HERLÂNDIA COTRIM SANTOS

BEBIDA FERMENTADA SUSTENTÁVEL “TIPO IOGURTE GREGO” À BASE DE LEITELHO

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

Orientadora: Ana Clarissa dos Santos Pires

Coorientadores: Jaqueline de Paula Resende
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Ana Clarissa dos Santos Pires
Orientadora

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RESUMO

SANTOS, Herlândia Cotrim, M.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Bebida fermentada sustentável "tipo iogurte grego" à base de leitelho**. Orientadora: Ana Clarissa dos Santos Pires. Coorientadores: Jaqueline de Paula Rezende e Maximiliano Soares Pinto.

O leitelho é um coproduto da fabricação da manteiga que possui alto valor nutricional e funcional, entretanto, muitas vezes, é subutilizado ou descartado, especialmente em pequenos e médios laticínios. Assim, a aplicação deste coproduto em diferentes matrizes alimentares é uma forma de contribuir com a preservação do meio ambiente e reduzir gastos com o tratamento de efluentes pelas indústrias. Adicionalmente, é uma maneira de disponibilizar produtos de alto valor nutritivo e menor custo para diferentes nichos de consumidores. Nesse sentido, esse trabalho objetivou desenvolver formulações de bebidas lácteas fermentadas, "tipo iogurte grego", com baixo teor de gordura à base de leitelho. Foram elaboradas cinco formulações de bebidas lácteas fermentadas, contendo proporções diferentes de leite desnatado:leitelho (em %, m/m): F1-100:0; F2-75:25; F3-50:50; F4-25:75 e F5-0:100. Estas formulações foram submetidas às análises de composição centesimal, pH, sinérese, capacidade de retenção de água, microbiológicas, sensoriais e reológicas. Os resultados demonstraram que, em geral, a composição centesimal e o pH de todas as formulações foram muito semelhantes e apresentaram parâmetros microbiológicos dentro dos padrões estabelecidos pela legislação Brasileira (< 3 MPN/mL para coliformes e < 10 UFC/mL para fungos filamentosos e leveduras). Ao longo dos 16 dias de armazenamento, a presença do leitelho nas formulações provocou melhora na capacidade de retenção de água (CRA) quando comparada à formulação com 100% de leite. A formulação com 25% de leitelho foi a que teve melhor CRA (62,72%). Comportamento semelhante foi observado para a sinérese, onde houve uma tendência decrescente da sinérese para todas as formulações, sendo a formulação com 25% de leitelho apresentou o menor valor de sinérese (11,50%). Os parâmetros reológicos mostraram que as bebidas lácteas fermentadas apresentaram comportamento de fluido não newtoniano, com características pseudoplásticas. O modelo reológico que melhor se ajustou aos dados experimentais na última curva foi o de Herschel-Bulkley, com o $R^2 > 0,9$. Nas duas temperaturas avaliadas (5 e 25°C), a formulação com adição de 25% de leitelho apresentou os maiores valores de viscosidade (1,18 e 0,98, respectivamente). Além disso, observou-se que o aumento da concentração de leitelho favoreceu a redução da viscosidade das formulações. As formulações com leitelho como substituto parcial (até 50%) do leite desnatado foram bem aceitas pelos consumidores (impressão global > 7,47) e obteve boa intenção de compra, não diferindo (p>0,05) da formulação com 100% de leite. Assim, é possível utilizar o leitelho como substituto parcial do leite para o desenvolvimento de bebida láctea fermentada "tipo iogurte grego", levando à obtenção de um produto nutritivo e com boa aceitação sensorial, fortalecendo a bioeconomia.

Palavras-chave: Coproduto. Bebida sustentável. Produtos inovadores.

ABSTRACT

SANTOS, Herlândia Cotrim, M.Sc., Universidade Federal de Viçosa, February, 2023. **Sustainable fermented beverage “Greek yogurt type” based on buttermilk**. Adviser: Ana Clarissa dos Santos Pires. Co-advisers: Jaqueline de Paula Rezende and Maximiliano Soares Pinto.

Buttermilk is a co-product of butter manufacturing that has high nutritional and functional value, however, it is often underutilized or discarded, especially in small and medium dairy products. Thus, the application of this co-product in different food matrices is a way of contributing to the preservation of the environment and reducing costs with the treatment of effluents by industries. Additionally, it is a way to make products with high nutritional value and lower cost available to different consumer niches. In this sense, this work aimed to develop formulations of fermented dairy beverage, “Greek yogurt type”, with low-fat content based on buttermilk. Five formulations of fermented dairy beverage were prepared, containing different proportions of skim milk:buttermilk (in %, m/m): F1-100:0; F2-75:25; F3-50:50; F4-25:75 and F5-0:100. These formulations were submitted to centesimal composition, pH, syneresis, water-holding capacity, microbiological, sensorial and rheological analyses. The results showed that, in general, the centesimal composition and pH of all formulations were very similar and presented microbiological parameters within the standards established by Brazilian legislation (< 3 MPN/mL for coliforms and < 10 CFU/mL for filamentous fungi and yeast). Over the 16 days of storage, the presence of buttermilk in the formulations led to an improvement in the water-holding capacity (WHC), when compared to the formulation with 100% milk. The formulation with 25% buttermilk had the best WHC (62.72%). A similar behavior was observed for syneresis, where there was a decreasing trend of syneresis for all formulations, with the formulation with 25% buttermilk having the lowest syneresis value (11.50%). The rheological parameters showed that the fermented dairy beverage presented non-Newtonian fluid behavior, with pseudoplastic characteristics. The rheological model that best fitted the experimental data on the last curve was the Herschel-Bulkley model, with R^2 >0.9. At the two evaluated temperatures (5 and 25°C), the formulation with the addition of 25% buttermilk showed the highest viscosity values (1.18 and 0.98, respectively). In addition, it was observed that the increase in the buttermilk concentration favored the reduction of the viscosity of the formulations. Formulations with buttermilk as a partial substitute (up to 50%) for skim milk were well accepted by consumers (global impression > 7.47) and obtained good purchase intent, not differing ($p>0.05$) from the formulation with 100% milk. Thus, it is possible to use buttermilk as a partial milk substitute for the development of a fermented dairy beverage "Greek yogurt type", leading to obtaining a nutritious product with good sensory acceptance, strengthening the bioeconomy.

Keywords: Co-product. Sustainable beverage. Innovative products.

SUMÁRIO

1. INTRODUÇÃO GERAL

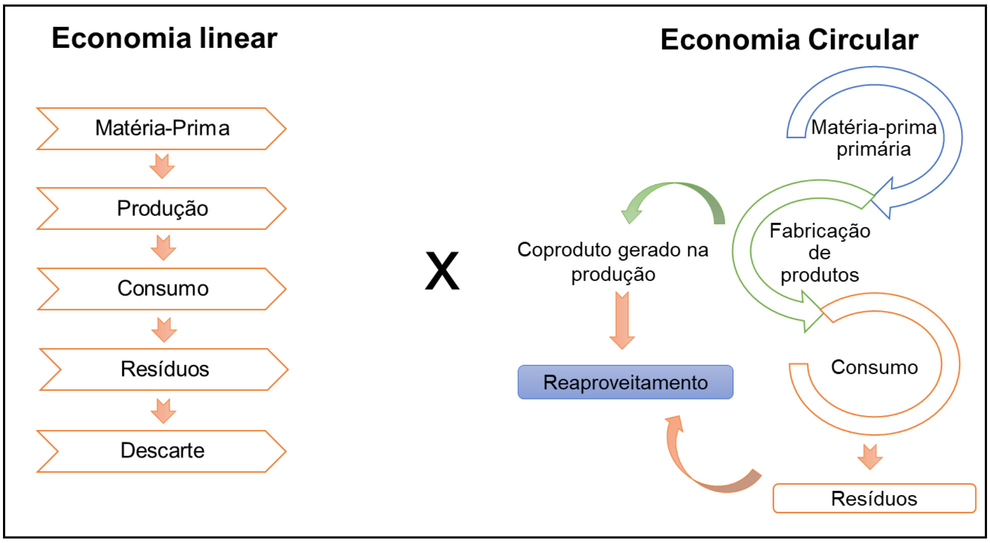
A indústria de laticínios gera uma grande quantidade de resíduos sólidos e líquidos, além de emitir gases poluentes para a atmosfera, impactando negativamente o meio ambiente. Por exemplo, para cada kg de leite processado, cerca de 2,0-2,5 L de água residual, contendo produtos químicos, gordura, entre outros, são gerados (ROTZ et al., 2021). Deste modo, a legislação ambiental exige que todas as empresas, independentemente do tamanho e potencial poluidor, tratem e disponham de forma adequada seus resíduos (SIVAPRAKASAM; BALAJI, 2021).

O custo ambiental da indústria de laticínios também decorre do consumo substancial de energia e refrigeração dos alimentos (AKINYEMI et al., 2021). Por exemplo, para produzir 1 kg de manteiga são necessários aproximadamente 5.500 L de água e 4 megajoules de energia. De forma adicional, para o funcionamento da indústria de laticínios a água é utilizada em outras diversas atividades, como na lavagem de carros, transporte de mercadorias, geração de vapor, uso de banheiros, lavagem e desinfecção de superfícies. Devido a grande quantidade de processos de aquecimento e resfriamento, a indústria de laticínios utiliza uma quantidade significativa de energia. As duas principais fontes de energia na indústria de laticínios são a eletricidade e o gás natural, que juntos representam 85% do consumo de energia (RAD; LEWIS, 2014).

Como o leite e seus derivados são considerados alimentos de elevado valor nutricional, importantes para a manutenção da saúde humana, seu consumo vem crescendo. No ano de 2020, houve um aumento de 5,3% no consumo de leite e seus derivados no Brasil (SIQUEIRA et al., 2021). Nos próximos anos, estima-se uma maior demanda por produtos lácteos no país, uma vez que, houve um crescimento da demanda nas nações em desenvolvimento, impulsionada pelo aumento da população e mudanças de estilo de vida (FAO, 2021). Portanto, o aumento do consumo e consequente produção de lácteos podem contribuir para agravar os problemas ambientais nos próximos anos (CHALERMTHAI et al., 2021).

Em vista disso, é de grande importância que as indústrias de laticínios invistam em sustentabilidade de forma a aproveitar ao máximo todos os recursos naturais utilizados e minimizar a geração de resíduos. É estratégico que a indústria de laticínios incorpore a economia circular (EC) (Figura 1) para a produção de lácteos, minimizando problemas ambientais, principalmente aqueles que impactam diretamente no desenvolvimento social e na saúde dos seres humanos.

Figura 1: Comparação esquemática entre a economia circular e a economia linear na indústria de laticínios.



Fonte: A autora

Desta forma, é essencial implementar mecanismos para induzir transformações industriais regenerativas, em substituição aos processos industriais lineares, possibilitando, assim, formas mais sustentáveis de produção e consumo (KIRCHHERR; REIKE; HEKKERT, 2017).

A fabricação de manteiga, por exemplo, gera a liberação do leitelho, um coproduto que, no Brasil, principalmente em pequenos e médios laticínios, não é plenamente aproveitado. Considerando-se que entre 2006 e 2017, o consumo nacional de manteiga aumentou 75% (FERREIRA; SIQUEIRA; STOCK, 2021), e que, de acordo com o Departamento de Agricultura dos Estados Unidos (USDA), em 2022, foram produzidas no Brasil 83 Ton de manteiga, a geração de leitelho também foi elevada, visto que, para cada kg de manteiga fabricada, uma quantidade similar de leitelho é produzido. Por se tratar de um coproduto rico em nutrientes, uma vez descartado sem tratamento, o leitelho é altamente poluidor (AHMAD et al., 2019). Por outro lado, mesmo que o descarte seja ambientalmente correto, há desperdício de constituintes importantes para a nutrição humana e impacto econômico para a indústria.

O leitelho corresponde à fase aquosa liberada durante a etapa de bateção do creme. Neste processo, ocorre a coalescência parcial dos glóbulos de gordura, resultando no rompimento da membrana do glóbulo de gordura do leite (MGGL).

Assim, o leitelho contém todos os componentes hidrossolúveis do creme, como proteínas, lactose, minerais, além de incluir o material da MGGL (ALI, 2019).

Os fosfolipídios advindos da MGGL proporcionam ao leitelho propriedades funcionais, atuando na redução da incidência de doenças como mal de Alzheimer (HORROCKS; FAROOQUI, 2004), câncer (VISSAC et al., 2002), estresse (MCDANIEL; MAIER; EINSTEIN, 2002), atividade bactericida e supressão de patógenos gastrointestinais (SPRONG; HULSTEIN; VAN DER MEER, 2002).

Além disso, os fosfolipídios são excelentes emulsificantes e espumantes naturais, devido à sua estrutura anfifílica (DEWETTINCK et al., 2008). Desta forma, o leitelho pode ser utilizado como ingrediente em diversos alimentos, como, por exemplo, em bebidas lácteas, sorvetes, produtos de panificação, dentre outros (GARCZEWSKA-MURZYN et al., 2022; MADENCI; BILGIÇLI, 2014; SZKOLNICKA; DMYTRÓW; MITUNIEWICZ-MAŁEK, 2020).

Garczewska-Murzyn et al. (2022) avaliaram a aplicação de leitelho em pó e leite em pó desnatado para padronizar o teor de matéria seca na produção de iogurte desnatado. Os resultados demonstraram que os perfis de ácidos graxos dos iogurtes enriquecidos com leite em pó desnatado e com leitelho em pó não diferiram entre si. Além disso, a adição do leitelho em pó não interferiu no processo de fermentação.

Bassi et al. (2012) demostraram que uma formulação de iogurte com adição de 30% de leitelho fluido na produção de iogurte apresentou resultados semelhantes para acidez, pH e viscosidade, em comparação com a formulação à base de leite. Além disso, houve boa aceitação sensorial. No entanto, as formulações foram adicionadas de purê de morango, e isso provavelmente contribuiu para a aceitação do produto. Nesta perspectiva, avaliar a incorporação de leitelho fluido em bebidas lácteas fermentadas sem adição de ingredientes aromatizantes é estratégico, pois visa a redução de gastos operacionais e conhecimento das características sensoriais que esse ingrediente proporciona ao produto desenvolvido.

Outro fator importante é a investigação do efeito da substituição de leite por leitelho no comportamento reológico de bebidas lácteas fermentadas, visto que este fator ainda não foi muito explorado. Isto é importante especialmente no desenvolvimento de um produto “tipo iogurte grego”, cujo consumo é crescente e os parâmetros reológicos e de textura têm impacto relevante na sua qualidade e aceitação pelos consumidores.

Assim, o desenvolvimento e análise de uma bebida láctea fermentada “tipo iogurte grego” à base de leitelho tem grande potencial para contribuir para o desenvolvimento sustentável da indústria de laticínios, propiciando um destino adequado e lucrativo para esse coproduto. Além disso, vislumbra-se agregar valor do ponto de vista tecnológico e nutricional/funcional para o leitelho e fornecer aos consumidores um alimento nutritivo e sustentável, fortalecendo, portanto, a bioeconomia no setor lácteo.

2. OBJETIVOS

2.1. Objetivo geral

Desenvolver formulações de bebidas lácteas fermentadas “tipo iogurte grego”, com baixo teor de gordura e diferentes concentrações de leitelho.

2.2. Objetivos específicos

Determinar a concentração lipídica média do leitelho obtido da fabricação da manteiga do Laticínios Escola - FUNARBE;

Desenvolver cinco formulações de bebidas lácteas fermentadas à base de leitelho;

Analisar a influência da porcentagem de substituição de leite desnatado pelo leitelho na composição centesimal, sensoriais e reologia das bebidas desenvolvidas;

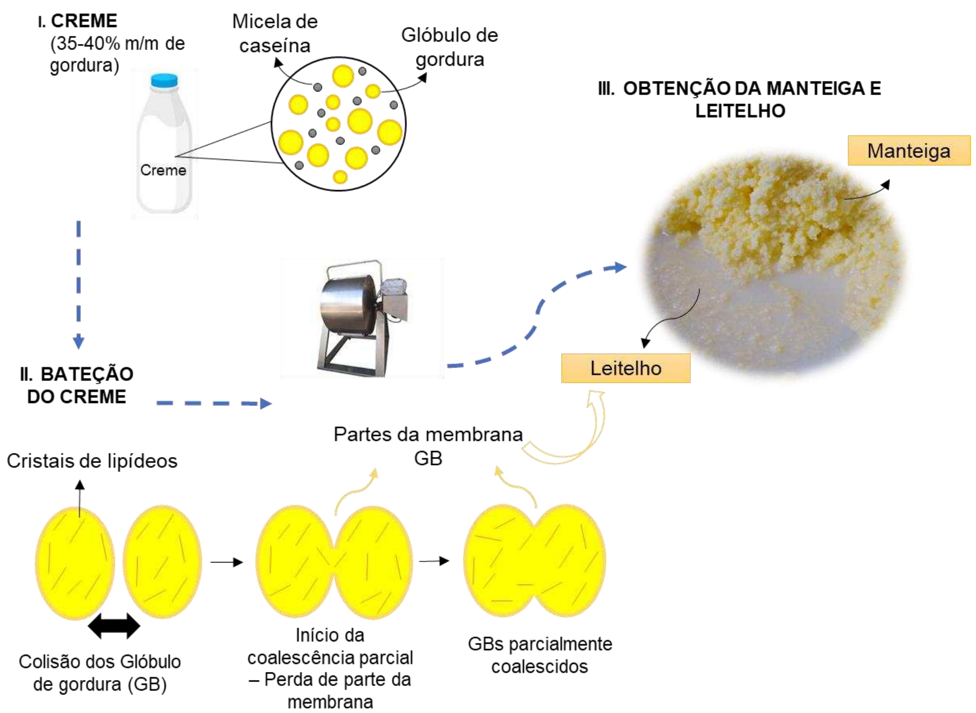
Avaliar, ao longo de 16 dias, as características microbiológicas, físico-químicas (pH, capacidade de retenção de água e sinérese) das bebidas formuladas.

3. CAPÍTULO 1: REFERENCIAL TEÓRICO

3.1 Leitelho

O leitelho é um coproduto lácteo produzido quando se transforma o creme de leite em manteiga. Esse processo ocorre por meio do batimento de creme de leite fresco (35-40% m/m de gordura) em baixa temperatura (8-12 ºC), provocando a coalescência parcial dos glóbulos de gordura, o que resulta na liberação dos triglicerídeos para formação da manteiga, e a separação de uma fase aquosa, denominada de leitelho (BRASIL, 2017).

Figura 2: Representação esquemática do processo de fabricação de manteiga e obtenção do leitelho.



Fonte: A autora

O consumo *per capita* de manteiga no Brasil aumentou 75%, entre 2006 e 2017, passando de 290 gramas/habitante, em 2006, para 460 gramas/habitante, em 2017 (FERREIRA et al., 2021). Assim, como a geração de leitelho, é vinculada à produção de manteiga se dá na proporção de 1:1, o volume de leitelho gerado também aumentou consideravelmente nesse período.

Este coproduto, além de conter a maior parte das proteínas encontrada no leite, possui vitaminas, minerais e propriedades funcionais devido à presença de fosfolipídios. Estes fosfolipídios, são provenientes dos fragmentos da MGGL, que são liberados durante a coalescência parcial dos glóbulos na etapa de bateção do creme. Este constituinte do leitelho confere propriedades tecnológicas e bioativas, de importância tanto para a indústria de alimentos quanto para a saúde do consumidor (ALI, 2019).

Os fosfolipídios da MGGL têm demonstrado auxiliar na redução da incidência de doenças neurodegenerativas, como Alzheimer (HORROCKS; FAROOQUI, 2004) e de alguns tipos de câncer (ITO et al., 1992; VISSAC et al., 2002). Alguns desses compostos também apresentam ação bactericida (MARTIN et al., 2004) e supressão de patógenos gastrointestinais (SPRONG; HULSTEIN; VAN DER MEER, 2002). Deste modo, por apresentar várias propriedades benéficas para a saúde do consumidor, o leitelho torna-se um coproduto promissor para o desenvolvimento de novos produtos alimentícios.

A composição centesimal do leitelho é similar à do leite desnatado, exceto pela maior quantidade de constituintes derivados do material membranoso do glóbulo de gordura, como fosfolipídios e lipoproteínas. Desta forma, a concentração de fosfolipídios totais no leitelho é cerca de sete a nove vezes maior do que no leite desnatado (BOURLIEU et al., 2018; LOPEZ et al., 2017). A Tabela 1 mostra a composição do leitelho comparada à composição do leite desnatado.

Tabela 1. Composição do leitelho comparada à composição do leite desnatado.

COMPONENTES	LEITE DESNATADO	LEITELHO
Água (%, m/m)	90,9	91
Extrato seco total (%, m/m)	9,1	9,0
Lactose (%, m/m)	4,8	3,9
Proteína (%, m/m)	3,4	3,2
Gordura (%, m/m)	<0,5	0,4-0,7
Sais (%, m/m)	0,9	0,9
Fosfolipídios (%, m/m)	0,015-0,020	0,12-018

Fonte adaptada: Teixeira, 2013

Os fosfolipídios são moléculas anfifílicas, isto é, em sua estrutura química, possuem uma parte hidrofóbica (cauda), formada por cadeias de hidrocarbonetos e outra parte hidrofílica (cabeça), formada por glicerol, fosfato e colina (SODINI et al., 2006). Além de possuírem características nutricionais e biológicas, os fosfolipídios apresentam funções tecnológicas, podendo atuar como agentes emulsionantes e/ou espumantes (DEWETTINCK et al., 2008). Um exemplo é na produção de sorvete, os emulsificantes presentem no leitelho, podem ajudar a melhorar a textura e a estabilidade do sorvete, além de contribuir para a redução da formação de cristais de gelo.

O aproveitamento total do leitelho ainda é um grande desafio para as indústrias brasileiras, especialmente para as pequenas e médias empresas, onde, na maioria das vezes, esse coproduto é descartado como resíduo ou destinado para a alimentação animal. Entretanto, no mercado internacional, o leitelho é um coproduto muito popular e consumido como um tipo de bebida fermentada que, normalmente, é consumida acompanhada de cereais, ou como substituto do leite fresco (LERAYER; BROLAZO; TALEB, 2001). Além disso, o leitelho é empregado em diversas receitas e formulações de produtos, como por exemplo em bolos, panquecas, sorvetes e bebidas fermentadas.

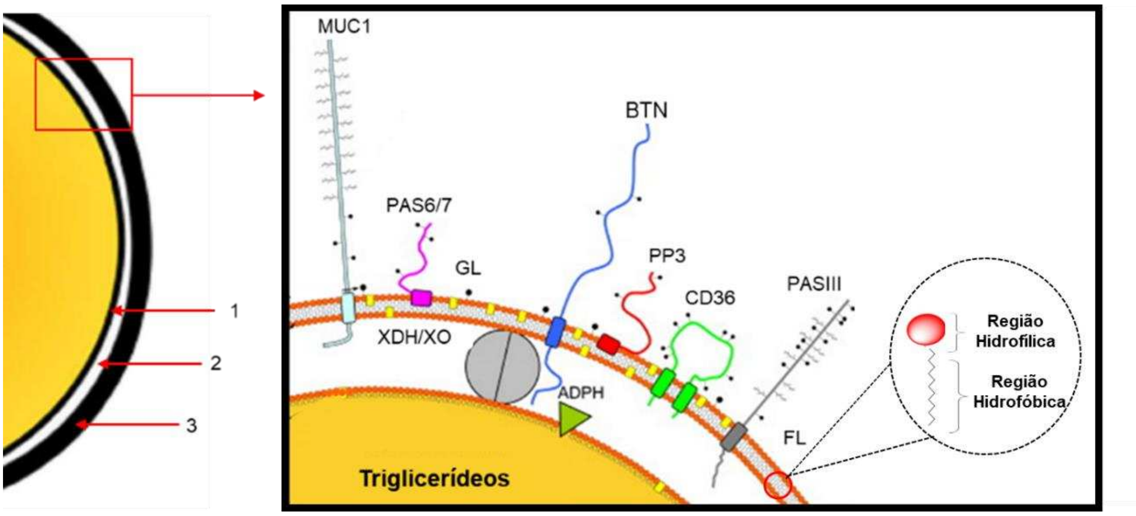
Em resumo, o leitelho apresenta uma considerável quantidade de nutrientes em sua composição e, portanto, uma elevada demanda biológica de oxigênio (DBO). Assim, este não deve ser misturado aos demais efluentes da indústria. Desta forma, o aproveitamento desse coproduto como ingrediente alimentício deve ser mais explorado por apresentar grande potencial do ponto de vista tecnológico, econômico e sustentável para a indústria, e funcional e nutritivo para os consumidores (PFRIMER, 2018).

3.2 Membrana do glóbulo de gordura do leite (MGGL)

A MGGL é sintetizada e secretada pelas células epiteliais da glândula mamária. Essa membrana é composta por uma mistura complexa de proteínas, glicoproteínas, enzimas, lipídios neutros e lipídios polares, como fosfolipídios (DEWETTINCK et al., 2008).

Da camada mais interna para a mais externa da MGGL (Figura 3), primeiramente, observa-se uma monocamada composta por lipídios polares e proteínas envolvendo os triglicerídeos (monocamada interna - 1). A seguir, nota-se uma monocamada intermediária (2), composta por uma cobertura proteica de elevada carga superficial e, por fim, uma bicamada externa (3), constituída por lipídios polares e proteínas (EVERS, 2004).

Figura 3. Estrutura da membrana de um glóbulo de gordura mostrando a disposição de seus principais componentes: 1- monocamada interna; 2- camada intermediária; 3- bicamada externa; adipofilina (ADPH); butirofilina (BTN); antígenos de diferenciação (CD36); mucina 1 (MUC1); ácido periódico de Schiff III (PASIII); ácido periódico de Schiff 6/7 (PAS 6/7); proteose peptona 3 (PP3); xantino dehidrogenase/oxidase (XDH/XO); glicolipídeos (GL); fosfolipídeos (FL).



Fonte adaptada: Dewettinck et al. (2008).

A composição e estrutura da MGGL sofrem influência de muitos fatores, mas, de forma geral, é composta por cerca de 25% de proteínas, principalmente de glicoproteínas, e 70% de lipídios, destes em torno de 55-70% são lipídios neutros e 40% lipídeos mais hidrofílicos (FONG; NORRIS; MACGIBBON, 2007).

Dentre os fatores que podem influenciar na composição e na estrutura da MGGL, incluem-se: a espécie, em relação à raça; o estágio de lactação; alimentação e frequência de ordenha; tratamento do leite e derivados, como resfriamento, congelamento, tratamento com alta pressão, tratamento térmico, homogeneização; a qualidade microbiológica do leite e derivados; métodos de isolamento e análise da MGGL (GRAVES; BEAULIEU; DRACKLEY, 2007; MCPHERSON; DASH; KITCHEN, 1984; YE; ANEMA; SINGH, 2007).

As proteínas da MGGL bovino são divididas em 7-8 bandas principais quando separadas por eletroforese em gel de poliacrilamida de dodecil sulfato de sódio

(SDS-PAGE). No entanto, cada uma das principais bandas compreende apenas uma das principais proteínas. Portanto, algumas proteínas menores apresentam a mesma mobilidade eletroforética aparente e desse modo, não podem ser separadas por meio de técnicas unidimensionais, somente por meio da eletroforese em duas dimensões (MATHER, 2000).

Dentre as principais proteínas já identificadas da MGGL incluem-se as: adipofilina (ADPH), butirofilina (BTN), antígenos de diferenciação (CD36), mucina 1 (MUC1), ácido periódico de Schiff III (PASIII), ácido periódico de Schiff 6/7 (PAS 6/7), proteose peptona 3 (PP3), xantina dehidrogenase/ oxidase (XDH/XO), além de proteína ligante de ácidos graxos (FABP), BTN e XDH/XO, que são as mais abundantes, representando cerca de 40 e 12% do total de proteínas da MGGL, respectivamente (SPITSBERG, 2005).

Na MGGL ainda contém diversas outras proteínas de menor concentração que ainda não foram identificadas. Em preparações para análise da MGGL, diversas outras proteínas podem ser observadas, sendo que a maioria corresponde às proteínas periféricas fracamente adsorvidas na membrana, incluindo as enzimas, imunoglobulinas e elementos derivados de leucócitos do citoplasma das células secretoras da glândula mamária e da fração desnatada do leite (DEWETTINCK et al., 2008).

Dentre os lipídios neutros presente no MGGL, destacam-se, principalmente, os triglicerídeos (≈95%) e os di- e monoglicerídeos em menor concentração, além do colesterol e seus ésteres. Os triglicerídeos da MGGL possuem maior proporção de ácidos palmítico (C16:0) e esteárico (C18:0) e menor de ácidos graxos insaturados, como miristoleico (C14:1), palmitoleico (C16:1), oleico (C18:1) e linoleico (C18:2), quando comparados com os triglicerídeos encontrados na manteiga (FONG; NORRIS; MACGIBBON, 2007).

Já os lipídios polares da MGGL do leite são compostos por diversos grupos de fosfolipídios, principalmente glicerofosfolipídios e esfingofosfolípidios. Dentre os principais fosfolipídios encontrados na MGGL destacam-se: fosfatidilcolina (25-40%), fosfatidiletanolamina (27-37%), esfingomielina (20-25%), fosfatidilinositol (≈5%) e fosfatidilserina (≈3%) (DEWETTINCK et al., 2008), além de pequenas quantidades de lisofosfatidilcolina, etanolamina, e traços de difosfatidilglicerol. Os glicolípidos representam 0,01 a 0,70% do total de lipídeos e também fazem parte da gordura do leite bovino e essa fração compõe principalmente cerebrosídeos, como glucosilceramida e lactosilceramida (FONG; NORRIS; MACGIBBON, 2007).

Esses lipídios polares são importantes na produção de alimentos, pois possuem propriedades nutricionais e são mais estáveis durante o processamento e armazenamento dos alimentos. Além disso, os fosfolipídios contribuem para a emulsificação na fabricação de diversos produtos por serem moléculas anfifílicas.

3.3 Bebida láctea fermentada

Os produtos lácteos são obtidos mediante o processamento do leite, podendo incluir aditivos alimentícios e ingredientes funcionalmente necessários para a sua fabricação (BRASIL, 2005). Pode-se observar um aumento notável na demanda por alimentos que apresentam propriedades nutricionais e funcionais e que seja sustentável (POLZIN; LUSK; WAHDAT, 2022; RAN et al., 2022). Assim, as indústrias alimentícias estão cada vez mais empenhadas em promover uma alimentação mais saudável e ajudar na saúde humana, desta forma têm desenvolvido e aplicado tecnologias na produção de alimentos funcionais, vitaminados, fortificados e que apresentam baixas calorias (SOUZA & BRUNARI, 2017).

Deste modo, dada a importância destes alimentos, os produtos lácteos fermentados são uma boa alternativa para a indústria alimentícia, pois, tratam-se de produtos de grande valor nutricional, por serem uma boa fonte de diversos macro e micronutrientes, incluindo proteínas, gorduras, vitaminas e minerais (fósforo e potássio e cálcio) (PEREIRA, 2014). Dentre estes produtos têm-se as bebidas lácteas fermentadas, cuja mercado vem crescendo mundialmente devido a sua praticidade de produção, baixo custo e grande aceitação pelos consumidores (NG et al., 2022).

Segundo o Regulamento Técnico de Identidade e Qualidade (RTIQ) de Bebida Láctea (BRASIL, 2005), bebida láctea é definida como o produto lácteo resultante da mistura do leite *in natura*, pasteurizado, esterilizado, Ultra High Temperature (UHT), reconstituído, concentrado, em pó, integral, semidesnatado ou parcialmente desnatado e soro lácteo (líquido, concentrado e em pó) com adição ou não de produto(s) ou substância(s) alimentícia(s), gordura vegetal, leite(s) fermentado(s), fermentos lácteos selecionados e outros produtos lácteos. A base láctea deve caracterizar pelo menos 51% massa/massa (m/m) do total de ingredientes do produto.

A bebida láctea fermentada apresenta a mesma definição de bebida láctea, acrescido do processo de fermentação, que consiste da ação de cultivo de microrganismos específicos e/ou adicionado de leite(s) fermentado(s) e não deve ser submetida a tratamento térmico posterior à fermentação (BRASIL, 2005). A legislação ainda define, que a contagem total de bactérias ácido lácticas viáveis deve ser no mínimo de 10^6 Unidades Formadoras de Colônias/grama (UFC/g), no produto final, para o(s) cultivo(s) láctico(s) específico(s) empregado(s), no decorrer de todo o prazo de validade do produto (BRASIL, 2013).

O leitelho por apresentar composição semelhante à do leite desnatado, além de possuir fosfolipídios e lipoproteínas, que são moléculas que possuem propriedades técnico-funcionais de interesse industrial, como emulsificante e espumante, assim este coproduto é uma boa opção para ser aplicado em diversas matrizes alimentares, incluindo em bebidas fermentadas.

Zhao et al. (2018) avaliaram as propriedades sensoriais e vários compostos voláteis de iogurte integral, iogurte desnatado e iogurte desnatado com adição de leitelho em pó em diferentes concentrações (0,5%, 1%, 2% e 4%). Segundo os autores, a adição de leitelho em pó a 1% ao iogurte desnatado melhorou o sabor, exibindo características semelhantes ao iogurte integral. Além disso, a adição de leitelho aumentou o conteúdo dos principais compostos voláteis (ésteres, aldeídos, álcoois e ácidos) que eram menos expressivos no iogurte desnatado.

Ao avaliar a substituição parcial ou total de leite desnatado por leitelho em formulações de sorvete, Ramos et al. (2021) mostraram que a substituição de 100% de leite por leitelho apresentou os melhores resultados para as análises de incorporação de ar (*overrun*) e derretimento, quando comparado ao sorvete tradicional. Além disso, sensorialmente, foi a formulação que obteve maior aceitação e com melhor intenção de compra.

Assim, o desenvolvimento de produtos à base de leitelho é muito promissor para indústrias de laticínios. A semelhança entre as composições do leitelho e do leite desnatado, torna esse coproduto uma boa matriz para a produção de bebidas lácteas fermentadas, reduzindo assim, os impactos ambientais causados por esse coproduto quando descartado de forma incorreta. Além disso, o aproveitamento desse coproduto oferece a oportunidade de fornecer aos consumidores um produto com alto valor nutricional (CRUZ et al., 2017).

3.4 Reologia

A reologia de alimentos é a ciência que estuda o comportamento de escoamento e deformação da matéria. Os estudos reológicos normalmente incluem uma relação entre a taxa de deformação e a tensão de cisalhamento. Os fluidos Newtonianos são uma classe de fluidos que apresentam uma relação linear entre a tensão aplicada e a taxa de deformação, ou seja, a viscosidade do fluido permanece constante independentemente da força aplicada. No entanto, em muitos casos, essa relação não é linear e os fluidos são classificados como não-Newtonianos, cuja viscosidade do fluido pode mudar com a taxa de deformação ou a tensão aplicada (SATO e CUNHA, 2007).

Esses fluidos podem ser pseudoplásticos, apresentando uma diminuição da viscosidade à medida que a taxa de cisalhamento aplicada aumenta (SCHRAMM, 2006). Além disso, os fluidos pseudoplásticos podem ser tixotrópicos e também dependentes do tempo de cisalhamento. Nesse caso, a viscosidade diminui em função do tempo, embora a taxa de cisalhamento seja constante. As curvas de fluxo de fluidos tixotrópicos apresentam diferença entre curvas de taxa ascendentes e de taxa descendentes, este é um fenômeno conhecido como área de histerese.

Os modelos comumente utilizados para descrever o comportamento dos fluidos não-Newtonianos são: Hershel-Bulkley, Ostwald-de-Waelle, Casson, Bingham e Mizrahi-Berk. A tabela 2 apresenta os modelos reológicos mais utilizados para fluidos pseudoplásticos e os valores dos parâmetros τ_0(tensão inicial) k (índice de consistência) n (índice de comportamento ao escoamento).

Tabela 2: Modelos reológicos utilizados nos ajustes das curvas de escoamento os valores de τ_0, k e n.

Modelos Reológicos	Equações	τ_0	K	n
Hershel-Bulkley	$\tau = \tau o + k.\dot{y}^n$	>0	>0	$0<n<\infty$
Ostwald-de-Waelle	$\tau = k.\dot{y}^n$	0	>0	0<n<1
Casson	$\tau^{0,5} = k_{oc} + k_c (\dot{y})^{0,5}$	>0	>0	0,5
Bingham	$\tau = \sigma_o + \mu_p. \dot{y}$	>0	>0	1

*onde τ_0 é a tensão inicial; k é o índice de consistência, n é o índice de comportamento ao escoamento.

Compreender o comportamento reológico de um fluido permite que a indústria de alimentos melhore as etapas de fabricação e desenvolva novos produtos. Uma das características reológicas mais significativas é a viscosidade, podendo ser correlacionada com a análise sensorial, possibilitando a produção de alimentos com as características de textura desejadas pelo consumidor. Além disso, a medição contínua da viscosidade permite que a consistência e as propriedades de textura sejam monitoradas em vários estágios durante o processamento de alimentos. Portanto, entender as características reológicas dos alimentos é essencial para o controle de qualidade, desenvolvimento de novos produtos e satisfação dos consumidores (VIDIGAL, 2009).

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CAPÍTULO 2: ARTIGO

Buttermilk as an ingredient of sustainable fermented dairy beverage: rheological, sensory, and physical-chemical properties

Article submitted to the Journal of Cleaner Production

Abstract

Buttermilk is a co-product of butter manufacturing, which has important technofunctional and nutritional properties, but which is often discarded or underused by the industry. Thus, the aim of this study was to develop five formulations of low-fat fermented dairy beverage were prepared from the mixing different proportions of skim milk:buttermilk: F1(100:0); F2(75:25); F3(50:50); F4(25:75); and F5(0:100). These formulations were submitted to centesimal composition, pH, syneresis, water holding-capacity (WHC), microbiological, sensorial and rheological analyses. The total replacement of milk by buttermilk did not influence the centesimal composition and pH of the beverages. Furthermore, addition of buttermilk decreased whey separation and increased the WHC of beverages during storage. The F3 formulation had good acceptance (global impression = 7.47) and good purchase intention (2.27), not differing from the sample with 100% milk (F1). On the other hand, the Rate-All-That-Apply (RATA) test demonstrated that the perception of characteristic sensory attributes of butter (such as taste, flavour, and greasy) reduced the acceptance of beverages with higher concentrations of buttermilk (F4 and F5). Thus, the use of buttermilk as a partial substitute (≤50%) for milk proved to be a good alternative ingredient for the production of nutritious foods, with sensory quality and low cost, thus strengthening the bioeconomy.

Keywords: Sustainable food. Co-product. Sensory attributes. Nutritional benefits

1 INTRODUCTION

Currently, consumers are increasingly concerned about health and environmental sustainability, encouraging the food market to expand to meet this demand (POLZIN; LUSK; WAHDAT, 2022; RAN et al., 2022; TORÁN-PEREG et al., 2023). This consumer behavior is motivated by the intensifying challenges of climate change, economic inequality, biodiversity loss and diet-related diseases (POLZIN; LUSK; WAHDAT, 2022).

Dairy products constitute a food group of great nutritional value, as they are a good source of various macro and micronutrients, including proteins, calcium, vitamins A, B, and D, phosphorus, potassium, magnesium, and zinc (PEREIRA, 2014). The consumption of dairy products is expected to increase substantially in the coming decade due to strong demand growth in developing nations, driven by a surge in population, increases in income, and lifestyle changes (FAO, 2021). However, despite the health benefits of milk and dairy products to most people, the dairy industry is responsible for a large use of natural resources and generation of waste with a high content of organic matter (ROTZ et al., 2021). Therefore, investing in the bio-circular strategies may solve some environmental problems that affect human health and social development, as well as an alternative to economically strengthen industrial sectors by reducing waste and environmental impact (OLIVEIRA et al., 2021). For instance, the bio-circular economy concept encourages the optimization of natural resources and the reuse of waste, especially co-products generated from the processing of raw materials. In dairy industries, the use of whey (from cheese) is better established, however other coproducts, such as buttermilk, is not used at all or very poorly (BARUKČIĆ et al., 2019).

Buttermilk is the co-product generated from the butter production. During the manufacture of butter, the churning of cooled cream results in the partial coalescence of the milk fat globules. Then, a three-dimensional network of fat is formed, obtaining butter and releasing an aqueous phase, called buttermilk. Hence, buttermilk has most of the water-soluble components of cream, such as proteins, lactose, and minerals, in addition to including some components of the milk fat globule membrane (MFGM) (ROESCH; RINCON; CORREDIG, 2004). The MFGM is constituted of several molecules such as phospholipids and lipoproteins, which act as emulsifying and foaming agents in food systems. Based on these properties, buttermilk can be used as an ingredient in various products, such as yogurt (GARCZEWSKA-MURZYN et al., 2022), breads (MADENCI; BILGIÇLI, 2014), cheeses (HICKEY et al., 2018), and ice cream (SZKOLNICKA; DMYTRÓW; MITUNIEWICZ-MAŁEK, 2020). Besides the techno-functional properties, the MFGM molecules have also shown bioactive properties, helping to reduce the incidence of Alzheimer disease, depression (HORROCKS; FAROOQUI, 2004), Helicobacter pylori or prevents gastrointestinal infections (BARUKČIĆ; JAKOPOVIĆ; BOŽANIĆ, 2019) and colon (CASTRO-GÓMEZ et al., 2016); (ZANABRIA; GRIFFITHS; CORREDIG, 2020) and breast cancer (VISSAC et al., 2002). Therefore, buttermilk is a dairy coproduct with great nutritional, technological, and biofunctional properties that need to be better explored.

Among dairy products, fermented beverages, especially those with low-fat content, are considered well-accepted healthy and nutritious food (NG et al., 2022; PENNA; GURRAM; BARBOSA-CÁNOVAS, 2006). The manufacture of the low-fat fermented beverage uses skimmed milk as one of its main ingredients. Considering the similarity between skimmed milk and buttermilk compositions, buttermilk is a good alternative as a substitute for skimmed milk. Bassi et al. (2012) evaluated a fermented beverage formulation using buttermilk as a partial substitute for milk and whey. The formulation with 30% buttermilk showed similar results for pH, acidity, and viscosity, compared to the formulations containing milk and whey. In addition, the sensory analysis revealed that the beverage manufactured from this addition of buttermilk was well accepted by consumers, as they had an average acceptance score exceeding ‘liked moderately’.

Other researchers used buttermilk to produce yogurt, however, in these studies was used buttermilk powder (GARCZEWSKA-MURZYN et al., 2022; ZHAO et al., 2018; ZHAO; FENG; MAO, 2020) and MFGM material isolated from industrial buttermilk powder (LE et al., 2011). Therefore, investigating the replacement of fluid milk by fluid buttermilk in the formulation of fermented beverages would be a way for butter producers, especially those small and medium size, to be able to use this co-product without the need for costly pre-operations.

In this sense, our aim was to propose a strategy to improve the sustainability of butter production by utilizing the buttermilk in a simple way. Hence, we developed a low-fat fermented dairy beverage type “Greek yogurt” by replacing skimmed milk with buttermilk. Furthermore, we evaluated the effect of adding buttermilk on the rheological and sensory properties of the manufactured beverage formulations.

2 MATERIALS AND METHODS

2.1 Experiments Overview

The manufacturing and analysis of the fermented dairy beverage "Greek yogurt type" based on buttermilk, were carried out in the laboratory room of the Federal University of Viçosa (UFV) (Brazil). The ingredients used for the development of the beverage were: buttermilk, skim milk, skim milk powder, sugar and lactic culture. The buttermilk was obtained from dairy factory FUNARBE located in UFV. The commercial freeze-dried lactic culture composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was acquired from the company Chr. Hansen (YF-L903, direct vat set - DVS, YoFlex®), and the other ingredients were purchased in the local market.

The experiment was conducted in a completely randomized design with three replications. The treatments were the five formulations (F1, F2, F3, F4 and F5) produced containing different proportions of skim milk: buttermilk (in %, w/w): F1 - 100:0; F2 - 75:25; F3 - 50:50; F4 - 25:75; and F5 - 0:100. The protein concentration of all formulations was standardized to 5% (w/w) using skim milk powder. On the first day of storage, centesimal composition, rheology, and sensory analysis were performed of the five formulations. Furthermore, over 15 days, the formulations were submitted to syneresis analysis, water-holding capacity, and pH testing at different times (1, 6, 11, and 16 days). Ultimately, on the first and sixteenth days of storage, microbiological analysis of the beverages were carried out (coliforms, filamentous fungi and yeasts count).

2.2 Preparation of formulations

The formulations of the fermented dairy beverage “Greek yogurt type” based on buttermilk were manufactured according to the following description. Initially, the ingredients were weighed according to the concentration defined for each formulation shown in Table 1. The milk, buttermilk, and sugar mixture were heated for 2 minutes at 90 °C before being cooled to 43 °C. The yogurt culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was inoculated into the mixture at 43 °C, at the concentration recommended by the manufacturer. Afterwards, the fermentation process of the formulations (kept at 43 °C) was monitored through pH measurements. Upon reaching pH 4.5, the formulations were refrigerated for 12 h, and then the clot was broken using a spatula. After that, the formulations were kept at 8 °C until analysis.

Table 1: Composition of fermented dairy beverages "Greek yogurt type" based on buttermilk.

Ingredients (%)	F1	F2	F3	F4	F5
Buttermilk	0.0	20.7	40.9	60.6	79.8
Skim milk	84.8	62.1	40.9	20.2	0.0
Skim milk powder	7.0	9.0	10.0	11.0	12.0
Sugar	8.0	8.0	8.0	8.0	8.0
Lactic culture	0.2	0.2	0.2	0.2	0.2

The proportions of skim milk: buttermilk of the formulations are F1 - 100:0; F2 - 75:25; F3 - 50:50; F4 - 25:75; and F5 - 0:100.

2.3 Analysis of fermented dairy beverage "Greek yogurt type" based on buttermilk.

2.3.1 Determination of the centesimal composition

The centesimal composition of the samples was determined by quantifying the level of moisture, ashes, lipids and proteins according to the methodology established by Association of Official Analytical Chemist Methods (AOAC, 2012). The moisture content of the samples was determined by oven drying at 105 °C. The ash content was obtained by incinerating 5 g sample in a muffle at 550 °C until constant weight was obtained. For the lipid content, the Soxhlet method was used with ether solvent for extraction. The determination of the protein content was carried out from the quantification of nitrogen by the Kjeldahl method using the conversion factor of 6.38. The total carbohydrate content was calculated from the difference between 100 and the sum of the percentage of moisture, ash, lipid and protein.

2.3.2 Microbiological analyses

The formulations were analysed by microbiological tests, which included counting the most probable number per gram (MPN/g) of the total coliforms and thermotolerant coliforms, and colony forming units per gram (CFU/g) for total count of filamentous fungi and yeasts, according to Methods for Examination of Dairy Products recommended by the American Public Health Association (APHA, 2015).

2.3.3 Determination of water-holding capacity

The analysis of water-holding capacity (WHC) was performed according to Wang et al. (2022). Approximately 20 g of each sample were centrifuged at 4500 × g for 10 min at 4 °C (Heraeus Fresco Centrifuge 21, Thermo Fisher Scientific, Germany). The expressible moisture has harvested and weighed. The WHC was calculated using the following equation (Eq. (1)):

$$WHC\ (\%) = 1 - \frac{mass\ of\ supernatant\ (g)}{mass\ of\ sample\ (g)} x\ 100 \qquad Eq.(1)$$

2.3.4 Syneresis

Syneresis was estimated as described by Gomes et al. (2022). Approximately 10 g of sample were centrifuged at 500 x g for 20 minutes at 4 °C, and then the aqueous supernatant phase was collected and weighed. The syneresis was calculated using the Eq. (2):

$$Syneresis\ (\%) = \frac{mass\ of\ supernatant\ (g)}{mass\ of\ sample\ (g)} x\ 100 \qquad Eq.(2)$$

2.3.5 pH

The pH of the beverage samples was evaluated using a pH meter (Hanna Instruments, Model H1221, Woonsocket, United States) previously calibrated with commercial pH 4 and pH 7 standards (AOAC, 2012).

2.3.6 Rheology analysis

The rheological tests of the fermented dairy beverage “Greek yogurt type” based on buttermilk were carried out with a dynamic Haake Mars rheometer equipped with a stainless-steel cone plate C60/1°Ti sensor (diameter = 60 mm, gap = 0,051 mm), at 25 and 5 °C.

The flow curves were generated by a linearly increased shear rate of 0.01 to 200 s^{-1} in three curves (1 stup cycle, down cycle and 2 stup cycle), in a total time of 6 minutes. The Power Law and Herschel Bulkley rheological models were adjusted to the experimental data equations (Eq. 3 and Eq. 4), being the hysteresis area that represents the thixotropy measurement obtained by the difference between the cycles (1st up cycle, down cycle). The apparent viscosity was evaluated at a shear rate of 50 s^{-1} (WANG et al., 2022; YILMAZ et al., 2015).

$$\tau = k.\dot{y}^{n} \qquad \text{Eq. (3)}$$

$$\tau = \tau o + k.\dot{y}^{n} \qquad \text{Eq. (4)}$$

where τ is the shear stress (Pa), τ_0 is yield stress (Pa), k is the consistency coefficient ($Pa.s^n$), $\dot{y}$ is the shear rate (s^{-1}) and n is the flow behaviour index (dimensionless).

Dynamic oscillatory tests were used for evaluate the viscoelasticity of the beverages, where the linear viscoelastic region was determined by means a strain scan from 0.0001 to 1% at a constant frequency (1 Hz). Posteriorly, a frequency scan of 0.01 to 10 Hz with a constant shear stress of 5 Pa was performed on the linear viscoelastic range for all samples. The data collected at a frequency of 1 Hz were presented in terms of storage modulus (G') and loss modulus (G''), in which the angular phase tangent (δ) is determined by Tan (δ) = G” /G'.

2.3.7 Sensory analysis

Analysis of the sensory characteristics was conducted by 100 untrained panellists (66 women and 34 men, aged 18- 62 years) in isolated booths with the same lighting and temperature conditions. The five formulations coded with three-digit random numbers were analysed for appearance, taste, texture, flavour and global impression by the acceptance test employing 9-point hedonic scale, in which 1= dislike extremely; and 9= likely extremely. It was also performed the purchase intent test, which was composed of a 5-point scale, in ascending order: 5 = certainly buy and 1 = certainly would not buy.

Additionally, the acceptability index was evaluated using the Eq. (5):

$$AI\ (\%) = \frac{\underline{S}}{S_{max}} \times 100 \qquad \text{Eq. (5)}$$

where AI is the acceptance index, $\underline{S}$ is the average score, and S_{max} is the maximum score given to the product. A product with an AI ≥ 70% is considered acceptable.

Furthermore, was conducted the descriptive test Rate-All-That-Apply (RATA). RATA data were treated as continuous, where the scale points were scored from 0 to 3 (0 = None, 1 = Low, 2 = Medium, 3 = High) (ARES et al., 2014).

The sensory evaluation of the present study was analysed and approved by the Committee on Ethics in Human Beings Research of the Federal University of Viçosa (UFV, Viçosa, Minas Gerais State, Brazil), under Opinion N° 5470985.

2.3.8 Statistical analysis

The results of this study were evaluated using one-way analysis of variance (ANOVA), and Tukey's test at 0.05 significance level, using the software R. The acceptance test and rheological data was also submitted to principal component analysis (PCA) to obtain the Preference Map.

3 RESULTS AND DISCUSSION

3.1 Centesimal composition of fermented dairy beverages

In general, the centesimal compositions of the beverages developed were very similar to each other (Table S1, in Supplementary Material). The lipid (~ 0.56%), protein (~5.42%), and ash (~1.14%) contents did not differ between the formulations ($p>0,05$). These results are due to the similarity between the macro component compositions of skim milk and buttermilk (BAHRAMI et al., 2015). Only the moisture and carbohydrate contents showed significant difference, ranging from 77.99% (F1) to 75.85% (F5), and from 15.11% (F1) to 16.96% (F5), respectively. Therefore, the total replacement of skim milk by buttermilk has very little influence on the macronutrient composition of the developed beverages.

3.2 Microbiological quality of the fermented dairy beverages

According to Moh et al. (2017), contamination by coliforms can happen due to low microbiological quality in the milk used to prepare the product, or insufficient heat treatment. The quality of food products is compromised when the presence of pathogenic microorganisms causes food deterioration, reducing their shelf-life, in addition to compromising their safety.

The microbial counts in the five beverage formulations, analysed the day after the beverage was manufactured and 16 days of storage were negligible (< 3 MPN/g for coliforms and < 10 CFU/g for filamentous fungi and yeasts). These results were in line with the restrictions imposed by the milk and yoghurt laws of Brazil, which were in effect at the time the fermented dairy beverages were produced (BRASIL, 2007; BRASIL, 2001).

This means that the five beverage formulations were underwent an efficient heat treatment process and produced in hygiene and sanitary conditions that were appropriate about the handling of raw materials, utensils, and equipment used to obtain the product, assuring food safety. Therefore, the beverages developed were suitable for human consumption, free from the risk of causing gastrointestinal infection, and adequate for sensory analysis (CÂNDIDO DE SOUZA; SOUZA DO AMARAL; LIMA DA SILVA BERNARDINO, 2021).

3.3 Physical-chemical characteristics: pH, WHC, and syneresis

The evolution of pH is marked by the production of lactic acid, which is one of the primary functions of lactic acid bacteria in fermented dairy products (BENMEZIANE et al., 2021). As shown in Fig. 1a, the addition of buttermilk did not influence the fermentation process of the formulations since all reached pH ~ 4.50 (in the graph, the time day 0 represents the end of the fermentation stage of beverage manufacture).

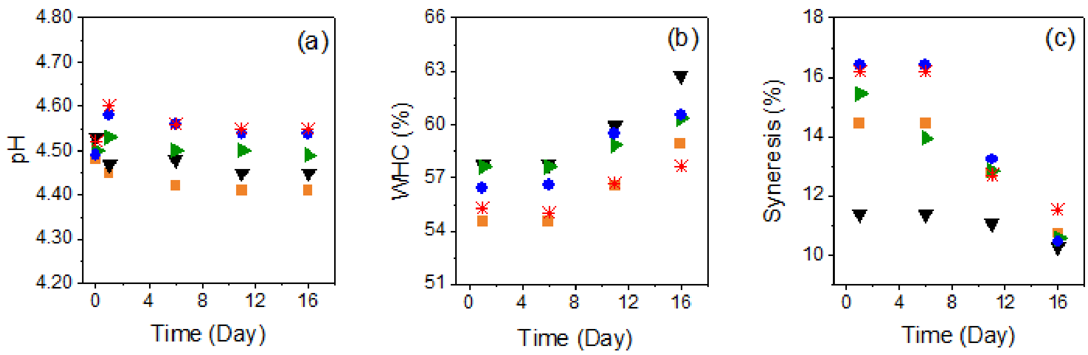

Fig. 1 Changes of pH (a), water-holding capacity (WHC) (b), and syneresis (c) of the 5 fermented dairy beverages formulations during 16 days of storage period at 8 °C. The proportions of skim milk: buttermilk of the formulations are (■) F1 - 100:0; (▼) F2 - 75:25; (▶) F3 - 50:50; (●) F4 - 25:75; and (✳) F5 - 0:100.

Furthermore, during the assessed storage periods, all formulations revealed a slight pH decrease, likely caused by the development of lactic yeast bacteria, which, even at low temperatures, their growth and acid latic production are not completely inhibited. After 16 days of storage, the average pH values of the beverages ranged from 4.41 to 4.55. These results are adequate since, pH values below 4.00 reduce the sensory acceptability of fermented lactic products (TAMIME; ROBINSON, 2007).

WHC is a very important evaluation parameter, as it provides information on the stability of the fermented beverage gels net and the tendency to syneresis (XU et al., 2019). Syneresis is an undesirable process that can be observed during the storage time of fermented dairy beverages, characterized by the separation of the liquid from the curd, thus reducing the overall acceptability of the product (JØRGENSEN et al., 2019).

The WHC of the formulations with varying buttermilk concentrations were monitored and quantified over a 16 day period. The Fig. 1b shows that, initially, the formulations presented WHC around 56% and remained stable until the sixth day of storage. Afterwards, all beverages showed an increase in WHC until the end of the storage period. In general, the presence of buttermilk in the formulations caused an improvement in WHC when compared to that without buttermilk (F1). However, this improvement was not proportional to the amount of buttermilk added, with F2 with 25% buttermilk having the best WHC (62.72% with 16 days of storage). Similar behaviour to WHC was verified for syneresis (Fig. 1c), which demonstrated stability until the sixth day (except for the F3). After the sixth day of storage, a decreasing trend for syneresis was observed for all formulations. The formulation F2 showed the lowest syneresis values (11.50%) throughout the entire storage period.

Zhao et al. (2020) developed five low-fat yogurt formulations added of 0.0%; 0.5%; 1.0%; 2.0% and 4.0% buttermilk powder and obtained the following WHC: 32.6%; 34.3%; 35.9%; 36.0%; and 34.5%, respectively. The formulations with 1.0% and 2.0% buttermilk powder were also the ones that showed the best syneresis rates, around 64.5% (with 14 days of storage). As we observed in our study, the addition of buttermilk increased the WHC of the products, but the increase was not proportional to the amount of coproduct incorporated. This study evaluates the microstructure of yogurts by confocal scanning laser microscopy and observed that as the buttermilk concentration increased, there were more phospholipids molecules in the pores of the cross-linked network. To make this observation, the phospholipids were previously labeled with a fluorescent dye. This WHC and syneresis behaviour observed as the buttermilk concentration increased in the formulations can be attributed to the phospholipids (ROMEIH; MOE; SKEIE, 2012). The concentration of this component in buttermilk is about seven times higher than in skim milk. Although phospholipids have amphiphilic characteristics, they have a lower WHC than proteins, which can transform the water retention of fermented beverage gels (DICKINSON; CHEN, 1999; ROMEIH; ABDEL-HAMID; AWAD, 2014).

3.4 Rheological properties

Rheometry is a practical technique that allows knowing the physical properties of a product before it is finally prepared for sensory testing. Furthermore, it plays an important role in understanding and determining food quality attributes such as taste, texture and stability (GOUSETI; WATSON; PACEK, 2020).

Understanding a fluid rheological behavior enables the food industry to improve production processes and develop new products. Viscosity is one of the more significant rheological characteristics that can be linked to sensory analysis to produce foods with the desired textural qualities for the consumer. In addition, continuous viscosity measurement enables consistency and textural properties to be monitored at various stages of the manufacturing process. Therefore, understanding the rheological characteristics of foods is crucial for quality control, the development of new products, and consumer satisfaction.

The Fig. 2 shows the graphical relation of strain rate versus shear stress for the 5 formulations.

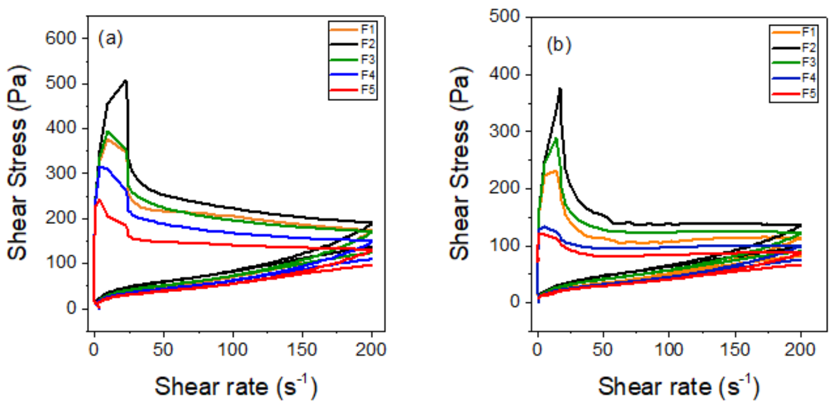

Fig. 2 Flow curves of the 5 dairy beverage formulations fermented at 5 °C (a) and 25 °C (b). The proportions of skim milk: buttermilk of the formulations are F1 - 100:0; F2 - 75:25; F3 - 50:50; F4 - 25:75; and F5 - 0:100.

The flow curves of the 5 formulations were carried out at temperatures of 5°C and 25°C because these temperatures represent typical storage and consumption conditions for fermented beverages. The temperature of 5°C is generally the storage temperature of fermented beverages, while the temperature of 25°C is the temperature that the chilled products reaches when consumed, furthermore for being the ambient temperature.

For the two temperatures evaluated, the relation of shear rate versus shear stress were not linear, which is typical of non-Newtonian fluid. Furthermore, the two graphs show an area of hysteresis, this indicates that the fermented beverage is a thixotropic product. The area of hysteresis is a measure of the difference between the upward and the downward curve, which is associated with structural or orientation changes of the molecules of the material in response to applied shear. This difference between the hysteresis area curves, the greater it is, indicates that the fluid has more weak interactions, which are broken during shear rate, that is, the material has less ability to recover and return to its original state.

The model that best fitted the experimental data on the last curve was on the last curve was Herschel Bulkley, with R2 >0.9. The rheological parameters of result initial stress (τ_0), consistency index (k (Pa.s)) and result behaviour index (n), in addition to the apparent strain rate of 50 s^{-1}(η_{50}), and the hysteresis area (ΔA (Pa s^{-1})), are presented in the Table 2.

Table 2. Rheological parameters of the 5 fermented beverage formulations at 5 and 25 °C.

Formulations	5 °C					
	τ_0 (Pa)	k (Pa.s)	n	R^2	ΔA (Pa s^{-1})	η_{50} (Pa.s)
F1	20.15^{c}	0.49^{b}	0.98^{a}	0.9939^{a}	27750.00^{b}	0.93^{bc}
F2	24.41^{a}	1.08^{a}	0.87^{bc}	0.9912^{a}	35735.00^{a}	1.17^{a}
F3	22.35^{b}	0.62^{b}	0.96^{a}	0.9919^{a}	27855.00^{b}	1.01^{b}
F4	20.24^{c}	0.37^{b}	0.99^{a}	0.9925^{a}	22845.00^{c}	0.85^{cd}
F5	17.99^{d}	0.35^{b}	0.94^{ac}	0.9936^{a}	17470.00^{d}	0.76^{d}
Formulations	25 °C					
	τ_0 (Pa)	K (Pa.s)	n	R^2	ΔA (Pa s^{-1})	η_{50} (Pa.s)
F1	9.99^{a}	0.94^{b}	0.82^{ab}	0.9968^{a}	13375.00^{b}	0.67^{bc}
F2	8.44^{a}	3.56^{a}	0.61^{c}	0.9960^{a}	21710.00^{a}	0.98^{a}
F3	10.27^{a}	2.78^{ab}	0.65^{bc}	0.9950^{a}	18520.00^{ac}	0.81^{ab}
F4	12.36^{a}	0.57^{b}	0.89^{a}	0.9942^{a}	10120.00^{bd}	0.62^{bc}
F5	11.33^{a}	0.61^{b}	0.85^{a}	0.9952^{a}	9301.00^{d}	0.57^{c}

The proportions of skim milk: buttermilk are F1 - 100:0; F2 - 75:25; F3 - 50:50; F4 - 25:75; and F5 - 0:100. The test was done by temperature and means with different lowercase letters in the column indicate a significant difference ($p < 0.05$).

The areas of hysteresis (area between the upward and the downward curve) that are associated with structure rupture during shear (HASSAN et al., 1996), increased with the insertion of buttermilk in the formulation. However, for the two evaluated temperatures, as the buttermilk concentration increased, the hysteresis area reduced (Fig. S1, in Supplementary Material). Probably, the molecules present in buttermilk from MFGM were able to provide stronger interactions, making the gel more stable, due to the formation of a greater number of interactions of the molecules responsible for the formation of the three-dimensional structure. But at higher concentrations of buttermilk, the phospholipids may have contributed to increase the steric repulsion between the gel-forming molecules.

Table 2 shows that the values of τ_0 at a temperature of 25°C did not differ significantly between the formulations, on the other hand at a temperature of 5°C there was a difference between the formulations. Flow behaviour index values (n) confirmed that all fermented beverage formulations showed pseudo plastic behaviour ($n<1$), as the beverage viscosity decreased with the increasing strain rate. Being that the formulation with the addition of 25% buttermilk (F2) at both evaluated temperatures, had a flow behaviour index (0.87 and 0.61), thus, it was the formulation that most moved away from a Newtonian fluid. Furthermore, F2 obtained the highest viscosity results (1.18 and 0.98 Pa.s), and the highest k values (1.08 and 3.56 Pa.s), respectively. In general, replacing up to 50% of the milk with buttermilk had little change in the consistency and viscosity of the beverages.

The study developed by Zhao et al. (2020) reported that the addition of (1-2%) buttermilk powder in yogurt formulations increased the viscosity (~60%). However, higher concentration of buttermilk powder (4%) caused a loss of viscosity (~13%). This indicates that the MFGM components that are present in buttermilk, in greater amounts, contributed to decreasing the viscosity of the yogurt. This increase in the viscosity of the formulations at lower concentrations is probably related to the emulsifier and amphiphilic properties of the phospholipids and proteins, however the interaction of these molecules in larger amounts decreases the viscosity, as was observed in our study, where the formulations with 75% and 100 % buttermilk had lower viscosity at both temperatures.

To further investigate the internal structure of the fermented beverage, dynamic rheological parameters, including storage modulus (G') (elasticity) and loss modulus (G") (viscosity) and tangent (tan δ), were determined. The elasticity of viscoelastic products is represented by the elastic modulus (G'). The viscous modulus (G") is related to the viscosity of the material. When there is an increase in the G' modulus is strongly related to molecular interactions or physical crosslinks that are capable of generating firmer materials. A materials elastic and viscous moduli are present when it is subjected to a specific strain level (ROCHA et al., 2022). The phase angle tangent (tan= G"/G') is the term used to describe this lag between the moduli response (WIDYATMOKO, 2016). The results of G', G" are in the Fig. S2, in Supplementary Material, and tan in Fig. 3.

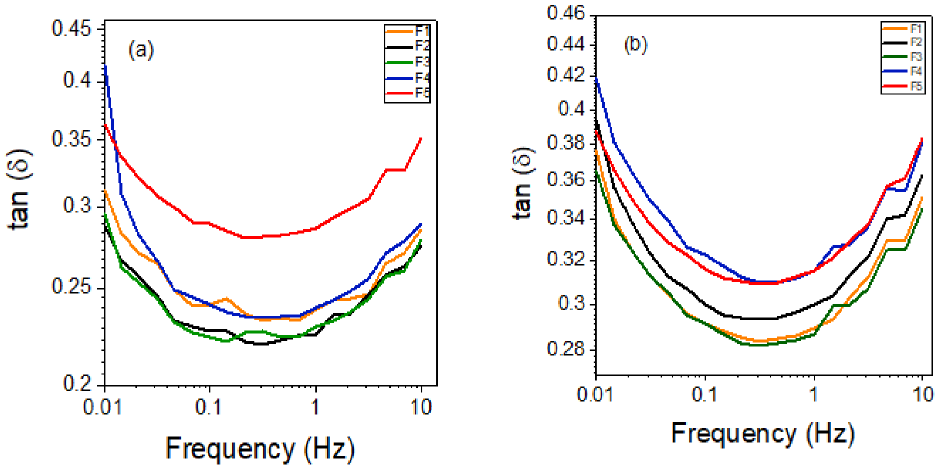

Fig. 3 Angular phase tangent (δ) vs. frequency (Hz) for the 5 beverage formulations fermented at 5°C (a) and 25°C (b).

All fermented beverage samples showed similar rheological behaviour and across the frequency range from 0.1 to 10 Hz, the G' value of all formulations was greater than the G" value, indicating that the elasticity response was stronger than the viscosity response and therefore exhibiting a food with semi-solid behaviour (Fig. S2, in Supplementary Material) (ZHONG; DAUBERT, 2013). This fact was confirmed with tan values, where all formulations had Tan δ < 1 (Fig. 3). Zhao et al. (2020) developed skim milk yogurt formulations added with buttermilk powder. According to the authors, the formulations with lower concentrations (1.0% and 2.0%) of buttermilk improved the textural rheological properties of yogurts. A similar result was obtained in the present study, where the addition of lower concentrations of buttermilk whey showed higher G' values.

For the rheological parameters, the analysis of principal components (PCA) was also performed. The first principal component explained 99.99% of the total data variance and the second principal component 0.01% (Fig. 4 a and b). The two principal components elucidated 100% of the data.

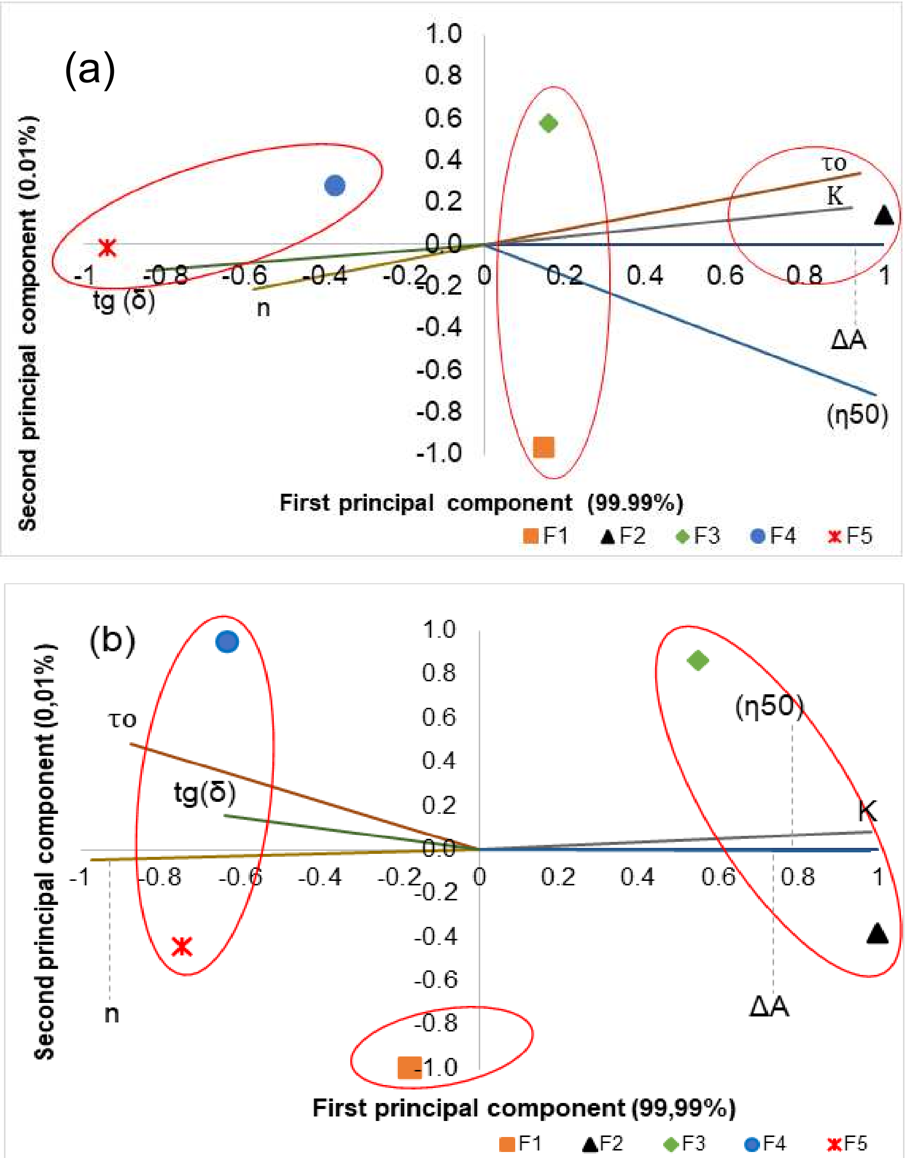

Fig. 4 Principal component analysis (PCA) of the rheological parameters of the 5 fermented beverage formulations (a) 5°C and (b) 25°C. The proportions of skim milk: buttermilk of the formulations are (■) F1 - 100:0; (▲) F2 - 75:25; (◆) F3 - 50:50; (●) F4 - 25:75; and (✳) F5 - 0:100.

As shown in Fig. 4 a and b, formed three groups at both temperatures (G) is notable: G1 – samples that are 100% milk, and 50% milk; G2 – sample that is 75% milk and G3 – samples that are 25% milk 100% buttermilk that differ from each other (5°C). The groups formed at 25°C were (G): (G) is notable: G1 – sample that is 100%

milk; G2 – samples that are 75% milk 50% milk and G3 – samples that are 25% milk 100% buttermilk.

Regarding the temperature 5°C, the group on the right had more intensity with the parameters (τ_o, K and ΔA), on the other hand the group on the left had a greater intensity with a (tgδ, η_{50} and n), whereas the group in the middle had intermediate intensity with all parameters (Fig. 4a). At the temperature of 25°C, the group on the right had more intensity with the parameters (η_{50}, K and ΔA), and the group on the left with (τ_o, tgδ and n) and the 100% milk formulation had intermediate intensity between the parameters (Fig 4b).

3.5 Sensory evaluation

It is fundamental for the food industry to understand how consumers perceive food products. This information is fundamental for the development of new products, reformulation of existing products and marketing (MEILGAARD; CARR; CIVILLE, 1999). Thus, sensory characteristics are very important to assess the acceptability of fermented dairy beverages by consumers (ABDEL-HAMID et al., 2020). The results of the sensory evaluation in relation to the acceptance and purchase intent tests of the formulations are shown in Table 3.

Table 3. Mean hedonic scores and purchase intent of fermented beverage formulations.

Formulations	Appearance	Taste	Texture	Flavour	Global impression	Purchase intent
F1	8.12^{a}	7.16^{ab}	7.86^{a}	7.59^{ab}	7.65^{a}	3.91^{bc}
F2	7.97^{a}	7.42^{a}	8.06^{a}	7.76^{a}	7.78^{a}	4.09^{c}
F3	8.03^{a}	7.03^{ac}	7.78^{a}	7.24^{b}	7.47^{a}	3.73^{b}
F4	7.88^{ab}	6.71^{bc}	7.66^{a}	6.67^{c}	6.91^{b}	3.37^{a}
F5	7.64^{b}	6.64^{c}	7.17^{b}	6.17^{c}	6.51^{b}	3.08^{a}

Means with different lowercase letters in the column indicate a significant difference ($p < 0.05$).

Appearance is an important attribute of quality and acceptance of food products, since, generally, the first impression of the food is obtained from a visual evaluation by the consumer (SALGADO et al., 2021). There was no significant difference between formulations F1, F2, F3 and F4 regarding the acceptability of appearance and texture attributes ($p > 0.05$). These observations, principally in relation to texture, may have been influenced by the standardization of the protein content of the formulations.

Although the centesimal compositions of macronutrients of the beverages were close to each other (Table S1, in Supplementary Material), we believe that the presence of some constituents of buttermilk contributed to the development of sensory characteristics that the panellists considered less pleasant. This fact would explain the lower hedonic scores attributed to the flavour and taste attributes for formulations F4 and F5 that contained the highest concentrations of buttermilk (75% and 100%, respectively).

The global impression of the formulations F1, F2, and F3 showed no difference between them. In this way, the substitution of up to 50% of skim milk by buttermilk did not affect the global acceptance of the developed products (F1, F2, and F3). Regarding the purchase intent, the F2 formulation that presented the best score of 4.09, corresponding to the concepts “certainly buy” and “probably buy”, was the same one that obtained the larger approval from consumers for all the attributes evaluated in the acceptance test (Table 3). In addition, formulations F4 and F5 had the lowest scores for global impression and scores of 3.37 and 3.08, respectively, for purchase intent (corresponding to the concepts "probably buy" and "maybe buy"), demonstrating that the flavour and taste attributes had a significant influence on the overall sensory evaluation.

The data from the acceptance test shown in Table 3 were evaluated using analysis of variance and means comparison test, that is, the individuality of the 100 evaluators who participated in the tests was not considered. However, knowing the behaviour of each evaluator in front of the samples is also useful information. For this, the preference map technique has been widely used. The Preference Map technique was created with the purpose of analysing affective data considering the individual response of the consumers instead of the mean of the group that evaluated the formulations (ARES et al., 2010; GUINARD; UOTANI; SCHLICH, 2001; VAN KLEEF; VAN TRIJP; LUNING, 2006).

To obtain the Preference Map, data from the acceptance test of the 5 formulations in relation to global impression were submitted to principal component analysis (PCA). Afterwards, the results were expressed in a scatterplot that correlates the acceptance data of each evaluator with the first and second principal components (DE CARVALHO; VALENTE; ASSUMPÇÃO, 2018; MORAIS et al., 2016) (Fig. 5).

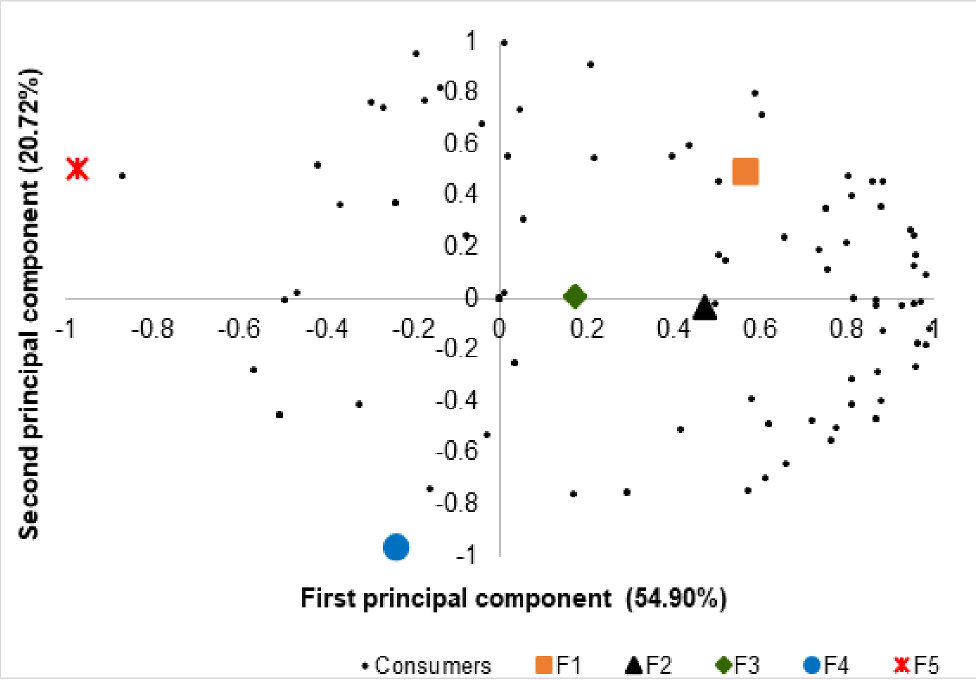

Fig. 5 Global impression preference map of the 5 fermented beverage formulations. The proportions of skim milk: buttermilk of the formulations are (■) F1 - 100:0; (▲) F2 - 75:25; (◆) F3 - 50:50; (●) F4 - 25:75; and (✳) F5 - 0:100.

As can be observed Fig. 5, the first principal component explained 54.90% of the total variation in acceptance between the formulations and the second component 20.72%. The two components together explain more than 70.00% of the total variation in the data, being sufficient to distinguish the formulations in relation to the global acceptability.

The spatial distribution of the 5 formulations indicates that there was the formation of three groups that differed from each other. Each point within the graph corresponds to a particular panellist and represents the correlation between your acceptance test response and the two principal components. Therefore, the correlation between the evaluator and at least one of the components indicates that he differed the samples. In the preference map, the evaluators are located close to the samples that they demonstrated greater acceptance. On the other hand, the points close to the central region of the graph represent the evaluators that did not present correlation with the principal components and, therefore, were not able to discriminate the formulations in relation acceptance. In this way, regarding global acceptance, the formulations prepared with milk (F1) and with up to 50% substitution of milk by buttermilk (F2 and F3) were the most accepted by the group. The F5 sample (located in the fourth quadrant) was the least accepted among the five evaluated formulations.

For a food product to be considered accepted, the AI must be ≥ 70. The appearance, taste, flavour and texture attributes presented an acceptability index above 70.00% for all formulations, except for the flavour attribute of F5, which the AI was of 68.56% (Fig. S3, in Supplementary Material). This can be explained by the buttery aftertaste of buttermilk attributed to F5, which was less accepted by consumers. On the other hand, when analysing the acceptability index in relation to the global impression, the results were greather than 70% for all formulations.

Carrying out a descriptive test helps us to understand which characteristics of the developed formulations influenced the evaluations obtained in the affective tests of acceptance and purchase intention. In this sense, the rate-all-that-apply (RATA) test is a descriptive test that allows consumers to assess descriptive attributes of samples. For the RATA test, the descriptive attributes were defined by the research team based on the sensory attributes of traditional fermented beverage and, as buttermilk originates from the manufacture of butter, some sensory attributes characteristic of this product were also considered. Thus, after surveying descriptive terms, sixteen attributes were selected as the best to characterize the formulations, namely: characteristic taste of natural yogurt; characteristic flavour of natural yogurt; characteristic colour of natural yogurt; sweet taste; creamy; firm, adhesiveness; sandy texture, homogeneous texture, brightness, milk taste; greasy, butter taste, butter flavour, unpleasant flavour; and sour taste. For the test, the consumers received evaluation sheets with the list of these attributes and reported whether they were applicable to describe the formulation, identifying the perceived intensity as: 'not applicable', 'low', 'medium' or ' high'. The RATA test results for the 5 beverage formulations are shown in Fig. 6.

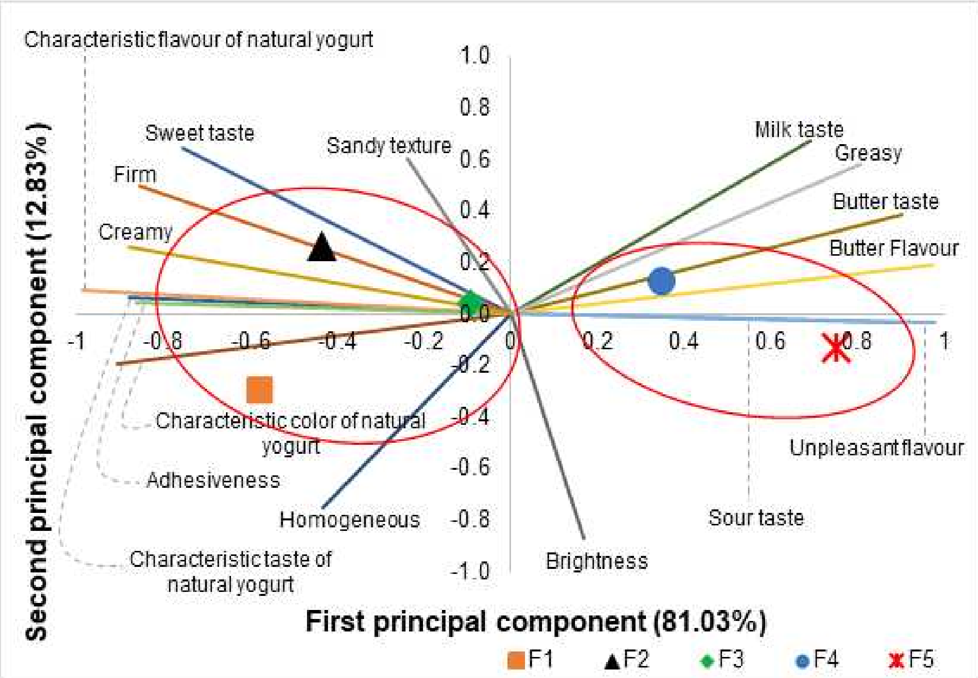

Fig. 6 RATA test results of the 5 fermented beverage formulations. The proportions of skim milk: buttermilk of the formulations are (■) F1 - 100:0; (▲) F2 - 75:25; (◆) F3 - 50:50; (●) F4 - 25:75; and (✳) F5 - 0:100.

As shown in Fig. 6, the formulations did not differ among themselves regarding the attributes "sandy texture", "homogeneous texture" and " brightness". However, it is notable that the formulations are distributed in two groups (G) that differed from each other: G1 – formulations with 100% milk (F1), 25% buttermilk (F2) and 50% buttermilk (F3) and G2 – formulations with 75% buttermilk (F4) and 100% buttermilk (F5) that differ from each other.

The G1 group was composed of the same formulations that presented the best scores for global impression in the acceptance test (F1, F2, and F3). The sensory characteristics related to the traditional beverage, such as sweet taste, creamy, firm, characteristics taste and flavour of natural yogurt, were attributed to the formulations in this group. On the other hand, the sensory attributes associated with butter, such as taste, flavour, and greasy were decisive to group the less accepted formulations in the G2 group (F4 and F5). These results demonstrated that molecules present in buttermilk associated with the sensory characteristics of the product from which it originates had an impact on the acceptance of the F4 and F5 formulations. In this way, the partial replacement of up to 50% of milk by buttermilk positively influenced on the sensory characteristics and enabled the production of fermented beverages that were well accepted by consumers.

4 CONCLUSIONS

In general, all formulations showed similar composition and pH and microbiological quality within the standards established by Brazilian legislation. The presence of buttermilk in the formulations caused an improvement in the WHC, when compared to the formulation without buttermilk (100% milk). The formulation with 25% buttermilk had the best WHC (62.72%). A similar behaviour was observed for syneresis, where it had a decreasing trend for all formulations, and the 25% buttermilk formulation had the lowest syneresis values (11.50%).

The rheological parameters demonstrated that the fermented beverages presented non-Newtonian fluid behaviour, with pseudo plastic characteristics. The formulation with the addition of 25% buttermilk showed the highest values of viscosity and consistency index, and lowest values of hysteresis and flow behaviour index. The G' value of all formulations was greater than the G'' value, indicating that the elasticity response was stronger than the viscosity response and, therefore, exhibiting a food with semi-solid behaviour.

The application of buttermilk as a partial substitute (up to 50%) for skimmed milk led to the production of fermented beverage formulations with good sensory acceptance. The RATA test showed that some sensory attributes characteristic of butter, such as taste, flavour, and greasy, were the main factors responsible for the reduced acceptance of formulations in which the buttermilk concentration was greater than 50% (F4 and F5).

The development of sustainable and healthy products is an important strategy for managing co-products and by-products generated by the food industry, especially in small and medium-sized industries. Therefore, buttermilk showed great potential to be explored as an ingredient by the food industries.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL FOR THE ARTICLE

Table S1. Composition of the fermented dairy beverage formulations produced with different proportions of skim milk:buttermilk

Formulations	Moisture	Protein	Lipid	Ash	Carbohydrate
F1	77.99^{a}	5.26^{a}	0.53^{a}	1.11^{a}	15.11^{a}
F2	77.13^{c}	5.47^{a}	0.55^{a}	1.10^{a}	15.74^{ab}
F3	77.26^{b}	5.36^{a}	0.56^{a}	1.12^{a}	15.69^{ab}
F4	76.05^{d}	5.57^{a}	0.57^{a}	1.18^{a}	16.63^{bc}
F5	75.85^{e}	5.45^{a}	0.57^{a}	1.18^{a}	16.96^{c}

The proportions of skim milk: buttermilk are F1 - 100:0; F2 - 75:25; F3 - 50:50; F4 - 25:75; and F5 - 0:100. Values with different superscript letters are significantly different ($p < 0.05$).

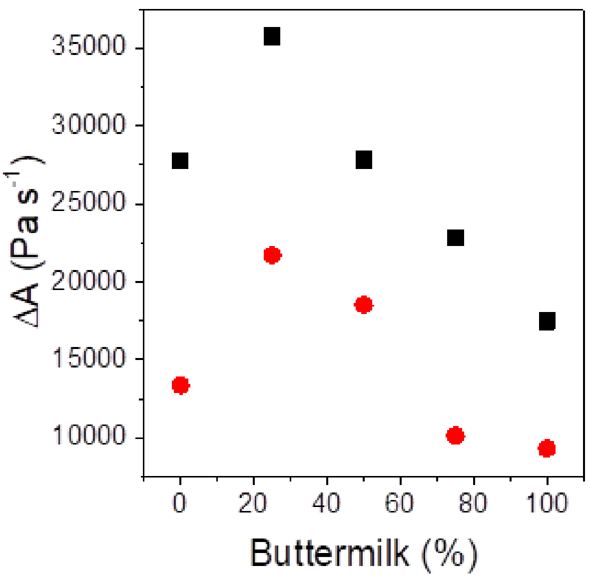

Fig. S1. Relationship between hysteresis area (ΔA (Pa s^{-1})) and added buttermilk concentration in beverage formulations, at 5°C (■) and 25°C (●).

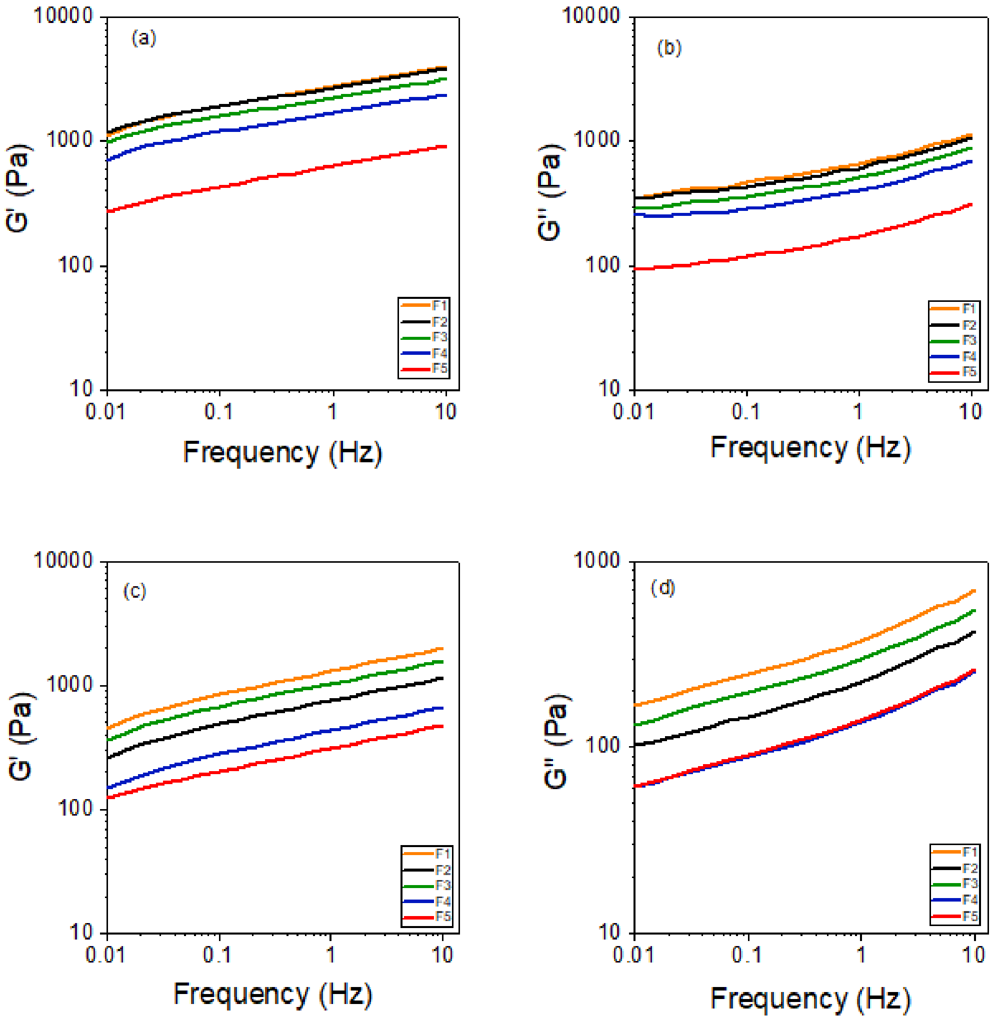

Fig. S2 Storage modulus (G') and loss modulus (G") vs. frequency (Hz) of the 5 beverage formulations fermented at 5 °C (a, b) and 25°C (c, d). The proportions of skim milk: buttermilk of the formulations are F1 - 100:0; F2 - 75:25; F3 - 50:50; F4 - 25:75; and F5 - 0:100.

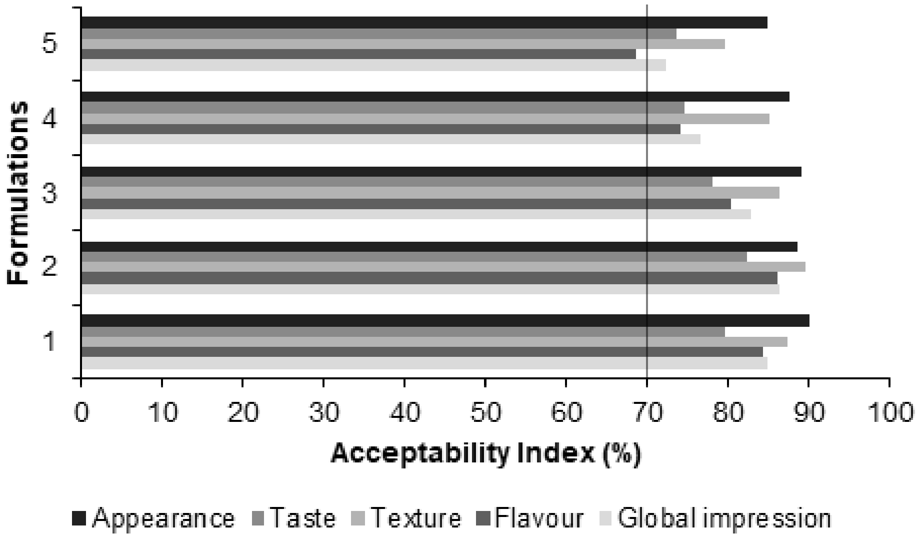

Fig. S3 Acceptability index (AI) results for appearance, taste, texture, flavour and global impression attributes of the 5 fermented beverage formulations.

CONCLUSÕES GERAIS

O presente estudo tratou-se do aproveitamento de um coproduto originado da fabricação da manteiga, denominado leitelho. Este coproduto foi usado para o desenvolvimento de cinco formulações de bebida láctea fermentada “tipo iogurte grego” com baixo teor de gordura.

Os resultados demostraram que as formulações de bebidas fermentadas apresentaram resultados semelhantes para a composição centesimal e pH. A presença de leitelho nas formulações ocasionou melhora na CRA, quando comparado a formulação com 100% de leite. A formulação com 25% de leitelho apresentou a melhor CRA (62,72%). Comportamento semelhante foi observado para a sinérese, onde todas as formulações apresentaram uma tendência decrescente e a formulação com adição de 25% de leitelho, apresentou o menor valor de sinérese (11,50%).

Os parâmetros reológicos demonstraram que as bebidas lácteas fermentadas apresentaram comportamento de fluido não newtoniano, com características pseudoplásticas. A formulação com adição de 25% de leitelho apresentou os maiores valores de viscosidade e índice de consistência, e menores valores da área de histerese e índice de comportamento de fluxo. O modelo que melhor se ajustou aos dados experimentais foi o de Herschel-Bulkley, com $R^2 > 0,9$. O valor G' de todas as formulações foi maior que o valor G'', indicando que a resposta de elasticidade foi mais forte que a resposta de viscosidade e, portanto, exibindo um alimento com comportamento semi-sólido.

Houve uma redução da aceitação nas formulações com adição de 75% e 100% leitelho, fato este que foi atribuído a alguns atributos característicos da manteiga, como sabor e aroma de manteiga e gorduroso. Portanto, a substituição parcial de leite por leitelho (até 50%) levou à produção de formulações de bebida fermentada tipo “iogurte grego” com boa aceitação sensorial.

Portanto, conclui-se que é possível utilizar o leitelho como substituto parcial do leite na produção de bebida fermentada “tipo iogurte grego”, levando à geração de produtos de alto valor nutricional e baixo custo, fortalecendo a bioeconomia.