83^{a}	6.02 ± 1.80^{ab}	5.02 ±2.12^{a}	2.23 ± 1.0
LPHF	6.36 ± 1.76^{ab}	6.59 ± 1.53^{ab}	6.61 ± 1.45^{a}	4.72 ± 2.31^{ab}	6.08 ± 1.78^{a}	6.31 ± 1.65^{a}	5.20 ± 2.08^{a}	2.20 ± 1.0

$^{a–d}$ Different lowercase superscripts indicate significant differences among treatments of artisanal fermented milk beverage ($p < 0.05$). [1)] Purchase intention was evaluated on a structured 5-point hedonic scale, whereas the other attributes were evaluated on a 9-point hedonic scale. HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour).

Additionally, for treatments with 1.5 % flour, higher pulp content improved taste, consistency, and purchase intention scores than average pulp levels. Indeed, HPLF was higher than MPLF for these sensory attributes. The results indicated that the 10% pulp concentration could have a positive sensory effect on the artisanal fermented milk beverages with 1.5 % flour. This acceptance can be attributed to the content of ascorbic acid and phenolic compounds that the pulp contains. Together with the volatile substances of the fermented beverage, they provide better odor and flavor to the product [44]. In terms of purchase intention, the low valuation obtained by the evaluated samples (2.13 to 2.71) were close to the term "maybe buy, maybe not buy", this can be attributed to the strong flavor that cupuassu pulp has and the lack of familiarity with the consumption of this type of fruit [13, 21].

Table 7 shows the values obtained on the JAR scale, used to identify the optimum intensity of each of the attributes of artisanal fermented milk beverages. The parameters of aroma (acid, alcoholic, cupuassu, and milk), color (white and beige), flavor (caramel), and texture (sandiness, consistency, viscosity, and mouthfeel) showed no significant difference ($p > 0.05$) among the samples. However, pulp concentration was a strong predictor for alcoholic aroma ($R_v = 0.837$; $p = 0.034$, Table S2), which at the same time, had an inverse correlation with the color parameter b^* (yellowness) the higher the perception of b^*, the lower the alcoholic aroma ($R_v = 0.875$; $p = 0.020$, Table S2; Figure S4A). For samples, pH was highly predictive of cupuassu aroma ($R_v = 0.985$; $p = 0.007$, Table S2), generated by the decrease of pulp and increase of flour in the beverage (Figure S4B). Furthermore, for a concentration of 1.5% flour, a higher percentage of pulp helps to reduce the bitterness (HPLF < LPLF; $p < 0.05$).

Table 7. Just-about-right (JAR) profile scores for the different formulations of Artisanal fermented milk beverage evaluated.

Sample	Aroma					Taste	
	Ácid	Alcoholic	Cupuassu	Milk	Sweet	ácid	Bitter
HPLF	3.25 ± 0.62^{a}	3.21 ± 0.68^{a}	3.03 ± 0.83^{a}	2.69 ± 0.78^{a}	2.28 ± 1.74^{a}	3.23 ± 0.62^{b}	3.25 ± 0.71^{c}
MPLF	3.38 ± 0.80^{a}	3.13 ± 0.79^{a}	3.03 ± 0.83^{a}	2.57 ± 0.77^{a}	1.98 ± 0.84^{ab}	3.51 ± 0.95^{ab}	3.46 ± 0.95^{abc}
LPLF	3.08 ± 0.69^{a}	3.07 ± 0.57^{a}	2.98 ± 0.85^{a}	2.57 ± 0.89^{a}	2.28 ± 0.85^{a}	3.33 ± 0.63^{b}	3.33 ± 0.82^{ab}
HPHF	3.26 ± 0.60^{a}	3.20 ± 0.87^{a}	3.00 ± 0.91^{a}	2.54 ± 0.87^{a}	1.85 ± 0.77^{ab}	3.39 ± 0.88^{ab}	3.85 ± 0.79^{a}
MPHF	3.43 ± 0.83^{a}	3.23 ± 0.91^{a}	3.10 ± 0.82^{a}	2.71 ± 0.80^{a}	1.74 ± 0.72^{b}	3.79 ± 0.86^{a}	3.74 ± 0.93^{ab}
LPHF	3.25 ± 0.65^{a}	3.07 ± 0.71^{a}	2.72 ± 0.93^{a}	2.59 ± 0.85^{a}	1.72 ± 0.76^{b}	3.39 ± 0.89^{ab}	3.69 ± 0.99^{abc}
Sample	Color			Flavor			
	White	Brown	Beige	Cupuassu	Caramel		
HPLF	2.97 ± 0.97^{a}	2.74 ± 1.03^{ab}	2.95 ± 0.83^{a}	3.23 ± 0.71^{ab}	2.03 ± 0.69^{a}		
MPLF	3.03 ± 1.02^{a}	2.54 ± 0.70^{b}	2.79 ± 0.78^{a}	3.16 ± 0.94^{ab}	2.03 ± 0.94^{a}		
LPLF	3.10 ± 1.08^{a}	2.62 ± 0.67^{b}	3.01 ± 0.77^{a}	3.08 ± 0.87^{ab}	2.00 ± 0.77^{a}		
HPHF	2.61 ± 0.94^{a}	3.08 ± 0.67^{a}	2.82 ± 0.67^{a}	3.39 ± 1.16^{a}	1.98 ± 0.99^{a}		
MPHF	2.71 ± 0.94^{a}	3.08 ± 0.46^{a}	2.84 ± 0.78^{a}	3.25 ± 0.99^{ab}	2.02 ± 0.89^{a}		
LPHF	2.69 ± 0.79^{a}	3.03 ± 0.61^{a}	2.89 ± 0.64^{a}	2.85 ± 1.12^{b}	2.10 ± 0.98^{a}		
Sample	Texture						
	Sandy	Consistency	Firmness	Viscosity	Mouthfeel		
HPLF	3.08 ± 0.82^{a}	2.82 ± 0.70^{a}	2.77 ± 0.59^{ab}	2.79 ± 0.72^{a}	2.85 ± 0.76^{a}		
MPLF	3.10 ± 0.87^{a}	2.49 ± 0.72^{a}	2.51 ± 0.72^{b}	2.69 ± 0.75^{a}	2.54 ± 0.98^{a}		
LPLF	3.08 ± 0.79^{a}	2.62 ± 0.64^{a}	2.57 ± 0.65^{ab}	2.71 ± 0.72^{a}	2.61 ± 0.92^{a}		
HPHF	3.20 ± 0.68^{a}	2.75 ± 0.75^{a}	2.80 ± 0.70^{ab}	2.85 ± 0.83^{a}	2.66 ± 1.01^{a}		
MPHF	3.08 ± 0.77^{a}	2.82 ± 0.62^{a}	2.85 ± 0.66^{a}	2.71 ± 0.65^{a}	2.56 ± 0.95^{a}		
LPHF	3.20 ± 0.64^{a}	2.72 ± 0.58^{a}	2.77 ± 0.58^{ab}	2.89 ± 0.63^{a}	2.56 ± 1.00^{a}		

$^{a–b}$ Different lowercase superscripts indicate significant differences among treatments of artisanal fermented milk beverage ($p < 0.05$). HPLF, beverage (10% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); HPHF, beverage (10% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour).

The pulp also can contribute to elevate the cupuassu flavor but significantly only at the concentration of 3 % flour (HPHF > LPHF; $p < 0.05$). On the other hand, the flour concentration contributed to a less sweet taste but a more acidic and bitter taste. Indeed, the acidic score of MPHF was higher than that of HPLF and LPLH. For bitter taste, scores of HPHF and MPHF were superior to that of HPLF. In contrast, MPHF and LPHF had sweet taste scores that were lower than those of LPLF and HPLF. This pattern is attributed to the correlation between flour concentration and bitter taste, where flour addition was a strong predictor of bitter taste ($R_v = 0.928$; $p = 0.006$, Table S2), which was also positively affected by WHC and reduced syneresis (Figure S4C, S4D).

The flour also favored the darkening of the beverages. The flour increased brown color perception scores and decreased white color perception scores ($p = 0.002$, Table S2), such that all treatments with 3% flour had higher browning scores than MPLF and LPLF, attributed to decreased syneresis and increased WHC (Figure S5A-5D). However, the flour did not influence the score obtained in the cupuassu flavor ($p > 0.05$), unlike the pulp percentages, which generated the increase in L^* and c^* color parameters, favoring cupuassu flavor ($p = 0.036$, Table S5) with increasing pulp percentage (Figure S5E, S5F).

For texture, the firmness attribute was the one that showed variation among samples ($p < 0.05$) but no correlation concerning pulp and flour concentration

(Table S2), in contrast to the mouthfeel attribute that was favored by the apparent viscosity, resulting from the consistency index K and the addition was inversely related to the behavior of a^* (Figure S6A, S6B, and S6C). In general, the results indicated that 3% cupuassu flour could increase the acid and bitter taste, brown color, and firm texture of the artisanal fermented milk beverage. Likewise, adding 1.5% cupuassu flour increased the sweet taste values.

According to the results, a penalty analysis was made with JAR scores (Table 8), to improve the formulations of the artisanal fermented milk beverage by adding cupuassu pulp and flour. Parameters with a penalty score > 0.5 and an incidence > 20 % were considered detrimental attributes for overall acceptability. All treatments were penalized for excessive acid aroma, acid taste, and bitter taste, as well as for lack of milk aroma, sweet taste, caramel taste, consistency, firmness, viscosity, and mouthfeel.

Table 8. Consumer penalty analysis of the just-about-right (JAR) diagnostic attributes for Artisanal fermented milk beverage.

Attributes		Samples											
		HPHF		LPHF		MPHF		HPLF		LPLF		MPLF	
		Not enough	Too much	Not enough	Too much	Not enough	Too much	Not enough	Too much	Not enough	Too much	Not enough	Too much
Aroma	Ácid	-[1]	24.59% (0.81)[2]	-	29.51 % (1.68)	-	37.70 % (1.01)	-	29.51 % (1.67)	-	21.31 % (1.11)	-	34.43 % (0.81)
	Alcoholic	-	31.15% (0.83)	-	-	-	31.15 % (1.01)	-	27.87 % (0.84)	-	-	-	24.59 % (1.60)
	Cupuassu	26.23 % (1.05)		37.70 % (1.23)	-	-	22.95 % (0.75)	-	-	27.87 % (1.39)	-	-	-
	Milk	47.54 % (0.9)	-	39.34 % (1.10)	-	34.43 % (1.33)	-	31.15 % (1.34)	-	40.98 % (1.33)	-	39.34% (0.70)	-
	Sweet	80.33 % (2.74)	-	81.97 % (2.53)	-	83.61 % (2.37)	-	68.85 % (1.81)	-	62.30 % (1.47)	-	73.77% (0.89)	-
Taste	Ácid	-	44.26% (1.03)	-	39.34 % (1.92)	-	57.38 % (1.46)	-	27.87 % (1.73)	-	36.07 % (1.46)	-	44.26 % (1.40)
	Bitter	-	67.21% (1.60)	-	54.10 % (2.42)	-	60.66 % (1.18)	-	29.51 % (0.95)	-	34.43 % (1.56)	-	45.90 % (0.29)
Color	White	-	-	-	-	-	-		-	-	-	-	-
	Brown	-	-	-	-	-	-	37.70 % (1.21)	-	37.70 % (1.02)	-	44.26% (1.23)	-
	Beige	-	-	-	-	-	-	-	-	-	-	-	-
Flavor	Cupuassu	-	44.26% (1.03)	36.07 % (1.55)	-	-	36.07 % (1.51)	-	-	-	26.23 % (1.32)	-	31.15 % (0.90)
	Caramel	75.41 % (1.93)	-	70.49 % (1.14)	-	75.41 % (1.87)	-	75.41 % (0.85)	-	77.05 % (2.17)	-	75.41% (1.01)	-
Texture	Sandy	-	-	-	24.59 % (1.08)	-	-	-	22.95 % (0.54)	-	-	-	24.59 % (1.61)
	Consistency	27.87 % (1.77)	-	27.87 % (1.56)	-	21.31 % (0.12)	-	27.87 % (1.91)	-	39.34 % (1.90)	-	49.18% (0.93)	-

Firmness	21.31 % (2.16)	-	27.87 % (1.43)	-	-	-	27.87 % (2.21)	-	40.98 % (1.97)	-	47.54% (1.21)	-							
Viscosity	26.23 % (1.90)	-	21.31 % (0.91)	-	29.51 % (0.94)	-	27.87 % (1.39)	-	31.15 % (1.39)	-	36.07% (1.46)	-							
Mouthfeel	45.90 % (1.61)	-	40.98 % (1.71)	-	44.26 % (2.00)	-	29.51 % (1.72)	-	39.34 % (1.76)	-	49.18% (1.81)	-							

[1] (-) indicates that less than 20% of consumers chose that JAR category. [2] percentage of consumers and mean decreases HPHF, beverage (10% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); HPLF, beverage (10% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour).

Samples HPHF, MPHF, HPLF, and MPLF with 10% or 7.5% pulp were penalized for excessive alcoholic aroma, which may be because cupuassu pulp represents an important source of substrates for the group of microorganisms (yeasts, lactic and acetic acid bacteria) of the cupuassu microbiota, which are involved in alcoholic fermentation [47]. Inline, MPHF was penalized for excessive cupuassu aroma, while LPHF and LPLF were penalized for the lack of this aroma. Similarly, HPHF, MPHF, and MPLF presented excessive cupuassu flavor, contrary to LPHF (5% pulp and 3% flour), which was penalized for lack of flavor. It indicates that an ideal pulp concentration has yet to be achieved. Samples with 1.5% flour (HPLF, LPLF, and MPLF) were penalized for lacking brown color. That is, for the brown color attribute, 3% flour was a more promising concentration.

However, it is important to note that a higher flour concentration increased acidity and bitterness, which were penalized attributes. Thus, an ideal flour concentration to harmonize these effects must be established LPHF, MPHF, and MPLF presented excessive sandiness. Despite the penalty results, most of the attributes evaluated were close to "moderately more than ideal" (JAR between 2.49 and 3.85). The exception was for sweetness and caramel flavor, which was less than "ideal" (JAR between 1.74 and 2.28) in all treatments. It can be attributed to the low percentage of sugar in the beverage (3%). The combination of the JAR profile data and the penalty analysis made it possible to establish an ideal product with an adjustment in the percentages of cupuassu pulp and flour, increasing the percentage of sweetener in the product, which would also reduce the acid aroma, acid taste, and bitter taste.

Figure 8 shows the projection of the CATA data of the artisanal fermented milk beverage samples. It explained 94.59% of the total variation of the data, by F1 with 87.06 % and F2 with 7.53%. According to the values obtained in the Cochran's Q test (data not shown), 7 terms were statistically different among the artisanal fermented milk beverages ($p < 0.05$). These terms were: homogeneous appearance, dark brown color, light brown, beige color, white color, bitter taste, and cocoa aroma. Samples containing 3% cupuassu flour (HPHF, MPHF, and LPHF) were described as having a bitter taste, dark brown color, and homogeneous appearance. MPHF and LPHF were also associated with the cocoa aroma, which can be attributed to the aromatic compounds present in the fermented cupuassu and cocoa seeds that impart an identical aroma to each other [18].

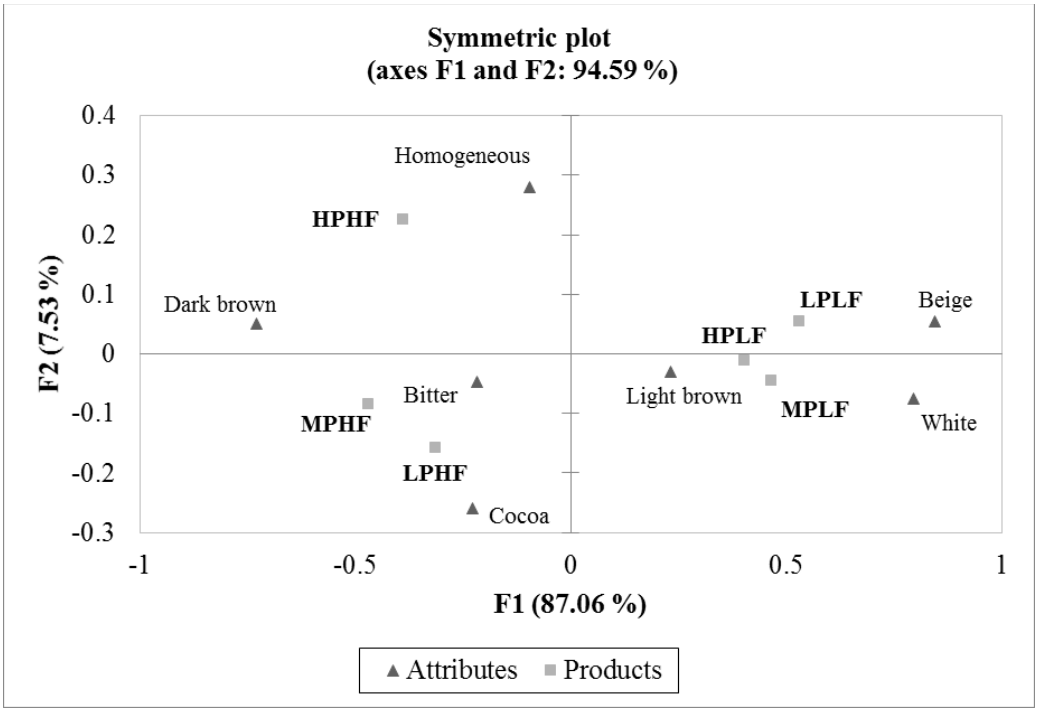


Figure 8. Correspondence analysis of the CATA attribute terms for artisanal fermented milk beverage with cupuassu pulp and flour.

On the other hand, samples containing 1.5% cupuassu flour (HPLF, MPLF, and LPLF) were described as white, light brown, and beige. These results showed similar behavior in the evaluators' scores, both for CATA and JAR, where the samples with the highest concentration of cupuassu flour (3%) were perceived as having the most bitter taste and brown color. Likewise, the white and beige colors were associated with the samples with the lowest concentration of cupuassu flour (1.5%).

4. Conclusions

Cupuassu pulp and flour had positive effects on some physicochemical parameters of artisanal fermented milk beverages by reducing syneresis and increasing the water retention capacity. The high pulp concentration (10%) improved rheological parameters, especially the consistency index and apparent viscosity. In addition, flour increased the protein and fiber content of the beverage. Likewise, these two ingredients affected the instrumental color parameters, where the flour decreased the values of L^*, b^*, and c^*, the pulp increased the values of a^* due to the pigmentation that each one presented as a result the technological process. However, this darkening of the drinking was sensorially favorable. This study established that higher flour concentration improved sensory appearance, color, consistency, and firmness in combined formulations, especially when containing 10% pulp. The pulp provided an of alcoholic aroma and cupuassu flavor. At the same time, the flour contributed to an excessively acidic and bitter taste. Despite the improvements in the rheological parameters by adding cupuassu, the beverages were still sensorially penalized regarding suboptimal firmness, consistency, and viscosity. Therefore, elaborating on an artisanal fermented milk beverages with functional potential using cupuassu pulp or seed flour as ingredients is promising due to physicochemical, nutritional, and microbial quality. However, it is recommended for future research, optimizing beverage formulations, mainly regarding the concentrations of cupuassu ingredients (e.g., using other

percentages of flour and sugar) and even forms of addition to improve the characteristics and achieve sensory fullness.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Optimism-corrected performance estimates through validation by bootstrap approach of significant models for prediction of physicochemical parameters from physicochemical variables; Table S2: Optimism-corrected performance estimates through validation by bootstrap approach of significant models for prediction of sensory parameters from physicochemical variables; Figure S1: Effect of shear rate on shear stress of artisanal fermented milk beverage; Figure S2: Spider plot of mean scores of sensory evaluation of artisanal fermented milk beverage with cupuassu flour and pulp; Figure S3 - Figure S6: Significant correlations ($p < 0.05$) and internally validated by Bootstrap method of sensory attributes in relation to physicochemical parameters of artisanal fermented milk beverages stored at 4 °C.

Author Contributions: For Conceptualization, K.G.-A.; formal analysis, K.G.-A. and C.P.V.; investigation, K.G.-A. and M.P.C.; resources, M.P.C., L.F.M, S.A.S., and C.O.d.S.; writing—original draft preparation, K.G.-A., A.I.L.S and R.L.C.d.J; writing—review and editing K.G.-A., C.P.V. and M.P.C.; supervision, M.P.C. All authors have read and agreed to the published version of the manuscript

Funding: This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Serviço Nacional de Aprendizagem do Cooperativismo (SESCOOP) Brazil — chamada CNPQ/SESCOOP number 11/2022, process [404190/2022-7].

Institutional Review Board Statement: The study was approved by the Ethics Committee of the Escola de Enfermagem da Universidade Federal da Bahia (sob CAAE 60414022.7.0000.5531).

Data Availability Statement: The data are available from the corresponding author.

Acknowledgments: The authors are thankful for the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil — grant [303074/2021-3, 404190.2022.7]. Katherine Gutiérrez-Alzate was financed by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

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

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Supplementary tables

Table S1. Optimism-corrected performance estimates through validation by bootstrap approach of significant models for prediction of physicochemical parameters from physicochemical variables in fermented milk beverage with cupuassu (*Theobroma grandiflorum*) pulp and flour and stored at 4 °C.

Parameter	Variable	Equation	R	R^2_{adj}	*p*	R^2_{app}	R^2_{boot}	R^2_{orig}	Optimism	R^2_v	R_v
Storage period	n	-0.002 *n* + 0.41	-0.32	0.105	0.012	0.548	-0.003	0.551	0.742	0.089	0.298
Pulp	pH	-0.03 pH + 4.47	-0.740	0.548	<0.0001	0.363	0.005	0.358	0.599	0.551	**0.742**
	Synereses	-0.24 Syn + 34.53	-0.480	0.230	0.0001	0.230	0.234	0.230	0.004	0.226	0.475
	WHC	0.24 WHC + 65.46	0.480	0.231	0.0001	0.231	0.235	0.231	0.004	0.227	0.476
	*a**	0.08 *a** + 1.51	0.430	0.189	0.001	0.189	0.183	0.188	-0.005	0.194	0.440
	K	46.91 *K* + 237.9	0.630	0.391	<0.0001	0.391	0.411	0.391	0.020	0.370	0.609
	n	-0.006 *n* + 0.42	-0.410	0.167	0.001	0.167	0.179	0.167	0.013	0.154	0.393
	App.viscosity	16.73 μ + 68.31	0.530	0.278	<0.0001	0.278	0.301	0.278	0.023	0.255	0.505
Flour	pH	0.08 pH + 4.180	0.6	0.364	<0.0001	0.364	0.368	0.364	0.005	0.359	0.599
	Synereses	-1.05 Syn + 34.75	-0.69	0.480	<0.0001	0.480	0.486	0.480	0.006	0.474	**0.689**
	WHC	1.05 WHC + 65.25	0.69	0.478	<0.0001	0.478	0.485	0.478	0.006	0.472	**0.687**
	*L**	-7.011 *L** + 32.83	-0.86	0.746	<0.0001	0.746	0.740	0.746	-0.006	0.752	**0.867**
	*a**	-0.19 *a** + 2.238	-0.36	0.133	0.004	0.133	0.159	0.133	0.027	0.106	0.326
	*b**	-9.52 *b** + 17.20	-0.32	0.101	0.013	0.101	0.291	0.101	0.190	-0.088	NA
	*c**	-7.82 *c** + 22.46	-0.87	0.751	<0.0001	0.751	0.745	0.750	-0.005	0.756	**0.869**
	h°	74.01 *h*° + 122.1	0.86	0.747	<0.0001	0.747	0.742	0.747	-0.005	0.753	**0.868**
pH	*L**	-33.49 *L** + 166.2	-0.53	0.28	<0.0001	0.280	0.290	0.280	0.011	0.269	0.519
	*a**	-2.216 *a** + 11.47	-0.53	0.283	<0.0001	0.283	0.286	0.283	0.003	0.280	0.529
	*c**	-36.26 *c** + 166.5	-0.52	0.266	<0.0001	0.266	0.275	0.265	0.010	0.256	0.505
	h°	353.1 *h*° - 1284	0.53	0.280	<0.0001	0.280	0.289	0.280	0.009	0.271	0.520
	K	-696.4 K + 3493	-0.39	0.155	0.002	0.155	0.172	0.155	0.017	0.139	0.373
	n	0.096 n - 0.02654	0.27	0.075	0.034	0.075	0.094	0.075	0.019	0.057	0.238
	App.viscosity	-270.8 μ + 1326	-0.36	0.132	0.004	0.132	0.146	0.131	0.015	0.117	0.342
Synereses	**WHC**	**-0.9994 WHC + 99.98**	**-1**	**1**	**<0.001**	**0.997**	**0.997**	**0.997**	**0.000**	**0.997**	0.998

	*L**	2.714 *L** - 67.72	0.51	0.26	<0.001	0.256	0.258	0.256	0.002	0.254	0.504
	*c**	3.015 *c** - 89.31	0.51	0.26	<0.001	0.256	0.258	0.256	0.002	0.253	0.503
	h°	-27.68 *h*° + 1152	-0.49	0.24	<0.001	0.240	0.243	0.239	0.003	0.236	0.486
WHC	*L**	-2.70 *L** + 203.0	-0.5	0.255	<0,0001	0.255	0.256	0.255	0.002	0.253	0.503
	*c**	-3.008 *c** + 211.7	-0.5	0.255	<0,0001	0.255	0.257	0.255	0.003	0.252	0.502
	h°	27.63 *h*° - 1613	0.49	0.239	<0,0001	0.239	0.242	0.239	0.004	0.235	0.485
L*	*a**	0.02459 *a** + 1.397	0.37	0.140	0.003	0.140	0.178	0.140	0.038	0.102	0.319
	*b**	1.670 *b** - 34.34	0.45	0.205	0.0003	0.205	0.437	0.205	0.232	-0.027	NA
	*c**	1.109 *c** - 14.02	1	0.994	<0.0001	0.994	0.994	0.994	0.000	0.994	**0.997**
	h°	-10.51 *h*° + 467.8	-1	0.994	<0.0001	0.994	0.994	0.994	0.000	0.993	**0.997**
a*	*c**	6.64 *c** - 2.19	0.39	0.154	0.002	0.154	0.192	0.153	0.039	0.115	0.340
	h°	-65.83 *h*° + 361.2	-0.41	0.169	0.001	0.169	0.204	0.167	0.036	0.132	0.364
b*	*c**	0.135 *c** + 10.33	0.45	0.201	<0.001	0.201	0.436	-0.249	0.685	-0.484	NA
	h°	-1.270 *h*° + 236.8	-0.44	0.197	<0.001	0.197	0.432	-0.255	0.687	-0.490	NA
c*	*h*°	-9.475 *h*° + 334.8	-1	0.998	<0.001	0.998	0.998	0.998	0.000	0.998	0.999
K	Syneresis	-0.0018 Syn + 34.09	-0.27	0.075	0.03	0.075	0.086	0.074	0.012	0.063	0.250
	WHC	0.0019 WHC + 65.89	0.28	0.076	0.03	0.076	0.088	0.076	0.012	0.064	0.253
n	K	-3998 K + 2047	-0.79	0.630	<0.001	0.630	0.641	0.630	0.012	0.618	**0.786**
Apparent viscosity	K	2.283 K + 131.0	0.97	0.932	<0.001	0.932	0.933	0.931	0.002	0.930	**0.964**
	n	-0.0003748 n + 0.447	-0.8	0.638	<0.001	0.638	0.651	-0.373	1.024	-0.387	NA

WHC: Water holding capacity; *L**: lightness; *a**: redness; *b**: yellowness; *c**: chroma; *h*°: hue angle; K: consistency index; n: flow behavior index; App.viscosity: Apparent viscosity(mPa s)**;** R: Pearson´s correlation coefficient; R^2_{adj}: adjusted coefficient of determination; *p*: probability value; R^2app: apparent coefficient of determination; R^2boot: bootstrap coefficient of determination; R^2orig: original coefficient of determination; R^2v: coefficient of determination of the model after validation. Rv: correlation coefficient of the model after validation.

Table S2. Optimism-corrected performance estimates through validation by bootstrap approach of significant models for prediction of sensory parameters from physicochemical variables in fermented milk beverage with cupuassu (*Theobroma grandiflorum*) pulp and flour and stored at 4 °C.

Sensory parameter	Variable	Equation	R	R^2_{adj}	*p*	R^2_{app}	R^2_{boot}	R^2_{orig}	Optimism	R^2_v	R_v
Appearance	Flour	0.67 Flour + 4.53	0.85	0.725	0.032	0.720	0.762	0.7224	0.039	0.681	0.825
	Syneresis	-0.29 Syn + 15.31	-0.87	0.757	0.024	0.760	0.821	0.748	0.073	0.687	0.829
	WHC	0.29 WHC - 13.58	0.87	0.755	0.025	0.760	0.820	0.745	0.076	0.684	0.827
Color	Flour	0.81 Flour + 4.26	0.94	0.89	0.005	0.890	0.899	0.8856	0.013	0.877	0.936
	Syneresis	-0.32 Syn + 16.31	-0.88	0.777	0.020	0.780	0.843	0.763	0.080	0.700	0.837
	WHC	0.32 WHC - 15.55	0.88	0.774	0.021	0.770	0.842	0.760	0.082	0.688	0.829
Flavor	Flour	0.34 Flour + 5.49	0.83	0.695	0.039	0.690	0.740	0.6943	0.046	0.644	0.803
	Syneresis	-0.15 Syn + 10.91	-0.86	0.743	0.027	0.740	0.801	0.739	0.063	0.677	0.823
	WHC	0.1451WHC - 3.625	0.86	0.740	0.028	0.740	0.800	0.735	0.064	0.676	0.822
Alcoholic(JAR)	Pulp	0.02780 Pulp + 2.942	0.85	0.714	0.034	0.710	0.717	0.7083	0.009	0.701	0.837
	*b**	-0.3603 *b** + 2.297	-0.88	0.781	0.020	0.780	0.789	0.775	0.014	0.766	0.875
Cupuassu JAR	pH	-1.488 pH + 9.356	-0.93	0.871	0.007	0.870	0.733	0.833	-0.100	0.970	0.985
	*L**	0.2730 *L** - 1.093	0.87	0.756	0.025	0.760	0.728	0.742	-0.014	0.774	0.880
Sweet JAR	Flour	-0.2736 Flour + 2.591	-0.89	0.788	0.018	0.790	0.793	0.7851	0.008	0.782	0.884
Bitter(JAR)	Flour	0.2769 Flour + 2.929	0.94	0.875	0.006	0.870	0.879	0.8702	0.009	0.861	0.928
	Syneresis	-0.1020 Syn + 6.811	-0.82	0.670	0.047	0.670	0.753	0.658	0.095	0.575	0.758
	WHC	0.1014 WHC - 3.348	0.82	0.668	0.047	0.670	0.753	0.654	0.098	0.572	0.756
White(JAR)	Flour	-0.2438 Flour + 3.398	-0.97	0.934	0.002	0.930	0.932	0.9319	0.000	0.930	0.964
	Syneresis	0.1014 Syn - 0.3917	0.96	0.912	0.003	0.910	0.935	0.907	0.028	0.882	0.939
	WHC	-0.1009 WHC + 9.714	-0.95	0.910	0.003	0.910	0.935	0.905	0.030	0.880	0.938
Brown(JAR)	Flour	0.2878 Flour + 2.202	0.96	0.930	0.002	0.930	0.935	0.9276	0.007	0.923	0.961
	Syneresis	-0.1108 Syn + 6.391	-0.88	0.777	0.020	0.780	0.822	0.769	0.053	0.727	0.853
	WHC	0.1101 WHC - 4.643	0.88	0.775	0.021	0.770	0.821	0.766	0.055	0.715	0.846
Cupuassu(JAR)	Pulp	0.06890 Pulp + 2.644	0.84	0.708	0.036	0.710	0.735	0.7044	0.031	0.679	0.824
	*c**	1.785 *c** - 2.060	0.9	0.805	0.015	0.810	0.793	0.804	-0.011	0.821	0.906
Mouthfeel (JAR)	*a**	-0.7049 *a** + 3.856	-0.83	0.691	0.040	0.690	0.702	0.674	0.028	0.662	0.813
	K	0.0001972 K + 2.500	0.94	0.879	0.006	0.880	0.800	-1.180	1.980	-1.100	NA
	App.viscosity	0.0004728 μ + 2.518	0.9	0.810	0.015	0.810	0.707	0.627	0.080	0.730	0.854

WHC: Water holding capacity; *L*:* lightness; *a*:* redness; *b*:* yellowness; *c**: chroma; *h°*: hue angle; K: consistency index; n: flow behavior index; App.viscosity: Apparent viscosity(mPa s)**;** R: Pearson´s correlation coefficient; R^2_{adj}: adjusted coefficient of determination; *p*: probability value; R^2app: apparent coefficient of determination; R^2boot: bootstrap coefficient of determination; R^2orig: original coefficient of determination; R^2v: coefficient of determination of the model after validation. Rv: correlation coefficient of the model after validation.

Supplementary figures

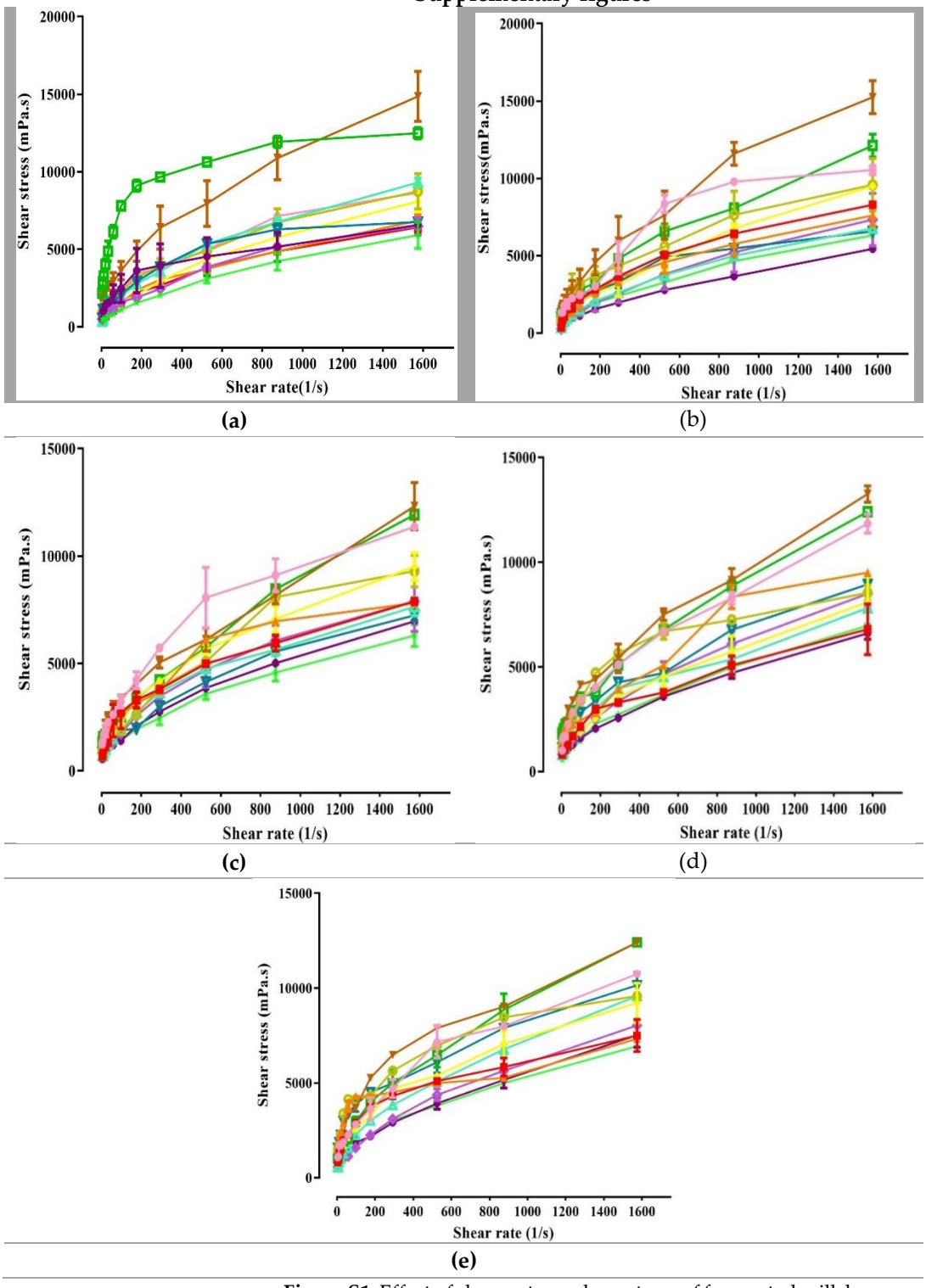

Figure S1. Effect of shear rate on shear stress of fermented milk beverage : (—●—) 10% pulp, (—■—) 5% pulp, (—▲—) 7.5% pulp, (—▼—) 10% pulp and 3% flour, (—★—) 5% pulp and 3% flour, (—●—) 7.5% pulp and 3% flour, (—■—) 10% pulp and 1.5% flour, (—▲—) 5% pulp and 3% flour, (—▼—) 7.5% pulp and 3% flour, (—◆—) control, (—●—) 3% flour, (—★—) 1.5% flour during storage a) day 0; b) day 7; c) day 14; d) day 21 and e) day 28.

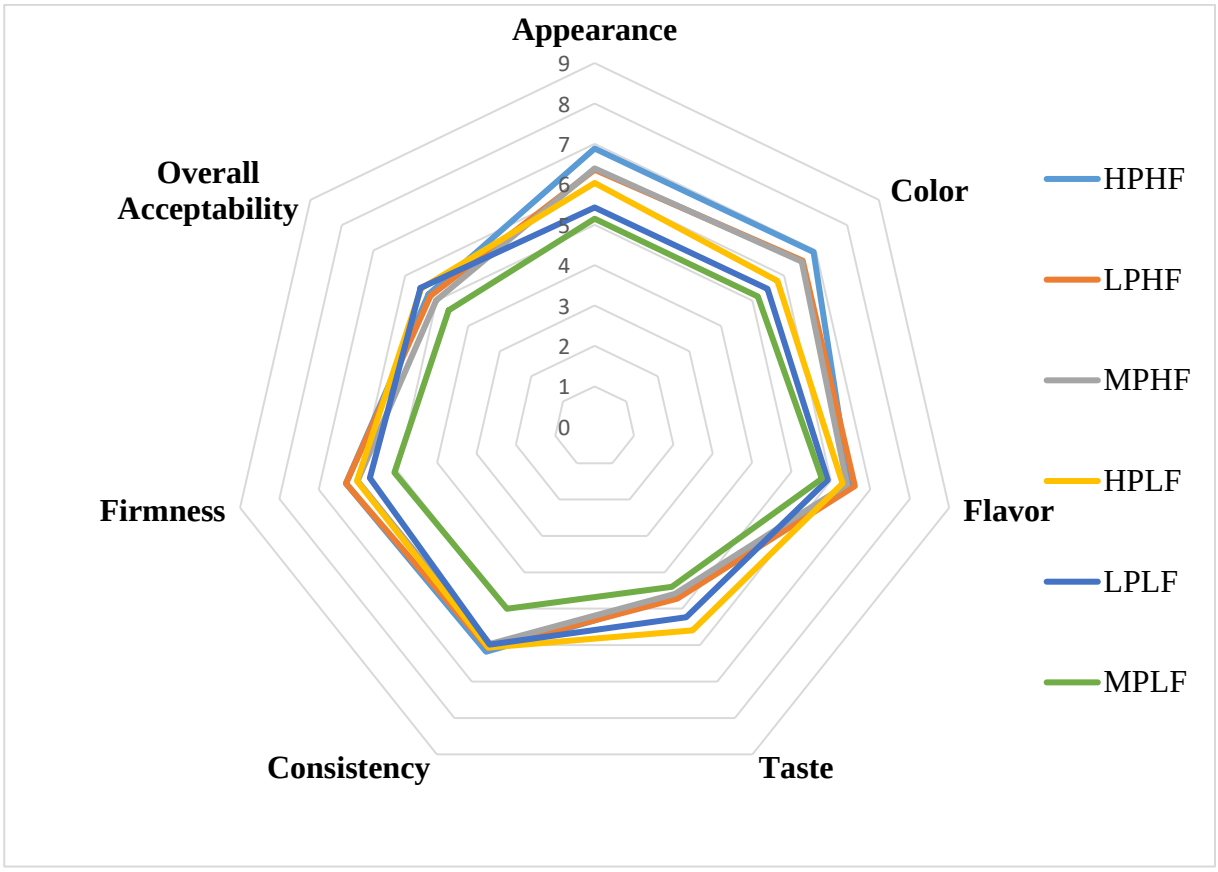

Figure S2. Spider plot of mean scores of sensory evaluation of fermented milk beverage with cupuassu flour and pulp. HPHF, beverage (10% pulp and 3% flour); LPHF, beverage (5% pulp and 3% flour); MPHF, beverage (7.5% pulp and 3% flour); HPLF, beverage (10% pulp and 1.5% flour); LPLF, beverage (5% pulp and 1.5% flour); MPLF, beverage (7.5% pulp and 1.5% flour).

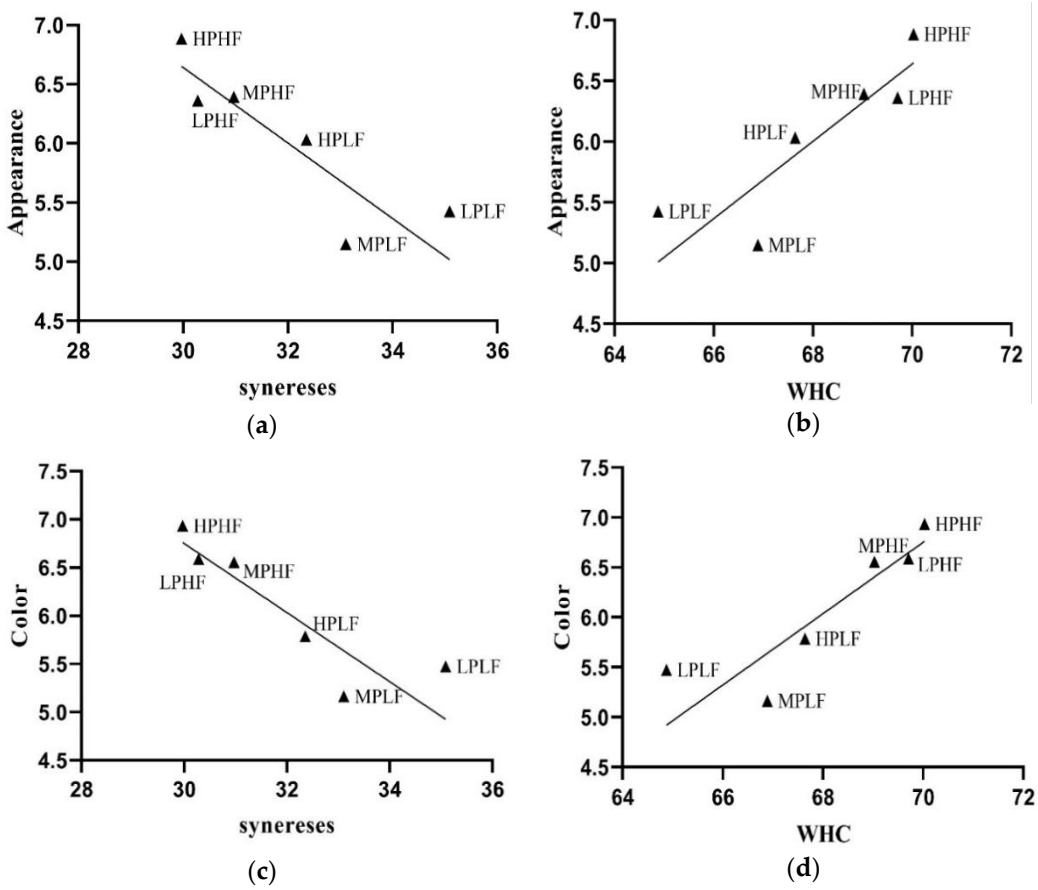

Figure S3. Significant correlations ($P < 0.05$) and internally validated by Bootstrap method of sensory attributes in relation to physicochemical parameters of fermented milk beverages stored at 4 °C. a) Appearance: syneresis; b) Appearance: water holding capacity (WHC); c) color: syneresis; d) color: water holding capacity (WHC).

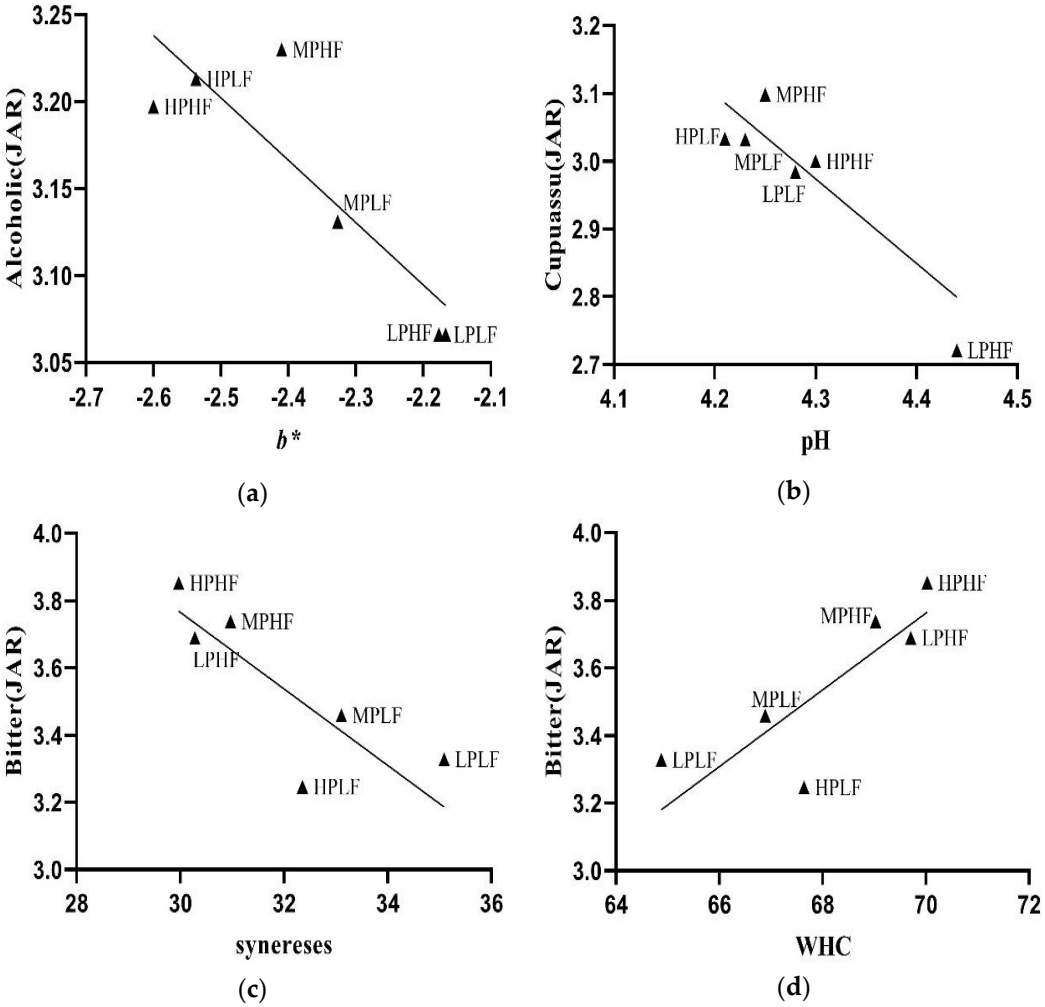

Figure S4. Significant correlations (P < 0.05) and internally validated by Bootstrap method of sensory attributes in relation to physicochemical parameters of fermented milk beverages stored at 4 °C. a) Alcoholic (JAR): *b*;* b) cupuassu (JAR): pH; c) Bitter (JAR): syneresis; d) Bitter (JAR): water holding capacity (WHC).

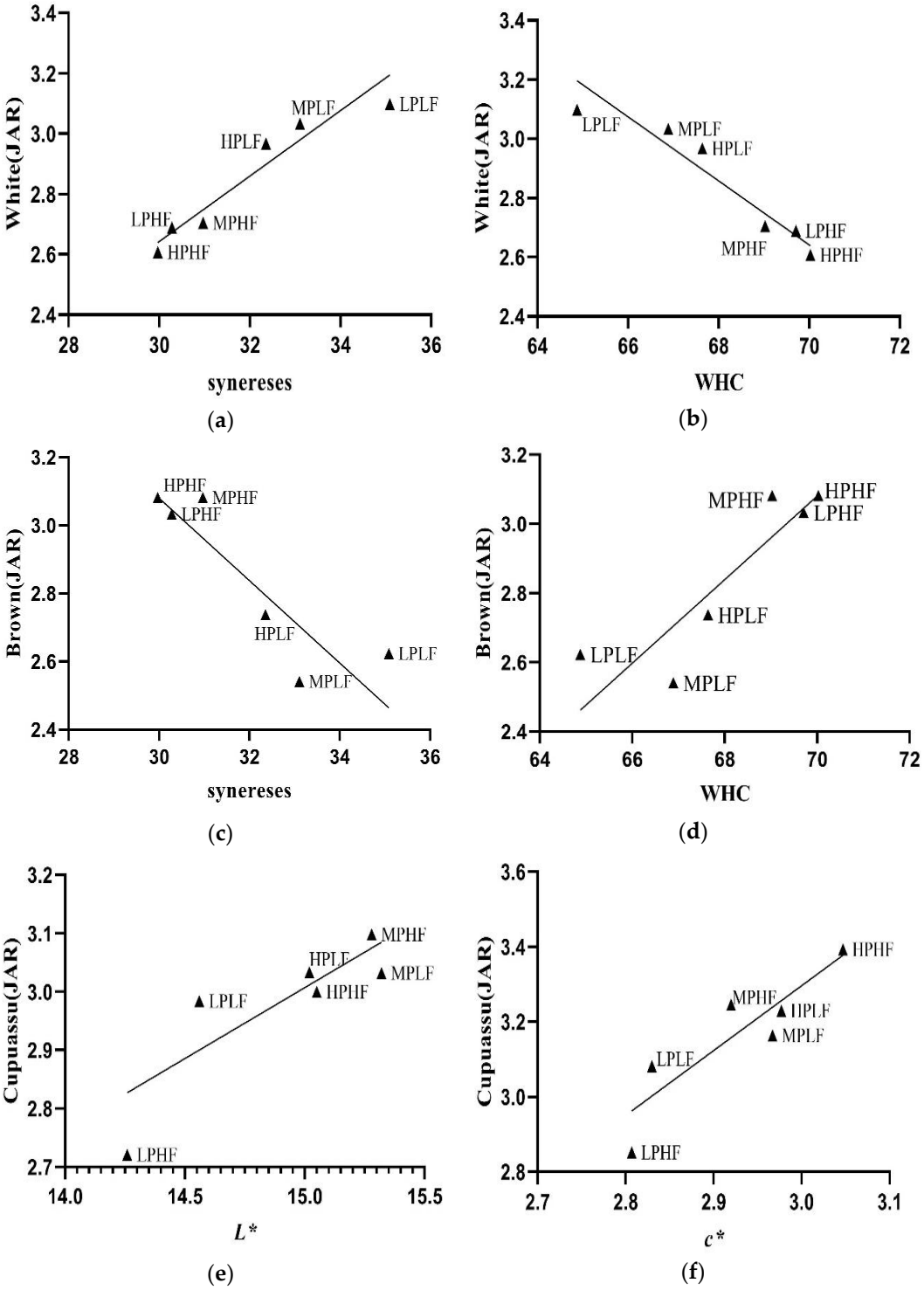

Figure S5. Significant correlations (P < 0.05) and internally validated by Bootstrap method of sensory attributes in relation to physicochemical parameters of fermented milk beverages stored at 4 °C. a) White (JAR): syneresis; b) White (JAR): water holding capacity (WHC); c) Brown (JAR): syneresis d) Brown (JAR): water holding capacity (WHC); e) Cupuassu (JAR): *L**; f) Cupuassu (JAR): *c**.

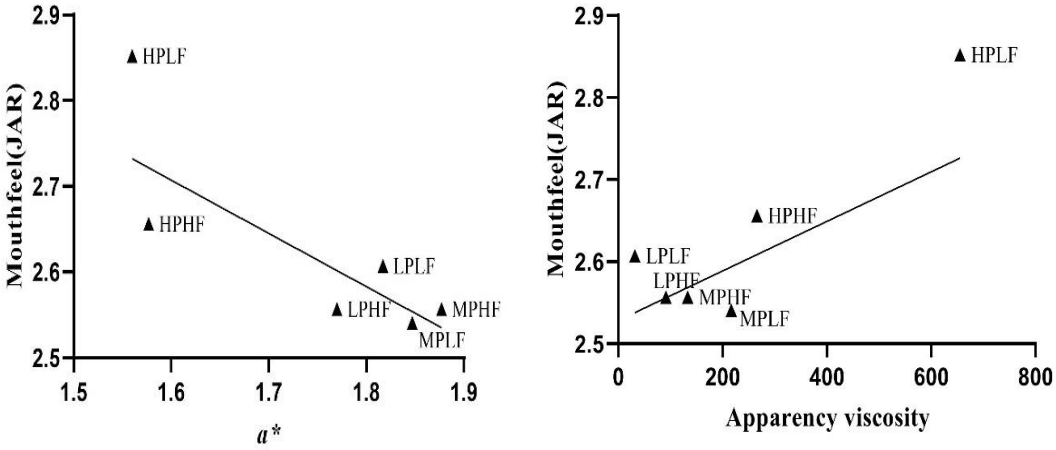

Figure S6. Significant correlations ($P < 0.05$) and internally validated by Bootstrap method of sensory attributes in relation to physicochemical parameters of fermented milk beverages stored at 4 °C. a) Mouthfeel (JAR): *a**; b) Mouthfeel (JAR): Apparency viscosity.

Capítulo III

Manuscrito: Micro and nanoencapsulation of probiotics: the impact on foods of animal origin

Micro and nanoencapsulation of probiotics: the impact on foods of animal origin

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Periódico a ser submetido (1ª submissão):	*Trends in Food Science and Technology; ISSN:0924-2244*
Maior percentil (Scopus):	*99%; 3/338; Food Science*

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Abstract

Background: Increased consumption of functional foods, including animal products enriched with probiotics, has had a significant impact on the market, allowing the growth of micro and nanoencapsulation in the food industry due to its functionality in the protection and viability of probiotic cells.

Scope and approach: Different studies that have been done in recent years on micro- and nanoencapsulation of probiotics in animal matrices are addressed to get different points of view on the influence that this topic generates. The topics discussed include the most commonly used micro- and nanoencapsulation techniques, the materials, the application done in dairy and meat products, how sensory attributes have been affected and what studies are being developed to reinforce the probiotics protection with micro- and nanoencapsulation.

Key findings and conclusions: In this review, it was described the most commonly used encapsulation techniques, such as spray drying, spray cooling, extrusion, emulsification, and electrospinning, with these main advantages related. Encapsulation materials such as alginate, starch, pectin, whey protein, and chitosan were discussed in the food industry and their benefits. In addition, they also entered into the latest research on dairy and meat products with the incorporation of probiotic micro- and nanocapsules and their effects on the final product. However, we can conclude that few studies evaluate the influence of probiotics and encapsulation techniques in meat products. In addition, there is a lack of research on the effectiveness of encapsulation materials associated with probiotic viability and maintenance of physicochemical and sensory characteristics from foods of animal origin.

Keywords: Capsules, Probiotics, Dairy products, Meat products, Functional foods.

1. Introduction

More and more consumers are concerned about their health, which creates concerns and demands for food choices that are healthy, safe, provide benefits to the human body, and are innovative to generate a variety of food products. Then, the food science is looking to evolve with the application of probiotics in different food matrices (Angiolillo et al., 2017; Ceylan et al., 2019; Arslan-Tontul, 2020; Dantas et al., 2021). These were developed to benefit the intestinal microbiota, which allows the effective absorption of probiotics in the colon. According to the World Health Organization (WHO) definition, probiotics are living organisms, such as bacteria and yeasts that confer health benefits to the host when administered in adequate amounts. These microorganisms are classified as GRAS (generally recognized as safe), characterized by a very low probability of infection (Fao, 2001; Cassani et al., 2020). Among the diversity of probiotics currently in use, the most common are Gram-positive genera, including strains such as *Lactobacillus, Bifidobacterium, Leuconostoc, Pediococcus, Enterococcus, Streptococcus,* and *Bacillus* (Ranadheera et al., 2015; Song et al., 2018; Han et al., 2020).

Probiotics provide health benefits due to the production of metabolites that inhibit the adhesion and prevalence of pathogenic microorganisms. This intestinal microbiota modification helps to protect the gut against damage related to the immune system, prevents intestinal infections, and acts as an immunomodulator to improve the absorption of micronutrients and stimulate the generation of organic and amino acids (Shori et al., 2019). Moreover, probiotic metabolites can promote the inactivation of toxic compounds by degrading toxins, reducing the absorption of toxic substances such as ammonia, amines, and indol (Sotoudegan et al., 2019), and prevent the production of compounds with anticarcinogenic activity, such as short-chain fatty acids and conjugated linoleic acid (Markowiak & Śliżewska, 2017).

Therefore, probiotic cell viability should be maintained during the different production processes of meat and dairy products, their whole shelf life and under gastrointestinal conditions after consumption. Consequently, one of the main requirements for obtaining health benefits is maintaining cell survival until reaching the gastrointestinal tract (GIT). Thus, the number of active probiotics to fulfill their physiological function in the GIT recommended by the International Dairy Federation (IDF) should reach at least 10^7 UFC/g of product consumed. However, these microorganisms must be able to withstand the acidic conditions of the stomach and the high

concentration of bile acids present in the small intestine, as well as the adverse factors that frequently affect probiotics and that may occur during processing, manufacturing, and storage of the products (Sukumar et al., 2021; Phoem et al., 2015).

By the multiple limitations that can be found to ensure the viability of probiotics, encapsulation has been used, the process of which consists of trapping a substance (active agent) inside another (wall material) that produces particles with diameters of a few nm to mm (Bratovcic & Suljagic, 2019). Here, the active agent is also known as the core material, filler, internal phase, or payload phase, and the wall material or substance to be encapsulated in the coating, shell, membrane, support material, external phase, or matrix, which can be modified in size on a nanometer scale (nanoencapsulation) or micrometer scale (microencapsulation). These structures are rapidly expanding in the food industry to deliver bioavailability solutions and protect the living cells against harmful environmental conditions, processing operations, food matrix effects, gastrointestinal transit, prevent losses, protect against reactions with other food compounds, prevent oxidation reactions, maintain storage stability and inclusively improve bacterial performance or to overcome other processing challenges (Mahdavi et al., 2016; Burgain et al. 2011). Encapsulation has been a helpful tool, especially micro- and nanoencapsulation technology that has grown in the food industry and will have a substantial impact on a wide variety of products such as functional foods and can improve the supply of bioactive compounds, mainly probiotics (Paredes et al., 2016; Jafari, 2017).

Microencapsulation is a technology for packaging microparticles (colloidal particles between 3 and 800 μm diameter) that can provide a physical barrier to protect the probiotics against adverse environmental conditions, which may occur during processing (biomass production, freeze-drying, storage, food application), food storage and during its passage through the GIT (Bratovcic & Suljagic, 2019). Microencapsulation can offer many advantages to improving the handling of probiotic cultures, as well as masking the flavor and aroma given by the production of different metabolic compounds produced during fermentation in food (for example, acetic acid), allowing to improve the yield of the fermentation process (Westman et al., 2012). Another important aspect is the effectiveness of microencapsulation to protect and enhance the probiotics survival with higher cell density after their inclusion in powder formulations, where there is the detrimental effect of low water activity and oxygen exposure (Gao et al., 2016; Mirzaei et al., 2012). The application

of microencapsulation has been an advantageous method for the food industry, especially for the dairy sector (Avila-Reyes et al., 2014; Silva et al., 2017) because spraying or freeze-drying the culture has made it possible to maintain the viability of the cells, allowing the development of new functional foods.

Nanoencapsulation is known as the technology of packing nanoparticles (colloidal particles between 10 and 1000 nm in diameter) of core or active (solid, liquid or gas) inside a secondary material (matrix or shell) to form nanocapsules or protective covers on food ingredients (Bratovcic & Suljagic, 2019; Paredes et al., 2016). This technique is of great interest as a promising alternative approach as it has been used to control the interaction of active ingredients with the food matrix due to its potential to optimize controlled release, improve bioavailability and allow precise alignment of living organisms in larger quantities compared to microencapsulation techniques (Minh-Hiep et al., 2014; Xie et al., 2015).

In this sense, micro and nanoencapsulation are techniques that allow the incorporation of probiotic microorganisms in different matrices, which has been the focus of multiple research projects due to the effectiveness this process provides when applied mainly in the food industry. Thus, this review aimed to describe the most common techniques, trending materials, and the application of micro- and nanoencapsulation of probiotics in matrices of animal origin.

2. Microstructure of nano- and microencapsulation

The microstructure for micro and nanoencapsulation tends to vary according to the method and material used in their preparation (Shori, 2017; Šipailienė & Petraitytė, 2018). It can affect the particle size, which is considered one of the essential characteristics for stability and potentiation of encapsulated active ingredients, affecting the viscosity of the prepared formulations and the texture and feel of the food ingredients. These factors and the fact that microbial cells are approximately 1 to 5 μm in size tend to cause some complications when making capsules due to the possibility of adverse effects on the structural and sensory properties of the product to which they are added (Kailasapathy, 2006). In addition to size, micro- and nanocapsules are evaluated according to particle size distribution (PSD), zeta potential, morphology, flowability, encapsulation efficiency, and stability (Minh-Hiep et al., 2014).

2.1.Technology applied to micro- and nanoencapsulation in food.

The micro- and nanoencapsulation of probiotics have allowed them to immobilize these bacteria inside semi-permeable and biocompatible materials, which allows their release in the GIT to be protected against adverse environments in place of controlled release (Kwak, et al., 2014; Yasmin et al., 2019). The selection of a particular technique will determine the efficiency of the capsule according to the viability of the probiotics, conditions during food processing, the storage conditions, the size, shape, solubility, and density of the encapsulation required, the release mechanism, and, the economic restrictions; therefore it is vital to choose the appropriate technique according to the need for micro or nanoencapsulation (Rodrigues et al., 2020). The nanoencapsulation techniques have been shown in several studies to be more complex than the microencapsulation process due to the size of the capsules, which tends to cause more significant inconvenience when subjected to treatment.

2.1.1. Spray-drying

The spray drying system is one of the most efficient techniques used for micro- and nanoencapsulation of probiotic microorganisms, requiring atomization of the feed medium in the hot air-drying chamber, where water is evaporated from the atomized droplets to create a dry powder (Anandharamakrishnan et al., 2008; Martín et al., 2015). This technique provides rapid water evaporation and maintains a low temperature in the particles (Busch et al., 2017). This technique has many advantages for the management of probiotic cultures since no cold chain is required for storage and transport once encapsulated, which reduces handling costs, helps to have an extensive selection of coating material, controls particle size, extends large scale production in continuous mode and fast drying (Santivarangkna et al., 2008). The different steps (pre-drying, spray drying, and post-drying) are crucial for an excellent probiotic encapsulation. Specific factors (bacterial strain, medium, temperature, pH, growth phase, initial cell concentration, and culture condition) and adverse stresses (acid, metabolic, and starvation stress, production strategy, and tolerance) must be considered. It is paramount to select suitable bacterial strains before drying as stresses such as heat, osmotic stress, and desiccation are the detrimental transcendental factors that can inactivate probiotics during or after spray drying (Huang et al., 2017).

During the whole spray-drying process and storage of the encapsulated microorganisms, the survival of the probiotic cells can be improved by the composition of the encapsulating material, so it is vital that it has emulsifying properties, which can be biodegradable, has TGI resistance with

low viscosity and high solid content, has low hygroscopicity and is low cost (Silva et al., 2017). Due to the limited number of commercially available materialsfor use as an encapsulating agent, Liu et al. (2018) and Busch et al. (2017) indicate that the use of particles containing probiotic bacteria produced by this technique in foods with high water activity and humidity is complex because they are water-soluble, which allows the cells contained in the particles to migrate into the product under hydrated conditions easily. However, spray drying presents some limitations for use in the nanoencapsulation of probiotics due to their larger size (1 - 5 μm) compared to nanocapsule size produced by conventional spray drying (2 - 25 μm), making this method a challenge for probiotic viability (Sosnik et al., 2015). Consequently, many studies have been conducted to evaluate probiotic culture formulation-based parameters, spray-drying conditions, and storage stability to obtain a suitable encapsulation and produce probiotic-loaded powder particles with the highest efficacy and viability.

2.1.2. Spray-Chilling

This technique produces microparticles, similar to spray drying in producing fine droplets, which differs mainly by atomizing the materials from the wall to the cooling chamber (Kwak, 2014; Lopes et al., 2015). In spray chilling, the mixtures of core and shell are atomized in the cooled or chilled air, which causes the shell to solidify around the core; the somewhat tiny liquid droplet is converted into microcapsules containing the probiotic, thus avoiding damage to the bacterial cells (Pelissari et al., 2016). This technique does not involve water evaporation; therefore, the matrix must be a thermogelating compound (Kwak, 2014).

It is considered promising and convenient encapsulation methods because it is a low-cost continuous process that is easy to scale up and requires no solvents, which leads to the formation of solid powder particles at room temperature, which has been studied to preserve the viability of encapsulated cells in different thermal situations and under gastric and enteric conditions (Sillick & Gregson, 2012; Kwak, 2014).

2.1.3. Extrusion

It is a process of mixing probiotic cells with a hydrocolloid solution, followed by expulsion into a solution through a syringe needle in the form of drops to fall freely into a polymerization or hardening bath and using high temperatures to dry the bacterial cells affecting the survival rate (Cook et al., 2011). The diameter of the needle and the free fall distance influence the size and

shape of the droplets, respectively, and the viscosity/flow rate of the hydrocolloid mixture with microbial cells. When comparing this technique with others, it proves to be more convenient and economical because it does not require high temperatures and solvents, it is simple, low cost, and ensures high retention of cell viability due to the gelling conditions; therefore, it is an efficient form to encapsulate probiotics even after the encapsulation process (Martín et al., 2015; Rodrigues et al., 2017). However, this technique present some disadvantages since it is a slow method, which hinders large-scale application, is ineffective for generating microspheres smaller than 500 μm, and it is necessary to use low- to moderately viscous hydrocolloid solutions (Ghasemnezhad et al., 2017).

2.1.4. Emulsification

It is a technique that has been implemented in the food industry to optimize the solubility, physiological activity, and stability of probiotics (Rodrigues et al., 2020). It consists of the relationship between a discontinuous phase (probiotic) and a continuous phase (oily) (Serna-Cock & Vallejo-Castillo, 2013). For this, it requires an emulsifier, which can prevent the spheres from being extruded before breaking the emulsion and decrease the surface tension between the oil and water interfaces; and also requires a surfactant agent to reduce the surface tension in the coating matrix to reduce the size of the spheres (Kwak, 2014). The size of the microparticles is highly dependent on the particle size of the internal phase, being directly proportional, as well as the agitation speed and the oil/water relationship, which influence, due to this, the size of the capsules is between 25 μm and 2 mm approximately (Martín et al., 2015). This technique is characterized by its low cost, high cell retention, small particle diameter, high encapsulation yield, and not requiring heat treatments (Holkem et al., 2017).

2.1.5. Electrospinning

The encapsulation technique, also known as electrospinning, emerged as an alternative to producing capsules on micro and nanoscale, with high relation between surface and volume, through the action of an external electric field applied between two electrodes and imposed on a polymer solution or melt, where the size of the capsules can be controlled through the flow velocities of the working fluids and the applied voltage (López-Rubio et al., 2012; Lim et al., 2019). This technique works through the base of a charged polymer jet that is extracted by applying an electrostatic force, and then the jet is extended in a straight line and exposed to an agitation

movement as it passes from a spinneret to the collector, finally collecting in the form of mats of ultra-thin non-woven fibers on the plate (Librán et al., 2017).

The success of the implementation of the technique lies both in the capsule obtained as well as in its effectiveness in protecting probiotics, which is generating high potential in the food industry for the development of new functional products because it does not require the use of temperature during processing, another advantage is that the mucoadhesive properties that can generate electrospun structures and electrospray structures, which can extend the residence time of probiotics in the GIT, and their interactions with the intestinal microbiome (Moreno et al., 2018; Sukumar et al., 2021).

2.2.Material of nano- and microencapsulation

The material used for the elaboration of the external and internal structure of the capsule must be carefully chosen because it plays a significant role in the viability of the probiotic, and it depends on it the protection of the encapsulated nucleus and, therefore, the greater the effectiveness of the capsule. Khan et al., (2013) and Deng et al. (2021) establish that compact microstructures present higher efficiency than dented, hollow, shrunk or thinner-walled microstructures. The latter has disadvantages regarding protectability and lower encapsulation efficiency and encapsulants with cracked porous microstructures or blowholes. Therefore, it is suggested to consider the properties of the encapsulant, the properties of the core material, it is loading, and the solidification conditions.

The probiotic bacteria are sensitive microorganisms that may be exposed to heat stress and other conditions in processing dairy products due to pasteurization, acidic media, mixing-pumping, freeze-thaw operations, and long-term refrigerated storage. Despite that, the probiotic strains must maintain their viability and functionality to ensure adequate protection; therefore, the structure, selection, and application of the capsule materials are essential and must be non-toxic and guarantee the stability of the particles, being a protective barrier for the cells and at the same time allowing a controlled release on passage through the TGI, taking into account that the type of material can be reflected in the final result of the capsules (Burgain et al., 2011; Rodrigues et al., 2020). Some researchers have worked with alginate, xanthan gum, gellan gum, starch, pectin, whey protein, chitosan, gelatin, and collagen (Frakolaki et al., 2021). Table 1 shows some studies in which probiotic viability was analyzed according to the type of encapsulating material.

2.2.1. Alginate

One of the most commonly used natural materials in probiotics is alginate (linear polymer derived from bacteria and algae). It is considered safe as it is non-toxic and suitable for encapsulation of bacterial strains due to the desired structural and biological properties, as the required gelling conditions are simple. Microcapsules made from this polymer can form calcium chloride matrices to retain sensitive materials, especially in living microbial cells, increase the survival of probiotic bacteria, including under acidic, bilious, heat, and storage conditions (Liu et al., 2018). The study of Afzaal et al. (2019a) evaluated the effect of alginate and carrageenan as wall materials on the microencapsulation of *Lactobacillus acidophilus ATCC 4356* in ice cream production and under simulated gastrointestinal conditions. The researchers established that encapsulation improved the survival of the probiotic in ice cream compared to non-encapsulated cells under cold storage and passage through a simulated TGI, where alginate microcapsules exhibited a better release profile carrageenan. The results were similar in traditional fermented yogurt samples containing immobilized *Lactobacillus casei*, where both wall materials were effective, but alginate showed a better release profile (Afzaal et al., 2019b)

Alginate is generally cross-linked with other polymers to mitigate its limitations since it has the disadvantage of high porosity, which causes the microspheres to rupture under acidic conditions (Khan et al., 2013). Another disadvantage of this polymer, when used by extrusion, is that capsules can present large size and poor shape due to its higher bioconversion rates, tending to favor smaller diameter capsules (Poncelet et al. 1995). So, it was suggested to encapsulate with other compounds, such as fatty acids, glycerol, or chitosan, as cryoprotectants to enhance the survival of probiotic bacteria by reducing acid diffusion and whey protein isolates (Amine et al., 2014). One such study was Han et al. (2020), who obtained a higher encapsulation efficiency with whey protein isolate in the coencapsulation of *Lactobacillus bulgaricus* and *Lactobacillus paracasei* using the internal/external gelation method. Respect to the structure was intensified with a regular morphology in the calcium alginate-coated microcapsules, improving the protection of probiotics in thermal and simulated gastrointestinal conditions since whey protein isolate can improve the buffering capacity of the environment and protect the probiotics from the adverse impact of the acidic environment. At the same time, alginate forms a continuous three-dimensional network, thermo-reversible and stable when it interacts with calcium, thus avoiding thermal shock to the probiotics (Rather et al.,2017). The efficacy of alginate was demonstrated in the study conducted

by Muthukumarasamy et al. (2006). They searched for the most suitable method and wall material for microencapsulation of the probiotic bacterium *Lactobacillus reuteri* to maintain cell viability during the gastric challenge. They found that alginate microencapsulation and alginate with starch formed regular spherical capsules by extrusion; this technique and the phase separation technique provided more excellent protection to the bacteria against simulated gastric juice.

Atraki & Azizkhani (2021) developed electrospun nanofibre mats using a combination of maize starch and sodium alginate to encapsulate probiotic strains of *Lactobacillus* (*L. acidophilus* (LA5) and *L. rhamnosus 23.527 LGG*) and B*ifidobactérias (B. bifidum* and *B. animalis)* to improve their survival in simulated gastrointestinal fluids. This study established that the viability of probiotic bacteria in simulated gastrointestinal conditions is significantly increased (81-100% of the initial population) through nanoencapsulation within processed nanofibre mats, the nanofibre mats produced from the mixture of sodium alginate and cornstarch showed a clean, basin-free structure with uniform size without probiotic charge, in contrast with the nanofibres with probiotic cells which presented a structure with basins increasing in size and diameter, the authors indicate that this is a promising approach to encapsulate probiotic bacteria in nanofibres. The results of the study are concordant with other works in the area (Škrlec et al., 2019; Yilmaz et al., 2020), where the incorporation of the probiotic in the nanofibres produces changes in the structure and the size of the diameter of the nanofibres, which can be attributed to the effect that the strain generates on the size of the fiber and to the materials used in the production of the nanofibres (Atraki & Azizkhani, 2021).

2.2.2. Starch

Starch is considered a natural biodegradable polymer, highly used for its biocompatibility, non-immunogenicity, low toxicity, and availability, which allows it to be a suitable material for the elaboration of micro and nanocapsules that protect probiotics against stomach acidity and improve their survival, being an industrial approach handy for the transport and administration of bacteria (Yang et al.,2015; Qi & Tester, 2019), which has been used as native starch (preserving its natural physical and chemical properties) obtained from different primary sources such as hylon starch, which has been studied, as well as wheat and corn starch, whose starch according to studies (Zanjani et al., 2018) allows increasing the firmness and integration of the structure in the microcapsules improving the viability and survival of probiotics, which can be attributed to the

cross-linked structure. The use of starches showed greater effectiveness in microencapsulation than nanoencapsulation since the latter has disadvantages when subjected to high temperatures, which is attributed to the size of the molecules (Ahmad et al., 2019).

Another type of starch used for the encapsulation of probiotics is the modified starch, which is given by altering the structure with or without chemical, physical, or enzymatic agents to obtain the desired properties for encapsulation in order to increase its resistance and gel-forming capacity, which allows to facilitate some types of encapsulation, such as electrospinning and spray drying (Zhu, 2017). Chemically modified starches have been successfully used for the encapsulation of *Bifidobacterium*, which improves both the survival of probiotics under stress conditions and the adverse effects on the sensory properties of fermented products due to the fragmentation of the starch during extrusion, the microcapsules containing phosphorylated starch obtained by this method present a smaller size structure and irregular forms compared to those containing phosphorylated starch by the conventional method, (Murúa-Pagola et al., 2020). Some studies have shown that capsules containing prebiotics such as resistant starch (Ashwar et al., 2021; Ta et al., 2021) tend to provide better protection against low pH conditions and bile salts, considering that they are those non-digested components, which as part of the starch molecule have the particularity to resist enzymatic digestion in the small intestine and reach the colon almost intact (Jiang et al., 2020).

2.2.3. Chitosan

It is a polysaccharide considered a versatile and bioadhesive natural polymer composed of glucosamine copolymers linked (1,4) with N-acetylglucosamine (a deacetylated derivative of chitin); in terms of its availability in nature, this polysaccharide is in second place only to cellulose, in the same form that it is one widely used polymers as wall material for the production of micro- and nanoencapsulation, thanks to its physicochemical characteristics that include positive charges through its amino groups, making it the only commercially available water-soluble cationic polymer, with low toxicity and non-allergenic, short-term biodegradability and biocompatibility with the human body, and antimicrobial actions and antifungal characteristics (Jang & Lee, 2008; Islam et al., 2017). Therefore, chitosan has a high potential for microencapsulation applied to the food industry positively influencing the survival rates and stability of different probiotic bacteria under storage conditions and in vitro gastrointestinal conditions (Ebrahimnezhad et al., 2017).

One of the many studies where the efficacy of chitosan for microencapsulation of probiotic bacteria was proven was realized by El-Abd et al. (2018), who prepared fermented camel milk in order to evaluate the viability of *Lactobacillus plantarum* and *Bifidobacterium animalis*, and the acceptability of the product stored at 4 °C for 21 days. They found that the chitosan-coated symbiotic microcapsules compared to alginate and free cells had higher survival rates after simulated exposure to gastric conditions in the small and large intestines. It also improved probiotic stability during processing, storage, and milk acceptability.

Chitosan is also considered a copolymer of glucosamine and N-acetylated glucosamine, which degrades quickly into simple metabolic sugars. It dissolves easily at low pH, so many researchers apply it in combination with another polymer that resists the low pH of the stomach. Accordingly, chitosan has been implemented for the targeted release of probiotics due to its high compatibility with living cells (De Vos et al., 2010). According to some studies (Cook et al., 2011; Shori, 2017), it has been shown that alginate-chitosan microspheres can open the tight junctions of epithelial cells to allow the absorption of many valuable materials into the host body, making it an ideal transport medium for bacteria, since microspheres made of alginate-chitosan compared to alginate reduced porosity, allowing a reduction in the leakage of probiotics and being stable at low pH. For these reasons, alginate and chitosan have been widely used to fabricate delivery systems. Following this, De Farias et al. (2019) verified the behavior of *Lactobacillus* (*L. rhamnosus* and *L. casei*) added to ice cream as free viable cells or encapsulated with algin-chitosan to compare low-temperature resistance, encapsulation efficiency, and capacity of cell survival in a simulated gastrointestinal environment. The study showed that microcapsules reduce cell loss under frozen storage and simulated gastrointestinal conditions. However, encapsulated *L. rhamnosus* had a more significant reduction in cell viability than its free form, establishing that the encapsulation process is not advantageous for all probiotic species.

2.2.4. Pectin

Pectin is a non-toxic and inexpensive polysaccharide associated with the cell wall and intercellular wall of plants and fruits, which is obtained from the esterification of high methoxylation pectin, being a commercial anionic oligosaccharide with acidic D-galacturonic units, forming a gel structure in the presence of divalent metal ions such as calcium, this being an essential property at

the moment of encapsulation of probiotics (Yan & Zhang, 2014; Yang et al., 2018), functioning as an emulsion stabilizer, gelling properties, and binding capacity. As alginate, pectin is used to encapsulate probiotic bacteria as wall material through extrusion, emulsification, nanoaspersion drying, nano complex formation, and coacervation (Barragán-Martínez et al., 2020; Raddatz et al., 2020).

When encapsulating probiotics, it is crucial to consider the degree of esterification (the percentage of the carboxyl group esterified with methanol) because this influences several properties of the plant pectin (Hosseini et al., 2016). According to the esterification degree, low methoxyl and high methoxyl pectin appear; the latter is considered more efficient for encapsulation because it can interact with hydrophobic molecules, allow better incorporation of the nucleus in the matrix, providing a controlled release, besides the high molecular weight and high gelling power provide tiny microparticles (Cacicedo et al., 2018), giving capsules that are more resistant to gastric and intestinal media. Similarly, it has been shown that thanks to its prebiotic properties, this polysaccharide, increases the growth of *Bifidobacterium* and *Lactobacillus sp* and identifies the characteristics linked to its functionality (Raddatz et al., 2020; Dantas et al., 2021).

2.2.5. Whey proteins

Whey proteins constitute approximately 15-20% of total milk proteins, where the major components are βlactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulins (IG) (Carvalho et al., 2013). These proteins are considered a suitable biomaterial for protecting cells against damage through stabilization of membrane components used for encapsulation of probiotics, especially *Streptococcus thermophillus* and *Lactobacillus bulgaricus*, as it increases resistance against acids and bile salts. In addition, whey proteins have diverse functional properties, such as emulsification, gelation, and foam-forming stability, making them an ideal material for microencapsulation of probiotics (Anandharamakrishnan et al., 2008; Abd El-Salam & El-Shibiny 2015). Whey proteins have been used alone or in combination with various polysaccharides in the microencapsulation of probiotics.

Whey proteins are commonly used to produce probiotic capsules because of their high efficiency and high stability during storage, unlike other materials that may cause decreased release rates due to interaction with the encapsulated matrix (Pérez-Masiá et al., 2015). It has also been established in some studies (Su et al., 2018; Yasmin et al., 2019) that whey proteins have a large number of

specific amino acid residues that allow them to be more effective in their protective function, which demonstrated that whey proteins are a suitable and competent wall material due to their buffering capacity and low pH tolerance.

3. Application of nano or microencapsulation in food from animal origin

3.1.Dairy products

Dairy products are considered one of the main matrices for incorporating probiotics in the food industry because they are one of the most used vehicles for ingesting these microorganisms (Kailasapathy, 2014; Murúa-Pagola et al., 2020). According to Gao et al. (2021), between 2001 and 2020, fermented milk and yogurts were the leading carriers of probiotics in dairy products. However, dairy foods present difficulties for probiotic incorporation because they are acidic, so pH, post-acidification during storage, oxygen concentration (permeation through the container), hydrogen peroxide production, and refrigerated storage temperatures alter the viability of microorganisms (Shori et al., 2019; Rodrigues et al., 2020). For these reasons, they cannot be an ideal medium to ensure the stability of probiotics. As a result, techniques have emerged to protect and ensure the viability of probiotics, including in situations or processes that prevent their functionality. Table 2 shows some works on the application of micro and nanoencapsulation of probiotics in dairy products.

The development of new dairy products has allowed the research field to conduct studies through the implementation of microencapsulation to promote the viability and functionality of probiotics, bringing positive changes to the physicochemical and sensory properties of dairy products, as is the case of the work realized by Ranadheera et al. (2015) who evaluated the effect of microencapsulation of *Lactobacillus acidophilus LA-5*, *Bifidobacterium lactis BB-12* and *P. jensenii 702* using spray-drying in goat milk, where the quality of the powder and the viability of the probiotics were measured after spray-drying and subsequent storage. The three probiotics maintained satisfactory levels of viability (6-7 log UFC/g) after spray drying. They established that the *Lactobacillus* and *propionibacteria* were not affected during storage at 4 °C, allowing viable cells to be established in probiotic foods. This was different from *Bifidobacterium lactis BB-12*, which showed the highest loss of viability.

For their part, Dimitrellou et al. (2016) performed the microencapsulation of *Lactobacillus casei ATCC 393* using the spray drying technique to be incorporated into fermented milk. They

established that adding the microcapsules into the fermented milk during production and storage did not generate significant alterations in the physicochemical characteristics since titratable acidity increased. pH decreased continuously during storage and at the same time allowed cell viability in simulated gastrointestinal conditions, which indicated that the treatment was effective. Also, the same Dimitrelloua et al. (2019) investigated the microencapsulation of the probiotic *Lactobacillus casei ATCC 393* in alginates, this time using extrusion, and evaluated the survival of the cells under simulated gastrointestinal conditions during the production of fermented milk and its storage at 4 °C for up to four weeks. The results showed that alginate had great potential as an encapsulation material for probiotics and established that applying encapsulated cells in fermented milk products generates practical cell survival during refrigerated storage of the product.

Some authors have attributed the viability of microencapsulated probiotics in dairy products to the protection provided by the high levels of total solids present in milk, as they concluded in work by Homayouni et al. (2008), who attributed the survival of the microencapsulated (calcium alginate) bacteria *Lactobacillus casei (Lc-01)* and *Bifidobacterium lactis (Bb-12)* in two types of symbiotic ice cream to the level of solids in the ice cream; including fat and milk solids, because the product increased in 30% the survival about the addition of the probiotics in the free state, with the same storage period at the same temperature.

During the production of dairy products and the research to innovate, it is essential to preserve or improve the characteristics of the product itself so that some so some studies, in addition to evaluating the viability of the microencapsulated probiotic, analyses are carried out about the technical characteristics of the products, which usually show positive changes in the final product, some of the cases are evident in the work of Pinto et al. (2019) which indicated better physical properties (lower moisture, solubility, and hygroscopicity values and higher density), increased pH, firmness and adhesion of the lactose-free Greek-style yogurt, due to the incorporation of *Bifidobacterium lactis BB-12* microcapsules, which had good survival, which allowed establishing that the food matrix was ideal for the study.

Another study that demonstrated probiotic survival, microencapsulation of probiotics and that encapsulation could bring about technological improvements was that of Lopes et al. (2021) in the production of spreadable goat ricotta, which showed technological improvements (no moisture loss, less proteolysis, and organic acid content), texture (less gumming and adhesiveness), and

volatiles (compounds with floral and fruity notes and less goat aroma) and were able to conclude that microencapsulation improved the probiotic survival and quality parameters of the product. Likewise, Ningtyas et al. (2019) found that the addition of encapsulated probiotic led cream cheese they elaborated presented more firmness and thickness compared to the non-encapsulated form, which allowed them to observe that the probiotic *Lactobacillus rhamnosus* alters the texture of this product, but does not significantly change the pH, moisture, fat content, and protein, allowing the encapsulation to maintain viable cells with less reduction during 35 days of product storage.

Due to the innovation that has been presented in the elaboration of dairy products, Kavas et al. (2021) made white goat cheeses with symbiotic microcapsules, which contain probiotic microorganisms (*Lactobacillus casei* and *Bifidobacterium longum*) plus prebiotic (inulin, fructooligosaccharide), to examine the changes of volatile aromatic substances during production and storage, and to determine the effect of the symbiotic microencapsulation method on these changes, who found that most of the aromatic compounds determined during storage in cheeses are alcohols and acids, which increase in quantity about the use of probiotic supplement culture, which allowed them to establish that the addition of prebiotics and probiotics through the technique of microencapsulation by extrusion does not affect the formation of these compounds, allowing to have greater viability of probiotic bacteria after 180 days of storage.

However, the cell damages resulting from microencapsulation processes, such as spray drying, remain a significant drawback, and there are studies in which it has been more perceptible, such as that of Picot & Lacroix (2004) which mixed bifidobacterial cultures with whey protein solutions heat-treated at 40 °C, and then dried these mixtures by spraying to produce water-insoluble microcapsules, the application of high temperatures and the dehydration process resulted in bacterial losses. The microcapsules based on milk protein helped increase survival rates during yogurt storage and gastrointestinal transit compared to free cells. Coinciding with the work carried out by Eckert et al. (2017) which proposes the use of whey, whey permeate, and whey retentate as suitable wall materials for the spray microencapsulation process of *Lactobacillus plantarum ATCC 8014* because it protects the probiotic bacteria from high temperatures during the microencapsulation process, such capsules were evaluated in milk stored at 4°C for 42 days, showed cell counts above the minimum value for functional foods, as when exposed to conditions simulating TGI, in the latter the whey retentate showed the best survivability than the permeate.

Like microencapsulation, nanoencapsulation has become very popular in dairy products due to its effectiveness in protecting probiotics, as demonstrated in their study by Yilmaz et al., 2020, who measured the viability of *Lactobacillus paracasei KS-199* encapsulated inside alginate-based electric nanofiber mats, the authors found that nanoencapsulation of the strain allows for both improved survivals in simulated gastric juice and improved survival in kefir. . Where, inoculation of kefir with the encapsulated strain did not alter the rheology of the product, as the characteristic pseudoplastic flow behavior and viscoelastic nature were not affected. Based on the results and the material implemented for nanoencapsulation, the authors recommend alginate as an important biopolymer to achieve higher viability in encapsulated probiotics. For their part, Ghorbani & Maryam (2021) conducted a study to improve the survival and viability of some probiotic strains of lactic acid bacteria (LAB) and bifidobacteria nano encapsulated on cornstarch and sodium alginate nanofiber mats by electrospinning method, where yogurt was used as the food matrix. It was found that nanofibers have a higher protective effect on LAB compared to *bifidobacteria* in an acidic environment; in samples tested at 55, 60, and 65 °C, respectively, the counts of the probiotic strains inside the nanofiber mats were lower than the loss of free cells, directly proportional to the data obtained after 20 days of yogurt storage.

3.2. Meat products

Although dairy products are commonly recognized as food vehicles for probiotic delivery, the meat products were not left behind, as studies have demonstrated that this is a relevant matrix, especially fermented meat products, for the delivery of probiotics with potential health benefits (Burgain et al., 2011; Kailasapathy, 2014; Sirini et al., 2021). Compared to the dairy industry, little research has been done on the encapsulation of probiotics in the production of meat products, and studies have been developed to produce functional and modern products (Behera & Panda, 2020). This is taking into account that meat and meat products are sources of high biological value proteins and essential micronutrients such as vitamin B12, iron, and zinc, in addition to having considerable amounts of monounsaturated and polyunsaturated fatty acids, which make them essential foods in human nutrition and part of the diet of consumers in most parts of the world. Despite its benefits, it poses a health risk to red and processed meat consumers, as excessive consumption can lead to disease, primarily type 2 diabetes and colorectal cancer (Ekmekcioglu et al., 2018). Therefore, producers and researchers of processed meat are searching for new alternatives to mitigate or eliminate potentially toxic compounds and nutritional deficiencies in products and innovate and

develop healthier products without affecting the sensory quality and safety of meat products (Ursachi et al., 2020).

The attempt to generate meat products with better properties that allow them to be functional foods, through the addition of probiotic bacteria, has been established as a helpful tool for the development of healthy products, in which it must be taken into account that each probiotic bacterium has specific properties about morphology, physiology, and technological effects, so it is essential to conduct preliminary studies that allow establishing the characteristics of each microorganism, which would allow determining which bacteria are more appropriate for use in the industrial production of a fermented meat product specific (Šipailienė & Petraitytė, 2018). Accordingly, Song et al. (2018) investigated the use of the probiotic *Bifidobacterium longum KACC* 91563 microencapsulated to be compared with a commercial starter culture containing *Staphylococcus carnosus* and *Lactobacillus sakei* in the production of functional fermented sausages, and these authors found that the products inoculated with encapsulated *Bifidobacterium longum* presented the highest level of cell viability, total unsaturated fatty acid content and fatty acids, while lipid oxidation levels were the lowest; therefore microencapsulation of *Bifidobacterium longum* in the meat mixture could be used for the production of healthier fermented meat product.

Probiotics are used mainly in this meat industry because fermented products have conditions that favor and improve their survival rate due to the protective effect that lipids have on bacterial cells and can be strengthened with the implementation of new technologies that have been used as the encapsulation of probiotics used mainly in fermented sausages, taking into account that these are consumed without cooking, being ready-to-eat products, which allows them to be an efficient means of transport for the cellular viability of microorganisms; therefore they are a valuable strategy to preserve the quality of meat products, meet the growing demand for practicality, commodity and with beneficial properties for the health of the consume (Tripath & Girir, 2014; Cavalheiro et al., 2015).

According to the above, it can be indicated that probiotic encapsulation is an alternative that is gaining increasing strength in the industry and the research field for the elaboration of conventional meat products (Table 3), where the intrinsic characteristics of the meat food and probiotic strains must be taken into account, as well as the appropriate encapsulation approaches and food processing conditions (pH, salt and additives) that could limit the growth of the probiotic strain, in

addition to microbiological and matrix-specific parameters (Sirini et al., 2021). Considering that probiotic microorganisms can act as cytoprotective agents due to their capacity, they present when generating high yields of bacteriocins, proven in studies after the high resistance caused by the significant reduction of pseudomonas bacteria, staphylococcus, and enterobacteria, thus increasing the resistance to spoilage and shelf life, in dry fermented probiotic sausages, with the addition of *Lactobacillus casei ATCC 393*, immobilized, in wheat grains (Sidira et al., 2014), where the practice of addition and microencapsulation has allowed protecting probiotics such as *Lactobacillus reuteri* which is used for the preservation of products, especially in fermented foods and can be added in free form and microencapsulated as a co-culture allowing to obtain a reduction in the number of E*scherichia coli* in the processing during manufacturing and increase the shelf life of dry fermented sausages. (El-Ziney et al., 1999; Muthukumarasamy & Holley, 2007).

Another product in which the effectiveness of microencapsulation was evaluated was chorizo (Spanish dry fermented sausage), in which extrusion and emulsion techniques were used for the microencapsulation of *Lactobacillus plantarum* made by Cavalheiro et al. (2019). They found that the addition of probiotics to alginate beads produced by extrusion resulted in higher lactic acid bacteria counts and was the most effective strategy. In addition, the physicochemical characteristics of the meat products were considered adequate during the entire maturation, with lower *Enterobacteriaceae* counts. This extrusion technique and alginate were also employed by De Souza et al. (2015) for the microencapsulation of *Lactobacillus curvatus* (lactic acid bacterium with anti-*Listeria* activity), whose functionality was evaluated on a salami. The authors found that 2 log UFC/g and 1.5 log UFC/greductions in pathogen count were generated after 10 and 20 days of storage of the product, respectively; therefore, they consider it necessary to apply these bacteriocins to improve salami safety.

Pérez-Chabela et al. (2013) suggest encapsulation using the spray-drying technique as a means of protection of the bacteria during the emulsification process of cooked meat products since they determined the effect of inoculation of LAB and *Enterobacteriaceae* during eight days of storage at 4 °C, which allowed them to identify the survival of bacteria before, during and after processing. Thus, the particles obtained can be used in other emulsified cooked meat products as bioprotective cultures to improve the microbial safety of these products because the count of *Enterobacteriaceae*

decreased during storage, without significant changes in the textural or physicochemical properties of this type of food, improving the nutritional value.

Cavalheiro et al. (2020) evaluated the effect of *Lactobacillus plantarum* encapsulated as a probiotic in alginate beads contained in dried sausages during 60 days of refrigerated storage and found an increased number of live bacteria (8,34 log UFC/g) and a lower level of lipid oxidation (0,602 mg MDA/kg) compared with the addition of free *Lactobacillus plantarum* cells in the sausage samples (8,02 log UFC/g, 0,625 mg MDA/kg), which allowed them to establish that the strategy of encapsulating the probiotic appears to be the best strategy for delivering the probiotic during the refrigerated storage of dry fermented sausages. The same food matrix was evaluated by Cavalheiro et al. (2021) in the inoculation of *Enterococcus faecium CECT 410* on the maturation and storage of the dry fermented sausages, which did not affect the growth and allowed them to protect the microorganisms during the maturation period; similarly, the addition of the probiotic granules showed increased protein and fat but lower moisture content in the sausages, with minimal effect on the quality of fermented pork sausage, except for the texture (the probiotic sausages were harder than the non-inoculated samples), indicating that the addition of *E. faecium CECT 410*, was influential in the production of dry fermented probiotic sausages.

However, Mrkonjic Fuka et al. (2020) when conducted their study on the survival rate and efficiency of encapsulated and non-encapsulated native starter cultures (two indigenous strains of *Lactobacillus sakei* and one of *Leuconostoc mesenteroide*) for the standardization of artisanal game meat sausage production, who indicated that no clear and positive effect of encapsulation was observed on bacterial viability and sausage quality, so they do not recommend encapsulation for these strains applied, since neither the viability of the multiple starter cultures applied did not improve the quality of the sausages compared to the non-encapsulated counterpart.

3.3.Fish products

Fish products have been characterized as an essential food source due to their high nutritional value, which has been studied to make a fortified and novel product by adding probiotic bacteria to fish. However, very few studies have been developed, so there is a lack of knowledge about the ideal techniques; therefore, current studies seek to optimize microencapsulation techniques. Some studies have been conducted to provide information about the viability of encapsulated probiotics incorporated in different meat products, such as the study to evaluate the viability of probiotics in

cooking fish burgers containing *Lactobacillus rhamnosus GG* microcapsules, a work conducted by Angiolillo et al. (2017) using the water-in-oil emulsion technique to prepare the capsules, which demonstrated that appropriate microencapsulation conditions, together with an appropriate concentration of the microencapsulated bacteria in the fish formulation, would allow the production of probiotic-enriched burgers, with a good appreciation of microbiological and sensory conditions and a longer shelf life.

The meat industry has also implemented the use of nanoencapsulation through the use of biomaterials, mainly in fish meat. According to the research by Ceylan et al. (2016), it was observed that the nanofibers provided a larger contact area on the fillet surface compared to the micro- or macro-sized material. Ceylan et al. (2018) performed nanoencapsulation of *Lactobacillus rhamnosus* using polyvinyl alcohol and sodium alginate nanofibers to ensure the microbial stability of fish fillets; this study showed that the rapid growth of total aerobic mesophilic bacteria (TMABc) and psychrophilic bacteria (TPBc) could be retarded by the application of probiotic nanocapsules. Therefore, this is a natural and renovating technique, and the authors suggest using nanofibers of 60 to 580 nm for use in different types of food materials stored in cold conditions.

Another probiotic used to evaluate the functionality and success of nanoencapsulation development in nanofibers was *Lactobacillus reuteri E81*, whose nanocapsules were applied by Ceylan et al. (2019) on the surface of fish fillets, obtaining a significant increase in antioxidant characteristics and enhancing probiotic vitality; therefore nanoencapsulation of *Lactobacillus reuteri* in nanofibers is suggested as an innovative approach to obtaining functional fish fillets. Since seafood products could be preserved for an extended period through the use of electrospun chitosan-based nanoscale materials, as they perform a fundamental role in limiting microbiological deterioration, the physical and chemical deterioration of these products has functional and structural advantages, in addition to providing high encapsulation efficiency, a high surface-to-volume ratio, and good stability of the encapsulated bioactive compounds, therefore, the application of nanotechnology-related to chitosan-based nanomaterials provides a low-cost method for seafood products (Ceylan et al., 2020).

4. Implications on sensory characteristics from foods of animal origin

In the innovation of dairy products with the addition of microencapsulated probiotics, studies have been presented in which the incidence of the capsules on the sensory properties of these products

is investigated. One of them was the study by Dimitrellou et al. (2016), where samples of fermented milk with microcapsules of *Lactobacillus casei,* control samples, and similar commercial products were compared. They found that the fermented milk with microencapsulated *Lactobacillus casei* had a higher Smoothness value than the commercial product. However, no significant differences were observed in other sensory attributes evaluated (color, sweet odor, sourness, sweetness, viscosity, aftertaste, and overall acceptability). Similarly, it has been observed that the appearance and color of dairy products, especially fermented milk with probiotics, are not affected by the addition of capsules, according to Dimitrellou et al. (2019), can be attributed to materials such as sodium alginate (as an encapsulating wall) in dairy products that do not affect the aspect and color. However, Kailasapathy (2006) reported that such material could influence the smoothness of fermented milk and yogurts by increasing the granularity of the clot by replacing sodium with calcium ions.

Likewise, Dimitrellou et al. (2019) highlighted the importance of capsule size, given that the products with capsule diameters of 587.46 ± 58.66 μm presented a good texture since these have a significant influence when evaluating the mouthfeel attributes because the smaller the size of the capsule, it presents a smoother texture (Kailasapathy, 2006). This could be observed in the research of Ortakci & Sert (2012), who made capsules of approximately 2 mm in diameter, which generated an undesirable texture in the yogurt in which it was incorporated, so they recommended using micro and nano-sized capsules in this type of beverage. The same situation was presented in the work of Kavas et al. (2021) when evaluating the sensory effect of white goat cheeses with the addition of microencapsulated *Lactobacillus casei* and *Bifidobacterium longum*, finding that the microcapsules were perceived on the tongue at the time of sensory testing, however, in this case, it was not a nuisance for the evaluators, because the structure of the capsules was gelatinous. Also, these provided better sensory characteristics and more significant appreciation in terms of mass and structure in the dry matter without generating alterations in the flavor and odor characteristic of white cheeses. All of the above has allowed improving and preserving the sensory properties of dairy products, allowing them to have a great potential for acceptance.

The sensory evaluation of a product can vary according to the ingredients and the technological processes to which a food may be subjected, which can be seen evidenced in the elaboration of chocolate milk by Ghasemnezhad et al. (2017) who incorporated microcapsules of *Lactobacillus*

casei and *Bifidobacterium Lactis*, using sodium alginate, resistant starch and chitosan for the elaboration of the capsules (in different combinations), this by the extrusion method, where they could see the effect on their sensory acceptability for 21 days, where they could find that the microcapsules did not alter the color of the samples, which was attributed to the fact that the wall materials were colorless compounds. However, the technique used affected the score when evaluating the texture since the capsules had a size between 300-500 μm, lower in acceptability than the samples containing free bacteria. In general, the highest acceptability was obtained in *Bifidobacterium Lactis* microencapsulated with sodium alginate and chitosan. Murúa-Pagola et al. (2020), in their work, proposed the use of modified starch by spray drying as a wall material in microencapsulation to reduce the adverse effects on the sensory properties of the final products due to overfermentation.

As with dairy products, meat products can also change due to incorporating capsules, which makes sensory parameters a critical point when unwrapping a product since they are the main factors responsible for acceptance. Therefore, when working with food matrices, the aim is to know the impact on people's palates, considering attributes such as flavor, odor, color, texture, appearance, and general acceptance. Based on these attributes, De Oliveira et al. (2021) established after microencapsulation of *Bifidobacterium BB-12* in Italian salami that the addition of the capsules did not alter the characteristic sensory attributes compared to commercial products, allowing to obtain good acceptability, according to the high percentage in the purchase intention of this salami.

Sensory analyses in meat products have been fundamental in determining the quality of products, which can also allow the identification of possible microbial growth, thanks to changes mainly in texture, color, flavor, and aroma, where probiotics can act as protective agents, in turn producing positive sensory alterations, mainly in fermented meat products, when this process takes place at higher temperatures. What has been evidenced in Turhan et al. (2017) research, which evaluated the effect of microencapsulated *Lactobacillus rhamnosus* and storage period on aroma and other quality characteristics of sucuk (dry fermented Turkish sausage). It was found that the storage period influences the sensory quality of the sucuk samples, given that after 6 months, the color of the external surface of the slices and the unpleasant odor of the sucuk decreased, confirming that the production of this sausage with microencapsulated probiotic cultures could be similar to the sensory properties of the traditional product, concluding that the addition of probiotics could

promote health and protective benefits associated with lactic acid bacteria and contribute to increased consumption of these products.

Angiolillo et al. (2017) also evaluated the sensory quality in fish burgers (*Dicentrarchus labrax*) fortified with *Lactobacillus rhamnosus GG* in gelatin microcapsules added to 10% and 32% (w/w) microcapsules in an uncooked and cooked fish burger, evaluating the attributes of color, odor, appearance, and texture. They found that cooked hamburger samples containing 32% microcapsules were affected due to the large number of microcapsules added to the product. The hamburger with the lowest concentration of capsules was the most appreciated since it improved the final consistency of the cooked product and favored juiciness, being less dry due to the gelatin consistency of the microcapsules.

5. Perspectives and recent innovations of micro or nanoencapsulation in food of animal origin

Currently, micro and nanoencapsulation continue to be studied for the protection of probiotic bacteria due to the effectiveness that they exercise. For this reason, some studies are being developed to mitigate the technical problems presented by the morphology of the capsules and the greater probiotic viability, which generates greater satisfaction to the needs of the different food matrices, as has been the case of dairy and meat products. Allowing innovation and implementation of new technologies such as electropolymerization and electrospinning, whose technologies have been used since they allow obtaining capsules of smaller sizes and during processing do not require extreme temperature conditions, do not require emulsion preparation for microencapsulation, and are not exposed to chemical solvents, which has led to the employ in development of functional foods that incorporate probiotics with greater viability and stability (Librán et al., 2017).

Advances in microencapsulation have allowed the development of new techniques such as electrohydrodynamics using electrohydrodynamic atomization or electrical spraying, a gentle encapsulation process that allows highly viscous solutions to form micrometer-sized capsules (Xie et al., 2015). It has been used in dairy and meat products to improve the cell viability of probiotics and obtain a smaller encapsulation size (Atraki & Azizkhani, 2021; Ceylan et al., 2020; Yilmaz et al., 2020).

Due to the multiple challenges that still arise when working with the microencapsulation of probiotics to improve the viability rate in the TGI, many studies have been developed with novel

alternatives to improve the technique of encapsulation of microorganisms further in the case of co-encapsulation. This has generated growth in the application of fibers and prebiotics to generate a symbiotic effect that can help further the growth and multiplication of probiotics and, in turn, becomes a novel approach for the development of potential functional foods (Apiwattanasiri et al., 2022). With the appearance of new techniques for micro- and nanoencapsulation, the demand for wall materials has also increased. However, scarce research has been done in this area, in which the use of emerging polysaccharides with preponderant properties that may be suitable for the encapsulation of bacteria, such as chemically modified cellulose, new exopolysaccharides, natural polysaccharides obtained from food processing wastes, among others, that allow increasing the area of application of micro- and nanocapsules, and the range of selection of wall material presenting properties such as biocompatibility, biodegradability and suitable mechanical properties (Liu et al., 2020; Rodrigues et al., 2020).

Barragán-Martínez et al. (2019) analyzed the effects of different agro-industrial co-products, cactus peel flour or apple pomace flour, as prebiotic co-encapsulants in the microencapsulation of thermotolerant probiotic lactic acid bacteria, *E. faecium AM1* and *P. pentosaceus UAM2*. It was found that the use of co-products is a good alternative for improving the nutritional properties of cooked meat products with minor changes in color and texture (greater cohesiveness); in terms of the lactic bacteria count was higher, including the samples containing inulin, inhibiting pathogens such as coliforms; the oxidative rancidity of the lipids decreased for storage, due to the natural antioxidants of the prebiotics. Combining these with lactic acid bacteria is a good alternative for formulating symbiotic functional ingredients that improve the nutritional properties of cooked emulsified meat products.

Zaeim et al. (2020) realized calcium alginate and chitosan microcapsules through electrohydrodynamic processing to be incorporated into an ice cream formulation. For which *Lactobacillus plantarum PTCC1896* was co-encapsulated with inulin or starch in Ca alginate/chitosan, finding that this technique allowed the creation of complex microstructures from highly viscous feed solutions, where polysaccharide matrices significantly improved the viability of probiotics during storage, especially at room temperature. Here, microcapsules containing inulin improved the survival of *Lactobacillus plantarum* during storage more efficiently than those containing starch, the latter prebiotic improving cell survival once the microcapsules were

incorporated into the ice cream. The capsules were micrometer-sized spherical capsules loaded with a high concentration of probiotics and prebiotics and could be used as an ingredient for functional foods.

6. Conclusion

The encapsulation of probiotics continues to be a challenge for the food industry and researchers where efforts are made to improve the primary function of the bacteria through micro and nanocapsules, facing multiple challenges that lead to innovative technologies to ensure the functionality of probiotics in the GIT, without drastically affecting the physicochemical and sensory properties of the food matrix to which it is added. According to this, it has been shown that probiotic nanoencapsulation has significant limitations compared to microencapsulation due to the influence of the size of the bacteria at the time of forming the nanocapsules and the conditions to which the probiotic is subjected to achieve an ideal encapsulation with high viability. Due to the review of different works, the growing use of probiotic nanocapsules in meat products can be evidenced through electrospun and microcapsules in dairy foods using spray drying and alginate as capsule material, alone or in company with other materials to improve the capsule structure. The micro and nanoencapsulation of probiotic bacteria have shown to be extensive and, at the same time unfinished path, given that there is still a lack of studies in which the probiotic behavior and the interaction of different polymeric materials for encapsulation in meat products are evaluated in-depth, taking into account the wide range that is currently available in the market.

Acknowledgments

The authors are thankful for the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil — grant [303074/2021-3]. Katherine Gutiérrez-Alzate was financed by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

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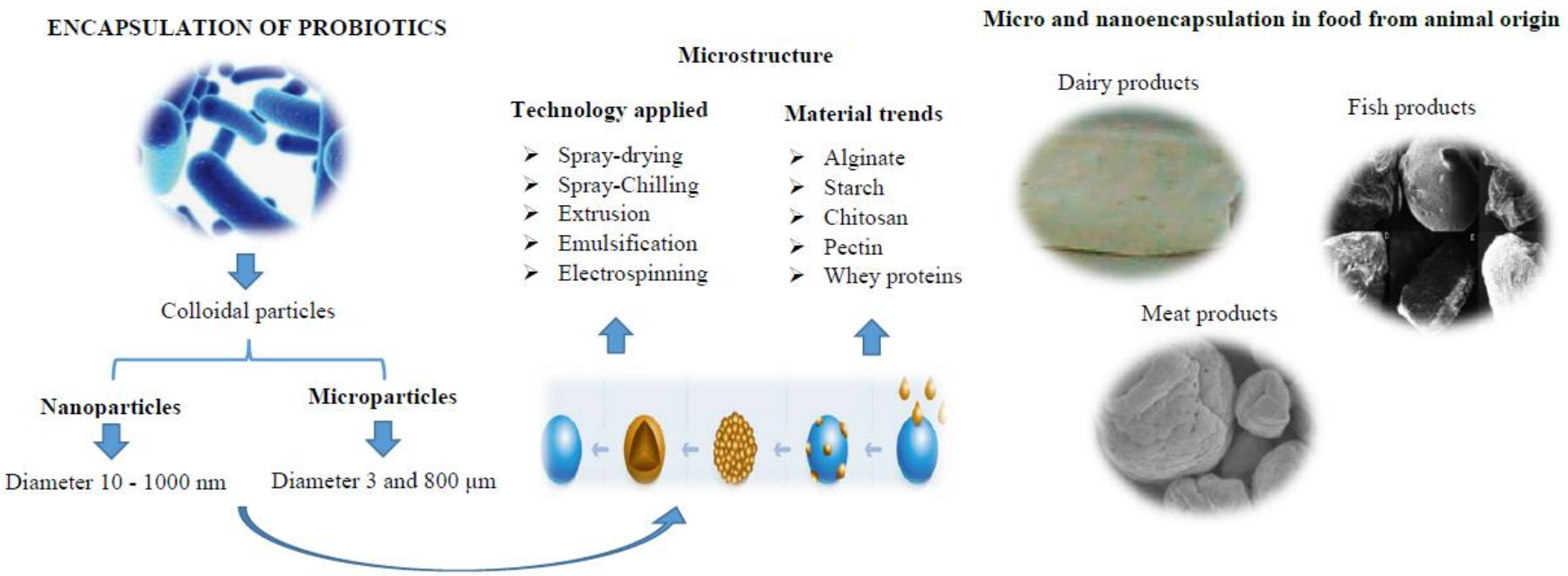

Figure 1. Graphical abstract

Table 1. Wall materials used in micro - and nanoencapsulation of probiotics.

Wall materials	Probiotics	Simulation	Results	Author
Alginate and Chitosan	*Lactobacillus. plantarum* and *Bifidobacterium animalis*	Fermented camel milk and SGC	Maximum survival bacterial counts were found in symbiotic chitosan coated beads containing 1% ginger or 10% beet root aqueous extract	El-Abd et al., 2018
Alginate and carrageenan	*Lactobacillus acidophilus ATCC 4356*	Ice cream and SGC	Alginate showed a better release profile than carrageenan.	Afzaal et al., 2019a-
Alginate and carrageenan	*Lactobacillus casei*	Yogurt and SGC		Afzaal et al., 2019b
Calcium alginate-chitosan	*Lactobacillus rhamnosus ASCC 290* and *Lactobacillus casei ATCC 334*	Ice cream and SGC	Alginate-chitosan is more efficient in preserving microencapsulated *L. Rhamnosus ASCC 290* at below −18 °C	De Farias et al., 2019
Alginate and coencapsulation with protein isolate	*Lactobacillus Bulgaricus* and *L. Paracasei*	SGC	Regular morphology in calcium alginate coated microcapsules and improved probiotic protection.	Han et al., 2020
Corn starch and sodium alginate	*Lactobacillus Bifidobactérias*	SGC	Nanofibers mats with clean, basin-free structure with uniform size without probiotic charge.	Atraki & Azizkhani, 2021
Chemically cross-linked starches	*Bifidobacterium breve ATCC 1570*	Yogurt and SGC	Smaller size structure and irregular shapes compared to those containing phosphorylated starch by the conventional method.	Murúa-Pagola et al., 2020
Pectin	*Lactobacillus acidophilus LA-5*	SGC	pectin microcapsules containing the prebiotics hi-maize, inulin and rice bran increased the survival of the microorganism	Raddatz et al., 2020
Whey protein concentrate and pectin	*Bifidobacterium longum BL-05*	Simulated intestinal fluid and SGC	Improves encapsulation efficiency and sphericity of beads with the highest value for hardness, cohesiveness, springiness, and resilience.	Yasmin et al., 2019

SGC simulated gastrointestinal conditions

Table 2. Application of micro - and nanoencapsulation in dairy products.

Product Technique	Probiotics	Results	Author
Ice cream Extrusion	*Lactobacillus casei (Lc-01) and B. lactis (Bb-12*	Encapsulation can significantly increase the survival rate of probiotic bacteria in ice cream over an extended shelf-life	Homayouni et al., (2008)
Goat´s milk Spray-drying	*Lactobacillus acidophilus LA-5, Bifidobacterium animalis subsp. lactis BB-12* and *Propionibacterium jensenii 702*	Spray drying process resulted in a significant viability loss, encapsulated *L. Acidophilus LA-5* and *P. Jensenii 702* were able to maintain a satisfactory viability ($\sim 10^7$ cfu/g) during storage.	Ranadheera et al., (2015)
Fermented milk Spray-drying	*Lactobacillus casei ATCC 393*	No significant alterations in the physicochemical characteristics, and at the same time allowed cell viability in simulated gastrointestinal conditions.	Dimitrellou et al., (2016)
Fermented milk Extrusion	*Lactobacillus casei ATCC 393*	Application of encapsulated cells in fermented dairy products generates an effective maintenance of cell survival during refrigerated storage of the product.	Dimitrelloua et al., (2019)
cream cheese Extrusion	*Lactobacillus rhamnosus*	The addition of microencapsulated probiotic generated greater firmness and thickness in the cream cheese compared to the non-encapsulated form	Ningtyas et al., (2019)
Lactose-free greek-style yogurt Spray-drying	*Bifidobacteria lactis BB-12*	Indicated better physical properties (lower moisture, solubility and hygroscopicity values and higher density), increased ph, firmness and adhesion.	Pinto et al., (2019)
Kefir Electrospinning	*Lactobacillus paracasei KS-199*	Nanoencapsulation of the strain allowed increased survival in simulated gastric juice and kefir.	Yilmaz et al., (2020)
Yogurt Electrospinning	Bifidobacteria and LAB	Nanofibers had a greater protective effect on LAB compared to bifidobacteria in an acidic environment	Ghorbani & Maryam (2021)
Goat cheeses Extrusion	*Lactobacillus casei* and *Bifidobacterium longum*	Microcapsules improved the viability of probiotic bacteria during 180 days of storage and did not affect the formation of aromatic compounds.	Kavas et al., (2021)
Spreadable goat ricotta Extrusion	*Lactobacillus acidophilus La-05*	Technological improvements (no moisture loss, less proteolysis and organic acid content), texture (less gumming and adhesiveness), and volatiles (compounds with floral and fruity notes and less goat aroma).	Lopes et al., (2021)

Table 3. Application of micro - and nanoencapsulation in meat products.

Product Technique	Probiotics	Results	Author
Salami Spray drying	*Bifidobacteria* and LAB	The reductions in pathogen counts in the product were generated after 10 and 20 days, respectively, so the application of these bacteriocins is considered important to improve the safety of salami.	De Souza et al. (2015)
Cooking fish burgers Extrusion	*Lactobacillus rhamnosus GG*	Adequate concentration of microencapsulated bacteria in the fish formulation would allow the production of probiotic-enriched patties with good microbiological and sensory conditions and longer shelf life	Angiolillo et al. (2017)
Fish fillets Electrospinning	*Lactobacillus rhamnosus*	Showed that the rapid growth of total aerobic mesophilic bacteria and psychrophilic bacteria can be retarded by the application of probiotic nanocapsules.	Ceylan et al. (2018)
Fermented sausages Extrusion	*Bifidobacteria longum KACC 91563*	The products presented the highest level of cell viability, total unsaturated fatty acid content and fatty acids, while lipid oxidation levels were the lowest.	Song et al., (2018)
Fermented sausage Extrusion	*Lactobacillus plantarum*	The addition of probiotics to alginate beads resulted in higher lactic acid bacteria counts and was the most effective strategy.	Cavalheiro et al., (2019)
Dry fermented sausages Extrusion	*Lactobacillus Plantarum*	Microcapsules increased number of live bacteria and lower level of lipid oxidation compared to the addition of free cells.	Cavalheiro et al. (2020)
Fish fillets Electrospinning	*Lactobacillus reuteri E81*	Significant increase in antioxidant characteristics and improved probiotic vitality.	Ceylan et al. (2019)
Artisanal game meat sausage Extrusion	*Lactobacillus sakei* and *Leuconostoc mesenteroide*	Microencapsulation prolonged the viability of *Leuconostoc mesenteroide*; this effect was not observed in the treatments in which *Lactobacillus sakei* was used alone or in combination.	Mrkonjic Fuka et al. (2020)
Dry fermented sausages Extrusion	*Enterococcus faecium CECT 410*	Increased protein and fat but lower moisture content in the sausages, with minimal effect on the quality of a fermented pork sausage, except for the texture (the probiotic sausages were harder than the non-inoculated samples).	Cavalheiro et al. (2021)