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TAMIRES MARQUES PAES DA CUNHA

CONCENTRAÇÃO DE PRODUTOS LÁCTEOS E POTENCIAL FÍSICO, QUÍMICO E NUTRICIONAL DE COMPOSTO LÁCTEO ADICIONADO DE EXTRATO DE SOJA

Florianópolis, SC

2022

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O presente trabalho em nível de mestrado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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Dedico...

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“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê”.

Arthur Schopenhauer

RESUMO

O desenvolvimento de novos produtos lácteos vem crescendo no setor alimentício, e a adição do extrato de soja é uma alternativa que visa enriquecer nutricionalmente o produto com compostos bioativos, além disso, a substituição parcial do leite em pó por extrato de soja permite reduzir custos de produção, pois o extrato de soja apresenta menor valor comercial em relação ao leite. A presente dissertação está estruturada em capítulos, sendo o primeiro capítulo referente à revisão bibliográfica, o segundo capítulo referente à revisão de tecnologias de membranas utilizadas para a concentração de lácteos, e o terceiro capítulo referentes aos resultados da pesquisa experimental. O objetivo deste trabalho foi revisar as tecnologias de membranas aplicadas na concentração de produtos lácteos, bem como desenvolver um composto lácteo adicionado de extrato de soja a fim de enriquecer nutricionalmente este produto. As tecnologias de membranas são utilizadas para a filtração, separação, purificação e concentração de alimentos, e tais tecnologias podem ser aplicadas no setor de produtos lácteos. A aplicação de tecnologias de microfiltração, nanofiltração, ultrafiltração, diafiltração e osmose reversa pode ser aplicada em diferentes derivados lácteos, como no leite em pó, onde atua na etapa de concentração do leite. Na parte experimental deste estudo foi desenvolvida cinco formulações de composto lácteo, onde houve a substituição do leite em pó desnatado por 10, 20, 30, 40 e 49% m/m de extrato de soja. Todas as amostras foram avaliadas quanto aos parâmetros físico-químicos, propriedades físicas e de reidratação, potencial polifenólico e perfil multielementar. A adição de extrato de soja nas formulações de compostos lácteos promoveu o aumento no teor de lipídeos, polifenóis totais, flavonóis totais, atividade antioxidante e minerais. Os minerais majoritários em todas as amostras foram o Ca, K, P, Mg e Na. Todas as formulações de compostos lácteos apresentaram maiores concentração de minerais, principalmente de Fe, Mn, P, Cu e Mg em relação a amostra controle (leite em pó desnatado). A análise de componentes principais revelou que a adição de extrato de soja ao leite em pó promoveu o enriquecimento do composto lácteo com minerais e polifenóis, principalmente quando se utiliza concentrações de 30, 40 e 49% de extrato de soja nas formulações. Quanto as propriedades físicas houve redução na densidade e fluidez das formulações de acordo com o aumento do percentual de extrato de soja. Os compostos lácteos formulados com extrato de soja apresentaram alta solubilidade (>69%). O composto lácteo formulado com leite em pó e extrato de soja é uma excelente alternativa ao tradicional composto lácteo adicionado de soro de leite em pó, pois a adição de extrato de soja promoveu o enriquecimento de compostos fenólicos e minerais essenciais sem prejudicar o percentual proteico do produto.

Palavras-chave: Concentração, Membranas, Composto Lácteo, Enriquecimento Mineral, Compostos Bioativos.

ABSTRACT

The development of new dairy products has been growing in the food sector, and the addition of soy extract is an alternative that aims to nutritionally enrich the product with bioactive compounds. production, since soy extract has a lower commercial value compared to milk. This dissertation is structured in chapters, the first chapter referring to the bibliographic review, the second chapter referring to the review of membrane technologies used for the concentration of dairy products, and the third chapter referring to the results of the experimental research. The aim of this work WAS to review membrane technologies applied in the concentration of dairy products, as well as to develop a dairy compound added with soy extract to nutritionally enrich this product. Membrane technologies are used for the filtration, separation, purification, and concentration of foods, and such technologies can be applied in the dairy sector. The application of microfiltration, nanofiltration, ultrafiltration, diafiltration and reverse osmosis technologies can be applied to different dairy products, such as powdered milk, where it acts in the milk concentration stage. In the experimental part of this study, five milk compound formulations were developed, where skimmed milk powder was replaced by 10, 20, 30, 40 and 49% w/w of soy extract. All samples were evaluated for physicochemical parameters, physical and rehydration properties, polyphenolic potential and multielement profile. The addition of soy extract in the formulations of dairy compounds promoted an increase in the content of lipids, total polyphenols, total flavonols, antioxidant activity and minerals. The major minerals in all samples were Ca, K, P, Mg and Na. All formulations of dairy compounds showed higher concentrations of minerals, mainly Fe, Mn, P, Cu and Mg in relation to the control sample (skimmed milk powder). Principal component analysis revealed that the addition of soy extract to powdered milk promoted the enrichment of the dairy compound with minerals and polyphenols, especially when using concentrations of 30, 40 and 49% of soy extract in the formulations. Regarding the physical properties, there was a reduction in the density and fluidity of the formulations according to the increase in the percentage of soybean extract. Dairy compounds formulated with soy extract showed high solubility (>69%). The dairy compound formulated with powdered milk and soy extract is an excellent alternative to the traditional dairy compound added with whey powder, as the addition of soy extract promoted the enrichment of phenolic compounds and essential minerals without harming the protein percentage of the product.

Keywords: Concentration, Membranes, Dairy Compound, Mineral Enrichment, Bioactive Compounds.

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INTRODUÇÃO

O leite é caracterizado como um alimento de excelente qualidade nutricional e indispensável para a alimentação humana, por ter em sua composição química, um vasto aporte de proteínas, lactose, gorduras, carboidratos, vitaminas D, B e A e minerais (RAYMUNDO *et al.*, 2014; LUCAS, 2015; INMETRO, 2020). Entre as alternativas para conservar o leite pode-se realizar sua concentração e desidratação. Entretanto, apesar do leite em pó ser largamente desenvolvido na indústria láctea e popularmente consumido (INMETRO, 2020), é considerado um produto de alto valor de produção, pois requer alta gasto energético, passado por três etapas que exige energia, como a pasteurização, concentração e secagem (YILDIRIM; GENC, 2017).

A produção de leite é sem dúvida uma importante fonte de renda e constitui-se uma das grandes potencialidades da região sul do Brasil. Assim, a concentração e desidratação de leite é uma alternativa viável para o seu aproveitamento e comercialização. A tecnologia de secagem contribui para estender a validade dos produtos lácteos, promovendo uma menor alteração química e microbiológica dos alimentos (KITAMURA *et al.*, 2009; SHARMA *et al*., 2012).

Para obtenção do leite desidratado ou leite em pó são necessárias duas etapas, a concentração e desidratação. A desidratação do leite é realizada pelo método *spray drying*, que é amplamente empregado pela indústria. Já a concentração tradicionalmente é realizada por evaporação, sendo a tecnologia de membranas uma alternativa para esta etapa. O leite em pó é um produto importante por ser reconstituído e utilizado na forma líquida, além de ser considerado um ingrediente, por exemplo, empregado na confeitaria e panificação (SHARMA *et al*., 2012), e como base para compostos lácteos.

A indústria de alimentos vem se destacando na produção de compostos lácteos, mistura de leite e outros ingredientes, como o soro lácteo, a fim de aproveitar a conservação creditada ao processo de desidratação. A legislação brasileira determina que composto lácteo deve conter 51% de leite em pó, sendo assim outros ingredientes podem compor até 49% (BRASIL, 2007). Uma alternativa seria o uso do extrato de soja como forma de enriquecer o composto lácteo, já que se trata de uma leguminosa com excelente fonte de proteína, cerca de 40% (GIRI; MANGARAJ, 2012).

A soja é descrita como um alimento com teores elevados de compostos bioativos, como daidzeína, genisteína, ácido gálico, entre outros (TYUG *et al*., 2010). Os compostos bioativos podem prevenir ou inibir doenças, como câncer, diabetes, doenças cardiovasculares, parkinson, alzheimer, inflamações, entre outras (SHAHIDI; YEO, 2018). A soja também possui altas concentrações de minerais, sendo estes essenciais na dieta humana atuando em diversos sistemas do corpo, soja possui valores expressivos de P, K, Mg, Ca, Mn, Na e Fe (DA SILVA SCHERER *et al*., 2021, VIEIRA *et al.,* 1999). Da soja, o seu extrato solúvel vem se destacando como um alimento nutritivo e viável economicamente (CARRÃO-PANIZZI; MANDARINO, 1998; GIRI; MANGARAJ, 2012).

Assim, este trabalho teve como objetivo realizar uma revisão bibliográfica sobre a concentração de lácteos, bem como elaborar e avaliar o potencial físico, químico e nutricional de composto lácteo com adição de extrato de soja em pó. Desta forma, esta dissertação está apresentada nos três seguintes capítulos: (a) capítulo 1, referente à revisão bibliográfica sobre leite, leite em pó, extrato de soja, compostos bioativos, perfil multielementar, obtenção tecnológica do leite em pó, obtenção tecnológica de bebidas de soja, propriedades físicas dos pós e reidratação de pós; (b) capítulo 2, sobre um artigo de revisão bibliográfica abordando a concentração de lácteos empregando os processos de separação por membranas; e o (c) capítulo 3, contendo os resultados e as discussões sobre composto lácteo em pó enriquecidos com extrato de soja (*Glycine max*), e a determinação dos seus parâmetros físico-químicos, suas propriedades físicas e de reidratação, além do seu potencial polifenólico e perfil multielementar.

CAPÍTULO 1

1 REVISÃO BIBLIOGRÁFICA

1.1 O leite

Leite é o produto de origem animal, procedente de uma contínua e completa ordenha de uma fêmea leiteira submetida a condições higiênico-sanitárias adequadas. Sensorialmente descrito com coloração branco uniforme, odor peculiar e sabor adocicado. Conforme a Instrução Normativa (MAPA) nº 76/2018, o leite cru precisa seguir parâmetros físico-químicos, mantendo em conformidade teores mínimos e máximos de gordura, proteína total, sólidos totais, lactose anidra, entre outros (BRASIL, 2018b).

A constituição do leite, com sua complexa combinação de macro e micronutrientes, é o que torna um alimento tão particular. Segundo Damodaran, Parkin e Fennema (2010), os constituintes básicos do leite são água, lipídeos, proteínas, carboidratos, vitaminas e minerais. A água é o componente majoritário do leite, cerca de 87%, podendo estar em sua forma livre, ou quimicamente ligada formando emulsões, dispersões e dissoluções com outros componentes (SILVA, 1997).

Os lipídeos representam de 3,5 e 5,3% a composição total do leite, e sua concentração pode ser diretamente influenciada por fatores como a dieta e a raça do animal. A composição lipídica do leite bovino é representada predominantemente por triglicerídeos, com teores de 96% a 98% do total lipídico, representados como glóbulos envoltos por uma membrana lipoproteica, mantidos em suspensão. Os teores restantes da composição de lipídeos no leite bovino podem ser definidos por diglicerídeos e monoglicerídeos, ácidos graxos livres, fosfolipídeos e colesterol (ALOTHMAN *et al.*, 2019; DAMODARAN; PARKIN; FENNEMA, 2010).

As proteínas compreendem de 3 a 4% da composição do leite. No leite, as proteínas majoritárias encontradas são as caseínas (αS1-caseínas, αS2-caseínas, β-caseínas, κ-caseínas), albuminas (α-lactoalbuminas) e globulinas (β-lactoglobulinas), sendo que o nitrogênio caseínico representa 80% do total proteico do leite, enquanto os nitrogênios não caseínicos representam apenas 20% (ALOTHMAN *et al.*, 2019; SILVA, 1997; DAMODARAN; PARKIN; FENNEMA, 2010).

Com relação aos carboidratos presentes no leite, a lactose é açúcar predominante, sendo composta pelos monossacarídeos glicose e galactose. A lactose pode atingir 50% do total de sólidos em um leite desnatado, o que favorece o sabor adocicado peculiar do leite (ALOTHMAN *et al*., 2019; SILVA, 1997; DAMODARAN; PARKIN; FENNEMA, 2010).

As vitaminas e minerais são os constituintes básicos do leite encontrados em menores concentrações. As vitaminas presentes no leite podem ser divididas em lipossolúveis (vitaminas A, E, D, K) e hidrossolúveis (vitaminas do complexo B e vitamina C) (SILVA, 1997; DAMODARAN; PARKIN; FENNEMA, 2010). Algumas dessas vitaminas podem sofrer degradação com o tratamento térmico aplicado no leite. Conforme Damodaran, Parkin e Fennema (2010), vitaminas como ácido ascórbico (vitamina C), tiamina (vitamina B1), piridoxina (vitamina B6), ácido fólico (vitamina B9) e cobalamina (vitamina B12), por exemplo, estão sujeitos a degradação oxidativa e a degradação por calor, sucedido por pasteurização e esterilização UHT (*Ultra High Temperature*). Os minerais representam em média 6,5%, sendo os principais fósforo, potássio, sódio, cálcio e magnésio e em menores concentrações ferro, alumínio, bromo, zinco e manganês (SILVA, 1997; ALOTHMAN *et al*., 2019).

1.2 Leite em pó

De acordo com a Instrução Normativa (MAPA) nº 53/2018, o leite em pó é definido como o produto resultante de processos tecnologicamente apropriados, para a desidratação do leite, sem que haja mudanças na proporção dos constituintes proteicos e gordurosos da matéria-prima, podendo estes ser ajustados caso seja necessário atingir os padrões estipulados de sua composição. Entretanto, deve ser imprescindível que se mantenha as proporções entre a proteína do leite (caseína) e a proteína do soro (BRASIL, 2018a).

O leite em pó é classificado segundo a Instrução Normativa (MAPA) nº 53/2018, por seus teores de gordura em integral, parcialmente desnatado e desnatado. Para o leite em pó integral os teores de gordura precisam ser maiores ou igual a 26,0%. Já para o leite em pó parcialmente desnatado os teores de gordura precisam ser maiores que 1,5% e menores que 26,0%. Por fim, os teores de gordura do leite em pó descrito como desnatado devem ser menores ou igual a 1,5%. Conforme a legislação corrente, as características sensoriais imprescindíveis são em relação ao aspecto, que devem apresentar uniformidade e ausência de grumos, coloração, e caracterizado como branco amarelado, e, em relação ao sabor e odor, que deve ser conforme o leite fluido, sem rancidez e aprazível ao paladar.

Somente alguns componentes podem estar presentes no leite em pó, como proteínas, gorduras, açúcares e minerais (BRASIL, 2018a).

Essas características físico-químicas dos produtos são as mais importantes para o consumidor, pois reflete na qualidade sensorial e de conservação do produto, o que inferirá na intenção de compra e aceitação do consumidor (HAMMES, 2013). Segundo Hammes (2013), para a obtenção do leite em pó são necessários vários processos tecnológicos como: padronização (desnate), pasteurização, atomização e instantaneização. Dentre essas operações unitárias, destaca-se que a mais importante para a obtenção do leite em pó, é a atomização (*spray drying*), momento em que ocorre a secagem transformando o líquido em pó (HAMMES, 2013). Bilge *et al*. (2016) relataram que a adição de soro lácteo em pó ao leite em pó tem sido amplamente utilizada pelas indústrias lácteas. Estas adições apresentam benefícios econômicos e nutricionais, mas, no entanto, devem respeitar a legislação vigente. Estas misturas resultam nos compostos lácteos (BILGE *et al*., 2016).

O Composto lácteo é definido pela Instrução Normativa (MAPA) 28/2007 como o produto em pó consequente da combinação do leite com derivados lácteos ou não lácteos, ou ambos, desde que sejam adequados para uso na alimentação humana, submetidos a processos apropriados tecnologicamente. A composição final do composto lácteo deve ser representada por um volume (massa/massa) de no mínimo 51% provenientes da matéria-prima leite, somados a substâncias alimentícias lácteas, como por exemplo, creme de leite, soro de leite, proteína concentrada de leite, manteiga, leites fermentados, entre outros. A composição remanescente deve conter um volume (massa/massa) de no máximo 49% de substâncias alimentícias não lácteas, tais como açúcares, amidos polpas/pedaços/suco de frutas, edulcorantes, entre outros (BRASIL, 2007).

1.3 Extrato de soja

A soja é uma das culturas mais importante do mundo, devido a sua ampla aplicação como fonte de lipídeos e proteínas na dieta humana (CHEN *et al*., 2012; DE BARROS; VENTURINI FILHO, 2016). A produção mundial de soja em 2010 foi de 261,6 milhões de toneladas (CHEN *et al*., 2012), e na safra de 2020/2021 a produção de soja foi de 362, 947 milhões de toneladas. O Brasil é o maior produtor mundial de soja, sendo que na safra de 2022 foram produzidas 135,409 milhões de toneladas de soja (EMBRAPA, 2022). Além da alta produção, a riqueza nutricional e aumento da popularidade (GIRI; MARAGAJ, 2012) faz da soja uma matéria prima com potencial de processamento e participação inovação de produtos. A bebida a base de soja (extrato de soja) é um derivado da soja que já se popularizou como um alimento saudável (GIRI; MARAGAJ, 2012).

O extrato de soja pode ser descrito como o produto resultante de uma emulsão aquosa obtida de grãos de soja hidratados, previamente limpos e posteriormente submetidos a processos tecnologicamente adequados (moagem, filtração, tratamento térmico). Pode ser encontrado em sua forma líquida, ou em pó, submetido à concentração e desidratação (GIRI; MARAGAJ, 2012).

Conforme Benassi, Benassi e Mandarino (2012), o extrato de soja é considerado uma opção saudável para a alimentação, por ser livre de colesterol, e uma alternativa aos intolerantes de produtos lácteos, já que este é um alimento sem lactose. Na composição química do grão de soja, se destaca como componente majoritário a proteína, com valores em média de 40%, seguido dos carboidratos (30%), lipídios (20%), fibras (5%) e cinzas (5%) (ROSSI; ROSSI, 2010; CHEN et al., 2012).

O extrato solúvel de soja apresenta elevada digestibilidade, e possui uma boa composição aminoacídica. Nos extratos solúveis comercializados os aminoácidos essenciais com maior concentração é leucina seguida lisina, valina, isoleucina, treonina, metionina, triptofano e Cistina (SALGADO, 2017; CARRÃO-PANIZZI; MANDARINO, 1998). Observa-se menor teor de aminoácidos sulfurados (cistina e metionina), comumente observados em leguminosas como a soja e uma carência de cálcio e vitaminas C e A. Além disso, o extrato de soja contém minerais como magnésio e fósforo, que são importantes para manutenção das funções básicas do organismo (CARRÃO-PANIZZI; MANDARINO, 1998)

Baseado nas características nutricionais da soja e produtos derivados nota-se que, o consumidor brasileiro, encontra-se mais disposto a incluir na dieta produtos de fonte vegetais, como à base de soja (DE BARROS; VENTURINI FILHO, 2016; ROSSI; ROSSI, 2010). Com isso, cresce em qualidade e variedade alimentos como bebidas, sorvetes, iogurtes, formulados infantis, entre outros que possuem extrato de soja em sua formulação (ROSSI; ROSSI, 2010).

A ação positiva da soja para corpo humano está afiliada ao seu perfil de compostos bioativos, a soja tem em sua composição isoflavonas, saponinas, peptídeos e fitosteróis. A soja é associada a uma gama de benefícios a saúde humana, o consumo deste grão, já foi verificado sua eficiência na prevenção da incidência de câncer, doenças cardíacas, osteoporose, colesterol e diabetes (ISANGA; ZHANG, 2008; CHEN *et al*., 2012).

1.4 Compostos bioativos

Resolução da ANVISA (Agência Nacional de Vigilância Sanitária) nº 243/2018 definiu que compostos bioativos são nutrientes ou não nutrientes consumido normalmente como componente de um alimento, que possui ação metabólica ou fisiológica específica no organismo humano (BRASIL, 2018). Os compostos bioativos são encontrados em diversas fontes alimentares, com destaque para os vegetais (O'CONEEL; FOX, 2001).

1.4.1 *Polifenóis*

Os compostos fenólicos possuem ao menos um anel benzênico agrupado a um ou mais substituintes hidroxilas, provenientes do metabolismo secundário das plantas (O'CONEEL; FOX, 2001). Os compostos polifenóis tem como principais grupos flavonas, flavonóis, flavan-3-ols, isoflavonas, flavononas, antocianidinas e lignanas (HOOPER; CASSIDY, 2006). Em leguminosas, os compostos fenólicos são mais expressivos no tegumento (SINGH *et al*., 2017), já no leite estes compostos têm origem da alimentação das matrizes (O'CONEEL; FOX, 2001).

No caso das leguminosas, como a soja, a composição bioativa sofre variações de acordo com fatores ambientais como cultivar, temperatura, ano de cultivo e por fatores genéticos do grão (LIN; LAI, 2006). Devi *et al.* (2009) encontraram cerca de 30mg GAE/g de fenólicos totais na soja, 22 mg GAE/g na farinha de soja e 9 mg GAE/g no extrato de soja, já no extrato de soja foi relatado concentração de 75,0 ± 1,8 mg/g de polifenóis totais (ISANGA; ZHANG, 2008). Quanto a isoflavonas, a soja é considerada uma fonte, é composto o fenólico mais relevante deste grão, sendo que já foi relatado valores entre 0,4 mg a 9,5 mg /g de soja (ANDRÉS *et al*., 2016; DEVI *et al.,* 2009; ISANGA; ZHANG, 2008). Já foram identificados na soja 12 isoflavonas, como as genisteína e daidzeína, as outras são gliciteína, daidzina, genistina, glicitina, 6ÿ-O-acetildaidzina, 6ÿ-O-acetilgenistina, 6ÿ-O acetilglicitina, 6ÿ-O-malonildaidzina, 6ÿ-O-malonilgenistina, 6ÿ-O-malonilglicitina (ISANGA; ZHANG, 2008).

Devi *et al*. (2009) avaliaram vários produtos de soja, grãos, brotos, farinha, molho e bebida, e foi evidenciado que o processamento do grão soja pode interferir nos valores de compostos bioativos. Os autores relataram que o conteúdo de polifenóis e flavonóis no extrato de soja foi menor que o teor de polifenóis encontrado para grãos. Shin *et al*. (2013) encontraram maiores quantidades de fenólicos em farinhas produzidas com grãos de soja que passaram por processamento térmico antes a moagem, isso porque o tratamento térmico gera ruptura paredes das células vegetais e hidrólise dos compostos ligados contribuindo para maior extração dos compostos fenólicos (SHIN *et al*., 2013).

Os compostos fenólicos presentes no leite, em sua maioria, têm origem da dieta dos animais. No leite bovino estão presentes os seguintes polifenóis: Fenol, *o* -Cresol, *p* -Cresol, *m* -Cresol, 2-etilfenol, Timol e Carvacrol. (DE FEO *et al*., 2006; O'CONEEL; FOX, 2001).

Os flavonóides são o principal grupo de fenólicos encontrados em leguminosas, (SINGH *et al*., 2017). Sua estrutura é formada núcleo que consiste em três anéis fenólicos representados na Figura 1.1 (anéis A, B e C). Quando o anel C é pirano heterocíclico ou pirona podem ser originados os flavonóis (AHERNE; O'BRIEN, 2002). Neste grupo a quercetina, kaempferol e miricetina, são os polifenóis mais identificados em alimentos (HOOPER; CASSIDY, 2006).

A quantidade de flavonóides na soja pode variar de 1,06 a 4,04 mg CE / g (SINGH *et al*., 2017). Chung *et al*. (2011) encontraram 3,345 mg/g de flavonóides na soja, onde a catequina e quercetina, apresentaram as maiores concentrações nos grãos de soja.

Figura 1. 1 Estrutura dos Flavonóides

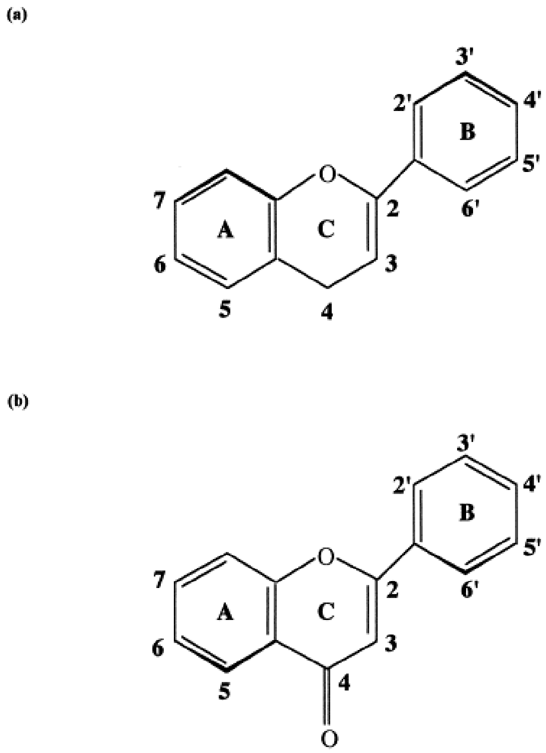


Figura 1.1 (a) Estrutura Flavonóides (b) Estrutura *4-oxo-flavonóides* (flavanóis, flavanonas, flavonóis e flavonas)

Fonte: AHERNE; O'BRIEN, (2002)

1.4.2 *Atividade Antioxidante*

Os radicais livres estão associados a uma série de doenças, como câncer, distúrbios neurais e doenças cardiovasculares, desta forma o consumo de compostos com capacidades antioxidantes vem sendo relacionado com a prevenção destas doenças (ALAM; BRISTI; RAFIQUZZAMAN, 2013). A atividade antioxidante é capacidade de eliminar os radicais de oxigênio e outras espécies reativas (EL GHARRAS, 2009). Os antioxidantes podem agir por diferentes mecanismos diminuindo a concentração de oxigênio, evitando a fase de iniciação da oxidação, quelando íons metálicos, decompondo produtos primários (SUCUPIRA *et al.*, 2012), devido a essas diferentes formas de ação é importante utilizar diferentes ensaios para determinar a capacidade antioxidante de uma amostra. Existem algumas metodologias *in vitro* habilitadas a avaliar a atividade antioxidante de amostras de alimentos, como os métodos DPPH, FRAP e ABTS (ALAM; BRISTI; RAFIQUZZAMAN, 2013).

O método DPPH tem como princípio a redução da molécula DPPH (1, 1-difenil-2-picrilhidrazil), esta é uma molécula considerada um radical livre, caracterizada por coloração violeta , está coloração é perdida ao interagir e receber um hidrogênio sofrendo redução. Este é o método mais utilizado para determinar atividade antioxidante, pois se trata de um método rápido, simples, não envolve muitas etapas e reagentes, é barato em comparação com outros (ALAM; BRISTI; RAFIQUZZAMAN, 2013). A análise de ABTS consegue quantificar a atividade antioxidante pela perda de cor Azul-verde, quando a amostra é misturada a solução de ABTS · + (ácido 2,2-azino-bis (ácido 3-etilbenztiazolina-6-sulfônico). As vantagens que o método ABTS apresenta é de ser rápido, boa estabilidade, oferece resultados reprodutíveis e pode ser feito tanto com amostras hidrossolúveis como com lipossolúveis. (ALAM; BRISTI; RAFIQUZZAMAN, 2013; SUCUPIRA *et al.*, 2012). Estes métodos têm como princípio a eliminação de radicais livres pela amostra adicionada. O método FRAP tem como princípio determinar a capacidade da amostra de reduzir complexo de ferro férrico e cloreto de 2,3,5-trifenil-1,3,4-triaza-2-azoniaciclopenta-1,4-dieno (ALAM; BRISTI; RAFIQUZZAMAN, 2013; SUCUPIRA *et al.*, 2012).

A atividade antioxidante do leite está associada principalmente a aminoácidos sulfurados, proteínas do soro do leite (especialmente β-lactoglobulina), vitaminas A, E e C, ou β-caroteno (STOBIECKA; KRÓL; BRODZIAK, 2022). Tagliazucchi *et al*. (2016) relataram em seu estudo os componentes encontrados no leite com capacidade de agir como antioxidantes, os compostos que mais influíram nas simulações para atividade antioxidante foram os aminoácidos triptofano, tirosina e alguns peptídeos contendo-a, fenilalanina e peptídeos contendo histidina (TAGLIAZUCCHI *et al*., 2016). A espécie, a dieta e a fase de lactação irão interferir na capacidade antioxidante do leite (STOBIECKA; KRÓL; BRODZIAK, 2022).

Os compostos fenólicos, revisados na seção anterior polifenóis, flavonóis e isoflavonas, tem capacidade antioxidante (EL GHARRAS, 2009). Segundo Sucupira *et al.* (2012) os fenólicos conseguem agir com diferentes mecanismos, doação de um átomo de hidrogênio de um grupo hidroxila, quelando metais de transição, interrompendo a reação de propagação dos radicais livres na oxidação lipídica, modificando o potencial redox do meio e reparando a lesão a moléculas atacadas por radicais livres. A isoflavonas, composto fenólico com grande destaque da soja, e outros polifenóis, são os grandes responsáveis pela capacidade antioxidante deste grão e seus derivados (TYUG *et al.*, 2010).

1.5 Perfil multielementar

Os minerais são essenciais na dieta humana, pois tem diversas reponsabilidades no funcionamento dos sistemas do corpo. Os minerais participam do desenvolvimento dos ossos e dentes, funções musculares, sistema imunológico, regulação pressão arterial, sistema digestivo, coagulação sangue, sistema nervoso, produção energética, atividades metabólicas e equilíbrio eletrolítico. São classificados em microminerais e macrominerais, sendo que a necessidade de ingestão dos microminerais e macrominerais são menores que 100 mg e maior que 100 mg dia, respectivamente (GHARIBZAHEDI; JAFARI, 2017). A resolução de n 269/2005 da Anvisa (Agência nacional de vigilância sanitária) determina os valores diários de consumo de nutrientes. Para adultos o consumo de Ca, P e Mg é 1000 mg, 700 mg e 260 mg, respectivamente. O Fe é um dos minerais que apresenta grande deficiência nas dietas em todo o mundo (GHARIBZAHEDI; JAFARI, 2017), sua recomendação diária é de 14 mg (BRASIL, 2005).

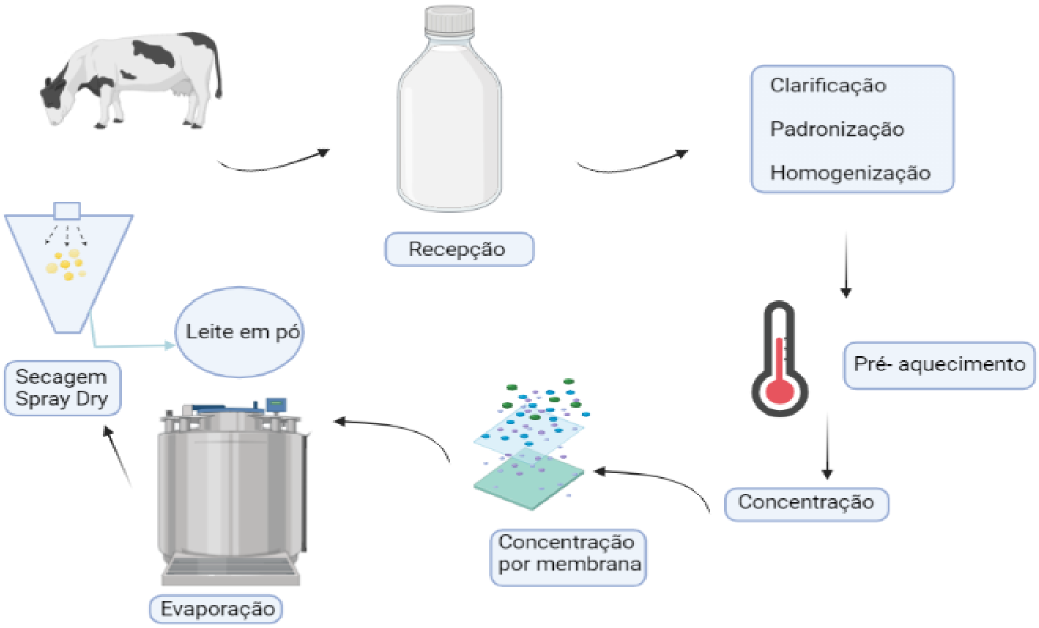
A soja possui valores elevados de minerais, como o K, seguido P, também está presente, em menores quantidades, no grão o Mg, Ca, Fe, Na e Mn (DA SILVA SCHERER *et al.*, 2021, VIEIRA et al., 1999). O perfil mineral dos grãos de soja pode sofrer variação de acordo com o cultivar. A soja processada, em sua forma de farinha, permanece com valores expressivos de K e P. Porter e Jones (2003), relataram na farinha de soja concentração de 2333mg/100g para K e 7.72 mg/100g, para P, os autores também verificara concentração de 321 mg/100g de Ca e 320mg/100g de Mg. Também se encontra na farinha em pequenas quantidades Zn, Al, Cu, Cr, Mo e Ni (PORTER; JONES, 2003).

Os minerais majoritários encontrados no leite são o Ca, P, Na, K e Mg e em menores quantidades o Al, Fe, Zn, Mn e Br. A representação dos sais pode ser em sua forma de íons livres ou íons complexados, como no caso do Ca e do P, que além da contribuição nutricional, são importantes por associarem-se com as proteínas, formando estruturas micelares com a caseína (ALOTHMAN *et al.*, 2019; SILVA, 1997; DAMODARAN; PARKIN; FENNEMA, 2010). Akpanyung (2006) ao analisar diferentes amostras de leite em pó reportou que o K foi o mineral majoritário (1500 e 1710 mg/100g), seguido do Ca (1080 e 1120 mg/100g), P (610 e 690 mg/100g) e Mg (100 e 110 mg/100g) (AKPANYUNG, 2006).

1.6 Obtenção tecnológica do leite em pó

A Figura 1.2 apresenta um resumo das etapas necessárias para obtenção de leite em pó. Após a recepção de um leite de qualidade, ou seja, dentro dos parâmetros adequados físicos, químicos e microbiológicos, o leite passa pela clarificação, padronização do percentual de gordura, homogeneização e depois o leite deve passar por pré-aquecimento, este tratamento térmico prévio deve ser equivalente a pasteurização, e seguir para concentração e desidratação, retirada de água (CHAVES *et al*., 2017).

Figura 1.2 Etapas para obtenção de leite em pó



Fonte: Autor

As etapas de clarificação, padronização, homogeneização e pré-aquecimento são pré-tratamento, elas têm funções respectivamente de retirada de impurezas, adequação de gordura conforme determinação (integral, semidesnatado e desnatado), diminuição dos glóbulos de gordura e redução da carga bacteriana (MOEJES; BOXTEL, 2017). A etapa de pré-aquecimento, é referente a pasteurização que pode ser feito em três formas, baixa intensidade (74ºC/30 segundos), média intensidade (76,5º-85ºC/ 15 a 30 segundos) e alta intensidade (90º-120ºC/1 segundo) (CHAVES *et al*., 2017). O tratamento térmico do leite utilizado determina a classificação de leite em pó como alto, médio, ou baixo tratamento, que está associado a quantidade de proteínas dos soros desnaturadas (BRASIL, 1996; CHAVES *et al*., 2017).

1.6.1 Concentração

A concentração do leite é feita comumente através de evaporadores, mas há a possibilidade de utilizar tecnologias de membranas, estás podem ser uma alternativa como uma forma de concentração, está tecnologia é descrita em detalhes no artigo do CAPÍTULO 2. A concentração tem por objetivo reduzir em 50% a umidade presente no leite. A concentração não pode ser excessiva, mas também não pode ser inferior a 30%, pois traz problemas tecnológicos ao produto devido dificuldades nas etapas de desidratação (CHAVES *et al*., 2017).

Existem vários tipos de evaporadores utilizados para a concentração de leite, e o evaporador de múltiplos efeitos é o mais utilizado. Os evaporadores de múltiplo efeito permitem o uso do vapor gerada na etapa anterior em uma pressão mais baixa, desta forma a uma economia de energia (MOEJES; BOXTEL, 2017). Para evitar modificações indesejadas e perda de nutrientes no leite, os evaporadores são operados em pressão abaixo da atmosférica podendo alcançar a ebulição em temperaturas inferiores a 100 ºC (CHAVES *et al.,* 2017).

1.6.2. Desidratação

A desidratação do leite concentrado é a etapa seguinte a evaporação na produção de leite em pó, o objetivo da desidratação é que o produto chegue em média a 4% umidade sendo permitido pela legislação brasileira um máximo de 5% de umidade no produto final (BRASIL, 2018a; CHAVES *et al*., 2017). Segundo Sharma *et al*., (2012) a secagem consiste em passar gostas de leite líquido para partículas sólidas formatados em massa contínua de lactose amorfa e outros componentes incorporados aos glóbulos de gordura, micelas de caseína e proteínas séricas (SHARMA *et al*., 2012).

A utilização do *spray dryer* é a forma mais usada na produção de leite em pó. A liofilização resulta em um produto de alta qualidade, porém tem custos muitos altos. A secagem de tambor é eficiente energeticamente, mas prejudica a qualidade final do leite em pó. (MOEJES; BOXTEL, 2017). O *spray drying* é uma metodologia com diversas aplicações, microencapsulação, estabilização de componentes, emulsões e para preservação de alimentos (TORRES *et al.*, 2017). Está metodologia tem por característica ser rápida e promover características no produto desejáveis para indústria (SHARMA *et al*., 2012). A desidratação por *spray drying* irá passar por três etapas atomização do produto, evaporação da umidade e separação das partículas (MOEJES; BOXTEL, 2017)

O *spray dryer* conta com a torre de secagem onde o leite concentrado pode ser liberado por bicos ou sistemas rotativos. Na tecnologia de pressão o bico libera o leite sob pressão entre 100 a 600kgf/cm², no caso dos centrífugos o leite concentrado é constantemente acelerado liberando o leite em pequenas gotículas e no sistema duplo menor pressão é utilizado. O leite concentrado é atomizado, separado em pequenas gotículas, o ar quente entrar na torre de secagem entre 180ºC até 230ºC, o pó seco sai pela parte inferior da torre e o ar umidificado sai pelo topo (CHAVES *et al*., 2017; MOEJES; BOXTEL, 2017)

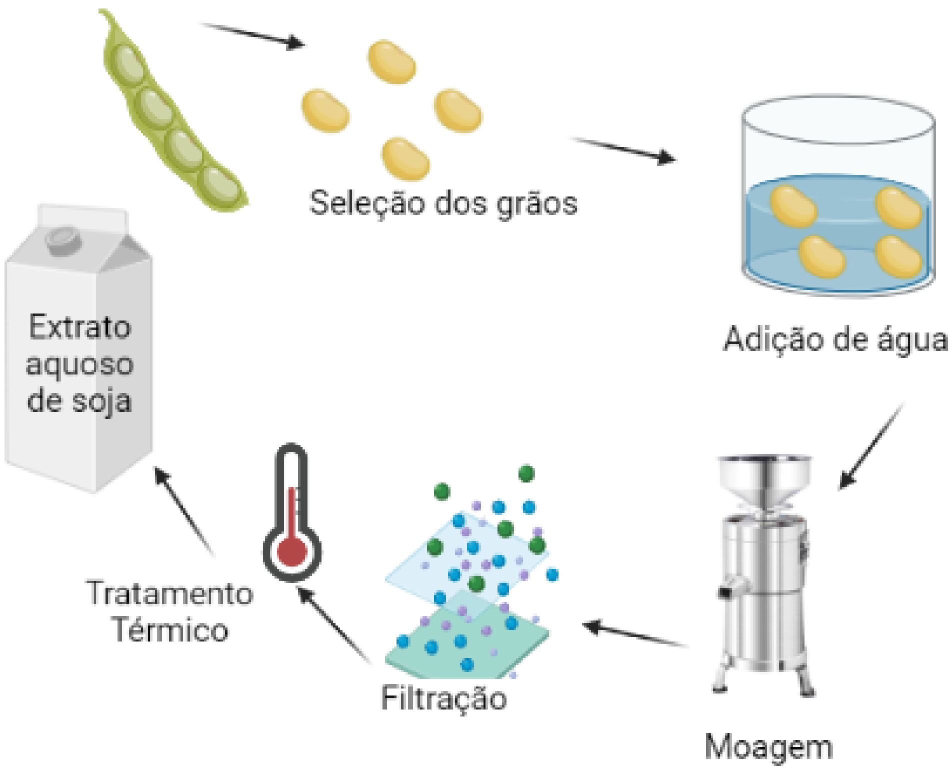
1. 7 Obtenção tecnológica de bebidas de soja

A Figura 1.3 apresenta as principais etapas para obtenção de extrato aquoso de soja. A bebida de soja na sua forma fluida se trata de um extrato aquoso, este extrato pode passar por secagem se transformando em um pó de soja, a farinha diluída em água é outro meio de produzir bebida de soja. As bebidas de soja ainda podem ser enriquecidas com adição de proteínas isoladas e óleo deste grão (GIRI; MANGARAJ, 2012).

Apesar de ser usada essa expressão “Leite de soja” na literatura, a legislação brasileira vetou seu uso na RDC Nº 91, DE 18 DE OUTUBRO DE 2000, desta forma em produtos nacionais será encontrado o termo “alimento de soja” (BRASIL, 2000)

Tyug *et al*. (2010) relatam a produção da bebida de soja com as seguintes etapas soja é embebida em água para remover a casca de soja, após a retirada da casca da soja, os grãos são secos, moídos, peneirados e cozidos até formar pasta, a pasta cozida é submetida a secagem a vácuo para se obter extrato de soja em pó. Boatright (2002) preparou a bebida de soja deixando os grãos de molho por 10 horas, fazendo moagem em uma parte de água para dez de grão em liquidificador por 1 minuto, o resultado desta moagem foi levado a frasco de vidro e mantido em banho a 85ºC por 8 minutos, foi resfriado e filtrado e armazenado a 4ºC. Lorenzo *et al.* (2007) usaram processos semelhantes aos de Boatright (2002), as diferenças foram que o tempo que os grãos ficaram de molho foram 14horas, na moagem foram utilizados sete partes de água para uma parte de soja e foi utilizado tratamento térmico UHT (*ultrahigh-temperature*) e HSTS (*High temperature−short time*). Giri e Mangaraj, (2012) explicam que há diversas formas e condições para preparação de bebidas de soja, a metodologia e os parâmetros determinados influenciam as caracteríscas sensorias, nutricionais e funcionais do produto.

Figura 1.3 Etapas para obtenção de Extrato Aquoso de Soja



Fonte: Autor

Segundo Giri e Mangaraj, (2012) a produção de extrato aquoso de soja pode ser preparada com quatro variações: extrato de soja pode ser obtido pela moagem a frio seguido de tratamento térmico, moagem a quente, com soja escaldada onde a moagem a quente após a soja passar por um branqueamento e moagem a frio sem ar (canadense). Como resultado tem-se o extrato aquoso, mas para a obtenção do extrato em pó deve ser realizado processos para retirada de água (GIRI; MANGARAJ, 2012).

Para obtenção do extrato de soja em pó o extrato aquoso passa por concentração chegando em 22-24% de sólidos, está pode ser realizada por evaporação ou por meio das tecnologias de membranas. Na desidratação é utilizado o método de *spray drying* para realizar a secagem. Por característica o extrato de soja em pó deve ter no mínimo 90% de sólidos totais, sendo pelo menos 38% de proteínas e 13% de lipídeos, deve ter coloração de banco a marrom claro, e deve ter facilidade em se dissolver em água (GIRI; MANGARAJ, 2012).

1.8 Propriedades físicas de pós

Os pós lácteos podem ter validade de até 24 meses, as propriedades físicas podem sofrer alterações ao longo do armazenamento afetando solubilidade e reações de escurecimento, tornando essencial o acompanhamento da estabilidade dos mesmos ao longo do tempo (CHENG *et al*., 2017). Industrialmente, existem muitos alimentos produzidos em conformação física de pó. Para tal é preciso conhecer e compreender suas propriedades físicas e químicas a fim de determinar as características intrínsecas que refletirão diretamente no comportamento de processamento, manipulação e armazenamento (FITZPATRICK; BARRINGERB; IGBALA, 2004).

Conforme Escudeiro e Ferreira (2014) os pós possuem propriedades físicas que podem ser divididas em relação à partícula individual, abrangendo massa específica e as dimensões. Quanto ao conjunto de partículas destacam-se propriedades como umidade; atividade da água; tempo de dissolução; densidade; quantidade de ar intersticial; fluidez e coesividade; escoamento (ESCUDEIRO; FERREIRA, 2014)

A densidade é referente à volume que a partícula de determinada massa ocupa, está característica dos pós é importante para operações que envolve seu transporte influenciando na funcionalidade e custos das mesmas (SHARMA *et al*., 2012). A fluidez é a propriedade que analisa movimento entre duas partículas, pode ser descrita como a resistência ao fluxo (SHARMA *et al*., 2012). O escoamento do pó, é uma propriedade física importante pois estabelece características de uniformidade e homogeneidade do produto (DE CAMPOS; FERREIRA, 2013).

Segundo Fatah (2009) as características dos pós, como sua morfologia, tamanho de partícula, porosidade, densidade, rugosidade, especificação, influenciam a estrutura local do agrupamento de partículas. Conforme Escudeiro e Ferreira (2014) essas propriedades da partícula e as suas características de escoamento relacionam-se, também, com o atrito e forças de coesão. Têm-se como parâmetro para a análise de escoabilidade, medidas simples que são empregadas por métodos experimentais (ESCUDEIRO; FERREIRA, 2014).

1.9 Reidratação de pós

Reidratação é uma propriedade muito importante para pós alimentícios, que é dependente de seus componentes, e deles e sua afinidade com a água (CHEVER *et al.*, 2017). Outro fator que influência na reidratação de pós é a tecnologia utilizado na desidratação, no caso do leite em pó, quando secos por tambor são menos solúveis (85%) do que quando secos por spray dryer (99%) (PUGLIESE *et al.,* 2017). A reidratação de um pó passa por três fases: molhabilidade, dispersibilidade e solubilidade. (CHEVER *et al.*, 2017).

O índice de molhabilidade é determinado pelo tempo necessário para todo, pó colocado em recipiente com água, seja umedecido e assim a amostra atinge o fundo do recipiente. A dispersibilidade diz respeito a separação das partículas dos pós e sua dispersão na água, que pode ser calculado através da equação 1. A solubilidade é a capacidade do pó de se dissolver na água, que pode ser calculado através da equação 2. (CHEVER *et al.*, 2017).

$$D(\%) = (m_2) \times \frac{100}{m_1} \qquad (1)$$

Onde D: dispersibilidade m_2: massa de amostra dispersa, m_1: massa de amostra utilizada na análise.

$$S(\%) = (m_2) \times \frac{100}{m_1} \qquad (2)$$

Onde S: solubilidade, m_2: massa de amostra solubilizada, m_1: massa de amostra utilizada na análise.

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CAPÍTULO 2

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*Anexo A: Comprovante de submissão artigo "**A theoretical approach to dairy products from membrane processes**"

A theoretical approach to dairy products from membrane processes

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Abstract

Milk is a food rich in nutrients and requires processing that promotes its conservation, such as concentration. The membrane process, as an emerging technology and lower energy consumption, has been applied to concentrate, purify, and adapt milk for fluid consumption or derivatives. The Microfiltration, Ultrafiltration, Nanofiltration, and Reverse Osmosis processes have already been applied to milk and the biggest challenge is in controlling phenomena such as fouling, and polarization influenced by the system's operating conditions. The membrane process has already been used in the production of cheese, ricotta, protein concentrates, powdered milk, and dairy beverages.

Keywords: milk; concentration; operation conditions; fouling; proteins.

Practical Application: The use of membrane process to produce dairy products.

1 Introduction

Milk is composed of the main macronutrients important in human nutrition, carbohydrates, proteins, and fats, in addition to the presence of vitamins and minerals (CARTER *et al*., 2021). It can be consumed in its fluid form or transformed into different products through concentration, coagulation, and fermentation process. As it is a nutritionally rich product with high water activity, it allows the action of microorganisms, its processing being essential to ensure longer shelf life and safety in consumption.

In addition to sterilization of milk, which allows for conservation at room temperature, concentration, that is, removal of water is another industrial process capable of improving the shelf life of milk. The most common technology used by the dairy industry is concentration by evaporators, which uses high temperatures and has high energy consumption (BLAIS *et al*., 2021). As an alternative to emerging concentration technologies, there are freeze concentration and membrane filtration.

Synthetic membranes for industrial application originate from observation and intent to reproduce biological membrane functions (HABERT *et al*., 2006; ULBRICHT *et al*., 2006). Membranes are selective barriers that separate fluids into two phases. Selection may be due to particle size or dissolution/diffusion across the membrane. Membranes can be made of different materials, having a wide variety of their characteristics according to the desired application. Membrane processes have already been studied and used in several areas such as health, water treatment, and the food industry.

Membrane processes allow the concentration and separation of components of interest. In the case of selecting particles according to their size, there are the processes of microfiltration, ultrafiltration, and nanofiltration, whereas the membranes used in the reverse osmosis process are not considered porous (HABERT *et al*., 2006). These processes generate retentate and permeate, and both can be used depending on the purpose.

These processes are the most used for dairy products (CARTER *et al*., 2021), which presents benefits such as lower operating temperatures, promoting conservation of thermosensitive components. This work sought to present a theoretical approach to processes with membranes, concepts, challenges, and advances in dairy processing.

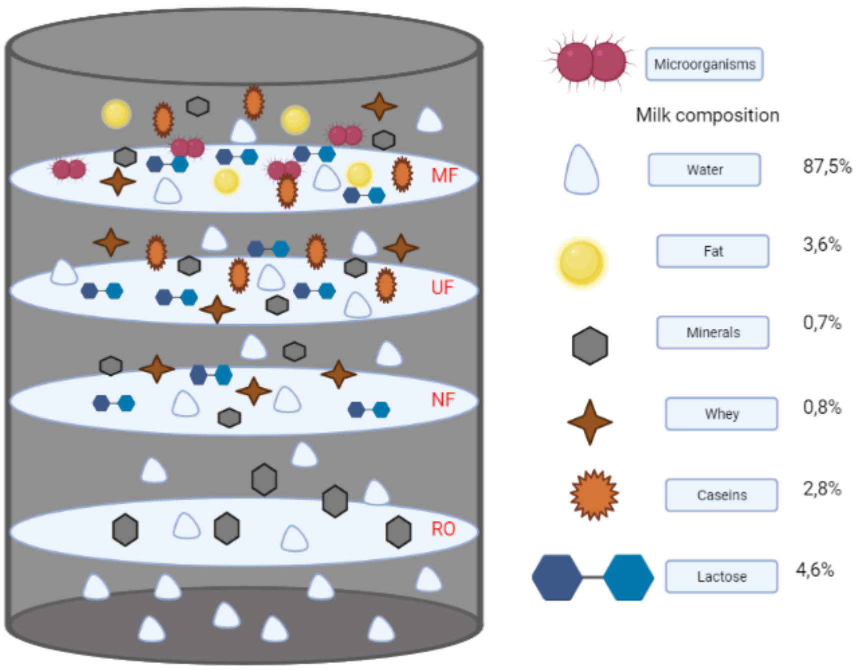
2 Membranes process for milk filtration

The use of membranes is possible due to their capabilities. The membrane can separate two phases of a solution, can be permeated, and can restrict passage, making it an excellent separation, concentration, and purification tool. Regarding the morphology of the membranes, they can be separated according to their porosity and density, symmetries, and asymmetries.

A driving force is necessary to occur the phase separation by the membrane. Membrane processes are associated with a driving force of pressure or concentration. The mass transfer mechanism across the membrane can be convection and/or diffusion (HABERT *et al*., 2006).

The whole milk presents a composition of 3.6% fat, 3.6% protein, 0.7% minerals, 4.6% carbohydrates, and 87.5% of water. Skimmed milk differs from whole milk in that it contains 2.9 to 0.5% fat. Each component of milk has a characteristic size and molecular weight, making membrane processes a good way of separating and fractionating compounds (Figure 2.1). Milk components have approximately the following sizes or molecular weights: fat globules 3.4 µm, caseins 110 nm, whey proteins 3-6 nm, and lactose 0.35 kDa (BRANS *et al*., 2004).

Figure 2.1 Membrane milk filtration - separation by component.



Source: Author

2.1 Microfiltration (MF)

Microfiltration (MF) presents membranes with pores between 0.1 and 10 µm. Thus, particles larger than 0.1 µm are part of the retentate, and the pore size may vary according to the purpose of use (CARTER *et al*., 2021). This process has as one of the applications for dairy the retention of bacteria and spores, thus it is necessary to control the size of the membrane pores, which should be small enough to retain microorganisms, but in a way, that does not compromise the composition of the permeated milk (DEBON *et al*., 2012; GRIEP *et al*., 2018). Kara & Sert (2022) verified that MF treatment of milk improved the microbiological quality without any significant change in the chemical composition of skim milk powder. On the other hand, Saha *et al*. (2022) evaluated the effect of di-sodium phosphate and calcium chloride salt concentrations (10 to 90 mM) on the isolation of casein micelles from buffalo milk by MF process (0.20 µm). Based on the results found by these authors, no aggregation was recorded for buffalo micellar casein and size varied from 30 to 80 nm. In the study realized by Hooda *et al*. (2020) was the physicochemical properties of micellar casein concentrate prepared from skim milk (buffalo and cow), using microfiltration (MF), were assessed. In this study was not observed difference ($P > 0.05$) between the average particle size of skim milk casein micelles (cow and buffalo) and their respective micellar casein concentrate. The casein-to-whey protein ratio increased from 4.91 and 4.75 in skim milk to 135.16 and 159.22 in micellar casein concentrate of buffalo and cow, respectively (HOODA *et al*., 2020). Vieira *et al*. (2020a) confirmed advantages of microfiltration processing of goat whey orange juice beverage using mild temperatures (between 30° and 40 °C) to preserve consistency and also obtain a desirable microbial quality, beyond the preservation of many functional properties and volatile compounds. Vieira *et al*. (2020b) also investigated the sensory profiling using free listing task and consumer acceptance of the goat whey orange juice beverage processed by microfiltration (20, 30, 40, and 50 °C) and by conventional pasteurization. Free listing task allowed the discrimination of the beverages based on the type of processing (pasteurization or microfiltration) and microfiltration temperature. Overall, free listing task was a suitable methodology for discriminating beverages submitted to different processing, and microfiltration (20 or 30 °C) is an interesting option for goat whey orange juice beverages, resulting in products with adequate sensory properties (VIEIRA *et al*., 2020b).

The MF process has also been applied as a pre-treatment to increase the stability of UHT milk, which is increasing during long-term storage (D'INCECCO *et al*., 2018). MF can generate dairy raw material, permeate, with adequate quality and characteristics for processing derivatives (DEBON *et al*., 2012).

2.2 Ultrafiltration (UF)

Ultrafiltration (UF) can prevent the passage of molecules larger than 0.001 µm, as they have membranes with pores between 0.01 – 0.001 µm (CARTER *et al*., 2021). UF can retain proteins (OLIVEIRA *et al*., 2021) and fat, and allows the passage of vitamins, minerals, and lactose. The use of UF to develop dairy products benefits yield, nutritional functionality, and sensory characteristics (FAION *et al*., 2019). This process can be applied for protein concentration and purification (NG *et al*., 2018) and stands out in cheese production, providing higher protein concentration and better nutritional characteristics to this product (FAION *et al*., 2019; GAVAZZI-APRIL *et al*., 2018). Another common application for UF is the production of milk protein concentrate (MPC) (GAVAZZI-APRIL *et al*., 2018).

Based on experimental data obtained by whey protein hydrolysate from UF process can be used in the technology of various Melnikova *et al*. (2022) dairy products to replace skimmed milk when making a normalized mixture and also as a main ingredient of beverages for sports nutrition taking into account sensory profiling of the products. This allows expanding the assortment line of domestic products for preventive nutrition of people suffering from allergies to cow's milk proteins, as well as ensuring import substitution in the segment of functional food products (MELNIKOVA *et al*., 2022).

2.3 Nanofiltration (NF)

For Nanofiltration (NF) processes, the membranes used have pores between 0.001-0.0001 µm (CARTER *et al*., 2021). NF is capable to concentrate small components of molecular weight equal to or greater than 100 Da. NF membranes can retain sugars, amino acids, dyes, and salts (CHEN *et al*., 2018). It can also be used in the concentration of whey proteins in milk to produce derivatives. The NF process is capable of high retention of organic compounds resulting from the interaction between the membrane, the solution to be filtered, and electrostatic repulsion (PRUDÊNCIO *et al*., 2014).

2.4 Reverse osmosis

Reverse osmosis (RO) began in the 1960s with the creation of the first membrane for this process. Osmosis is the process that uses membranes with pores smaller than 0.0001 µm (CARTER *et al*., 2021). These membranes can retain larger ions and compounds releasing water into the permeate and can be applied for pre-concentration of milk (BLAIS *et al*., 2021). Syrios *et al*. (2011) compare this process to evaporation, both of which promote water removal and osmosis may contain traces of other low molecular weight components. This process presents an increased osmotic pressure and feed stream viscosity (BLAIS *et al*., 2021) facing severe problems with fouling and permeate flow reduction. The disadvantage of this process has become the focus of several studies to promote optimization.

3 Cascade process

These processes can be joined together to form cascades to increase the efficiency of each process. Diafiltration (DF) is always used in association with one of the processes mentioned above, where water is added to the feed to overcome viscosity and allow the system to continue filtering to increase efficiency (GAVAZZI-APRIL *et al*., 2018), where DF also allows obtaining a purer retentate (PRUDÊNCIO *et al*., 2014). The union of several processes with membrane can promote different flows with different compositions that can be applied for different purposes, such as the standardization of milk to produce derivatives (XIA *et al*., 2020).

For the RO process, being inserted in a cascade is interesting due to its difficulties with encrustations. The addition of processes such as UF and MF before RO in milk filtration can improve its efficiency to retain somatic cells, fat globules, and protein aggregates. Due to the retention of microorganisms during the MF step, it allows RO to be performed at higher temperatures without microbiological compromise due to the multiplication temperature of microorganisms (BLAIS *et al*., 2021).

4 Membrane materials and operations conditions for milk processing

Knowledge of physical and chemical data about the solution to be filtered is essential for proper and efficient choices regarding membrane material and process operating conditions to try to predict the behavior of the solution, applied conditions, and interaction with the membrane. In the case of milk, there are proteins, fats, lactose, minerals, and a small number of vitamins. Verruck *et al*. (2019) related that dairy products are considered as products with substantial health benefits, such as cheese, cheese whey, dairy beverage, and powder milk. These products could be consideredand essential for the equilibrated diet from a nutritional and functional point of view. It is noteworthy that they are also excellent sources of proteins and minerals, especially calcium in a highly bioavailable form. Therefore, dairy products can improve health or well-being and, when consumed at recommended levels, their benefits include improved immune system function, reduced risk of cardiovascular, reduced risk of bone mass loss, and protection against free radical damage (VERRUCK *et al*., 2019). For this reason the use of milk to generate dairy products with new nutritional values justified its use in the membrane process.

Membranes can be separated into organic (polymers) and inorganic (metals and ceramics) (HABERT *et al*., 2006). In dairy processing, membranes are used made of polymers or ceramics. The most used polymers for filtration in dairy products are polyvinylidene fluoride and polypropylene for UF and MF and polyamide for RO, while ceramic membranes are commonly used for MF (CARTER *et al*., 2021). Ceramic membranes have a longer service life, but polymeric membranes are cheaper (HABERT *et al*., 2006).

Ceramic membranes are more expensive due to the higher raw material value for fabrication and more complex production processes, but this balances out when presented with its advantages. This material allows a membrane with greater durability, high mechanical strength, resistance to chemicals and solvents, and thermal stability (MESTRE *et al*., 2019). The difficulty in using a ceramic membrane for milk filtration is because whey proteins tend to adsorb on the surface, while caseins contribute to the encrustation layer proportionally to the applied pressure (BLAIS *et al*., 2021). Ceramic membranes are applied in the microfiltration process as an alternative for pasteurizing milk and for valuing whey (MESTRE *et al*., 2019).

Polymer membranes are cheaper, considering that membranes represent 40% of the cost of systems is an important consideration. Although other materials are also used, polymeric membranes are the majority. Polymers provide the possibility of abundant variations in structure and barrier properties (ULBRICHT, 2006).

As for the operating conditions, it is necessary to control the temperature, pressure, the feed direction being frontal or tangential, and the feed speed. Influences of these parameters on membrane filtration efficiency have already been reported. Feed speed can be important for running the process, especially when have a tangential feed. In this type of feeding, solids deposited on the membrane are dragged, making the polarization and incrustation phenomena more controlled. When using higher speeds, this drag is continuous, and the filtration resistance is lower (ATRA *et al*., 2005).

Within the data collected in Table 1, it is observed that the temperatures for processing milk and whey, with membranes, occur between 10 ºC and 55 ºC. In general, higher temperatures generate lower viscosity, which benefits the permeation flow. Ng *et al*. (2018) applied UF in skimmed milk found flows with an increase of 250% when the temperature was increased from 10 ºC to 50 ºC, but this variation increased the filtration resistance. In the case of RO, Blais *et al*. (2021) achieved an 89% increase in filtration flow when going from 15 °C to 50 °C.

Pressure acts as a driving force in the MF, UF, NF, and RO processes. It can be seen from Table 1 that the operating values are up to 3 bar for MF, between 2 and 8 bar for UF, 2 up to 20 bar for NC, and RO is operated at much higher values and may reach 40 bar. The flow is related to the applied driving force, requiring higher pressures in tighter membranes. RO membranes are not considered porous, so they are operated at high-pressure values to have a satisfactory flow (HABERT *et al*., 2006).

Table 1. Membrane materials, geometries, objectives, and operating conditions for milk filtration.

Process	Materials	Pores/Molecular weight	Geometry/ Modules	Objective	Operating temperature	Operating pressure	Source
MF	Ceramics	0.1 µm		Protein fractionation	10 °C		Schiffer *et al*., 2020
MF	Ceramics	1.4/1.2 µm	Tubular	Removal of microorganisms and spores	6 °C		Griep *et al*., 2018
MF	Organic poly(imide)	1.4 µm	Hollow fiber	Fermented Milk Production	45 ºC	1-3 bar	Debon *et al*., 2012
MF	Ceramics	1.4 µm	Tubular	Concentrated skimmed milk (milk powder)			Blais *et al*., 2021
MF	Ceramics	1.4 µm		Increases UHT stability			D'Incecco *et al*., 2018
UF	Zirconium oxide	50 kDa	Tubular	Concentration (cheese production)	50-55 ºC		Deshwal *et al*., 2020
UF	Polyethersulfone (PES)	10 kDa	Spiral	Temperature X Flow (skimmed milk)	10/30/50 ºC		Ng *et al*., 2018
UF	Polysulfone amide	10 kDa	Hollow fiber	Sheep cheese production	22 ºC	1-2 bar	Faion *et al*., 2019

UF+DF	Polyethersulfone (PES)	10 kDa/50kDA	Spiral	Protein concentrate (skimmed milk)	50 ºC	4.65 bar	Gavazzi-April *et al*., 2018
UF	Polyethersulfone (PES)	10 kDa		Biofilm investigation	15/50 ºC		Chamberland *et al*., 2019
UF	Polyvinyl difluoride	6-8 kDa	Flat	Milk and whey concentration	30-50 ºC	1-5 bar	Chen *et al*., 2018
UF	Polyethersulfone (PES)	15-20 kDa	Flat	Milk and whey concentration	30-50 ºC	1-5 bar	Chen *et al*., 2018
UF	Polyethersulfone (PES)	10 kDa	Spiral	Skimmed milk concentration	10-50 ºC	2.76-7.58 bar	Méthot-Hains *et al*., 2016
UF	Polyethersulfone (PES)	5/30 kDa	Flat	Fouling analysis in whey processing	25 ºC	1-3 bar	Luján-Facundo *et al*., 2017
UF+DF	Polyethersulfone (PES)	10 kDa	Spiral	Production of milk protein concentrate			Eshpari *et al*., 2017
UF	Polysulfone	25 kDa		Koummis production	52 ºC	2.6-3.6 bar	Kücükcetin *et al*., 2003
NF	Polyamide			Milk and whey concentration			Atra *et al*., 2005
NF	Polyamide		Spiral	Treatment of dairy waste	30-50 ºC	10-20 bar	Chen *et al*., 2018

NF+DF		150/300 Da	Spiral	Whey filtration (ricotta production)	24 ºC	6.9 bar	Prudêncio *et al*., 2014
RO	Polyamide		Spiral	Pre-concentration of milk	15/50ºC	30.05 bar	Blais *et al*., 2021
RO	Polyamide		Spiral	Skimmed milk concentration (cheese production)	50 ºC	26.6 bar	Dussault-Chouinard *et al*., 2019
RO	Polyamide			Skimmed milk concentration	10 ºC	40 bar	Artemi *et al*., 2020

Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF), Reverse osmosis (RO), Diafiltration (DF).

5 Development of dairy products using membranes

5.1 Cheese

Cheese is an important product of the dairy industry and is part of many traditional food preparations. Membrane filtration of milk for cheese preparation brings benefits such as increased casein and total solids in milk, raw material, and product with greater standardization and increased yield (CARTER *et al*., 2021), however, it can influence texture parameters in cheese (DESHWAL *et al*., 2020). The microstructure of cheeses is also affected when the raw material is the result of filtration, being more compact, influencing the behavior of caseins, and interference from fats (DESHWAL *et al*., 2020).

Ultrafiltration was used in the process of making pecorino cheese from sheep's milk. Faion *et al*. (2019) compared two formulations, one with low-fat sheep's milk and the second formulation with concentrated non-fat sheep's milk per UF. The performance of the UF process and the physicochemical characteristics of the raw material and the cheeses were evaluated. The UF favored the presence of proteins and fat in the raw material and consequently presented cheeses with higher yields.

The UF was also present in the manufacture of Halloumi cheeses made with goat's milk. From the retentate with different percentages of fat, the cheeses were produced and analyzed for physical-chemical, rheological, and sensory aspects. The researchers observed an interesting relation regarding the amount of protein, fat, and minerals in cheese, that is, cheeses with lower fat and higher protein content also have higher amounts of minerals. With an increase in milk fat, the retention of proteins by the membrane consequently also decreases the levels of minerals that bind to them and can pass to permeate during the UF process (DESHWAL *et al*., 2020).

5.2 Cheese whey

Whey is a by-product of the cheese industry. To produce 1 kg of cheese, 9 kg of whey are generated (LUJÁN-FACUNDO *et al*., 2017), with a large amount of waste to be treated, obtaining essential processing and utilization of it. Whey is liquid rich in proteins and lactose and can be used in processes with membranes for the concentration and purification of these components and application in dairy derivatives. However, there is a great difficulty because whey proteins influence the incrustation, reducing the efficiency of the process. Luján-Facundo *et al*., (2017) carried out a study focused on the analysis of 5.000-30.000 Da cutting membrane fouling in the filtration of whey. They related the percentage of calcium in the filtration feed solution with the increase in scale.

The use of NF associated with diafiltration was analyzed for ricotta production as an alternative to traditional production. Prudêncio *et al*. (2014) collected the whey from the result of the production of Minas fresh cheese, applied the membrane process to the collected by-product and the retentate was applied in the manufacture of ricotta cheese. Physical, chemical, rheological characteristics and images were evaluated to verify ricotta quality. Membranes, for 150-300 Da, were used for NF in spiral modules with tangential feed. The study shows that NF from whey had a decrease in permeate flux over time, which may be justified by polarization concentration, encrustation, and adsorption, effects already expected in processes with membranes. The serum underwent two different treatments, only NF and NF plus diafiltration, which were compared to the control serum. The ricotta cheeses produced with retentate from the processes with membranes had a lower quantity of total solids and proteins, which may be associated with the operating conditions and sensitivity to undergo protein alterations, and more affected coloration when compared to the control.

5.3 Dairy beverages

A fermented milk drink has already been developed from MF permeate. Using hollow fiber membranes with an average pore size of 1.4 µm, Debon *et al*. (2012) aimed to generate an adequate raw material that would allow the stability of a prebiotic drink. MF allowed acceptable microbiological quality and few nutritional losses for the raw material, generating a dairy beverage without the need for heat treatment.

Koumiss is an alcoholic drink traditionally made with mare's milk widely consumed in central Asia. Kücükcetin *et al*. (2003) applied membrane technology when adapting the production of the Koumiss drink with cow's milk. Due to the differences between the milk of the species, MF, UF, and NF were used to make the cow's milk suitable to produce the drink and, in this way, compare the characteristics with the traditional drink. Performing sensory analysis with mare's milk and modified cow's milk Koumiss showed no significant differences between samples. The use of processes with membranes proves to be effective for the compositional manipulation of milk to produce derivatives.

5.4 Powder milk

The production of powdered milk requires a great deal of water withdrawal, and approximately 87% of the milk is composed of water, making the membrane process unfeasible as a single manufacturing step. However, this does not prevent membrane technology from being applied as a form of pre-concentration, exposing the milk to heat treatments for shorter periods. Blais *et al*. (2021) analyzed the use of RO and MF in the concentration of skimmed milk for powdered milk production. The main objective of the research was to analyze the performance of the individual use of OR and together with MF. Another factor analyzed was the temperature (15 ºC and 50 °C). MF was used to remove microorganisms and other scales and RO to remove water. As for the permeate flux, there was no great difference between the use of RO and MF combined with RO at 15 ºC. However, when the process took place at 50 °C the flux values were a little higher, that is, the higher temperature delayed the inlay process. This improvement could be due to the lower viscosity of the milk at a higher temperature. The authors also studied the costs of the process and reported that RO can generate savings in the milk concentration process when compared to the traditional methodology.

Syrios *et al*. (2011) compared the UF, NF, and RO retentates for spray drying in the production of skimmed milk powder. The authors related the membrane process, as a pre-treatment, with ionic calcium retained and heat stability when powdered milk was reconstituted at 25%. The three processes showed a percentage increase of solids in the retentate, including total and ionic calcium, but it was only possible to reach the reconstituted milk powder with satisfactory stability to heat with NF treatment, at the sterilization temperature. The authors noted the difficulty of generating reconstituted powdered milk without prior heat application.

Prestes *et al*. (2022) reported that emerging non-thermal Technologies, such as membrane process, are promising alternatives that have been developed and explored in powder milk producing. With a purpose to decrease the negative effects of the conventional concentration processes and contribute with powder milk with high quality. These alternative procedures preserve sensory and flavor properties and maintain food pigments, original volatile compounds, vitamins, enzymes, and proteins (PRESTES *et al*., 2022).

5.5 Protein concentrate

Protein concentrates are important products because they can be applied to different types of food, such as infant formula and bakery products (CARTER *et al*., 2021). Milk has high levels of protein which makes it suitable raw material to produce concentrate and protein isolates. One of the difficulties in handling milk proteins is their thermal sensitivity, which can affect stability in the reconstitution of concentrates (ESHPARI *et al*., 2017; CARTER *et al*., 2021).

Milk protein concentrates are commonly produced using membrane processes such as UF and diafiltration followed by evaporation and a drying step, varying the percentage of protein concentration (ESHPARI *et al*., 2017; GAVAZZI-APRIL *et al*., 2018). Gavazzi-april *et al*. (2018) evaluated the performance of UF and diafiltration in the production of milk protein concentrate. They reported favoring the flow of the system by diafiltration and considered the use of membranes with a 10 kDa cutoff as suitable to produce milk protein concentrate.

6 Performance of membrane processes in dairy processing

For the results of the membrane process to be viable, it is necessary to evaluate its performance. Good performance will depend on the combination of membrane characteristics and operating conditions that, consequently, will interfere with the characteristics and flows of currents (supply, retentate and permeate). To assess the performance of a membrane process, it is important to observe the parameters of permeate flux, hydraulic resistance (GAVAZZI-APRIL *et al*., 2018; FAION *et al*., 2019; MÉTHOT-HAINS *et al*., 2016), feed composition versus permeate and retentate (BLAIS *et al*., 2021; GAVAZZI-APRIL *et al*., 2018), retention index (PRUDÊNCIO *et al*., 2014; ATRA *et al*., 2005), solute yield and concentration factor (ATRA *et al*., 2005).

The permeate flux (J) is related to the permeate volume (V) during a given time (t) through an area of membrane (A) (GAVAZZI-APRIL *et al*., 2018) (Equation 1).

$$J = \frac{V}{t \cdot A} \quad \text{(L/h.m2)} \qquad (1)$$

For the UF + skim milk diafiltration process, Gavazzi-April *et al*. (2018) found values between 5.6 and 8.7 kg/hm2 at a temperature of 50 ºC and a pressure of 4.65 bar. Faion *et al*. (2019) had used the UF for the concentration of sheep milk, at pressures of 1 and 2 bar found initial values of 15.38 and 18.71 L/hm2 respectively, so they opted for the process at 2 bar, but this flow was decreasing throughout the process and stabilized at 0.85 L/hm2 after 6 min of filtration.

The total resistance (R_t) of the process can be obtained by summing the membrane resistance (R_m) the reversible resistance (R_R) and the irreversible resistance (R_i) (MÉTHOT-HAINS *et al*., 2016) (Equation 2, 3, 4, 5).

$$R_t = R_m + R_R + R_i \quad (2)$$

$$R_m = \frac{P}{\mu J_w} \quad (3)$$

$$R_R = \frac{P}{\mu J_E} - R_m - R_i \quad (4)$$

$$R_i = \frac{P}{\mu J_R} - R_m \quad (5)$$

where P is the transmembrane pressure (Pa), μ is the permeate viscosity (Pa.s), J w is the water flux (m3/m2.s), J R is the water flux (m3/m2.s) after membrane rinsing, and J E is the permeation flux (m3/m2.s) at the end of UF.

Faion *et al*. (2019) observed that when doubling the pressure during the UF process (1 bar and 2 bar), the hydraulic resistance to permeation dropped by half. Méthod-Hains *et al*. (2016) reported the influence of temperature on the UF resistances of skimmed milk, at a temperature of 50 °C compared to 10 °C obtained higher membrane resistance values, but lower irreversible resistance.

To evaluate the composition of the RO and MF/RO filtration process currents, Blais *et al*. (2021) quantified percentages of total solids, fat, proteins, casein, lactose, and somatic cells of skimmed milk, which was used as feed, during all studied processes Like previous authors, Gavazzi-April *et al*. (2018) evaluating the UF and UF/diafiltration currents in the production of protein concentrate (retentate), obtained a considerable loss of protein in the permeate. The compositional evaluation of currents is essential to verify the efficiency of the process, after all, it will determine the nutritional quality of the developed products and concentrate yields, in addition to indicating problematic points and the need for process optimization. Faion *et al*. (2019) reported differences in composition with a change in operating pressure, when a pressure of 2 bar was used, the retentate contained more values of protein, fat, and minerals than the retentate at 1 bar.

The retention index (R) is a parameter to evaluate the relation between the amounts of the compound of interest in the permeate solutions and the retentate solutions, thus determining the capacity of the process (membrane and operating conditions) to retain the component of interest (PRUDÊNCIO *et al*., 2014). Prudencio *et al*., 2014 found an R of 0.9 for protein when applying NF and diafiltration on whey from the production of Minas Frescal cheese. Atra *et al*. (2005) observed values between 0.92-0.98 of protein retention when they used UF to filter whey (Equation 6).

$$R = 1 - \frac{c_\rho}{c_R} \qquad (6)$$

where C P is the solute concentration in the permeate (g/L) and C R is the solute concentration in the retentate (g/L).

The concentration factor (F) is related to the volume of solution in the feed (V_F) and the volume of retentate (V_R) (Equation 7). The solute yield (Y) is the concentration of the components in the feed by the components in the retentate (ATRA *et al*., 2005). Atra *et al*. (2005) found that it is possible to achieve a Y greater than 90% in lactose recovery by applying NF (Equation 8).

$$F = \frac{V_F}{V_R} \qquad (7)$$

$$Y = \frac{V_{RC_R}}{V_F c_F} \qquad (8)$$

where C_R is the solute concentration in the retentate (g/L) and c_F solute concentration in the feed (g/L).

Membrane characteristics are important for the final efficiency of the process, as it is related to the selectivity and composition of permeate and retentate. Uniformity of both pore size and pore distribution across the membrane is important for the containment of the components in question (BRANS *et al*., 2004; GAVAZZI-APRIL *et al*., 2018). The thinner the membrane, the easier it will be to permeate it. Very thick membranes are commercially unfeasible due to extremely low flows, but they have enough structure for adequate mechanical strength (HABERT *et al*., 2006).

The permeate flux parameter is important for evaluating the efficiency of a membrane process. Several studies involving milk processing look at the flow behavior.

The permeate flux in milk filtration tends to follow a pattern. At the beginning of filtration, the permeate flux is the highest in the process, after a while, membrane compaction and system adjustment may occur, leading to a decrease in this flux. After these steps, the flow tends to decrease over time due to incrustations, pore obstruction, adsorption of components by the membrane, increased feed viscosity, among other effects that lead to increased filtration resistance (BLAIS *et al*., 2021).

High permeate fluxes can be seen as successful for the membrane process, but caution is needed to manipulate operating conditions to increase the flux. An example of this is the increase in pressure, which is proportional to the increase in flow in the short term, as the common effect of high pressure is severe incrustations on the membrane (ARTEMI *et al*., 2020). Permeate flow is directly proportional to pressure, but above the critical transmembrane pressure, the flow becomes pressure-independent (CHEN *et al*., 2018). Artemi *et al*. (2020) observed the onset of incrustation linked to linearity with the flow, when it no longer exists, they characterize it as a critical flow achieved, this was reported for the RO process.

Higher temperatures favor the flow performance, due to the lower viscosity and greater diffusivity that promotes an improvement in polarization (ATRA *et al*., 2005). However, higher temperatures may facilitate the growth of thermophilic microorganisms and the decomposition of sensitive components (NG *et al*., 2018). The flux has a common behavior of linear rise combined with temperature. Atra *et al*., 2005 observed an increase of 0.51 m2h at each degree ºC for UF of skimmed milk. Blais *et al*. (2021) found that by increasing the temperature by 1ºC there is a 3% improvement in the permeate flow in the RO process in the concentration of skimmed milk.

7 Challenges in milk filtration: fouling and polarization

Fouling can be subdivided into organic and inorganic. Organic fouling is caused by proteins, lactose, and organic acids, while inorganic fouling is mainly caused by calcium phosphate precipitation and biofouling (BLAIS *et al*., 2021). This phenomenon can be reversible when there is the possibility of recovering the membrane through cleaning, and irreversible when the incrustation is permanent. This phenomenon can make the process unfeasible depending on the severity (HABERT *et al*., 2006).

Fouling can be generated due to adsorption of components across the membrane, clogging of pores, and deposition of components on the membrane surface, including concentration polarization (HABERT *et al*., 2006). The responsibility for membrane encrustations during milk filtration has been largely associated with protein adsorption and calcium precipitation. Calcium phosphate behaves differently from other components, and its solubility decreases when the temperature of the process increases (NG *et al*., 2018).

Concentration polarization will occur in all membrane filtration processes and this is due to the concentration of components close to the membrane surface. This phenomenon can be influenced by the concentration of components in the feed, flow conditions, pressures above the limiting flow, and selective capacity of the membrane (HABERT *et al*., 2006). Protein-rich liquids, such as milk, can be polarized by high concentration, as the proteins are deposited on the surface of the membrane, favoring this phenomenon (FAION *et al*., 2019).

Both incrustation and polarization give resistance to the filtration process and are the focus of several studies on manipulations in membrane operations to reduce these problems. Blais *et al*. (2021) observed that operations using higher temperatures can reduce this phenomenon due to the association with viscosity. Using a tangential feed with higher speeds is one more way to improve the effects of polarization (HABERT *et al*., 2006; BRANS *et al*., 2004). Brans *et al*. (2004) reviewed features to reduce fouling phenomena using vibrating modules, cleaning particles, ultrasonic waves, pulsating, or high-speed crossover feed stream. Furthermore, the choice of membranes should be considered, as the more hydrophilic and negatively charged, the more they will contribute to the reduction of protein encrustation (CARTER *et al*., 2021).

8 Costs of membrane process

The concentration of milk is commonly carried out by evaporation and drying, which are energy-demanding processes. In the quest for greater sustainability in industrial operations, membrane processes are known to save energy when compared to other concentration technologies. The energy-related cost comes mainly from the feed pumps and depends on the pressures used in the process (BLAIS *et al*., 2021).

A multi-stage evaporator has an average consumption of 83.3 Wh L−1 of water removed (BLAIS *et al*., 2021), depending on the combination of processes and mode of operation, membranes can be an option for reducing energy consumption in dairy products. To evaluate energy consumption in the process of concentrating milk proteins by UF and diafiltration Gavazzi-April *et al*. (2018) used a voltmeter connected to the system power motor, calculated Wh/kg of retentate, and reached values between 36 × 103 kWh and 114 × 103 kWh depending on diafiltration variations. Blais *et al*. (2021) achieved a value of 110 Wh L−1 of water removed from energy expenditure for the RO process in the concentration of skimmed milk, and when associating the MF before RO and raising the temperature from 15 ºC to 50 ºC, the spent started to be 49.4 Wh L−1, generating energy savings.

Membrane costs are calculated per m2. The values of ceramic membranes based on alumina and zirconia can vary between 500 and 3000 $/m2 and polymeric membranes vary between 20 and 200 $/m2 (MESTRE *et al*., 2019). In addition to the value of membranes, Gavazzi-April *et al*. (2018) point out costs with cleaning and replacement of membranes, which have a useful life limit.

9 Conclusion

The membranes are a technology appropriate to milk processing and dairy milk product. The MF, UF, NF, and RO are the process used in the dairy industry. For MF, ceramic membranes are the most used for dairy products, according to the pore size range they can be used for microbiological standardization of milk, whey processing, and increased stability of fluid milk. UF has great application in cheese production, with the advantage of increased yield due to greater protein retention, it can also be applied in the filtration of whey. NF can concentrate whey proteins and recover lactose. In RO, polyamide membranes stand out, whose function is to remove water from either milk or its serum, forming a retentate rich in total solids.

To evaluate the performance of the milk filtration process with membranes, the permeate flow is an important parameter. Due to the composition of milk, a source of protein, the biggest difficulties in its filtration through membranes are related to scale and concentration polarization. These problems are the focus of several studies to evaluate manipulations in processes to reduce their effects that affect performance. The main membrane filtration costs to be analyzed are the energy costs of pumping, the membrane itself, cleaning, and maintenance.

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CAPÍTULO 3

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*Anexo B: Comprovante de submissão artigo "**Powder dairy enriched with a soy extract (Glycine max) as an innovative product: physicochemical parameters, physical and rehydration properties, polyphenolic potential, and multielement profile"**

Powder dairy enriched with a soy extract (Glycine max) as an innovative product: physicochemical parameters, physical and rehydration properties, polyphenolic potential, and multielement profile

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Abstract

This work presents the enrichment of powdered dairy compounds with soy extract, and the determination of its physical properties, rehydration characteristics, multielement profile, and polyphenolic potential. Five dairy compound formulations were developed, where milk powder was replaced by 10, 20, 30, 40, and 49% w/w of soy extract.. Multivariate analyzes using combined PCA analyzes were used to group the samples and, thus, reveal the main characteristics associated with their physicochemical properties, bioactive composition, and multi-element profile. The protein content in the samples was not significantly affected by the addition of soy extract. There was a gradual increase in the total fat as the concentration of soy extract increased. Furthermore, with the increase in the percentage of soy extract in the dairy compounds, there was an increase in the levels of total polyphenols, total flavonols, and antioxidant capacity, and of some minerals, such as Fe, Mn, P, Cu, and Mg. The DC49 sample showed the highest values for total polyphenols (178.65 mg of gallic acid (GAE)/100 g) and total flavonols (1.51 mg of catechin/100 g). The addition of soy extract promoted the enrichment of important minerals in the samples, with an increase of up to 55 times in the Fe content and up to 40 times in the Mn content. Physical properties (density and fluidity) and rehydration properties (wetting, dispersibility, and solubility) also were affected as the percentage of soy extract in the samples increased. When there was an addition of up to 20% soy extract, the samples are still wettable. All dairy compounds showed solubility above 69%. The use of soy extract in the polyphenolic and mineral enrichment of the dairy compounds is important to add nutritional value to powdered milk, we emphasize that this product has enormous potential to be used in diets that require mineral supplementation.

Keywords: Bioactive compounds; Elemental composition; Nutritional enrichment; Dairy compound, Soy.

1. Introduction

Milk is composed of carbohydrates, proteins, fats, and high humidity, its dehydration transforms it into a product concentrated in nutrients, increases its shelf life, allows its conservation at room temperature, facilitates its transport, and adds value to the product (SHARMA; JANA; CHAVAN, 2012). The fluid milk, after confirmation of its quality, is received and goes through a stage of concentration, dehydration, and agglomeration. The processing required to dry the milk raises the cost of production. An alternative to delivering powdered milk to the consumer at a more affordable price is the formulation of the dairy compounds.

The normative Instruction No. 28 of June 2007 of MAPA (Ministério da Agricultura, Pecuária e Abastecimento) defines as a dairy compound the powder product that contains at least 51% of milk and can be added with dairy and non-dairy food ingredients (BRASIL, 2007). The development of the dairy compound, in addition to collaborating with the reduction of the price of powder milk, can generate nutritional enrichment, according to the raw material chosen for addition to powdered milk. They have already been studied as a form of enrichment/mixing/fortification/partial replacement of powder milk, the following materials: grape extract (MILINČIĆ *et al*., 2021), honey (BANSAL *et al*., 2017), sucrose, and lactose (CHEVER *et al*., 2017), salep tubers (DEVELI IŞIKLI *et al*., 2015), powder whey (MARTÍNEZ-PADILLA *et al*., 2014). Therefore, an alternative to the commercialization of powdered milk, aiming to reduce costs and nutritionally enrich this product, is the addition of powdered soy extract to powdered milk for the elaboration of dairy compounds. Soymilk is already a popular product and is associated with healthy eating. Soy is recognized as an excellent source of protein and high amounts of bioactive compounds and minerals (GIRI; MANGARAJ, 2012).

Bioactive compounds are nutrients or non-nutrient normally consumed as a component of a food, which has a specific metabolic or physiological action in the human body (BRASIL, 2018), these fall into the group of compounds phenolics. The phenolic acids and flavonoids are phenolic compounds present in high concentrations in soybean extract, which have the excellent antioxidant capacity. The antioxidants compounds reduce the risk of several chronic diseases such as cancer, inflammation, bacterial disorders, diabetes, cardiovascular diseases, and neurodegenerative diseases (SHAHIDI; YEO 2018).

Over the last few years, with the increase in awareness among consumers and by experts in the field of food science have considered the consumption of functional foods, i. e., foods containing bioactive compounds, such as polyphenols with health benefits. Milk and milk-based products are among the most consumed foods in various daily meals in different countries and can be a suitable option for delivering bioactive to the body (ADINEPOUR *et al*., 2022). The soybean grain is one of the main products to consider when generating added value due to the produced volume, and its excellent nutritional characteristics. Standing out is its flavonoid concentration, which acts like powerful antioxidants that have been associated with the prevention of chronic diseases. Soy powder extracts could be incorporated into milk as a natural ingredient in the formulation of functional foods (RODRÍGUEZ-RUIZ *et al*., 2022). Consequently, the fortification of powder milk with soy extract is a method to enhance health properties and develop novel functional dairy products (PERNA *et al*., 2018).

Perna *et al*. (2018) have shown a strong correlation between antioxidant capacity and phenol content for goat milk yogurt fortified with Rhus coriaria leaf powder. Therefore, the behavior of bioactive compounds is important in dairy products because according to Helal *et al*. (2014) observed variations in the recovery of the different compounds, which were attributable to the binding affinity between individual phenols and protein. Prigent and Dimitrov (2003) showed that the effect of the interaction between proteins or peptides (or both) and phenolic compounds on antioxidant capacity depends on the amino acid composition of the proteins and type of phenols, i. e., the type of bioactive compounds presents.

Soy also contains high levels of minerals, among which the majority are K, P, Mg, Ca, Fe, Na, and Mn (VIEIRA *et al*., 1999; GIRI; MANGARAJ, 2012; GREMBECKA; SZEFER, 2022.). Minerals have several responsibilities in the functioning of the body participate in the structure of bones and teeth, muscle contraction, immune system, blood pressure regulation, nervous system, cardiac functions, metabolic activities, brain functions, formation of red blood cells and hemoglobin, reproduction system and oxygen transport (GHARIBZAHEDI; JAFARI,2017), therefore their presence is essential in the human diet.

The soy extract has already been studied as a substitute for milk in dairy products to be an alternative for nutritional enrichment, especially protein, and cost reduction, its extract or flour is considered a cheap substitute for milk. The partial/total replacement of cow's milk in the following dairy products has already been evaluated, as follows: yogurt

(EHIRIM; NDIMANTANG, 2005; BRISTONE *et al*., 2015; PONKA *et al*., 2022), Kefir (HARUN, 2011), ice cream (PEREIRA *et al*., 2011), and smoothie (ANDRÉS *et al*., 2016). However, to the best of our knowledge, there are no reports on the substitution of milk powder for soy extract, nor the investigation of physicochemical composition, physical properties, rehydration characteristics, multielement profile, and polyphenolic potential of this product. In addition, multivariate statistical data were used through main component analysis (PCA) to at the grouping of the samples and unravel the chemical characteristics associated with the substitution of milk powder for soy extract. This study provides important information on the nutritional potential of dairy compounds enriched with soy extract.

2. Material and methods

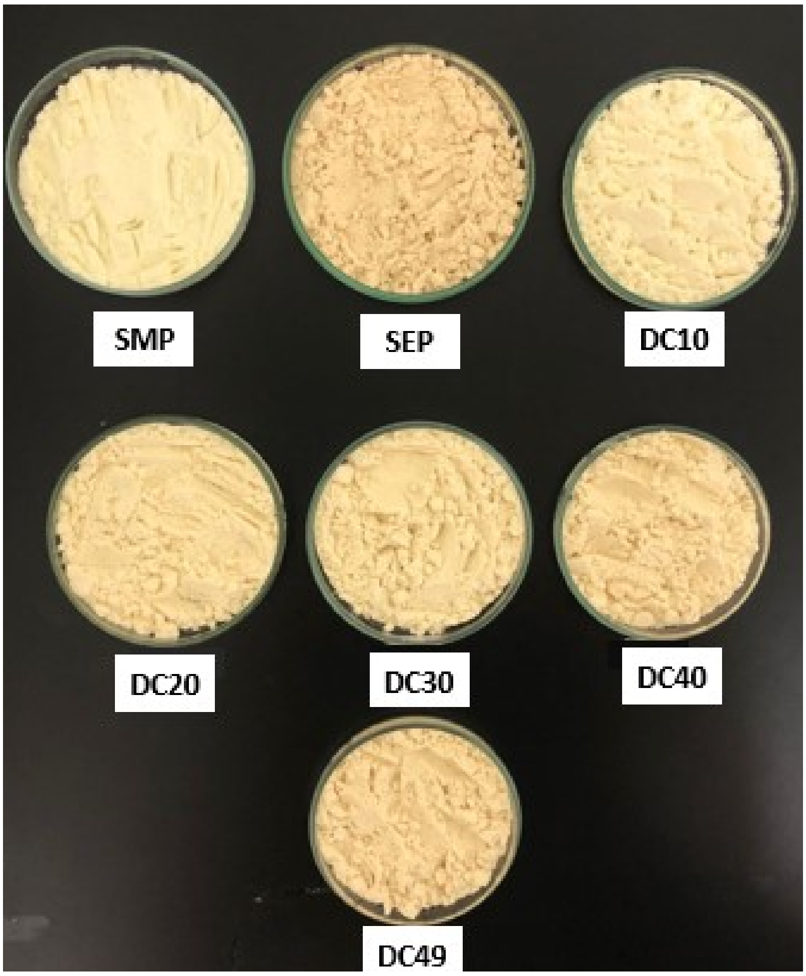
2.1. Samples

The soy extract powder was prepared from the soybeans (Glycine max). Therefore, soybeans were washed three times using tap water and then deionized water before being soaked in soybean: water ratio of 1:5 (w/w) for 10 h at room temperature in a dark room. The swollen soybeans were sieved, rinsed with distilled water, and ground using a blender (Walita®, São Paulo, SP, Brazil), at room temperature with the conventional grinding method, where soybeans were ground with distilled water in soybean: water ratio of 1:8.

The slurry from the grinding method was filtered through a three-layer cheesecloth and heated at 95 °C for 15 min and cooled to room temperature using an ice bath for about 20 min. Finally, the soymilk was dehydrated in a laboratory scale spray dryer (B-290 mini spray dryer, Buchi, Flawil, Switzerland) using the operating parameters described by Fritzen-Freire *et al*. (2012). Drying was performed at a constant air inlet temperature of 150 ± 1 °C and outlet temperature of 50 ± 4 °C. Each drying assay was carried out in triplicate. The soy extract powder was collected from the base of the cyclone, placed in sterile vials, and stored at room temperature.

Five formulations of dairy compounds were prepared, where 10, 20, 30, 40, and 49% (m/m) of commercial skim milk powder were replaced by soy extract powder, being denoted as DC10, DC20, DC30, D40 e DC49, respectively (Table 3.1). These contents were defined according to Normative Instruction No. 28 of June 12, 2007, of the Ministry of Agriculture, Livestock, and Supply. This Normative Instruction establishes that dairy ingredients must represent at least 51% (m/m) of the total ingredients (mandatory or raw material) of the product called a dairy compound. However, two control samples were used, 100% skim milk powder (SMP) and 100% soy extract powder (SEP). The visual characteristics of the samples are shown in Fig. 3.1.

Figure 3.1 Visual characteristics of samples of the milk powder, soy extract, and dairy compound enriched with soy extract powder.



SMP- Skim milk powder, SEP- Soy extract powder, DC10- Dairy compound with 10% soy extract, DC20- Dairy compound with 20% soy extract, DC30- Dairy compound with 30% soy extract, DC40- Dairy compound with 40% soy extract, DC49- Dairy compound with 49% soy extract.

Source: Author

Table 3.1. Five formulations of dairy compounds (DC) samples prepared with commercial skim milk powder (SMP) and soy extract powder (SEP).

Samples	Commercial skim milk powder (%) (m/m)	Soy extract powder (%) (m/m)
DC10	90	10
DC20	80	20
DC30	70	30
DC40	60	40
DC49	51	49
SMP (Control 1)	100	0
SEP (Control 2)	0	100

2.2. Physicochemical analysis

The samples were analyzed for moisture, water activity, total fat, crude protein content, carbohydrates, pH, titratable acidity, and mineral salts. The moisture content of the samples (g/100 g) was evaluated by drying in an oven at 105 °C until constant weight (IDF, 1993). The samples of powdered milk and powdered milk compound were reconstituted with distilled water and then analyzed for pH and titratable acidity. The pH analysis was performed using a digital pH meter (Tecnal tec-7, São Paulo, Brazil). The titratable acidity was determined according to Brasil (1981), and the results were expressed in g of lactic acid /100 g. The pH analysis was performed using a digital pH meter (Tecnal tec-7, São Paulo, Brazil). Crude protein content (g/100 g) was determined using the Kjeldahl method, which determines the total nitrogen content in samples (CHEVER *et al*., 2017). The total fat (g/100 g) in samples was determined by the Soxhlet method according to IAL, 2008. Carbohydrates were quantified by difference, according to Chever *et al*., (2017). The mineral salts content (g/100 g) was determined by incineration at 550 °C for 5 h (CHEVER *et al*., 2017). All analyzes were performed in triplicate.

2.3. Color parameters

Colorimetric parameters were measured using a Minolta Chroma Meter CR-400 colorimeter (Konica Minolta, Osaka, Japan). The instrument was calibrated with a white reference block before measurements. The CIELab color scale was used to measure the color parameters, where: L^* represents luminosity, ranging from 0 (black) to 100 (white); a^* indicates the variation from red ($+a^*$) to green ($-a^*$); and the parameter b^* indicates the variation from yellow ($+b^*$) to blue ($-b^*$). The total color difference (ΔE) (Equation 1) was calculated according to Saricoban and Yilmaz (2010) and Himmetagaoglu and Erbay (2019).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Where ΔL^* is the difference in luminosity, Δa^* is the difference in parameter a^*, and Δb^* represents the difference in parameter b^* between two types of samples. The ΔE^* values calculates were ΔE^*1 and ΔE^*2. In this case, ΔE^*1 represents the color parameters difference between SEP sample with color parameters of other samples SMP, DC10, DC20, DC30, DC40, and DC49, respectively; and ΔE^*2, demonstrates the color parameters difference between the SMP sample with color parameters of other samples SMP, DC10, DC20, DC30, DC40, and DC49, respectively.

2.4. Determination of the phenolic content and antioxidant capacity

The extracts were prepared according to the method proposed by Carvalho et al., (2019), with some modifications. For the extraction process, 2 g of sample was mixed with 15 mL of 80 % (v/v) acetone solution acidified with 0.1 % (v/v) HCl. The mixture was kept in a stirring bath (Dist DI950M, Florianópolis, SC, Brazil) at 50 ± 2 °C for 60 min and centrifuged at 3000 g for 5 min (Hermle Z200A, Germany). The supernatants were collected and then the analyzes of total polyphenols, total flavonols, and in vitro antioxidant capacity were performed.

2.4.1 Total polyphenols and total flavonols

Total polyphenols were determined according to the Folin-Ciocalteu assay, (Singleton, Joseph & Rossi, 1965). The absorbance of the samples was measured at 765 nm and the results were expressed in mg of gallic acid (GAE)/100 g. The total flavonols were determined according to the DMACA method (*p*-dimethylaminocinnamaldehyde) as described by Arnous, Makris & Kefalas (2002). Absorbance was measured at 640 nm and results were expressed in mg of catechin/100 g.

2.4.2 In vitro antioxidant capacity

The antioxidant capacity was determined according to the free radical scavenging methods ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) (KIM; GUO; PACKER, 2002) with absorbance readings at 734 and 517 nm, respectively. The FRAP method was performed according to Arnous, Makris and Kefalas, 2002, this method is based on the reducing power of the ferric complex Fe3+ -2,4,6-tripyridyl-s-triazine by antioxidant compounds. Absorbance was measured at 620 nm. The results were expressed as equivalent antioxidant capacity of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (μM TEAC/100 g). The analyzes were performed in a UV-VIS spectrophotometer (model U-1800, Hitachi, Japan). All analyzes were performed in triplicate.

2.5. Physical Properties

2.5.1. Density

The density of the samples was measured by the values for the aerated and compacted bulk density, as performed by Lebrun *et al*. (2012). The aerated bulk density was obtained by deposition of approximately 2 g of sample in a beaker and was calculated using Equation 2.

To determine the compacted density, after deposition of the sample inside the test tube, mechanical movements were performed (100 times) to evaluate its compaction. The tapped density was calculated using Equation 3.

$$\text{Aerated bulk density} = \frac{\text{sample mass (g)}}{\text{sample volume}(cm^3)} \quad (2)$$

$$\text{Compacted density} = \frac{\text{sample mass (g)}}{\text{volume sample after mechanical movements }(cm^3)} \quad (3)$$

2.5.2. Amount of interstitial air

The amount of interstitial air (cm³/100g) in the samples was determined using the values of aerated and compacted bulk density, as proposed by Chever *et al*., (2017) (Equation 4).

$$\text{Interstitial air} = \left(\frac{1}{\text{aerated bulk density } (kg\ m^{-3})} - \frac{1}{\text{compacted density}(kgm^{-3})}\right) \text{X } 100000 \quad (4)$$

2.5.3. Fluidity and cohesiveness

The samples were evaluated for fluidity and cohesiveness in terms of the Carr index (CI) and the Hausner rate (HR), respectively. Both IC and TH will be calculated from the aerated and compacted bulk densities using Equations 5 and 6, provided by Reddy *et al*., (2014).

$$\text{Carr index (CI)} = \frac{\text{aerated bulk density} - \text{compacted density}}{\text{aerated bulk density}} \text{ X } 100\ (\%) \quad (5)$$

$$\text{Hausner rate (TR)} = \frac{\text{aerated bulk density}}{\text{compacted density}} \quad (6)$$

2.6. Rehydration properties

2.6.1. Wettability

The wettability index (WI) was determined by adding 13 g of sample on a dry basis and 100 g of water at a temperature of 40 ºC without stirring. The wettability index is defined as the time required for a powder to become completely wet, that is, the time for the powder to reach the bottom of the beaker. For wettability results with a time greater than 120 s, the results should be considered merely informative (CHEVER et al., 2017).

2.6.2. Dispersibility

The dispersibility analysis was performed according to the procedure described by Bansal *et al*., (2015) with some modifications. 1 g of sample and 10 mL of water were used at a temperature of 40±1°C. This mixture was manually stirred for 15s, where approximately 25 complete movements were performed. The reconstituted sample was poured into a sieve (mesh size of 250 µm), the sample that passed through the sieve was collected and 2 mL was transferred to a crucible, previously weighed, and kept in an oven at 105±1°C until mass stabilization. The dispersibility of the samples was calculated by Equation 7.

$$D(\%) = (m_2 \times 5) \times \frac{100}{m_1} \quad (7)$$

Where D: dispersibility $the\ the\ m_2$: mass of sample dispersed, m_1: mass of sample used in the analysis.

2.6.3. Solubility

Solubility analysis was determined according to Amiri-Rigi *et al.*, (2011) with some modifications. 100 mL of water at a temperature of 40±1°C was used to solubilize 1 g of the sample by mixing at high speed in a magnetic stirrer (Kasvi, Paraná, Brazil) for 5 min. The milk suspension was then transferred to a centrifuge tube and centrifuged (Hermle z 200 A, Tuttlingen, Germany) at 3000 rpm for 5 min. 25 mL aliquot of the supernatant was transferred to a crucible, previously weighed, and placed in an oven at 105±1°C until the mass was stabilized. Finally, the solubility of the powder sample was calculated by Equation 8:

$$S(\%) = (m_2 \times 4) \times \frac{100}{m_1} \quad (8)$$

Where S: solubility, $the\ m_2$: mass of sample solubilized, m_1: mass of sample used in the analysis.

2.7. *Multi-elemental profile*

The multi-element profile was determined in ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) Model iCAP 6000 (Thermo Analytica, USA), high purity argon 99.95% (Air Liquide, Brazil) was used. The parameters used are Plasma Power 1300, Pump speed 65 rpm, Pump stabilization time 1 s, Auxiliary gas flow 1 L min^{-1}, Flow of nebulizing gas 0,39 L min^{-1}, Plasma view radial (Table S1). The traced minerals were Al, Cd, Cr, Cu, Fe, Mn, Zn, Ca, K, Mg, Na, and P (Table S2).

Table S1. ICP-OES parameters for analysis of minerals.

Parameter	
Plasma Power	1300
Pump speed (rpm)	65
Pump stabilization time (s)	1
Auxiliary gas flow (L min^{-1})	1
Flow of nebulizing gas (L min^{-1})	0.39
Plasma view	Radial

Table S2. Wavelength (nm) and analytical curves (μg mL^{-1}) were used for the determination of minerals.

Element	Wavelength (nm)	Analytical curve (μg/ mL)	Pattern mark
Al	308.215	0.10 - 2.00	Specsol®. Brasil
Cd	228.802	0.10 - 2.00	
Cr	283.563	0.10 - 2.00	
Cu	324.754	0.10 - 2.00	Specsol®. Brasil
Fe	238.204	0.10 - 2.00	SCP Science. Canadá
Mn	257.610	0.10 - 2.00	SCP Science. Canadá
Zn	213.856	0.10 - 2.00	Specsol®. Brasil
Ca	422.673	5.00 - 35.00	Specsol®. Brasil
K	766.490	5.00 - 35.00	Merck. Alemanha
Mg	280.270	5.00 - 35.00	SCP Science. Canadá
Na	589.592	5.00 - 35.00	VETEC. Brasil
P	213.618	5.00 - 35.00	Specsol®. Brasil

2.8 Statistical analysis

The results were expressed as mean ± standard deviation. To determine significant differences ($P < 0.05$), a one-way analysis of variance (ANOVA) and Tukey's test were performed. Principal component analysis (PCA) was used to elucidate the grouping of the samples and unravel the main chemical characteristics associated with the dairy compounds enriched with soy extract in different proportions. All statistical analyzes were performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, USA).

3. Results and discussion

3.1. Physicochemical properties

According to Adinepour *et al*. (2022), many researchers, especially in the food industry, have become increasingly interested in adding bioactive to dairy products because of their health-promoting properties. Cutrim and Cortez (2018) stated the enrichment of dairy foods with bioactive compounds from natural sources has become particularly widespread over ten years because of consumer demands for healthier and functional foods. Polyphenolic substances, predominantly herbal extracts, have been extensively investigated in recent studies as functional additives in food, but not for powder milk. Cutrim and Cortez (2018) cited that the functional additives are added into white cheese, cheese, yogurt, traditional Greek-type and European-type sheep's, yogurts, full-fat and nonfat yogurt, cheddar-type cheese, low-fat hard cheese, drinking yogurt, low-fat yogurt, low-fat hard cheese, Serra da Estrela cheese, ultrafiltered Feta cheese, fermented goat's milk, probiotic yogurt, and cottage cheese. This represents a considerable challenge to the food industry in terms of the suitability of these components and potential influences on the physicochemical, microbiological, and sensory aspects of the final products (CUTRIM ; CORTEZ, 2018). However, the dairy compounds market has been gaining relevance in recent years in Brazil. It stands out, mainly, for being a lower-cost substitute concerning powdered and fluid milk. Thus, the only existing dairy compound is the one that replaces part of the milk with cheese whey, further highlighting the novelty of the present study, including about to compare our findings with similar assays where dairy powders were enriched with soy or similar extracts.

The physicochemical properties of the samples are presented in Table 3. 2. The results of ANOVA and linear regression coefficients are listed in Table S3. Regarding crude protein content, no dairy compound differed significantly ($p \leq 0.05$) from the sample control (SMP, skim milk powder). The replacement, even in 49% of powdered milk with a soy extract (DC49, dairy compound with 49% soy extract), does not affect the crude protein content percentage of the product. The enrichment of skim milk powder with soy extract powder is interesting to maintain the crude protein content of the product since, once the soy extract contains about 40% of the crude protein content in its composition (GIRI; MANGARAJ, 2012).

There was a significant increase in total fat when powdered milk was replaced with soy extract since soy extract is rich in this component. The total fat of all dairy compound formulations differed significantly from skim milk powder. The SMP sample had shown a total fat content of 0.44±0.01 g/100 g, while in the sample DC49, the total fat (6.29 ± 0.57 g/100 g) increased considerably. The dairy compounds enriched with soy extract had 3.36-13.29 times more total fat than the SMP sample, it is noted that the SEP (soy extract powder) sample has 14.77 times more total fat, which justifies our results. These results show that the addition of soy extract to the skim milk powder reconstitutes the total fat contained in the skim milk. The soybean and its derivatives are important sources of lipids, and soy lipid fraction is mainly composed of unsaturated fatty acids. Lipid oxidation is a primary cause of quality deterioration in fat-containing dairy powders and is often used as an estimation of shelf-life products, and this may affect the use of soy extract in food formulations. The lipid oxidation produces numerous volatile organic compounds including aldehydes, ketones, and alcohols, which are known to contribute to the development of off-flavors in dairy powders (CLARKE *et al*., 2021).

Carbohydrates represent between 52 and 48% of the composition of the samples. Bristone *et al*., (2015) used aqueous soy extract to replace part of cow's milk in the production of yogurt and found the same behavior as in the present study, an increase in lipids and a decrease in carbohydrates in the product. Sucrose is the major soluble carbohydrate found in soybeans, followed by stachyose and raffinose, therefore. The insoluble carbohydrates present in soybeans include cellulose, hemicellulose, and lignin, which are found mainly in the cell wall (LEVEK *et al*., 2022). The high concentration of lactose and lysine-rich proteins makes dairy products very sensitive to non-enzymatic browning. In milk, non-enzymatic browning begins with the condensation of lactose with proteins, forming lactulosyl-lysine. The occurrence of lactosylation may not cause browning, but it may decrease the nutritional quality of the milk due to the blockage of lysine, which is no longer available for digestion. This reaction is mainly induced by heat treatment but can also occur during the storage of powdered milk (GONZALES *et al*., 2010).

The maximum moisture allowed for powdered milk, according to Brasil (2018), is 5%, so the formulations of dairy compounds have adequate moisture, and their results vary between 4.30% and 4.69%. Water activity is an important characteristic of powdered food products. In the case of powder milk and dairy compounds, due to the long shelf life, it is necessary to maintain the water activity at adequate values to maintain nutritional quality and prevent lactose crystallization (PUGLIESE *et al*., 2017). We observed that there was no significant difference for Aw of skimmed milk powder and dairy compounds enriched with soy extract, except for the DC20 sample DC20 sample (a dairy compound with 20% soy extract), which differs from the SMP sample for this parameter. Pugliese *et al*., (2017) evaluated eleven different commercial brands of powder, skim, and whole milk, finding values between 0.24 and 0.33, in agreement with those found in this study of the control samples of skim milk powder and dairy compounds enrichment of soy extract powder.

There was a reduction in titratable acidity, and all dairy compounds enriched with soy extract differed from sample control (SMP). The replacement of 49% of cow's milk with soy extract generated a decrease in titratable acidity in yogurts (PONKA *et al*., 2022), corroborating the results of this study (SMP 1.62 ± 0.10 for DC49 1.1 ± 0.09). The pH values of samples DC10 (dairy compound with 10% soy extract), DC20 (dairy compound with 20% soy extract), and DC30 (dairy compound with 30% soy extract) do not present a significant difference from the control sample (SMP). The formulations of dairy compounds enriched with soy extract showed no significant difference ($p \leq 0.05$) in the levels of mineral salts compared to the control sample (SMP).

The samples were evaluated for color parameters and the results are shown in Table 3.2. As expected, the L^* was significantly lower ($p < 0.05$) in the soy extract, so when increasing the percentage of soy extract in the dairy compound, the formulations became darker. The a^* parameter (red-green) showed negative values in all samples, except for soy extract, its addition to the compounds made them approach the red hue and move away from the green one. The parameter b^* (yellow-blue) did not show significant differences between the samples. The results for L and a* are close to those found by Pugliese et al., (2017), for different samples of skim milk powder, (between L^* 96.5 and 97.45; a^* -2.13 and -2.47; b^* 8.64 and 12.02), but this study presented higher numbers for the values of b^*. All formulations show a visible color difference from the control samples because, according to Lee and Coastes (2003), the total color difference above 2.00 is noticeable.

Table 3.2 Physicochemical properties and color parameters of control samples (skim milk powder and soy extract powder) and dairy compound enriched with soy extract powder.

	Samples						
	SMP	SEP	DC10	DC20	DC30	DC40	DC49
Physicochemical analysis (g/100g)							
Crude Proteins *(g/100g)*	35.20±1.50[a]	30.77±1.32[b]	34.76±1.39[a]	34.31±1.40[a]	33.87±1.01[a]	33.42±1.01[a]	33.02±1.14[ab]
Total fat *(g/100g)*	0.44±0.01[e]	6.94±0.21[a]	1.92±0.16[d]	3.26±0.11[c]	4.12±0.24[c]	5.33±0.27[b]	6.29±0.57[ab]
Carbohydrates *(g/100g)*	52.35±0.07[a]	50.76±0.10[b]	51.01±0.21[ab]	50.49±0.03[b]	50.75±0.38[b]	49.68±0.13[bc]	48.97±0.83[c]
Moisture *(g/100g)*	4.27±0.09[b]	5.27±0.21[a]	4.66±0.08[ab]	4.40±0.06[b]	4.30±0.32[b]	4.30±0.03[b]	4.69±0.19[ab]
Salts mineral *(g/100g)*	7.74±0.16[a]	6.26±0.09[b]	7.65±0.03[a]	7.53±0.22[a]	6.96±0.46[ab]	7.26±0.18[a]	7.03±0.05[ab]
Titratable acidity *(g/100g)*	1.62±0.10[a]	0.84±0.01[d]	1.39±0.02[b]	1.31±0.03[bc]	1.23±0.04[bc]	1.13±0.05[c]	1.1±0.09[c]
Water activity	0.34±0.01[a]	0.43±0.01[c]	0.33±0.01[a]	0.30±0.01[b]	0.32±0.01[a]	0.33±0.01[a]	0.32±0.01[a]
pH	6.43±0.02[c]	6.96±0.01[a]	6.51±0.02[c]	6.49±0.04[c]	6.54±0.01[c]	6.73±0.10[b]	6.51±0.02[a]
Color parameters							
a*	-5.64±0.25[f]	1.45±0.38[a]	-3.31±0.84[e]	-2.31±0.26[d]	-1.34±0.21[c]	-0.49±0.18[b]	-0.43±0.07[b]
b*	21.57±1.79[a]	21.32±1.43[a]	19.01±0.84[a]	19.73±1.96[a]	21.45±1.26[a]	22.22±1.29[a]	20.36±1.05[a]
L*	91.61±1.66[ab]	84.41±1.86[c]	92.17±1.63[a]	90.40±0.87[ab]	87.02±2.17[bc]	85.41±1.58[c]	87.13±0.73[abc]
ΔE*1	10.18±0.39[a]	*	9.41±0.45[a]	7.29±0.75[b]	4.29±1.21[c]	2.92±0.23[c]	3.78±0.96[c]
ΔE*2	*	10.18±0.39[a]	3.57±0.99[d]	4.13±0.32[cd]	6.36±1.34[bc]	8.39±1.30[ab]	7.00±0.21[b]

Results expressed as mean ± SD of dw (n=3). Different letters in the same line indicate statistical difference between samples ($p < 0.05$). SMP- Skim milk powder. SEP- Soy extract powder. DC10- Dairy compound with 10% soy extract. DC20- Dairy compound with 20% soy extract. DC30- Dairy compound with 30% soy extract. DC40- Dairy compound with 40% soy extract. DC49- Dairy compound with 49% soy extract. ΔE*1 is color parameters difference between SEP sample with other samples (SMP, DC10, DC20, Dc30, DC40, DC49). $\Delta E^{*}2$ is color parameters difference between SMP sample with other samples (SEP, DC10, DC20, Dc30, DC40, DC49). Titratable acidity- results expressed in g of lactic acid /100 g.

Table S3. Analysis of variance and linear regression coefficients of physicochemical analysis, color parameters, physical and rehydration properties, antioxidant capacity, polyphenolic potential and multielement profile.

Source of variation	SS	df	MS	F Value	*p*-Value
Intercept	228.6500	1	228.6500	2879.755	0.000000
Lipids	66.4726	6	11.0788	139.533	0.000001
Error	0.5558	7	0.0794		
Intercept	35811.43	1	35811.43	271376.8	0.000000
Carbohydrates	13.59	6	2.26	17.2	0.000728
Error	0.92	7	0.13		
Intercept	290.6535	1	290.6535	9574.370	0.000000
Moisture	1.5400	6	0.2567	8.455	0.006306
Error	0.2125	7	0.0304		
Intercept	726.6833	1	726.6833	15087.44	0.000000
Minerals	3.1477	6	0.5246	10.89	0.002982
Error	0.3372	7	0.0482		
Intercept	21.29817	1	21.29817	6475.069	0.000000
Titratable acidity	0.72691	6	0.12115	36.833	0.000060
Error	0.02302	7	0.00329		
Intercept	2.415511	1	2.415511	83971.88	0.000000
Aw	0.029868	6	0.004978	173.05	0.000000
Error	0.000403	14	0.000029		
Intercept	608.7845	1	608.7845	266343.2	0.000000
pH	0.4133	6	0.0689	30.1	0.000117
Error	0.0160	7	0.0023		
Intercept	62.67802	1	62.67802	1119.060	0.000000
a*	94.60145	6	15.76691	281.504	0.000000
Error	0.78413	14	0.05601		
Intercept	9050.945	1	9050.945	4481.912	0.000000
b*	24.663	6	4.111	2.035	0.128171
Error	28.272	14	2.019		
Intercept	163858.3	1	163858.3	66129.56	0.000000
L*	169.5	6	28.2	11.40	0.000107
Error	34.7	14	2.5		
Intercept	614.8570	1	614.8570	1271.798	0.000000
ΔE*SEP	244.9525	6	40.8254	84.445	0.000000
Error	6.7684	14	0.4835		
Intercept	673.3027	1	673.3027	979.4872	0.000000
ΔE* SMP	206.6248	6	34.4375	50.0979	0.000000
Error	9.6236	14	0.6874		
Intercept	89.60119	1	89.60119	5500.747	0.00
Polyphenols	28.24178	6	4.70696	288.967	0.00
Error	0.52125	32	0.01629		
Intercept	47.98487	1	47.98487	2189.833	0.000000
Flavonols	4.37700	6	0.72950	33.291	0.000000

Error	0.70120	32	0.02191		

Source of variation	SS	df	MS	F Value	*p*-Value
ABTS	104.240	6	17.373	112.998	0.00
Error	4.920	32	0.154		
Intercept	78.84313	1	78.84313	1460.931	0.000000
DPPH	13.46627	6	2.24438	41.587	0.000000
Error	1.67300	31	0.05397		
Intercept	87.61827	1	87.61827	3595.074	0.000000
FRAP	9.91097	6	1.65183	67.776	0.000000
Error	0.75552	31	0.02437		
Intercept	2.732441	1	2.732441	10939.50	0.000000
Density	0.070052	6	0.011675	46.74	0.000000
Error	0.003497	14	0.000250		
Intercept	6.389356	1	6.389356	13470.89	0.000000
Compacted density	0.064526	6	0.010754	22.67	0.000002
Error	0.006640	14	0.000474		
Intercept	214638.2	1	214638.2	658.6752	0.000000
Interstitial air	13319.9	6	2220.0	6.8126	0.001530
Error	4562.1	14	325.9		
Intercept	25531.68	1	25531.68	1686.367	0.000000
CI	376.36	6	62.73	4.143	0.013345
Error	211.96	14	15.14		
Intercept	50.15667	1	50.15667	4679.966	0.000000
HR	0.18109	6	0.03018	2.816	0.051779
Error	0.15004	14	0.01072		
Intercept	115741.6	1	115741.6	9296.998	0.000000
Wettability	59666.1	6	9944.4	798.785	0.000000
Error	174.3	14	12.4		
Intercept	124514.5	1	124514.5	5126.142	0.000000
Dispersibility	448.2	6	74.7	3.075	0.039028
Error	340.1	14	24.3		
Intercept	129427.6	1	129427.6	83766.22	0.000000
Solubility	1800.2	6	300.0	194.18	0.000000
Error	21.6	14	1.5		
Intercept		1	83990.04	10986.98	0.00
Al	29053.56	6	4842.26	633.43	0.00
Error	428.09	56	7.64		
Intercept	160.0133	1	160.0133	1651.268	0.000000
Cd	3.1432	6	0.5239	5.406	0.000183
Error	5.4266	56	0.0969		
Intercept	627.6785	1	627.6785	193.3509	0.000000
Cr	21.6408	6	3.6068	1.1110	0.367661
Error	181.7938	56	3.2463		
Intercept	991.2636	1	991.2636	16728.96	0.00
Cu	375.9702	6	62.6617	1057.50	0.00
Error	3.3182	56	0.0593		

Intercept	1951339	1	1951339	3938.652	0.00
Fe	1238153	6	206359	416.522	0.00
Error	25267	51	495		
Intercept	7285.209	1	7285.209	21033.54	0.00
Mn	4778.322	6	796.387	2299.29	0.00
Error	19.396	56	0.346		
Source of variation	**SS**	**df**	**MS**	**F Value**	***p*-Value**
Intercept	115098.7	1	115098.7	97225.87	0.00
Zn	739.6	6	123.3	104.12	0.00
Error	66.3	56	1.2		
Intercept	2.85E+09	1	2.85E+09	3169.256	0
Ca	3.72E+07	6	6.20E+06	6.907	0.000016
Error	5.03E+07	56	8.98E+05		
Intercept	7.09E+09	1	7.09E+09	3336.759	0
K	5.58E+07	6	9.30E+06	4.376	0.001083
Error	1.19E+08	56	2.13E+06		
Intercept	298051869	1	298051869	71712.13	0
Mg	14694689	6	2449115	589.26	0
Error	232749	56	4156		
Intercept	519733549	1	519733549	40586.41	0
Na	23692495	6	3948749	308.36	0
Error	717114	56	12806		
Intercept	8.22E+09	1	8.22E+09	7997.766	0
P	1.25E+07	6	2.09E+06	2.033	0.076318
Error	5.75E+07	56	1.03E+06		

SS – sums of squares; df – degrees of freedom; MS – mean square.

3.2. Total polyphenols, total flavonols, and antioxidant capacity of dairy compound enriched with soy extract

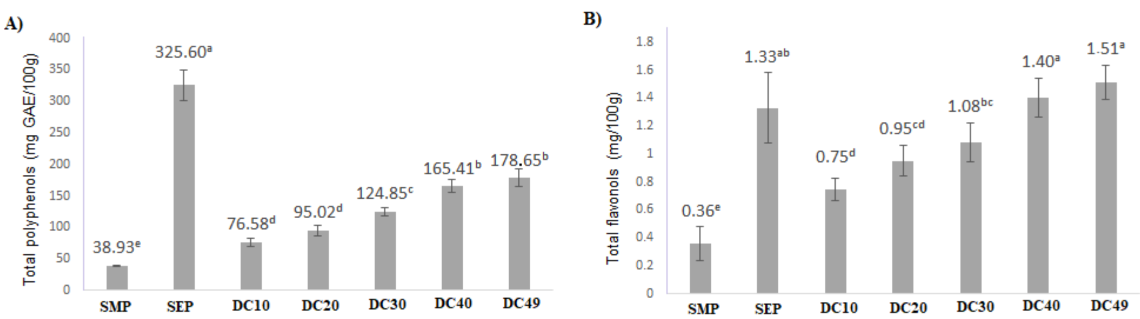
The levels of total polyphenols, total flavonols, and antioxidant capacity of the samples are shown in Fig. 2 and Fig. 3. The results of ANOVA and linear regression coefficients are listed in Table S3. The values found for total polyphenols were 38.93 mg/100 g for the control sample (SMP) and 325.60 mg/100 g for the SEP sample. The total polyphenols content in the dairy compounds enriched with soy extract varied from 76.8 to 178.65 mg/100 g, with a gradual increase along with the increase in the percentage of soy extract in the sample. Shin *et al*., (2013) verified the content of polyphenols and flavonols present in soy flour prepared with grains treated in different ways (toast, roast, crude) and found values between 85 and 105 mg/100 g for total phenolics and between 60 and 80 mg/100 g for total flavonols. Tyug *et al*. (2010) found 103.86 ± 5.29 mg/100 g of total phenolic compounds for powder soymilk. These results are lower than those reported in our study for soy extract (SEP). The SEP sample presented high values of polyphenols and flavonols, compared to the control sample (SMP), consequently, when increasing the values of soy extract powder in the formulations, there was a proportional enrichment of these bioactive components in the dairy compounds. There was a significant difference between the SMP sample and formulations of the dairy compounds (DC10, DC20, DC30, DC40, and DC49) for polyphenols and total flavanols.

According to Helal and Tagliazucchi (2018), this occurrence is because polyphenols have a high binding affinity for proteins, which leads to the formation of soluble or insoluble complexes. Frazier *et al*., (2010) highlighted the numerous hydrophobic interactions that occur particularly between polyphenols and proline-rich proteins, such as casein. The milk protein polymorphism affects the amino acid composition of protein and, consequently, the ability to bond with phenols. As the nitrogenous fraction of powder milk is characterized by the simultaneous presence of caseins and whey proteins and peptides of various molecular weights, these results could be due to a greater capacity of proteins and peptides to form stable complexes with phenols. The DC40 (dairy compound with 40% soy extract) and DC49 samples have no significant difference in the levels of these compounds. The bioactive compounds are very unstable to storage as they are sensitive to factors such as humidity, temperature, light, and oxygen. These factors can accelerate the degradation processes of certain nutrients and change the nutritional and sensory properties during the product's shelf life.

The 100% soy extract (SEP) sample showed the highest antioxidant capacity, the opposite was observed in the 100% skimmed milk powder (SMP) sample, which showed the lowest antioxidant capacity. The addition of soy extract promoted a gradual increase in antioxidant capacity in all formulations. The dairy compound enriched with 40 and 49% soy extract showed high levels of antioxidant capacity regardless of the method used. There was an increase of 87.92 and 62.46%, 119.79 and 100.37%, and 87.02 and 79.14% in the antioxidant capacity of samples DC40 and DC49 concerning the control sample (SMP) when evaluated by the ABTS, DPPH and FRAP methods respectively. Prigent and Dimitrov (2003) showed that the effect of the interaction between proteins or peptides (or both) and phenolic compounds on antioxidant capacity depends on the amino acid composition of the proteins, such as the casein, and phenols.

The different behavior detected among enriched powder milk with soymilk extract led us to hypothesize that the effects of the protein-polyphenol complex on antioxidant capacity are interactive, in agreement with those obtained by Perna *et al*. (2018). Therefore, the interaction between polyphenols and milk proteins could have a protective effect because this interaction may provide physical trapping, and according to Perna *et al*. (2018) increase the stability of polyphenols during digestion. Consequently, the fortification of powder milk with soymilk extract is a method to enhance health properties and develop novel functional dairy products.

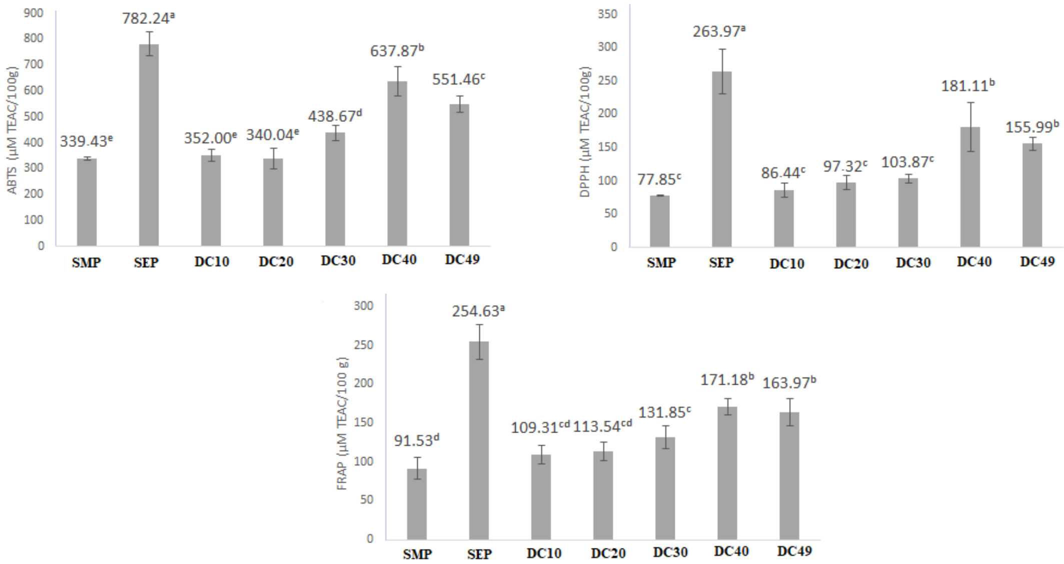
Figure 3.2 Total polyphenols (A) and total flavonols (B) of the milk powder, soy extract, and dairy compound enriched with soy extract powder.



Results are expressed as mean ± SD of dw (n=3). SMP- Skim milk powder, SEP- Soy extract powder, DC10- Dairy compound with 10% soy extract, DC20- Dairy compound with 20% soy extract, DC30- Dairy compound with 30% soy extract, DC40- Dairy compound with 40% soy extract, DC49- Dairy compound with 49% soy extract.

Source: Author

Figure 3.3 *In vitro* antioxidant capacity of the milk powder, soy extract, and dairy compound enriched with soy extract powder.



Results are expressed as mean ± SD of dw (n=3). SMP- Skim milk powder, SEP- Soy extract powder, DC10- Dairy compound with 10% soy extract, DC20- Dairy compound with 20% soy extract, DC30- Dairy compound with 30% soy extract, DC40- Dairy compound with 40% soy extract, DC49- Dairy compound with 49% soy extract.

Source: Author

3.3. Physical properties of dairy compound enriched with soy extract

The physical properties of the powders are reported in Table 3.3. The results of ANOVA and linear regression coefficients are listed in Table S3. The aerated density of the SEP sample is lower than that of the SMP sample. The density of the powders is determined by the density of the particles, which in turn is determined by the density of the solid and the porosity. Therefore, modification of particle size can result in changes in density, flowability, and appearance of the final product. The particle preparation method can have a profound effect on the physical properties of the powder (milk and soy extract), especially spray drying and milling (Section 2.1).

The DC10 sample showed a significant reduction in aerated density, but there is no statistical difference ($p \leq 0.05$) between dairy compounds of DC30, DC40, and DC49 for this parameter. For compacted density, the significant difference compared to the SMP sample from the 30% replacement of skim milk powder (DC30), with no difference between higher substitutions. As for the interstitial air of the samples, there is an increase, and all samples are similar to the SEP control, but DC30, DC40, and DC49 differ from the SMP sample. Density is an important characteristic for powders, mainly for storage, due to the determination of the volume occupied, this is important information for the economic and commercial part of the product (SHARMA *et al*., 2012).

The results found for density are lower than those reported by Pugliese *et al*., (2017), where found density between 0.504 g/cm³ and 0.666 g/cm³ and density compressed between 0.705 g/cm³ and 0.872 g/cm³ for samples of skimmed milk powder, but agree with those reports by Chever *et al*., (2017), which found density between 0.330 g/cm³ and 0.407g/cm³ and density compressed between 0.494 g/cm³ and 0.720 g/cm³ for milk powder with added sucrose and lactose. The values found for interstitial air are in agreement with Chever *et al*., (2017) for milk powder plus sucrose and lactose (between 66 ± 1 cm³/100 g and 107 ± 3 cm³/100 g) for the SMP, DC10, DC20, and DC30 samples, while the SEP, DC40 and DC49 samples are slightly higher.

The Carr index (CI) and Hausner Ratio (HR) assess the flowability and cohesiveness of samples. As for HR, there is a statistical difference ($p \leq 0.05$) only between the control samples (SMP and SEP), it is known that the total fat can impair the fluidity of the powders (SHARMA *et al*., 2012), and the SEP sample has high concentrations of total fat, which explains the lower fluidity of this sample. The HR and CI results for the SMP sample agree with Pugliese *et al*., (2017) who found RH values between 1.31 and 1.42 and found CI values between 23.5 and 29.5 for different samples of skimmed milk powder but are above those found by Bansal *et al*., (2015) who found HR values between 1.18 and 1.23 and found CI values between 15.56 and 19.15 for milk powder enriched with honey.

The dairy compounds enriched with soy extract did not differ between them or among the controls, despite the increase in rates. Dairy compounds (DC30, DC40, and DC49 samples) differ from the SMP sample for CI. According to the HR and IC classification, skim milk powder has acceptable flowability, dairy compounds have very poor flowability, and soy extract has poor flowability (LEBRUN *et al*., 2012). The increase of soy extract powder in dairy compounds increases the lipid total fat, which justifies the loss of flowability. The total fat can impair the flowability of powders (SHARMA *et al*., 2012), and the increase of soy extract powder in the compounds increased the total fat, which justifies the loss of flowability.

The rehydration characteristics of the samples were investigated through the three stages of reconstitution: wetting, dispersion, and solubilization (Table 3.3). Powders considered wettable must present complete wetting of the surface within 120 seconds (CHEVER *et al*., 2017). The soy extract does not fit into this classification, as the reconstitution of the powder is generally hampered by the physical and chemical changes that occur during the spray dry spray drying process. The dairy compounds with 30% or more of soy extract powder are not wettable. Bansal *et al*., (2015) found the wettability of powdered cow's milk equal to 24±2.68 s, above control samples and below DC20 sample, whereas for dispersibility these authors found higher values, that is, more dispersible. The dispersibility of dairy compounds is affected in the DC40 sample and the solubility in the DC30 sample. Chever *et al*., (2017) found dispersibility values ranging from 62 to 99.7% for dispersibility for whole milk powder added sucrose and lactose, all treatments and control were classified as wettable powders with 99.6% solubility.

Table 3.3 Physical and rehydration properties of control samples (skim milk powder and soy extract powder) and dairy compound enriched with soy extract powder. Results expressed as mean ± SD of dw (n=3).

	Samples						
	SMP	SEP	DC10	DC20	DC30	DC40	DC49
Physical Properties							
Density (g/cm³)	0.47±0.02^{a}	0.28±0.03^{e}	0.39±0.02^{b}	0.37±0.01^{bc}	0.35±0.01^{cd}	0.34±0.01^{cd}	0.32±0.01^{d}
D. compressed (g/cm³)	0.63±0.02^{a}	0.45±0.04^{e}	0.60±0.03^{ab}	0.58±0.02^{abc}	0.55±0.01^{bcd}	0.54±0.02^{cd}	0.51±0.01^{d}
Interstitial air (cm³/100g)	52,76±7,19^{b}	140,48±42,57^{a}	88,74±12,74^{ab}	92,78±10,26^{ab}	107,59±2,80^{a}	109,47±5,34^{a}	115,87±10,61^{a}
CI (%)	24.94±2.60^{b}	38.23±8.04^{a}	34.75±3.94^{ab}	34.73±3.13^{ab}	37.14±0.26^{a}	37.09±1.82^{a}	37.19±2.45^{a}
HR	1.33±0.05^{b}	1.64±0.23^{a}	1.54±0.09^{ab}	1.53±0.07^{ab}	1.59±0.01^{ab}	1.59±0.05^{ab}	1.59±0.06^{ab}
Rehydration Properties							
Wettability (s)	5.09±1.01^{b}	>120	6.22±1.56^{b}	28.37±9.14^{a}	>120	>120	>120
Dispersibility (%)	80.97±4.99^{a}	68.58±0.54^{b}	79.39±1.60^{a}	80.40±0.58^{a}	77.50±2.10^{a}	66.41±1.20^{b}	70.76±0.43^{b}
Solubility (%)	88.26±1.11^{a}	61.34±2.58^{d}	86.94±0.49^{a}	84.95±0.80^{ab}	81.92±1.31^{b}	76.68±0.33^{c}	69.43±0.47^{c}

Different letters in the same line indicate statistical difference between samples ($p < 0.05$). SMP- Skim milk powder. SEP- Soy extract powder. DC10- Dairy compound with 10% soy extract. DC20- Dairy compound with 20% soy extract. DC30- Dairy compound with 30% soy extract. DC40- Dairy compound with 40% soy extract. DC49- Dairy compound with 49% soy extract. CI- Carr index. HR- Hausner Ratio.

3.4. Mineral profile

All investigated minerals are shown in Table 4. The results of ANOVA and linear regression coefficients are listed in Table S3. The multielement profile was affected by the addition of soy extract powder in the formulations of dairy compounds, there was enrichment of minerals, such as Fe, Mn, P, Cu, and Mg. The major minerals in all samples evaluated were Ca, K, P, Mg, and Na. Cadmium, Cr, and K showed no significant difference in their levels between all formulations and control samples.

The (Fe) showed a concentration of 5.36±2.88 µg/g and 493.41±56.32 µg/g for SMP and SEP samples, respectively. All levels of replacement of skim milk powder by soy extract powder promoted Fe enrichment. Among the formulations evaluated, the DC10 sample had the lowest Fe contents (57.23±4.55 µg/g), in contrast, the DC49 sample had the highest this mineral (300.95±16.75 µg/g). There was an approximately 55-fold increase in Fe levels in the DC49 sample compared to the control sample (SMP). The SMP sample showed minimum levels (0.39 ±0.13 µg/g) of Mn, and the SEP sample showed a higher concentration (28.67 ± 1.13 µg/g) of this mineral. There was a gradual increase in Mn levels as soy extract was added to the dairy compound formulations. The DC49 sample showed a concentration of Mn (15.89±0.67 µg/g) 39 times higher than that reported in the SMP sample.

Andrés *et al*., 2016 analyzed the mineral profile of smoothies made with soy and found that the product obtained higher values of Fe and Mn milk when compared to a product made with cow's milk, corroborating the results of this study. The lack of iron (Fe) is one of the main mineral deficiencies in the world population, the lack of it causes anemia. Manganese (Mn) is related to brain and nerve activities and bone structure. (GHARIBZAHEDI; JAFARI, 2017). The mineral fortification of foods is an alternative to combat deficiencies of these nutrients in the human diet. Mineral food fortification is an alternative to combat mineral deficiencies such as Fe and Mn (GHARIBZAHEDI ; JAFARI, 2017).

As expected, the SMP sample had the highest levels of Zn (43.41±1.30 µg/g) while the SEP sample had the lowest levels of this mineral (34.7±0.58 µg/g). The addition of soy extract did not promote a large increase in the Zn content in the dairy compounds. The Zn contents reported for the SEP sample are higher than the levels reported by Etiosa *et al*., (2017) for soy seeds (27 µg/g), but it is lower than the value found for soy flour

(51.5 μg/g) (PORTER; JONES, 2003). Zinc participates in the structure of enzymes, the production of proteins in the immune, reproductive and digestive systems. Zinc is part of the micromineral group along with Cr, Cu, Fe, and Mn, so lower daily doses are needed in the diet when compared to macro minerals such as Ca, Mg, P, K, and Na (GHARIBZAHEDI; JAFARI, 2017).

Calcium is present in high concentrations in the samples (5108.25±608.08 μg/g and 7697.02±2077.64 μg/g). There was no significant difference ($p \leq 0.05$) in Ca levels between the formulations of dairy compounds and the control sample (SMP). Soy and milk are among the main sources of Ca. Calcium has important functions in bone structure, muscle contraction, blood pressure regulation, nervous and immunes system, and blood clotting (GHARIBZAHEDI; JAFARI, 2017).

Potassium was the second most abundant mineral in the samples (between 9295.81±668.27 μg/g and 11934.24±3115.57 μg/g). There was no significant difference in the content of K between the control samples and the dairy compounds enriched with soy extract. This is an important mineral for water balance, muscle contraction, blood pressure maintenance, and waste elimination (GHARIBZAHEDI; JAFARI,2017).

The addition of soy extract enriched the formulations of dairy compounds with high levels of Mg. The SMP sample had a concentration of 1480.34±117.07 μg/g of Mg and the SEP of 2992.26±69.35 μg/g, all dairy compounds differed from the controls. Magnesium varied in the dairy compounds between 1710.11±59.22 μg/g and 2631.70±24.31 μg/g, thus, the enrichment of Mg in the compounds reached up to 77% (DC49). The Mg values of the control SEP sample and the formulations of dairy compounds are next to those of soy seeds found by Etiosa *et al*., (2017) (2582,4 μg/g).

Phosphorus is the most abundant mineral in the samples, concentrations between 10041.47±790.37 μg/g and 11914.26±1902.45 μg/g were found. There was a significant increase in P in the DC20, DC30, DC40, and DC49 samples, which differ from the control sample (SMP). Phosphorus is an essential mineral for bone health. The values found for P in the samples of this study are superior to the findings for soy seed and soy flour respectively by Etiosa *et al*., (2017) and Porte and Jones (2003).

Sodium was observed in high concentrations in the SMP and DC10 samples (3127.41±214.84 μg/g and 3177.96±138.11), and lower levels were reported in the SEP sample (1601.55±62.59 μg/g). There was a reduction in Na contents in samples with 40% and 49% replacement of skim milk powder by soy extract. Cadmium was the mineral in the smallest amount in the formulations of dairy components. In addition, there was a significant increase in Al and Cu levels in dairy compounds enriched with soy extract. The enrichment of essential minerals in dairy compounds was an important result due to all the functions of this in the healthy functioning of the human body. There is a need to combat mineral deficiency, and the enrichment of formulations is among the potential strategies. The enrichment of dairy products with soy extract is an interesting alternative to increase their nutritional value, especially regarding the concentration of macro and micronutrients, such as Fe, Mn, Mg, and P. We emphasize that iron deficiency in the human diet has gained prominence in recent years, its deficiency is one of the main factors that lead to anemia, therefore, the consumption of the dairy compound enriched with soy extract may be a potential strategy to combat the deficiency of this mineral, especially in children.

Table 3.4 Multi-element profile of control samples (skim milk powder and soy extract powder) and dairy compound enriched with soy extract powder.

	Samples						
Mineral (µg/g)	SMP	SEP	DC10	DC20	DC30	DC40	DC49
Al	11.46±1.29[g]	82.07±3.60[a]	19.36±3.02[f]	25.46±2.28[e]	32.9±2.39[d]	38.63±2.94[c]	45.78±3.17[b]
Cd	1.75±0.40[a]	1.77±0.34[a]	1.73±0.25[a]	1.56±0.32[a]	1.49±0.30[a]	1.66±0.24[ab]	1.14±0.26[b]
Cr	3.44±1.36[a]	3.21±1.34[a]	3.84±1.85[a]	2.04±2.07[a]	3.16±2.23[a]	2.69±1.91[a]	3.82±1.63[a]
Cu	1.14±0.15[g]	9.02±0.30[a]	2.07±0.29[f]	2.53±0.28[e]	3.43±0.16[d]	4.29±0.22[c]	5.3±0.23[b]
Fe	5.36±2.88[f]	493.41±56.32[a]	57.23±4.55[e]	88.81±7.87[e]	136.3±12.50[d]	211.43±15.93[c]	300.95±16.75[b]
Mn	0.39±0.13[g]	28.67±1.13[a]	3.08±0.31[f]	5.83±0.40[e]	9.29±0.47[d]	12.41±0.41[c]	15.89±0.67[b]
Zn	43.41±1.30[ab]	34.7±0.58[c]	44.98±0.54[a]	42.86±1.53[b]	43.91±1.58[ab]	44.87±0.70[a]	43.97±0.77[ab]
Ca	6361.69±384.49[a]	5108.25±608.08[b]	6449.35±234.35[ab]	6818.89±68.51[a]	7697.02±2077.64[a]	7242.4±923.48[a]	7169.66±734.42[a]
K	9295.81±668.27[a]	9356.48±1124.75[a]	9556.52±358.91[a]	10233.52±122.70[ab]	11934.24±3115.57[a]	11485.14±1421.76[ab]	11562.96±1136.68[ab]
Mg	1480.34±117.07[g]	2992.26±69.35[a]	1710.11±59.22[f]	1895.04±59.48[e]	2066.82±39.88[d]	2383.01±36.73[c]	2631.70±24.31[b]
Na	3127.41±214.84[b]	1601.55±62.59[e]	3177.96±138.11[b]	3139.16±59.90[b]	3614.10±74.64[a]	2849.16±101.55[c]	2501.02±31.83[d]
P	10041.47±790.37[b]	11100.92±826.47[ab]	11341.93±265.20[ab]	11584.46±462.08[a]	11914.26±1902.45[a]	11700.89±1132.03[a]	11587.41±835.44[a]

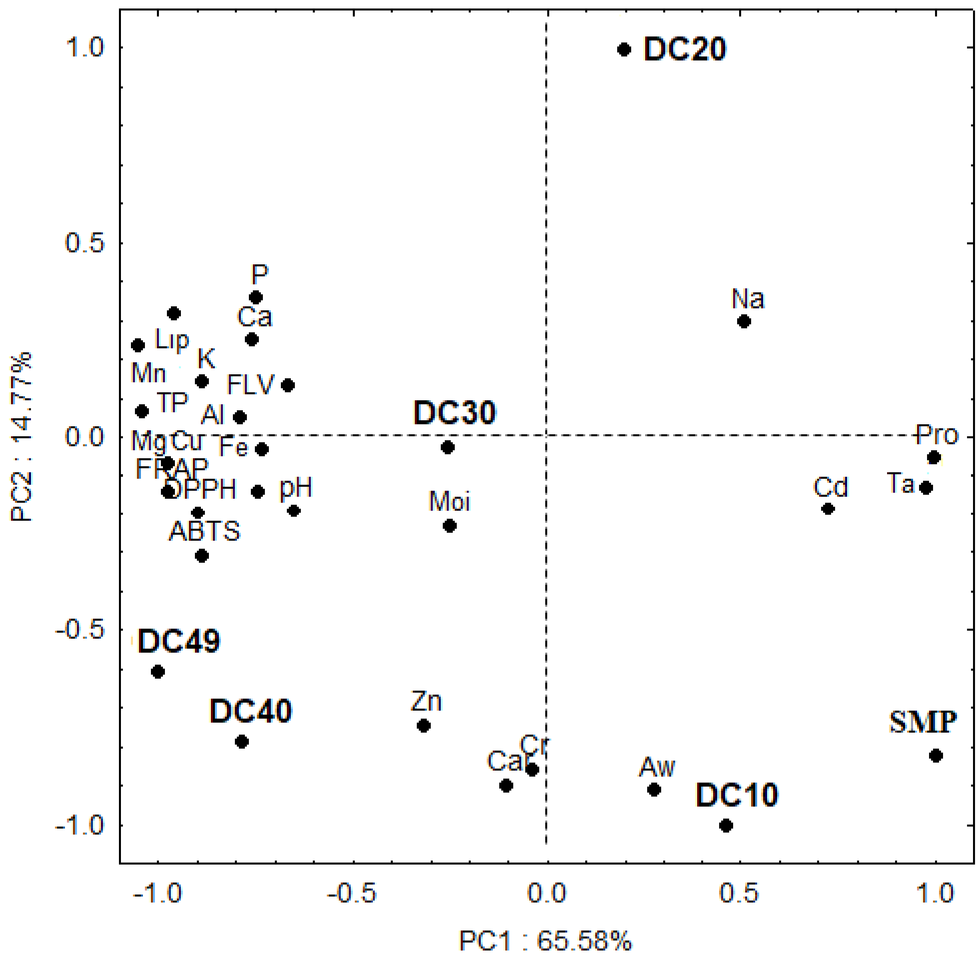
Results expressed as mean ± SD of dw (n=3). Different letters in the same line indicate statistical difference between samples ($p < 0.05$). SMP- Skim milk powder. SEP- Soy extract powder. DC10- Dairy compound with 10% soy extract. DC20- Dairy compound with 20% soy extract. DC30- Dairy compound with 30% soy extract. DC40- Dairy compound with 40% soy extract. DC49- Dairy compound with 49% soy extract.

3.5 Principal component analysis (PCA)

To understand the data set the differences in the chemical composition of milk powder and dairy compounds enriched with soy extract in different proportions, principal component analysis (PCA) was applied to the data obtained based on the physicochemical properties, polyphenolic composition, *in vitro* antioxidant capacity and multi-element profile (Fig.4). The sample dataset revealed that 80.35% of the total variability was represented in two main components (PC1 × PC2). PC1 explained 65.58% of data variability, while 14.77% were explained by PC2. The multivariate analysis revealed that the dairy compound enrichment with soy extract in the proportions of 30, 40, and 49% (DC30, DC40, and DC49) showed a strong association with most of the physical-chemical parameters, minerals (P, Ca, K, Mn, Al, Mg, Cu, Fe, Zn, and Cr), total polyphenols, total flavonols and antioxidant capacity (ABTS, DPPH, and FRAP method) in PC1. In contrast, the powdered milk (control sample) and dairy compounds enriched with soy extract in proportions of 10 and 20% (DC10 and DC20) were separated into PC2 and showed association only with Cd, Na, and crude protein content, acidity, and Aw.

Based on the results obtained, the multivariate approach revealed that the addition of soy extract to powdered milk promoted the enrichment of minerals and polyphenols, mainly when using concentrations of 30, 40, and 49% of soy extract in the formulations of dairy compounds.

Figure 3.4 Principal component analysis of physicochemical properties, total polyphenols, *in vitro* antioxidant capacity, total flavonols, and multi-element profile of the milk powder and dairy compound enriched with soy extract powder.



TP- total polyphenols, FLV- total flavonols, DPPH- antioxidant capacity, ABTS- antioxidant capacity, FRAP- antioxidant capacity, Pro- protein, Lip- lipids, Car- carbohydrates, Moi- moisture content, Ta- titratable acidity, Aw- activity of water, SMP- Skim milk powder, DC10- Dairy compound with 10% soy extract, DC20- Dairy compound with 20% soy extract, DC30- Dairy compound with 30% soy extract, DC40- Dairy compound with 40% soy extract, DC49- Dairy compound with 49% soy extract.

Source: Author

4. Conclusion

The dairy compounds enrichment with soy extract and powder milk showed no significant difference in the levels of crude protein content, moisture, and minerals salts. The dairy compounds enriched with soy extract had 3.36-13.29 times more total fat than the SMP sample. The total polyphenols content in the dairy compounds enriched with soy extract varied from 76.8 a 178.65 mg/100 g. The dairy compound enriched with 40 and 49% soy extract showed high levels of antioxidant capacity. The addition of soy extract to powdered milk promoted a significant increase in the levels of Fe, Mg, P, and Mg. The DC49 sample had the highest Fe content (300.95±16.75 µg/g).

The principal component analysis revealed that the addition of soy extract to powdered milk promoted the enrichment of dairy compounds with minerals and polyphenols, mainly when using concentrations of 30, 40, and 49% of soy extract in the formulations. The rehydration of the powders is harmful with the increase in the percentage of soy extract powder. The dispersibility of dairy compounds is affected in the DC40 sample and the solubility in the DC30 sample. The enrichment of dairy compounds with essential minerals and bioactive compounds demonstrates that replacing milk powder with powdered soy extract can be an alternative for nutritional enrichment of the dairy compound. It is suggested in future works to evaluate the chemical and physical stability of the dairy compound enriched with soy extract over the storage time. In addition, soy extract could be applied to other dairy products, aiming at nutritional enrichment.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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CONSIDERAÇÕES FINAIS E TRABALHOS FUTUROS

A transformação do leite fluido em pó prolonga sua validade, para este processo a concentração, por tecnologias de membranas e evaporadores, e desidratação, pelo método *spray drying*, são principais processos tecnológicos aplicados. As tecnologias de membranas têm diversas aplicações na indústria láctea. Já foram utilizados os processos de microfiltração, ultrafiltração, nanofiltração, osmose reversa e diafiltração em leite ou seus derivados. As membranas foram utilizadas em lácteos com os objetivos de concentração de leite e soro, remoção de microrganismos, fracionamento de proteínas, tratamento de resíduos lácteos e como etapa na produção de queijos, ricota, bebidas fermentadas e leite em pó. Todas as tecnologias de concentração citadas anteriormente foram minimamente detalhadas neste trabalho.

Para a formulação do composto lácteo foi adicionado diferentes percentuais de extrato de soja. A soja é uma matéria prima apresenta altos níveis de proteínas, compostos bioativos e minerais essenciais, e, portanto, é uma excelente alternativa para enriquecimento nutricional do composto lácteo. Foi possível verificar que a adição de extrato de soja ao leite em pó promove enriquecimento nutricional do produto. A adição de extrato de soja no leite promoveu o aumento significativo de polifenóis totais, flavonóis totais, atividade antioxidante e minerais essenciais (Fe, Mn, P, Cu e Mg). Algumas propriedades físicas, fluidez e densidade, dos compostos lácteos sofreram redução com o aumento de adição de extrato de soja ao leite em pó, assim como a solubilidade. O enriquecimento nutricional do composto lácteo mostra que o extrato de soja é um ingrediente em potencial para compor as formulações de compostos lácteos.

Sugere-se como trabalhos futuros avaliação destes produtos por métodos sensoriais, bem como, verificar a estabilidade química, física e bioativa ao longo do tempo de armazenamento do composto lácteo adicionado de extrato de soja em diferentes percentuais. Avaliar o uso de aditivos para melhorar características físicas e reidratação de compostos lácteos com adição de extrato de soja. Além disso, o extrato de soja poderia ser aplicado a outros produtos lácteos, visando o enriquecimento do produto com compostos bioativos.

ANEXOS

*Anexo A: Comprovante de submissão artigo "**A theoretical approach to dairy products from membrane processes**"

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*Anexo B: Comprovante de submissão artigo "**Powder dairy products enriched with a soy extract (*Glycine max*): physicochemical parameters, physical and rehydration properties, polyphenolic potential, and multielement profile**"

Food Research International

Powder dairy products enriched with a soy extract (Glycine max): physicochemical parameters, physical and rehydration properties, polyphenolic potential, and multielement profile

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	Bioactive compounds; Elemental composition; Nutritional enrichment; Dairy compound, Soy.
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	Elane S Prudencio, Dr.
Abstract:	The objective of this study was to (i) develop powder dairy compounds enrichment with soy extract, and (ii) determine its physical properties, rehydration characteristics, multielement profile, and polyphenolic potential. Multivariate analyzes using combined PCA analyzes were used to group the samples and, thus, reveal the main characteristics associated with their physicochemical properties, bioactive composition, and multi-element profile. The protein content in the samples was not significantly affected by the addition of soy extract. There was a gradual increase in the lipid content as the concentration of soy extract increased. Furthermore, with the increase in the percentage of soy extract in the dairy compounds, there was an increase in the levels of total polyphenols, total flavonols, and antioxidant activity, and of some minerals, such as Fe, Mn, P, Cu, and Mg. The DC49 sample showed the highest values for total polyphenols (178.65 mg/100g) and total flavonols (1.51 mg/100g). The addition of soy extract promoted the enrichment of important minerals in the samples, with an increase of up to 55 times in the Fe content and up to 40 times in the Mn content. Physical properties (density and fluidity) and rehydration properties (wetting, dispersibility, and solubility) also were affected as the percentage of soy extract in the samples increased. Note that even when you hear an addition of up to 20% soy extract, the samples are still wettable. All dairy compounds showed solubility above 69%. The use of soy extract in the polyphenolic and mineral enrichment of the dairy compounds is important to add nutritional value to powdered milk, we emphasize that this product has enormous potential to be used in diets that require mineral supplementation.
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