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TESE

Estudo das Propriedades Físicas e Tecnológicas de Farelos de Sorgo (*Sorghum bicolor (*L.) Moench) e Milheto Pérola (*Pennisetum glaucum* (L.) R. Br.) e Uso da Extrusão Termoplástica Como Pré-Tratamento Para Extração Supercrítica de Compostos Bioativos

André Leonardo dos Santos

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ESTUDO DAS PROPRIEDADES FÍSICAS E TECNOLÓGICAS DE FARELOS DE SORGO (S*orghum bicolor* (L.) Moench) E MILHETO PÉROLA (*Pennisetum glaucum* (L.) R. BR.) E USO DA EXTRUSÃO TERMOPLÁSTICA COMO PRÉ-TRATAMENTO PARA EXTRAÇÃO SUPERCRÍTICA DE COMPOSTOS BIOATIVOS

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Tese submetida como requisito parcial para obtenção do grau de **Doutor em Ciência e Tecnologia de Alimentos**, no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração em Tecnologia de Alimentos.

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RESUMO GERAL

SANTOS, André Leonardo dos**.** 2024. **Estudo das propriedades físicas e tecnológicas de farelos de Sorgo (S*orghum bicolor* (L.) Moench) e Milheto pérola (*Pennisetum glaucum* (L.) R. Br.) e uso da extrusão termoplástica como pré-tratamento para extração supercrítica de compostos bioativos.** Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2024.

Os compostos bioativos são encontrados em cereais e têm despertado o interesse das indústrias de alimentos e farmacêuticas. Esses compostos em sua maioria estão presentes nos grãos integrais e, em maior número, na fração do farelo (pericarpo). A espécie de milheto mais cultivada no mundo é *a Pennisetum glaucum* (L.) R. Br., sendo o sexto cereal mais produzido. Os grãos de milheto têm excelente qualidade nutricional e são comparáveis ou superiores à alguns cereais frequentemente consumidos, como arroz e trigo. O Sorgo (*Sorghum bicolor* (L.) Moench) está entre os cinco cereais mais importante do mundo, sendo uma fonte de compostos fitoquímicos, como fenóis, antocianinas, taninos e flavonoides. A extração desses compostos deve ser feita com o máximo de eficiência, sendo necessárias técnicas avançadas de pré-tratamento e extração, preferencialmente tecnologias verdes. A extrusão termoplástica (ET) é um processo versátil utilizado como pré-tratamento para liberação de compostos de interesse. Já a extração com fluido supercrítico (EFS) é utilizada para extração de composto bioativos de diferentes fontes. A literatura não reporta estudos em que a extrusão termoplástica seja utilizada como pré-tratamento para EFS em farelos de sorgo e milheto pérola. Essas duas técnicas foram utilizadas nesse estudo com objetivo de avaliar o processo de pré-tratamento e a extração supercrítica na obtenção de compostos bioativos a partir dos farelos de milheto pérola e sorgo para que as frações obtidas sejam concentradas em compostos bioativos de interesse. O brunimento influenciou o teor de amido e apresentou as maiores porcentagens de farelo (15,5%) e de taxa de extração (30,7%). A maior viscosidade final foi em farelo de milheto 82,9 cP. A recuperação do farelo foi positivamente correlacionada com o amido total (0,96). A análise de componentes principais (ACP) mostrou que os farelos foram associados a amido total. A análise HCPC formou dois grupos principais: MB25 e MB05-MB10 como cluster 1, e SB10 e SB05-SB07 como cluster 2. O maior teor de compostos fenólicos totais (TPC) foi de 1240,94 μg GAE / 100 g em farelo de milheto perola extrudado (MEB), e a maior capacidade antioxidante (AC) foi de 55,51 μmol TE / g em farelo de sorgo extrudado (SEB). A correlação de Pearson indicou fortes relações positivas entre TPC, AC-ABTS e SV. As amostras extrudadas apresentaram maior homogeneidade e densidade aparente (29,72%). A análise colorimétrica revelou um ΔE^* de 8,49 para EMB e 9,78 para ESB. SFE mostrou que a maior concentração de TPC foi de 21,34 mg GAE/g de extrato, obtido a 40 °C 10 MPa e 15% de cossolvente. EMB apresentou o maior AC, em ABTS foi de 4,47 μM TE/g de extrato e em FRAP foi de 157,92 μM TE/g de extrato, obtido a 50 °C, 10 MPa e 15% de cossolvente e 50 °C, 20 MPa e 0,5% de cossolvente, respectivamente. As variáveis temperatura (T), pressão (P) e porcentagem de cossolvente (C%) influenciaram diretamente o TPC e a atividade antioxidante (CA-ABTS/FRAP). No estudo cinético, o maior rendimento foi de 20 % para ESB, e a fração de extrato recuperada na etapa de CER foi maior em MB (79,71%), com maior recuperação total na amostra extrudada. Extratos de farelo de sorgo e milheto pérola, ricos em compostos bioativos, apresentaram rendimentos comparáveis ou superiores aos de outros cereais, destacando as tecnologias TE e SFE como alternativas sustentáveis e eficazes para extração de bioativos.

Palavras-chave: Extrusão termoplástica, fluido supercrítico, cereal, extração, compostos bioativos.

ABSTRACT

SANTOS, André Leonardo dos. **Study of physical and technological properties of Sorghum (*Sorghum bicolor* (L.) Moench) and Pearl millet (*Pennisetum glaucum* (L.) R. Br.) Bran and use of thermoplastic extrusion as pretreatment for supercritical extraction of bioactive compounds**. Thesis (Doctorate in Food Science and Technology). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2024

The bioactive compounds found in cereal have attracted the interest of the food and pharmaceutical industries. These compounds are mostly present in whole grains and, particularly, in the bran fraction (pericarp). Pearl millet and sorghum are grains with bioactive potential. The most cultivated species of millet in the world is *Pennisetum glaucum* (L.) R. Br., being the sixth most produced cereal. Millet grains have excellent nutritional quality and are comparable or superior to some frequently consumed cereals, such as rice and wheat. Sorghum (*Sorghum bicolor* (L.) Moench) is among the five most important cereals in the world, being a source of phytochemical compounds, such as phenols, anthocyanins, tannins, flavonoids and antioxidants. The extraction of these compounds must be done with maximum efficiency, requiring advanced pretreatment and extraction techniques, preferably using green solvents. Thermoplastic extrusion (TE) is a versatile process used as pretreatment for the release of compounds of interest. Supercritical fluid extraction (SFE) is used to extract bioactive compounds from different sources. The literature does not report studies in which thermoplastic extrusion is used as pretreatment for SFE in sorghum and pearl millet brans. These two techniques were used in this study to evaluate the pretreatment process and supercritical extraction in obtaining bioactive compounds from pearl millet and sorghum brans so that the obtained fractions are concentrated in bioactive compounds of interest. The pearl millet grains debranning for 25 minutes showed the highest bran percentages (15.5%) and (30.7%). The branning time influenced the starch content. The highest final viscosity was millet bran (MB) 80.0 cP. Bran recovery was positively correlated with total starch (0.96). Principal component analysis (PCA) showed that MB25 was associated with total starch. HCPC analysis formed two main groups: MB25 and MB05-MB10 as cluster 1, and SB10 and SB05-SB07 as cluster 2. The highest total phenolic compounds (TPC) content was 1240.94 μg GAE / 100 g in extruded pearl millet bran (MEB), and the highest antioxidant capacity (AC) was 55.51 μmol TE / g in extruded sorghum bran (SEB). Pearson correlation indicated strong positive relationships between TPC, AC-ABTS and SV. The extruded samples showed higher homogeneity and apparent density (29.72 %), but lower porosity. Colorimetric analysis revealed a ΔE^* of 8.49 for EMB and 9.78 for ESB. SFE showed that the highest TPC concentration was 21.34 mg GAE / g extract, obtained at 40 °C 10 MPa and 15% co-solvent. EMB showed the highest AC, in ABTS it was 4.47 μM TE / g of extract and in FRAP it was 157.92 μM TE / g of extract, obtained at 50 °C, 10 MPa and 15% co-solvent and 50 °C, 20 MPa and 0.5% co-solvent, respectively. The variables temperature (T), pressure (P) and percentage of co-solvent (C%) directly influenced the TPC and antioxidant activity (CA-ABTS/FRAP). In the kinetic study, the highest yield was 20 % for ESB, and the fraction of extract recovered in the CER step was higher in MB (79.71 %), with higher total recovery in the extruded sample. Extracts of sorghum and millet bran, rich in bioactive compounds, showed yields comparable to or higher than those of other cereals, highlighting TE and SFE technologies as sustainable and effective alternatives for bioactive extraction.

Keywords: Thermoplastic extrusion, supercritical fluid, cereal, extraction, bioactive compounds.

SUMÁRIO

INTRODUÇÃO

Os cereais são alimentos básicos, amplamente cultivados em todo o mundo. Eles fornecem nutrição e vários benefícios à saúde humana e de animais (OFOSU *et al.*, 2021). Dentre os grãos com potencial bioativo podemos citar o sorgo [*Sorghum bicolor* (L.) Moench] e milheto pérola [*Pennisetum glaucum* (L.) R. Br.].

O sorgo (*Sorghum bicolor* (L.) Moench) está entre os cinco cereais mais importantes do mundo (TYMCHAK; KUIANOV, 2018). Ele não contém glúten, cresce em ambientes adversos e é considerado, assim como o milheto pérola, uma planta resistente às adversidades causadas pelas mudanças climáticas, podendo ser utilizado para alimentação de humanos e animais (LEMLIOGLU-AUSTIN, 2014; OSEGUERA-TOLEDO *et al.*, 2020). O valor nutricional e a composição centesimal dos grãos de sorgo são semelhantes aos do milho, do arroz e do trigo, seus principais componentes são polissacarídeos, seguidos por proteínas e lipídeos, sendo também fonte de compostos fitoquímicos, como fenóis, antocianinas, taninos, flavonoides e antioxidantes (AROUNA; GABRIELE; PUCCI, 2020; DYKES *et al.*, 2011; SVENSSON *et al.*, 2010).

Milheto é o nome genérico dado a grãos de cereais de tamanho reduzidos. Em países como a Índia, o sorgo também é considerado uma das espécies de milhetos. No entanto, nos EUA e no Brasil, por exemplo, o sorgo não é conhecido como uma das diversas espécies de milheto. Das espécies botânicas de milheto, a *Pennisetum glaucum* (L.) R. Br., é a mais plantada no mundo, sendo o sexto cereal mais produzido, após milho, arroz, trigo e sorgo, com uma produção de 27,8 milhões de toneladas, da qual 96% de sua produção está compreendida em ambientes tropicais semiáridos da Ásia e África (FAOSTAT, 2023). Os grãos de milheto pérola têm excelente qualidade nutricional comparável ou mesmo superior a alguns cereais frequentemente consumidos, como o arroz e o trigo (DIAS-MARTINS *et al.*, 2019; SRIVASTAVA; SAINI; SINGH, 2020). Também é fonte de proteínas, compostos fenólicos, flavonoides e apresenta alta capacidade antioxidante (AGRAWAL; JOSHI; GUPTA, 2016; MUTSHINYANI; MASHAU; JIDEANI, 2020; SLAMA *et al.*, 2020).

As partes anatômicas mais importantes de um grão de cereal são endosperma, farelo e germe. O farelo representa cerca de 14–19% do peso do grão, sendo uma fonte rica em vitaminas, minerais, fitoquímicos e fibra alimentar, particularmente polissacarídeos não amiláceos, como celulose, arabinoxilanos e arabinogalactanos (AGIL; HOSSEINIAN, 2014; PASHA; AHMAD, 2021).

Os bioativos em sua maioria estão presentes nos grãos integrais, na fração do farelo e compreendem uma ampla gama de antioxidantes naturais. Técnicas de extração "verde" têm sido utilizadas para extrair bioativos em grãos (AQUINO-BOLAÑOS *et al.*, 2021; AROUNA; GABRIELE; PUCCI, 2020; NOGALA-KAŁUCKA *et al.*, 2020; SLAMA *et al.*, 2020). Os compostos bioativos presentes na fração farelo têm motivado pesquisas para o estudo e aprimoramento de técnicas de extração, concentração, purificação e aplicação (AFRIN; SIDHU, 2021; XIONG *et al.*, 2022).

A extração de compostos bioativos é de interesse para produção de alimentos funcionais, produtos farmacêuticos e suplementos dietéticos. Para isso, a extração desses compostos deve ser feita com o máximo de eficiência, sendo necessárias técnicas avançadas de pré-tratamento e extração, preferencialmente com a utilização de solventes verdes. As técnicas baseadas no uso de fluidos supercríticos e extrusão termoplástica claramente se enquadram nessa categoria (HERRERO; IBAÑEZ, 2018; VIGANÓ *et al.*, 2016).

A extração com fluido supercrítico (EFS) é uma tecnologia considerada "verde" com aplicações potenciais para a indústria alimentícia e tem sido amplamente utilizada para extração de compostos bioativos em diversas matérias-primas (PIMENTEL-MORAL *et al.*, 2019; TEJEDOR-CALVO *et al.*, 2021). A extrusão termoplástica, por sua vez, é um processo versátil que tem sido associada à melhor retenção de compostos fenólicos e atividade antioxidante, quando comparada ao cozimento convencional (CAMELO-MÉNDEZ; TOVAR; BELLO-PÉREZ, 2018).

Nesse contexto, o objetivo deste trabalho foi estudar o efeito da extrusão termoplástica (ET) como pré-tratamento para extração supercrítica (EFS) em farelos de sorgo e milheto pérola, para isso, os farelos extrudado e não extrudados foram avaliados quanto as suas propriedades, físicas, físico-químicas, tecnológicas, colorimétricas e de pasta. Os extratos obtidos por EFS foram avaliados quanto a seu teor fenólico e capacidade antioxidante. Foi ainda realizado um estudo cinético para otimização da EFS.

Este estudo teve por objetivo inicial realizar a revisão bibliográfica, como introdução geral, abordando aspectos teóricos relacionados aos grãos e farelos de sorgo e milheto, composição, processamento por extrusão termoplástica e extração com fluido supercrítico. Que serviram de base para os capítulos I ao III que foram organizados na forma de artigo (Capítulo

I – Influencia do brunimento no teor de amido e nas propriedades de pasta de sorgo (*Sorghum bicolor* (L.) Moench*)* e milheto pérola (*Pennisetum glaucum* (L.) R. Br.).; Capítulo II – Efeito da extrusão termoplástica nos composto fenólicos, capacidade antioxidante e propriedades físicas de sorgo (*Sorghum bicolor* (L.) Moench*)*, milheto pérola (*Pennisetum glaucum* (L.) R. Br.).; Capítulo III – Thermoplastic extrusion as a pre-treatment for supercritical extraction of bioactive compounds in sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum glaucum* (L.) R. Br.) bran. Por fim, foram descritas as considerações finais deste estudo.

JUSTIFICATIVA

O aproveitamento integral dos grãos de sorgo (*Sorghum bicolor* (L.) Moench) e de milheto pérola (*Pennisetum glaucum* (L.) R. Br.) é fundamentado no valor nutricional desses grãos e no potencial quase inexplorado de suas frações de farelo. Tanto o sorgo quanto o milheto pérola são cereais de grande importância econômica e cultural, principalmente em regiões da Ásia e da África, onde são cultivados para alimentação humana.

Milheto pérola e o sorgo estão entre os seis cereais mais produzidos no mundo, com produções anuais de 30,89 milhões e 61,36 milhões de toneladas, respectivamente (FAOSTAT, 2023). No Brasil, essas culturas ocupam áreas significativas, com o milheto cobrindo mais de 4 milhões de hectares e o sorgo cerca de pouco mais de 1 milhão de hectares (DIAS-MARTINS et al., 2018a; FAOSTAT, 2023).

Os grãos de milheto e sorgo possuem três camadas principais: pericarpo, endosperma e gérmen, o endosperma, maior porção do grão, corresponde a 75–80% do peso total, o germe compreende a maioria dos lipídios, minerais, além de alguns fenólicos, incluindo fatores antinutricionais, e o pericarpo destaca-se por sua riqueza em fibras, vitaminas, minerais e fitoquímicos (HAMA *et al.*, 2011; SRUTHI; RAO; RAO, 2021).

O processo de brunimento ou decorticação tem como objetivo a valorização dos grãos, melhorar a palatabilidade e o valor biológico para o consumo humano. Contudo, cerca de 35 % do peso total dos grãos é removido neste processo. O produto do brunimento ou decorticação dos grãos de cereais é o farelo, que representa aproximadamente 14-19% do peso do grão, sendo frequentemente descartado ou destinado à alimentação animal, mesmo sendo uma rica fonte de compostos antioxidantes e fibra alimentar (DHARMARAJ; RAVI; MALLESHI, 2014).

Técnicas avançadas, como a extrusão termoplástica e a extração por fluido supercrítico, são promissoras na recuperação desses compostos bioativos presentes nesse farelo.

Extrusão termoplástica permite modificar amidos e proteínas, aumentando a disponibilidade de compostos fenólicos em farelos extrudados (WU et al., 2020; CAMPELO et al., 2020). Já a SFE utiliza dióxido de carbono supercrítico (SC-CO_2) como solvente para extrair compostos de difícil acesso e pode ser potencializada com o uso de cossolventes como o etanol, ampliando a extração de compostos polares (PEREIRA; MEIRELES, 2010; FRATERRIGO GAROFALO et al., 2020).

Embora o uso combinado de ET e EFS em farelos de sorgo e milheto ainda não tenha sido explorado, estudos com outras matrizes indicam que essa abordagem pode aumentar a eficiência e sustentabilidade da extração de compostos bioativos melhorando as características nutricionais das farinhas destes cereais. Tais resultados alinham-se aos Objetivos de Desenvolvimento Sustentável (ODS) da ONU, que promovem práticas de produção sustentáveis (SDG 12).

OBJETIVOS

O objetivo principal desse estudo foi avaliar o processo de extrusão termoplástica de farelos de sorgo e de milheto pérola como pré-tratamento na extração com fluido supercrítico de compostos bioativos, bem como avaliar os efeitos da extração com fluido supercrítico nas características químicas dos extratos.

1 REVISÃO DE LITERATURA

1.1 Sorgo e milheto: aproveitamento do farelo como fonte de bioativos

O mercado de compostos bioativos tem motivado pesquisas em todo o mundo para o desenvolvimento de técnicas de obtenção, concentração, purificação e fornecimento de produtos que possam veicular esses compostos. Em particular, a extração de compostos bioativos é de grande interesse para alimentos funcionais e produtos fármacos, bem como para a produção de suplementos dietéticos. Dos alimentos funcionais que contêm alegações de promoção de saúde e bem-estar atualmente comercializados em todo o mundo, peptídeos, polifenóis, carotenoides, ácidos graxos poli-insaturados ou esteróis e o estanol são os compostos de maior interesse. As matérias-primas mais comuns são plantas, fungos e organismos marinhos, incluindo microalgas e algas marinhas, mas também subprodutos alimentares e agroalimentares (HERRERO; IBAÑEZ, 2018; VIGANÓ *et al.*, 2016).

Os compostos bioativos encontrados em grãos de cereais compreendem uma ampla gama de antioxidantes naturais hidrofílicos e lipofílicos. Esses compostos em sua maioria estão presentes nos grãos integrais e na fração farelo. Dentre os grãos de cereais com potencial bioativo, o sorgo [*Sorghum bicolor* (L.) Moench] e o milheto pérola [*Pennisetum glaucum* (L.) R. Br.] se destacam (AROUNA; GABRIELE; PUCCI, 2020; CARBAS *et al.*, 2020; NOGALA-KAŁUCKA *et al.*, 2020; SLAMA *et al.*, 2020).

A literatura reporta o sorgo e milheto pérola como potenciais fontes de compostos bioativos. Os compostos bioativos de interesse presentes nestes cereais estão dispostos na Tabela 1. O conteúdo de carotenoides totais em sorgo 0,66 mg/100 g e milheto pérola 0,52 mg/100 g são valores próximos ou bem superiores ao encontrado em outros grãos ou sementes, como chia 45,9 µg/100 g, amaranto 22,0 µg/100 g, quinoa branca 109,1 µg / 100 g e arroz selvagem 212,3 µg/100 g (NIRO *et al.*, 2019). Os carotenoides servem como precursores para a síntese de vitamina A e são essenciais para o funcionamento normal da retina do olho (KOTÍKOVÁ *et al.*, 2016).

Quanto à capacidade antioxidante, o sorgo apresenta valores mais expressivos tanto nos ensaios com ABTS 95,77 % quanto com DPPH 86,43 % em relação ao milheto pérola (MAWOUMA *et al.*, 2022a). Quanto ao conteúdo total de fenólicos, flavonoides e fibras, observa-se que o sorgo possui valores superiores aos encontrados em milheto, sendo 173,68 169,85 mg GAE / g para fenólicos, 27,40 e 9,14 mg CE/g para flavonoides, 35,20 e 7,15 % para fibras alimentar e 7,17 e 3,72 para fibra bruta, respectivamente. Esses compostos desempenham um papel importante na redução do risco da incidência de doenças como o câncer (OBADINA *et al.*, 2016).

Tabela 1: Compostos bioativos e capacidade antioxidante (CA) em sorgo e milheto pérola

composto bioativo	**Método de análise**	**Teor médio encontrado**	**Referência**
	Sorgo (*Sorghum bicolor* L.)		
Carotenoides (mg / 100 g)	Totais	0,66	(MAWOUMA *et al.*, 2022a)
Capacidade antioxidante Inibição (%)	ABTS DPPH	95,77 86.43	(MAWOUMA *et al.*, 2022a)
Flavonoides (mg CE /g)	Flavonoides totais	27,40	(ABDELHALIM *et al.*, 2021)
Compostos fenólicos (mg GAE /g)	Fenólicos totais	173,68	(PUNIA *et al.*, 2021)
Fibra (%)	Alimentar Bruta	35,20 7,17	(SRUTHI; RAO; RAO, 2021)
	Milheto pérola (*Pennisetum glaucum* (L.) R. Br)		
Carotenoides (mg / 100 g)	Totais	0,52	(MAWOUMA *et al.*, 2022a)
Capacidade antioxidante	ABTS DPPH	89,24 73,27	(MAWOUMA *et al.*, 2022a)
Flavonoides (mg CE / g extrato)	Flavonoides totais	9,73	(EL KOURCHI *et al.*, 2024)
Compostos fenólicos (mg GAE / 100g)	Fenólicos totais	169,85	(OBADINA *et al.*, 2016)
Fibra (g / 100 g)	Alimentar Bruta	7,15 3,72	(DIAS-MARTINS *et al.*, 2024; MAWOUMA *et al.*, 2022a)

1.2 Sorgo (*Sorghum bicolor* (L.) Moench)

O Sorgo é o quinto cereal mais importante do mundo, com uma produção 61,36 milhões de toneladas, onde EUA, Nigéria, Etiópia e México foram os maiores produtores em 2020 (FAOSTAT, 2023). Esse cereal não contém glúten, cresce em ambientes adversos e resiste às mudanças climáticas, pois resistem a altas temperaturas e escassez de água (LEMLIOGLU-AUSTIN, 2014). Os grãos permanecem dormentes na seca, mas diferentes enzimas que participam do processo de germinação são ativadas quando o grão é embebido, o sorgo possui um perfil nutricional equilibrado pode ser considerado um cereal “inteligente para o clima” (RUMLER *et al.*, 2021). O sorgo é utilizado para alimentação de humanos e animais (OSEGUERA-TOLEDO *et al.*, 2020). Para o consumo humano, é preferencialmente utilizado na forma de farinha devido a dureza do grão. Dessa forma, antes de ser consumido, o sorgo precisa ser processado ou submetido a diferentes técnicas ou preparações culinárias (PINHEIRO *et al.*, 2021).

A introdução deste cereal no Brasil aconteceu no início do século XX, sendo um dos poucos lugares no mundo onde se aumentou consistentemente a área plantada nas últimas décadas (DUARTE; MIRANDA, 2015). Segundo a FAO a produção brasileira de sorgo em 2020 foi estimada em 2,5 milhões de toneladas com produtividade de 2.756 kg/ha e uma área plantada de 888,534 mil hectares (CONAB, 2018; FAOSTAT, 2023). Esta produção é destinada principalmente para alimentação animal, na forma de grãos e silagem (massa verde) de alta qualidade em grandes quantidades (DO NASCIMENTO *et al.*, 2020).

O valor nutricional e a composição química dos grãos de sorgo são semelhantes aos do milho, arroz e trigo. Seus principais componentes são polissacarídeos, seguidos por proteínas e lipídeos. No entanto, entre os cereais, o sorgo tem a menor digestibilidade de amido e proteína, particularmente quando o sorgo possui taninos que se associam ao amido e às proteínas do cereal (AROUNA; GABRIELE; PUCCI, 2020). No entanto, no Brasil os genótipos de sorgo granífero plantados apresentam majoritariamente níveis baixos ou mesmo isentos de tanino, uma vez que são destinados preferencialmente à alimentação animal, já que o tanino é conhecido por reduzir ainda mais o nível de digestibilidade proteica, indesejável na produção animal (VARGAS-SOLÓRZANO *et al.,* 2014).

Nos últimos anos, o interesse pelo consumo de sorgo em humanos tem aumentado nos países desenvolvidos, devido à demanda por alimentos funcionais como fonte de compostos bioativos e livres de glúten (DE MORAIS CARDOSO et al., 2017). Além das propriedades nutricionais, o sorgo é uma fonte de compostos fitoquímicos, como fenóis, ácidos hidroxibenzóicos, ácidos hidroxicinâmicos,taninos, flavonóides e antioxidantes (Tabela 1) (PRZYBYLSKA-BALCEREK; FRANKOWSKI; STUPER-SZABLEWSKA, 2019). Esses compostos estão concentrados principalmente nas camadas externas do grão, o pericarpo ou no farelo, produzido a partir dessas camadas, (DYKES *et al.*, 2011; SVENSSON *et al.*, 2010). Embora o farelo de sorgo seja considerado subproduto da indústria, este possui alto valor e grande potencial comercial devido ao seu rico conteúdo fenólico, que é até seis vezes maiores que os do grão integral (AWIKA; MCDONOUGH; ROONEY, 2005; XIONG *et al.*, 2019). O farelo de sorgo também é uma fonte rica de outros ingredientes funcionais, como vitaminas e fibras, sendo utilizado para produção de pães suplementados e produtos cárneos (AFRIN; SIDHU, 2021; XIONG *et al.*, 2022).

1.3 Milheto Pérola (*Pennisetum glaucum* (L.) R. Br.)

Outros cereais de interesse como fonte de bioativos são os milhetos. O termo milheto é nome dado de forma genérica a grãos de cereais de tamanho reduzido, menor que o grão de sorgo, sendo que a maioria dos milhetos são nativos do continente africano, assim como o sorgo, que na Índia é tratado como um dos milhetos. A espécie de milheto mais cultivada no mundo é o milheto pérola *Pennisetum glaucum* (L.) R. Br., sendo o sexto cereal mais produzido, após milho, arroz, trigo e sorgo, com 96% da produção (27,8 milhões de toneladas) em ambientes tropicais semi-áridos da Ásia e África. O milheto pérola é um grão importante e nutritivo na Índia e na África Ocidental, onde é geralmente cultivado principalmente em solos pobres e condições climáticas adversas (SHIVHARE *et al.*, 2020).

No Brasil, por prevalecer o plantio da espécie *Pennisetum glaucum* (L.) R. Br., a qual é conhecida em inglês por *pearl millet*, e traduzido por milheto pérola, tem-se adotado o termo genérico milheto, porém limitando o termo milheto a outras espécies que aqui no Brasil poderiam ser exploradas na alimentação humana. Assim, para simplificar sua descrição no texto, este termo genérico será adotado. Para denominação de espécies de milheto será usado a palavra milheto combinada com outro termo que o identifique do milheto pérola. A utilidade do grão de milheto vai desde ingrediente para fabricação de rações e alimentos, forragem, material de construção, até a fabricação de cerveja e biocombustível, tornando-o uma espécie de cultivo importante para a segurança alimentar e de subsistência de mais de 500 milhões de pessoas pobres e nutricionalmente inseguras em todo o mundo(BOLLAM *et al.*, 2018; LI *et al.*, 2020b). Produtos de farinha de milheto, como muffins, pães e macarrão são amplamente populares em algumas regiões do mundo devido à sua boa capacidade de armazenamento, alta nutrição e propriedades hipoglicêmicas (LI *et al.*, 2020b).

Nos países em desenvolvimento, o processamento comercial desses grãos cultivados localmente em alimentos e bebidas de valor agregado é um importante motor para o desenvolvimento econômico (TAYLOR, 2004). O uso do milheto não só fornece aos agricultores um mercado para seus produtos, mas também economiza divisas, que de outra forma seriam necessárias para importar outros cereais. Particularmente nos países desenvolvidos, há hoje uma demanda crescente por alimentos e bebidas sem glúten, isso porque pessoas com doença celíaca e outras intolerâncias ao trigo que não podem consumir produtos de trigo, cevada ou centeio (TAYLOR; SCHOBER; BEAN, 2006).

Os primeiros relatos do plantio de milheto no Brasil são de 1929, sendo cultivado nas regiões Sul, Sudeste e Centro-Oeste do país. Não existem dados oficiais de produção no Brasil, porém a área estimada de plantio é de 5 milhões de ha (DIAS-MARTINS *et al.*, 2018a), área somente menor que a do milho e a soja. O milheto assume um papel importante no agronegócio brasileiro, sendo utilizado para produção de grãos para alimentação animal e de cobertura para o plantio direto (PEREIRA FILHO, 2016).

Os grãos de milheto têm excelente qualidade nutricional que são comparáveis ou superiores a alguns cereais frequentemente consumidos, como arroz e trigo (DIAS-MARTINS *et al.*, 2018a; SRIVASTAVA; SAINI; SINGH, 2020). Por serem naturalmente isentos de glúten, os grãos de milheto possuem esta vantagem, considerando que a ingestão da proteína do glúten pode promover diversos distúrbios metabólicos em certos indivíduos, causando alergias, intolerâncias, doenças autoimunes e permeabilidade intestinal (HOLLON *et al.*, 2015).

O milheto também é fonte de proteínas, compostos fenólicos, ácidos fenólicos, flavonoides e seus compostos apresentam alta capacidade antioxidante como observado na Tabela 1 (AGRAWAL; JOSHI; GUPTA, 2016; MUTSHINYANI; MASHAU; JIDEANI, 2020; SLAMA *et al.*, 2020). No entanto, o farelo e frações ricas em farelo são geralmente descartados durante os processos de descascamento, branqueamento, moagem e polimento com finalidade de melhorar os atributos sensoriais dos grãos (VAN DER KAMP *et al.*, 2014). O farelo contém uma grande variedade de compostos benéficos à saúde, incluindo compostos fenólicos, flavonoides e atividade antioxidante. Consequentemente, o farelo é uma fonte promissora para aplicações em processamento de alimentos ou produtos nutracêuticos (ZHENG *et al.*, 2022).

1.4 Extração com fluido supercrítico (EFS)

O estado supercrítico de um fluido pode ser definido como um estado em que tanto líquidos quanto gases são indistinguíveis um do outro (ANUKIRUTHIKA *et al.*, 2019). Dessa forma, os fluidos supercríticos (FS's) apresentam propriedades intermediárias entre um líquido e um gás. A viscosidade e a difusividade desses fluídos são semelhantes à de um gás, já a densidade se aproxima à de um líquido. Como consequência dessas propriedades físicas, os fluídos supercríticos atuam como excelentes solventes, uma vez que possuem alta capacidade de penetrar dentro de uma matriz sólida e solubilizar o soluto mesmo quando ele está fortemente ligado à parede celular (L. ZABOT; N. MORAES; ANGELA A. MEIRELES, 2014). As propriedades desses fluídos podem ser alteradas por meio da manipulação da temperatura e pressão, sendo possível modular a sua densidade e consequentemente o poder de solvatação e a seletividade dos fluidos supercríticos (FRATERRIGO GAROFALO; TOMMASI; FINO, 2020). Na Tabela 2 são apresentadas as propriedades de densidade, difusividade e viscosidade para gás, líquido e fluido supercrítico.

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Tabela 2: Valores de faixa das propriedades físico-químicas de gases, líquidos e fluidos supercríticos (HERRERO; CIFUENTES; IBAÑEZ, 2006): Adaptado

Estado de fluido	Densidade (ρ, g / cm ^{3})	Difusividade (D, cm ^{2} / s)	Viscosidade (μ, g s / cm)
Gás			
p= 1 atm; T = 21 ° C	10^{-3}	10^{-1}	10^{-5}
Líquido			
p = 1 atm; T = 15-30 ° C	1	$<10^{-5}$	10^{-3}
Supercrítico			
$p = p_c$; $T = T_c$	0,3 – 0,8	$10^{-3} – 10^{-4}$	$10^{-5} – 10^{-4}$

Devido a essas características os fluidos supercríticos podem ter várias aplicações, dentre elas em processos de extração (KNEZ *et al.*, 2019). Uma quantidade substancial de pesquisas sobre o uso dos FS como solvente alternativo para extrações de solutos tem sido realizada nas últimas décadas. Existem relatos sobre o uso de vários solventes, como propano, butano, hexano, etanol, metanol e água para extração em condições supercríticas (MANJARE; DHINGRA, 2019). Contudo, o uso de CO_2 como solvente tem recebido destaque na obtenção de extratos (Tabela 3)

Tabela 3: Propriedades físico-químicas de alguns fluidos supercríticos (HERRERO; CIFUENTES; IBAÑEZ, 2006).

	Propriedade Crítica			
Solvente	**Temperatura (°C)**	**Pressão (atm)**	**Densidade ρ SCF (g / mL)**	**Solubilidade σ (cal $^{-1/2}$ cm $^{-3/2}$)**
Dióxido de carbono	31,2	72,9	0,470	7,5
Etano	32,4	48,2	0,200	5,8
Eteno	10,1	50,5	0,200	5,8
Metanol	-34,4	79,9	0,272	8,9
Óxido nitroso	36,7	71,7	0,460	7,2
n-buteno	-139,9	36,0	0,221	5,2
n-pentano	-76,5	33,3	0,237	5,1
Hexafluoreto de enxofre	45,8	37,7	0,730	5,5
Água	101,1	217,6	0,322	13,5

O dióxido de carbono supercrítico (SC-CO2) é o fluido supercrítico mais utilizado devido à sua baixa temperatura e pressão críticas (31 ºC e 7,38 MPa, respectivamente), bem como por sua não inflamabilidade e não toxicidade. Além disso, é considerado um solvente de grau alimentício (rotulado como GRAS - Geralmente Reconhecido como seguro) e de baixo custo (TIRADO; LATINI; CALVO, 2021). Outra vantagem importante que aumenta o interesse pelo uso desse composto é que o CO_2 é um gás em condições ambiente. Na prática isso significa que após o processo de extração, quando a pressão é aliviada, o CO_2 é evaporado automaticamente deixando um extrato perfeitamente livre de solvente (MENDIOLA *et al.*, 2014). Algumas vantagens e desvantagens da extração usando CO_2 supercrítico como solvente são apresentadas na Tabela 4.

Tabela 4: Vantagens e desvantagens do processo de extração supercrítica (CVJETKO BUBALO et al., 2018).

Vantagens	Desvantagens
• Tratamento suave de materiais sensíveis ao calor (sua temperatura crítica moderada de 31,2 °C importante para a preservação de compostos bioativos em extratos). • Produtos sem solventes • O CO_2 não causa problemas ambientais e é fisiologicamente inofensivo, germicida e não inflamável. • CO_2 é um solvente geralmente reconhecido como seguro (GRAS). • CO_2 é um solvente barato • Devido à baixa viscosidade e à difusividade relativamente alta, o CO_2 supercrítico tem propriedades de transporte mais aprimoradas do que os líquidos, podendo se difundir facilmente através de materiais sólidos e, portanto, fornecer taxas de extração mais rápidas. • Fragrâncias e aromas podem permanecem inalterados. • Extração seletiva e separação fracionada. • Extratos puros por meio de algumas etapas do processo. • Poder de solvatação ajustável (possibilidade de modificar a densidade do fluido alterando sua pressão e/ou temperatura). • Alta solubilidade para substâncias não polares/baixas (por exemplo, compostos voláteis). • Possibilidade de acoplamento direto com técnicas analíticas cromatográficas, como cromatografia gasosa (CG) ou cromatografia de fluido supercrítico (CFS).	• Alto custo de investimento (requer um plano de negócios cuidadoso contemplando a análise custo/efetiva dos compostos desejados a serem extraídos). O uso de altas pressões acarreta custos de capital para a planta e os custos operacionais também podem ser altos, de modo que o número de processos comerciais que utilizam extração de fluido supercrítico ainda é relativamente pequeno. • Pode ser necessário um estudo do equilíbrio de fase do sistema solvente/soluto, o que pode dificultar as condições de extração do processo. • Substâncias altamente polares (açúcares, aminoácidos, proteínas) não são solubilizadas em SC-CO_2 necessitando de cossolventes para atuarem como modificadores de polaridade.

O SC-CO_2 tem uma característica não polar, o que limita sua utilização para extração de componentes polares. Contudo, esses constituintes polares ou de massa molecular elevada também podem ser extraídos por meio da adição de um modificador de polaridade ou cossolvente. Os cossolventes convencionais incluem pequenos álcoois como o etanol, acetato de etila, acetona, hexano, ácido fórmico e água. A adição de cossolvente torna mais fácil separar os compostos solúveis dos locais de adsorção da matriz ativa (JAFARIAN ASL; NIAZMAND; JAHANI, 2020).

Segundo o levantamento feito por De Melo et al., (2014), etanol, metanol, água e o diclorometano, são os principais cossolventes utilizados para auxiliar na extração supercrítica (ESF). O uso de cossolventes como modificadores de polaridade melhora o desempenho do processo de extração supercrítica (MANJARE; DHINGRA, 2019).

Outras variáveis, como temperatura, pressão e fluxo do solvente, desempenham papéis igualmente importantes no processo de extração com fluido supercrítico (EFS). Esse tipo de extração envolve dois processos distintos e incompatíveis relacionados à temperatura. Quando a temperatura aumenta sob pressão constante, a densidade do solvente diminui, o que pode impactar negativamente a recuperação dos compostos-alvo. Por outro lado, o aumento da pressão de vapor do soluto, especialmente no caso de compostos voláteis, em temperaturas mais elevadas pode compensar a redução da densidade do solvente, resultando em uma maior recuperação (BELLO *et al.*, 2023; WOZNIAK *et al.*, 2020)

O aumento da pressão em sistemas que utilizam SC-CO_2 promove uma elevação em sua densidade e poder de solvatação, intensificando a interação com compostos bioativos e facilitando sua extração. Sob temperatura constante, variações de pressão afetam significativamente a densidade do CO_2, influenciando diretamente sua capacidade de extrair os compostos desejados (CVJETKO BUBALO *et al.*, 2018). No entanto, ao utilizar cossolventes, o aumento da pressão tem menor impacto sobre a densidade do fluido, mas reduz a difusividade efetiva, podendo diminuir a taxa de transferência de massa da matriz para o solvente. (AILI *et al.*, 2024)

O ajuste adequado do fluxo de SC-CO_2 é essencial para alcançar um rendimento satisfatório no processo. O fluxo influencia os fenômenos de transferência de massa, como a dispersão axial, o coeficiente de transferência de massa convectiva e o acúmulo de compostos extraídos na fase supercrítica. Com o aumento do fluxo do fluido, a resistência à transferência de massa diminui até que o fluido de saída atinja a saturação, momento em que o equilíbrio de solubilidade do soluto é alcançado. Caso o fluxo de SC-CO_2 continue a aumentar, a resistência à transferência de massa continua a diminuir, levando a um aumento no rendimento da extração. Entretanto, taxas de fluxo excessivamente altas ou baixas podem reduzir a eficiência da extração (AILI *et al.*, 2024).

Atualmente existem diversos modelos e configurações de extratores supercríticos. A figura 1 apresenta o modelo proposto Viganó et al., (2016) com objetivo de explorar a seletividade do CO_2 supercrítico para obtenção de frações apolares de extratos de bagaço de maracujá.

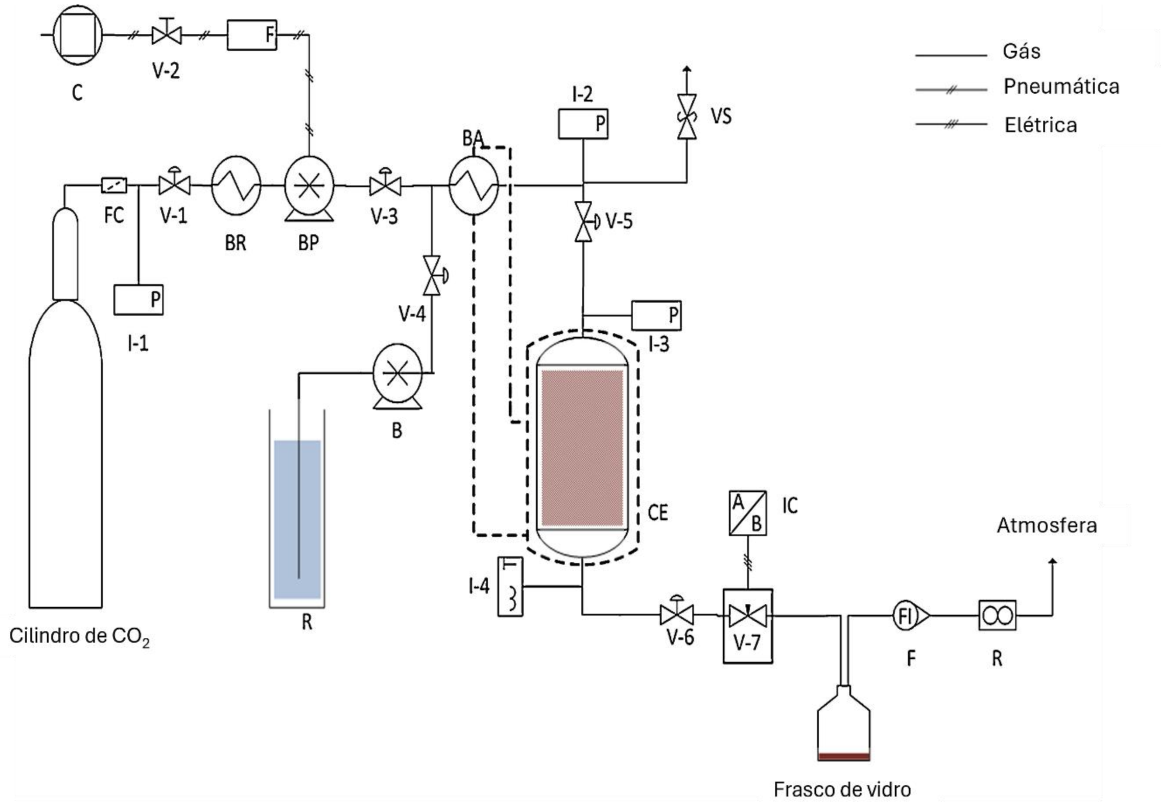

Figura 1: Fluxograma da unidade ESC;V-1 a V-6: válvulas de bloqueio;V-7: válvula micrométrica; VS: válvula de segurança; C: compressor; F: filtro de ar; FC: filtro de CO_2; BR: banho de resfriamento; BP: bomba de CO_2; BA: banho de aquecimento; CE: coluna de extração encamisada; R: reservatório de solvente; B: bomba de HPLC; I-1, I-2 e I-3: indicadores de pressão; I-4: indicadores de temperatura; IC: controlador de temperatura; F: medidor de vazão; R: totalizador de vazão. **Fonte:** *(*VIGANÓ et al., 2016*)*. Adaptado.

O CO_2 é liberado do cilindro, passa por um refrigerador para ser liquefeito, e assim, já em estado líquido e com auxílio de uma bomba compressora, ele é pressurizado por bombeamento para dentro do extrator. O solvente então é aquecido e entra em contato com a amostra presente no extrator. Em seguida o extrato é levado para um separador, onde o solvente será liberado para obtenção do extrato puro. Também é possível utilizar um cossolvente durante a EFS, sendo necessária mais uma bomba para impulsioná-lo até o extrator, onde encontrará o

CO_2 e a matriz sólida. O extrato obtido deve passar por mais uma etapa para separar o cossolvente do extrato (evaporação sob baixa temperatura), o que pode ocasionar um maior custo ao processo e dano à qualidade do extrato, mas o modificador também pode trazer vantagens como discutido anteriormente. Algumas etapas de pré-tratamento na matéria-prima podem otimizar o processo de extração, sendo o caso da secagem a qual pode ser feita em estufa, liofilizador ou energia solar. A retirada de água reduz a interferência entre o solvente o soluto levando à concentração dos compostos. Assim como a secagem, a trituração traz efeitos benéficos uma vez que quanto menor o tamanho da partícula, maior será a superfície de contato da amostra com o solvente.

A EFS pode ser otimizada e para isso requer conhecimentos fundamentais sobre dados termodinâmicos e cinéticos do processo. Segundo Hense et al., (2013) e Minozzo et al., (2012) a cinética de extração da EFS pode ser dividida em três etapas como ilustra a Figura 1.

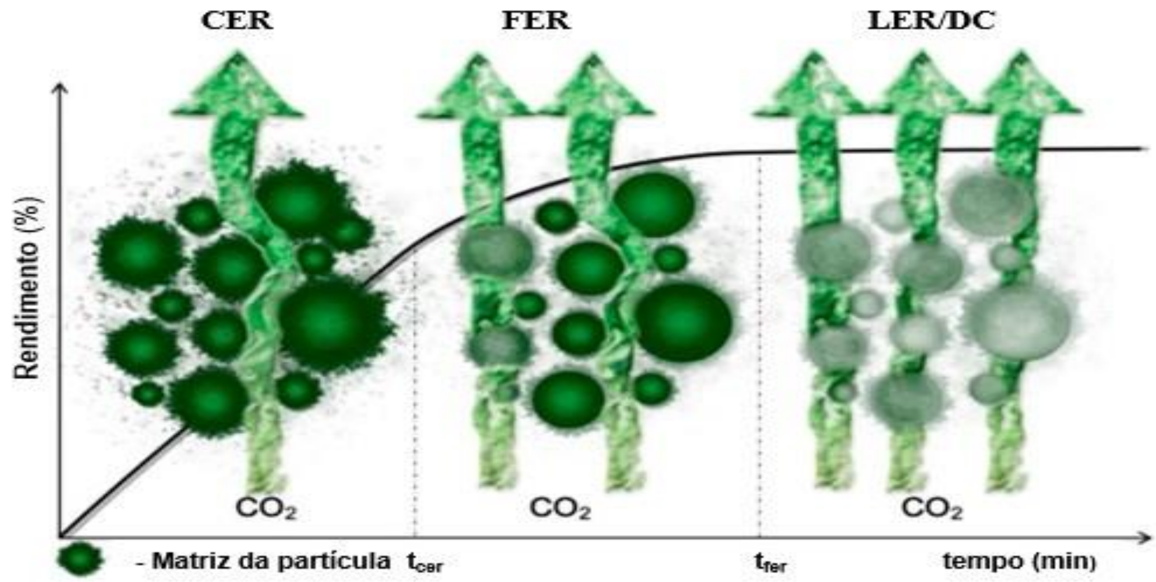

Figura 2:) Curva de extração global da cinética de EFS (rendimento mássico de extrato versus tempo de extração), a caracterização da curva em três etapas (CER, FER e LER/DC) e o arraste do soluto na partícula da matriz sólida pelo dióxido de carbono supercrítico. **Fonte:** (DA SILVA; ROCHA-SANTOS; DUARTE, 2016) Adaptado.

A primeira etapa é a CER (*Constant Extraction Rate*), onde a superfície externa das partículas é coberta com soluto (soluto facilmente acessível) e a convecção é o mecanismo dominante de transferência de massa. A etapa seguinte corresponde à FER (*Falling Extraction Rate*), isto é, etapa de velocidade decrescente, nessa etapa ocorre a exaustão da camada superficial do soluto e o processo difusional na matriz sólida começa a exercer maior influência sobre a convecção na parte fluida, já que o solvente encontra espaços livres para penetrar no sólido, solubilizar o soluto e difundir a mistura soluto-solvente para a superfície. A terceira e última etapa é a LER (*Low Extraction Rate*) ou DC, (*Diffusion Controlled*) na qual a extração é nula, isto é, todo o extrato já foi solubilizado nas etapas anteriores.

1.6 Extrusão termoplástica

A extrusão termoplástica é um processo versátil, que combina operações tais como mistura, cozimento, compressão, fricção, expansão, texturização e moldagem (SINGH GUJRAL; SINGH, 2002; XU *et al.*, 2016). Sendo uma técnica em que o amido e o material proteico presentes na matriz alimentar se modificam sob diferentes combinações de parâmetros de processo, o que proporciona uma oportunidade única para preparar um novo produto (LIU *et al.*, 2011).

O processo térmico associado ao mecânico (cisalhamento) utiliza alta temperatura, porém aplicado ao alimento em curto tempo, e é utilizado para produzir diferentes produtos como cereais matinais, *snacks* e farinhas instantâneas. No entanto, a qualidade nutricional e funcional de um produto extrudado é dependente diretamente do tipo da matéria-prima utilizada e as variáveis do processo como a temperatura, tipo de parafuso e velocidade de rotação do parafuso (LI et al., 2020a; VARGAS-SOLÓRZANO et al., 2014).

Efeitos positivos relacionados ao uso extrusão termoplástica foram relados por Wu et al., (2020) em seu trabalho com macarrão produzido com farelo de arroz extrudado, onde foi observado um aumento do conteúdo de ácido fenólicos, tais como, ácido vanílico, ferúlico,ácido cumárico e ácido protocatecuico. Semelhantemente, ao estudar deferentes genótipos de sorgo extrudado, Campelo et al., (2020) evidenciaram o aumento no conteúdo de fibra, compostos fenólicos e atividade antioxidante. Este processo tem sido utilizado não apenas como um processo único, mas também como pré-tratamento na preparação de diversos produtos, sendo caracterizado por sua capacidade de fornecer alto cisalhamento, rápida transferência de calor e mistura eficiente (KARUNANITHY; MUTHUKUMARAPPAN, 2010).

Estudo mostram que a utilização da extrusão termoplástica, como pré-tratamento, afeta positivamente os compostos fenólicos e a capacidade antioxidante em farelo de sorgo (ORTIZ-CRUZ *et al.*, 2020a). Ela tem sido associada, mais recentemente, à melhor retenção de compostos fenólicos e atividade antioxidante, quando comparada ao cozimento convencional (CAMELO-MÉNDEZ; TOVAR; BELLO-PÉREZ, 2018). Enquanto extração supercrítica tem se mostrado excelente tecnologia na recuperação desses compostos (CANABARRO *et al.*, 2020).

Tabela 5: Estudos recentes sobre extrusão com fluido supercrítico (EFS).

Matriz alimentas	Parâmetros do processo: extrusão	Parametros do Processo: CO2	Principais descobertas	Referências
Snacks à base de bagaço de fruta e soro de leite líquido	Taxa de alimentação (35 kg / h), velocidade do parafuso (100–120 rpm) Temperatura do barril (25 °C),	SC-CO_2 injetado a 25 ºC, (L/D) = 24	84 % dos fenólicos totais e 74 % dos antioxidantes do bagaço de maçã foram preservados. Preservação dos pigmentos de cor e dos compostos bioativos associados. A utilização dos resíduos da agroindústria oferece ingredientes funcionais e de preservação eficazes.	(PARAMAN *et al.*, 2015)
Extrudado grego à base de soro ácido	Taxa de alimentação (40 e 35 kg / h), velocidade do parafuso (120–100 rpm),	pressão de injeção de SC-CO_2 (17,2 MPa), taxa de injeção de SC-CO_2 (0,45 e 0,47 kg / h)	Potencial para substituir água durante a extrusão	(YOON; RIZVI, 2020)
Snack extrudado à base de bagaço de maçã e farinha de sorgo	Teor de umidade da alimentação (15, 20 e 25 g /100 g), Temperatura do cilindro (80, 120 95 e 160 °C), Velocidade do parafuso (100, 150 e 200 rpm),	Injeção do SC-CO_2 a 20 ºC	Extrudados com SC-CO_2 apresentaram maior teor fenólico (12 %) e atividade antioxidante (7 %), com 59% menos dureza, 70 % mais crocância, menor absorção e maior solubilidade em água. melhorou propriedades antioxidantes, texturais e funcionais em temperaturas abaixo de 100 °C.	(LOHANI; MUTHUKUMARAPPAN, 2017)
Bolos de avelã desengordurados, camelina, abóbora e cânhamo em snacks extrudados à base de milho	Temperatura (90–110 - 120 °C), velocidade do parafuso (100 rpm), velocidade de dosagem: 35 rpm	Pressão de SC-CO_2 (140 bar)	A adição do subproduto teve influência significativa na taxa de expansão, densidade aparente, fraturabilidade e crocância	(PANAK BALENTIĆ *et al.*, 2019)

Como mostrado, o $SC\text{-}CO_2$ e a extrusão termoplástica tem sido aplicado em conjunto, em uma técnica conhecida como extrusão de fluido supercrítico, que consiste na substituição do vapor de água, empregado no processo de extrusão, por um fluido supercrítico (GOPIRAJAH *et al.*, 2020). Porém o uso da extrusão termoplástica com o objetivo de aumentar o rendimento na extração de bioativos de cereais de reconhecida fonte, por exemplo de ácidos fenólicos, como o sorgo e milheto ainda não foi estudado.

1.7 CONCLUSÃO

A partir desta revisão, foi possível obter conhecimentos importantes relacionados aos grãos e aos farelos de sorgo e de milheto pérola, composição, processamento por extrusão termoplástica (ET) e extração de compostos bioativos com fluido supercrítico (EFS). Embora pesquisas extensivas tenham sido realizadas usando FSE para extrair bioativos de matrizes vegetais, o custo do processo para sua exploração comercial pode ser um desafio e, portanto, deve ser levado em consideração. Uma solução possível é o uso da ET como pré-tratamento das amostras para EFS, de forma a permitir maior rendimento da extração ideal de compostos bioativos.

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2 CAPÍTULO I

INFLUÊNCIA DO BRUNIMENTO NO TEOR DE AMIDO E NAS PROPRIEDADES DE PASTA DE SORGO (*Sorghum Bicolor* (L). Moench) E MILHETO PÉROLA (*Pennisetum Glaucum* (L.) R. BR.)

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ABSTRACT: Pearl millet followed by Sorghum are largely cultivated in Brazil, but not known as food. From their grains, bran can be extracted, which is a great source of minerals, phytochemicals (bioactive compounds) and dietary fiber. This study investigated the effect of debranning time on moisture, starch content, colorimetric analysis and paste properties. Pearl millet grain debranned for 25 min showed the highest percentages of bran (15.5 %) and dehulling (30.7%). Debranning time affected the starch content, ranging from 7.23 g / 100 g (5 minutes) to 22.16 g / 100 g (25 minutes) for pearl millet and from 11.6 g /100 g (5 minutes) to 20.7 g / 100 g (10 minutes) for sorghum, indicating its influence on pasting properties. The final viscosity for millet bran ranged from 63.5 to 82.9 cP and for sorghum bran from 47.5 to 80.0 cP, revealing that bran behaves similarly to wholemeal flour, even at lower levels. With coefficient values ranging from -1 to 0.99, bran recovery showed a strong positive correlation with total starch (0.96) debranning time (0.81), and moisture showed a high relationship with ΔE^* (0.95). In the colorimetric analyses, L^* correlated positively with minCV (0.91) and PV (0.87), and negatively with PT (-0.92). Principal component analysis (PCA) revealed that MB25 had proximity to total starch, bran recovery, IV, FV, minCV, and PV, while SB10 and SB07 showed proximity to moisture and colorimetric variables. HCPC analysis suggested two main groups: MB25 and MB05-MB10 formed cluster 1 and SB10 and SB05-SB07 formed cluster 2, the heatmap evidencing these relationships.

2.1 INTRODUCTION

The sorghum [*Sorghum bicolor* (L.) Moench] and pearl millet [*Pennisetum glaucum* (L.) R. Br) are cereals of African origin and were domesticated between 3,000 and 5,000 years ago. In addition, these cereals are among the five most important cereals in the world, preceded by wheat, rice, corn and barley (DE MORAIS CARDOSO *et al.*, 2017). Sorghum and millets grains are rich in bioactive and antioxidant compounds. These compounds are mostly present in whole grains, particularly in the bran fraction (AQUINO-BOLAÑOS *et al.*, 2021; AROUNA; GABRIELE; PUCCI, 2020; NOGALA-KAŁUCKA *et al.*, 2020; SLAMA *et al.*, 2020).

The sorghum pericarp is rich in non-starch polysaccharides, phenolic compounds (such as 3-deoxyanthocyanidins, tannins and phenolic acids) and carotenoids. The germ contains lipids, B vitamins and minerals, while the endosperm stands out for its high concentration of carbohydrates and proteins (DE MORAIS CARDOSO *et al.*, 2017).

Sorghum and millet grains are rich in bioactive and antioxidant compounds. These compounds are mostly present in whole grains, particularly in the bran fraction (AQUINO-BOLAÑOS *et al.*, 2021; AROUNA; GABRIELE; PUCCI, 2020; NOGALA-KAŁUCKA *et al.*, 2020; SLAMA *et al.*, 2020). These grains are usually decorticated in order to improve the taste and biological value of porridges and flours intended for human consumption. In this process, around 35% of the total weight of the grain is lost, generating a substantial amount of bran, a hard outer layer usually discarded as processing waste or used as animal feed (DHARMARAJ; RAVI; MALLESHI, 2014).

The process of decortication, also known as dehulling or debranning, is the removal of the bran (pericarp and partially germ) from the grains to promote improvements in quality attributes such as palatability, grain color, phytic acid reduction and fat content. Traditionally, in rural areas, decortication is carried out manually with a wooden pestle and mortar or, mechanically, with a dehuller, after which the grains are quickly washed and then sieved to separate the bran from the decorticated grains(HAMA *et al.*, 2011).

Several authors have reported a reduction in nutrients, fiber and antioxidant capacity of whole grains after removing the bran, suggesting that bran and bran-rich fractions (aleurone layer, and pericarp) are concentrated sources of nutrients (AGIL; HOSSEINIAN, 2014; PASHA; AHMAD, 2021). In addition to nutritional losses, debranning can cause nutrient losses and changes in physical properties. These changes depend on the moisture, the type of processing (mechanical or manual) and the degree of decortication (DHARMARAJ; RAVI; MALLESHI, 2014; DIAS-MARTINS *et al.*, 2019).

Although there are many studies on debranning, most of them are aimed at evaluating the influence of the decortication/polishing process on the nutritional attributes of millet and sorghum or to improve the process through different pretreatments. Literature reports few studies on these brans on the effect of debraning on the physical, chemical, physicochemical, colorimetric and pasting properties. Therefore, the objective of this study was to understand the characteristics of pearl millet and sorghum grains and the changes induced by debranning in the quality of pearl millet and sorghum brans.

2.2 MATERIALS AND METHODS

2.2.1 Sorghum and pearl millet

Grains of the pearl millet hybrid ADRg9070 were kindly donated by Atto Sementes (Rondonópolis, Brazil) and the brown sorghum grains provided by Embrapa Milho e Sorgo (Sete Lagoas, Brazil).

2.3 Sample preparations

The whole grains were cleaned to remove foreign material, broken grains and other types of grains in a Clipper Office Tester 400/B (A.T. Ferrell Co., Bluffton, USA). After cleaning, the grains were debranned in a bench test rice mill model MT-97, n. 3788-5 (Suzuki, Santa Cruz do Rio Pardo, Brazil). For millet, 150 g were used at times of 5 (MB5), 10 (MB10) and 25 min (MB25), whereas for sorghum, 100 g were processed at 5 (SB5), 7 (SB7) and 10 min (SB25). The differences in batch size and time were due to the size of the grains, as sorghum grains are at least twice the size of pearl millet. The brans were ground in a 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm aperture. Debranned grains were ground in the same mill and used as control samples (M0 and S0). The samples were vacuum-packed in polyethylene bags, protected from light and kept at freezing temperature until analysis.

2.3.1 Debranning characteristics and bran extraction

The bran recovered (%) and debranned grains (%) was calculated using the following Equation 1 e 2.

$$Bran\ recovered\ (\%) = \frac{Weight\ of\ bran\ obtained}{Weight\ of\ \ initial\ sample\ taken} x\ 100 \qquad (1)$$

$$Debranning\ (\%) = \frac{Weight\ of\ debranned\ grain}{Weight\ of\ initial\ sample\ taken} x\ 100 \qquad (2)$$

2.3.2 Total Starch and moisture

Total starch was determined using a Megazyme© Assay kit (Megazyme Intl. Ireland Ltd., Wicklow, Ireland), according to 79-13 method (AACC, 2000). The sorghum and pearl millet bran samples were analyzed for moisture using the methods, 931.04 (AOAC, 2010).

2.3.3 Paste properties

The paste viscosity was determined using a Rapid Visco Analyzer series 4 RVA (Newport Scientific Pty Ltd., Warriewood, Australia) using the method 76-21 (AACC, 2000) used for measuring paste viscosity of starch.

2.3.4 Colorimetric analysis

The color[*] analysis of the bran was carried out by transmittance on a Hunterlab Colorquest colorimeter, model XE (Reston, Virginia, USA). A CIELAB and CIELCh scale was used to measure Hunter's color parameters ($L*$, $a*$, $b*$ $C*$ and $h°$) according to The International Commission on Illumination (CIE, 2004). The color variation ($\Delta E*$) was calculated using Equation (3).

$$\Delta E = \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2} \quad (3)$$

where: $\Delta a*$, $\Delta b*$ and $\Delta L*$ are the differences between the standard and the sample in $a*$, $b*$ and $L*$, respectively.

2.3.5 Statistical analysis

Analyses of variance (ANOVA), Tukey's test at 5%, principal component analysis (PCA), Pearson's coefficient (r) and hierarchical clustering in principal components (HCPC) were carried out. The analyses were carried out using Minitab 22 (Minitab Ltd., State College, PA, USA) and XLSTAT 23 (Addinsoft, New York, USA) with a 95% confidence level (α = 0.05).

2.4 RESULTS AND DISCUSSION

The grain cleaning process is extremely important to remove dirt, straw and insects as it can be seen in Figure 1, that shows the dirt, husks and bran of sorghum and pearl millet.

Figure 1: Visual aspect of sorghum and pearl millet. A) Sorghum. B) Pearl millet

The figures A1 and B1 show the dirt removed during the cleaning process using an air-mechanical separator. During the dehulling process, the grain husk is the first part of the cereal to be removed. After separation by sieves, it is possible to see the sorghum husk (A2) and pearl millet husk (B2). The final sorghum (A3) and pearl millet (B3) brans are obtained after grinding in a hammer mill.

The yield of recovered bran and debranning are shown in Figure 2 and Table 1. The initial grain weight of millet and sorghum at 0 min (150 g and 100 g, respectively) was reduced to 103.99 g and 85.88 g at 25 and 10 (min). Millet showed the highest percentage of debranning and bran recovery that were 30.7 % and 15.22 %, respectively, at 25 min. Sorghum showed debranning of 14.13% and bran recovery of 12.80 %.

The debranning time influenced the bran yield and these results are in agreement with Barbhai and Hymavathi (2022). Different debranning processes have been reported in the literature for cereal grains. These differences are due to the origin of the grain and different conditions involved in the process, such as moisture content, decortication method (manual by friction or mechanical by abrasion) and type of machine (DIAS-MARTINS et al., 2019; HAMA et al., 2011).

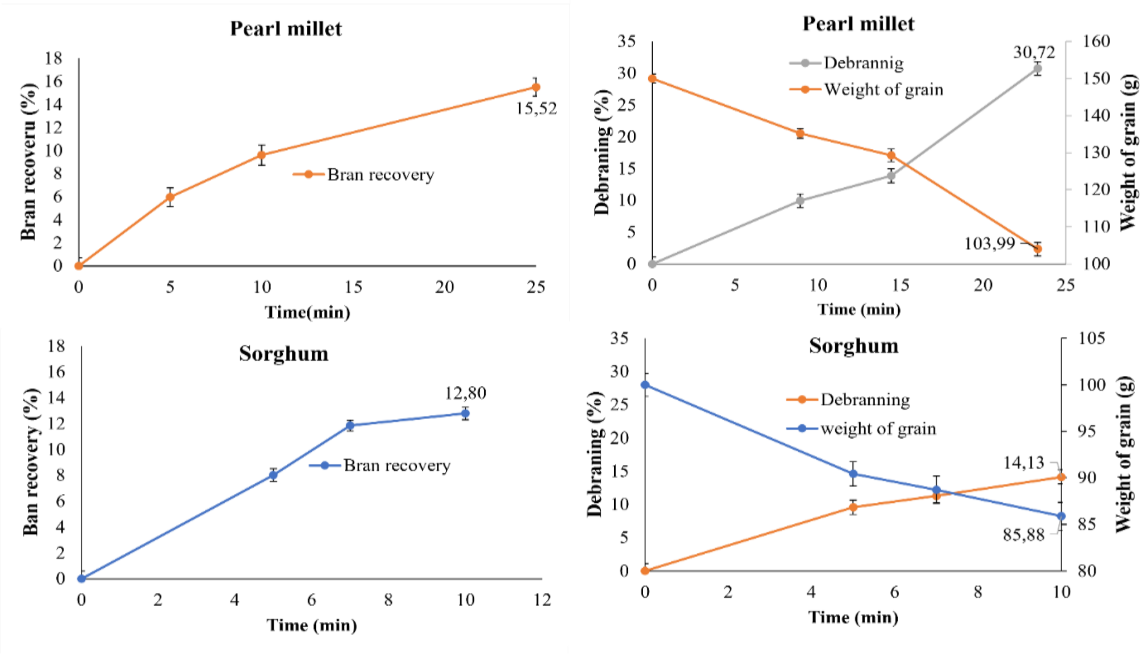

Figure 2: Bran recovery, debranning and weight of sorghum and millet grains as a function of processing time.

Chemical and physical analyses of pearl millet and sorghum brans are presented in Table 1. The moisture content of millet brans did not show any significant difference, while in sorghum the moisture content varied from 8.22 % (SB05) to 11.92 % (SB10).

Table 1: Moisture, starch content and colorimetry

Parameters	Millet			Sorghum		
	MB05	**MB10**	**MB25**	**SB05**	**SB07**	**SB10**
Moisture	8.56±0.1^{a}	8.42±0.0^{a}	8.54±0.0^{a}	8.20±0.0^{c}	10.63±0.0^{b}	11.92±0.0^{a}
Total Starch	7.23±0.0^{c}	11.84±0.0^{b}	22.16±0.0^{a}	11.35±0.0^{c}	14.35±0.0^{b}	20.70±0.0^{a}
Recovery bran	5.96±0.0^{c}	9.62±0.0^{b}	15.52±0.0^{a}	8.03±0.0^{c}	11.86±0.0^{b}	12.80±0.0^{a}
Debranning	9.95±0.4 ^{c}	13.87±0.0^{b}	30.72±0.0^{a}	9.58±0.0^{c}	12.29±0.0^{b}	14.13±0.0^{a}
$L*$	64.92±0.1^{c}	65.54±0.0^{b}	66.30±0.1^{a}	55.85±0.6^{c}	58.82±0.3^{b}	60.27±0.1^{a}
$a*$	1.31±0.0^{b}	1.44±0.0^{a}	1.29±0.0^{b}	14.86±0.3^{a}	13.38±0.1^{b}	12.28±0.1^{c}
$b*$	16.49±0.0^{a}	16.65±0.1^{a}	15.15±0.0^{b}	21.96±0.4^{a}	20.12±0.0^{b}	19.26±0.0^{c}
$C*$	16.54±0.0^{a}	16.71±0.1^{a}	15.21±0.3^{b}	26.52±0.5^{a}	24.16±0.1^{b}	22.84±0.1^{c}
$h°$	85.44±0.3^{a}	85.05±0.3^{b}	85.10±0.3^{b}	55.91±0.3^{b}	56.37±0.3^{b}	57.47±0.3^{a}
ΔE		1.63	1.92		3.79	5.78

Lowercase letters in the same line indicate differences between fractions within the same grain ($p<0.05$), by Tukey's test.

This phenomenon occurs due to the difference in moisture content between the grain fractions, with the endosperm being the fraction with the highest moisture content. As the debranning time increased, a more significant removal of the central part of the grain occurred, which resulted in an increase in the moisture content of the sorghum brans. This can be confirmed in the studies of Sruthi et al. (2021) e Galán et al., (2018)

The debranning time directly influenced the starch content, which varied between 7.23 (5 min) and 22.16 (25 min) for millet bran, and 1.6 (5 min) to 20.7 (10 min) for sorghum. Higher values (26.33 g/100 g) were found by Cukelj Mustač et al., (2020)in their study with proso millet (*Panicum miliaceum* L.). In their study with pearl millet, Lestienne et al., (2007) reported 12% of starch 30 % of iron, 10 % of fibers and 5% of phenolic compounds in the bran, which confirms that bran fraction is rich in these nutrients.

The color parameters (*L*, a*, b* C** and *h*°) of millet and sorghum bran are also shown in Table 1. It was possible to observe that the debranning process significantly affected color. Using MB05 and SB05 as standards for the other bran. The color difference (*ΔE**) between the millet bran was (*ΔE**) = 1.63 (MB10) and (ΔE*) = 1.92 (MB25), between the sorghum bran was (ΔE*) = 3.79 (MB07) and (*ΔE**) = 5.78 (MB10). All the brans presented (*ΔE**) >1 which means that they are differences that are perceptible to the human eye (FRANCIS; CLYDESDALE, 1975).

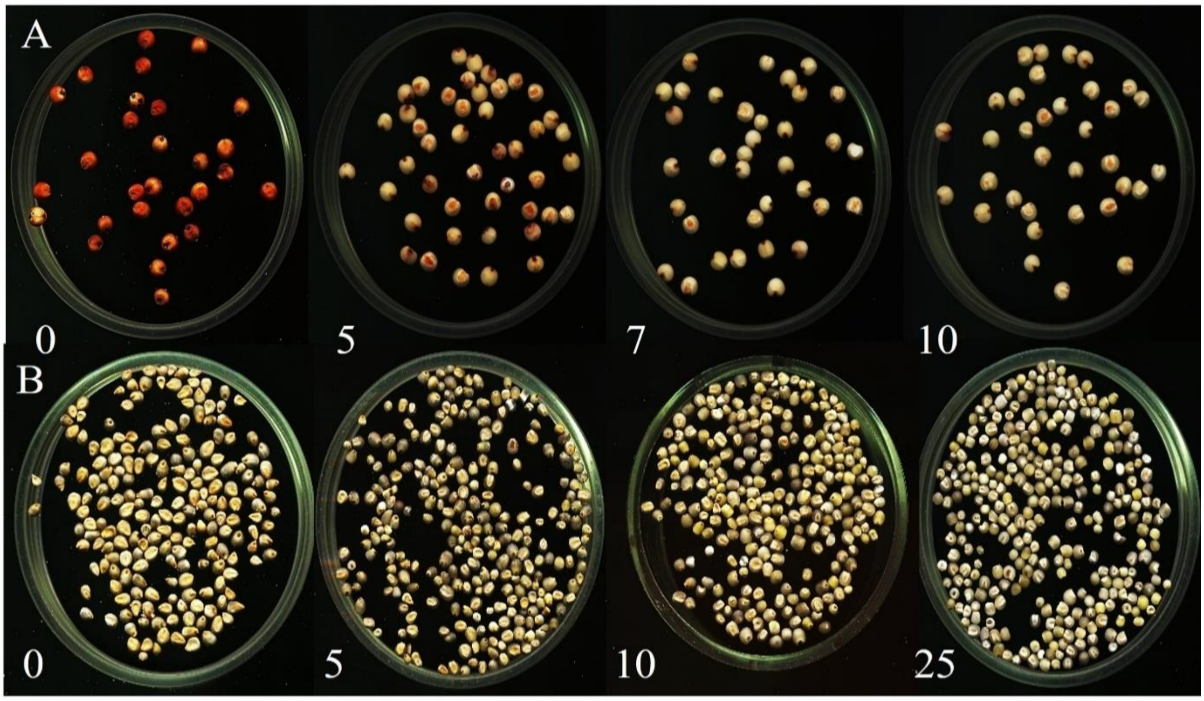

Figure 3: Visual aspect of sorghum and pearl millet A) Sorghum grain .0, 05,0 7,10 min. B) pearl millet grain 0, 05, 10, 25 min.

Although a colorimetric analysis was not performed on the grains, it is possible to observe a slight color change between the millet grains, while in the sorghum grains this change was more noticeable when the grains were compared at 0, 10 min (sorghum) and 25 min (millet) of debranning, as a result of bran extraction (Figure 3).

The starch content (Table 1) has a direct influence on the paste properties (Table 2 and Figure 4). The paste properties of sorghum and millet bran show that the results changed depending on the origin of the grain and the time debranning. The bran showed similar behavior to that of whole grain, although to a lesser extent.

Table 2: Paste properties of the brans

	Millet			Sorghum		
Parameters	**MB05**	**MB10**	**MB25**	**SB05**	**SB07**	**SB10**
Initial viscosity	52.00±2.1[a]	53.00±0,9[a]	47.00±0.0[a]	49.50±0.59[b]	38.16±0.0[c]	66.00±0.0[a]
PT (°C)	80.44±1.4[c]	83.70±0.0[a]	81.94±2,5[b]	95.04±0.0[a]	95,03±0.0[a]	95,01±0.0[a]
PV (cP) at 95 ºC	72.00±0.0[c]	88.16±6.3[a]	82.00±1.4[b]	36.53±4.9[b]	38.37±0.0[b]	66.00±0.0[a]
Min.CV (cP)	52.20±0.0[c]	67.10±2.8[a]	65.10±1.2[b]	24.41±4.9[c]	27.51±0.0[b]	55.15±0.0[a]
DB (cP)	15.51±0.0[c]	21.50±1.1[a]	17.06±0.0[b]	25.06±0.0[a]	11.60±0.0[b]	10.51±0.0[c]
Max.CV (cP)	68.52±0.0[c]	81.10±1.4[b]	84.50±6.3[a]	49.10±1.4[c]	53.33±0.0[b]	85,50±0.0[a]
Setback (cP)	12.50±0.0[c]	15.10±0.0[b]	19.51±0.0[a]	24.50±3.5[c]	26.40±0.0[b]	30,23±0.0[a]
FV (cP)	61.16±0.0[c]	75.16±2.1[b]	78.52±2.6[a]	49.23±0.5[c]	53,45±0.0[b]	81.13±0.0[a]

Lowercase letters in the same line indicate differences between fractions within the same grain ($p<0.05$), by Tukey's test. PT, Paste temperature; PV, Peak viscosity; Min.CV; minimum cooling viscosity; DB, Breakdown; Max.CV, Maximum cooling viscosity, FV; Final viscosity.

Overall, the paste viscosity of both brans shows very low viscosity profile when compared to whole grain flour (DIAS-MARTINS *et al.*, 2024). Peak viscosity up to 95 ºC was the highest in millet bran at 88.16 cP (MB10) while sorghum bran (SB05) had the lowest peak viscosity at 36.53 cP. The increase in peak viscosity is usually related to the presence of intact and even partially broken starch granules that are able to swelling, hence increase paste viscosity, The increase in peak viscosity is usually related to the presence of intact and even partially broken starch granules that are able to swelling, hence increase paste viscosity. The low viscosity in sorghum was probably due to the low content of starch and high in fiber leading to a reduction of the peak viscosity (AWOLU, 2017; SRUTHI; RAO; RAO, 2021). Paste stability is measured by breakdown viscosity (BD) and there was a statistical difference between all samples, ranging from 10.51 to 25.06 cP. The lowest BD was found in sorghum bran SB10, thus sorghum bran (SB10) showed greater resistance to thermal and mechanical treatment. This makes it useful for adding nutritional value to products that do not require high viscosity and foods that need heat treatment, such as extruded snacks (JAMES *et al.*, 2018; ORTIZ-CRUZ *et al.*, 2020b).

The setback viscosity is the value that represents the tendency to starch retrogradation, the setbacks were 12.50, 15.10 and 19.51 cP for MB05, MB10 and MB25 respectively, and 24.50, 26.40 and 30.23 cP for SB05, SB07 and SB10. Sorghum bran showed higher setback values compared to millet bran, which may be due to the approximation of amylose molecules and the formation of intermolecular hydrogen bonds (DIAS-MARTINS *et al.*, 2024).

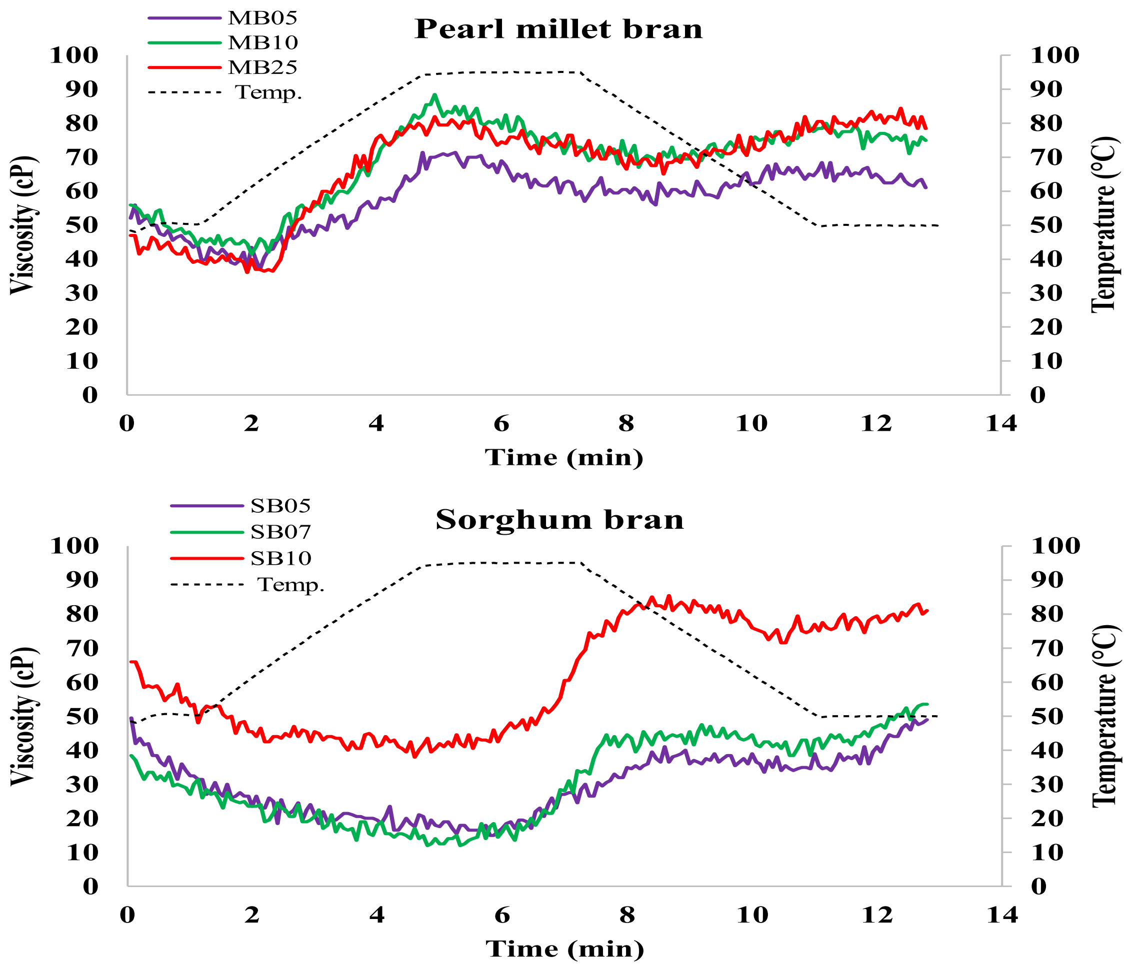

Figure 4: Viscosity curve for pearl millet and sorghum bran.

The greater the debranning, the greater the final viscosity. It is possible to observe that for millet bran the ranged from 63.5 to 82.99 cP and for sorghum bran it ranged from 47.5 to 80.00 cP. The variation between the values may be related to the non-uniformity of the debranning process which can vary according to the structure, thickness of the pericarp and the percentage of endosperm and germ of each grain. As the starch granules are located mainly in the endosperm of the grain, their content is proportionally higher when the pericarp is removed (HAMA *et al.*, 2011).

In general, debranning promotes the extraction of various compounds beneficial to health, so sorghum and pearl millet bran can be used to enrich food products with low viscosity requirements. Viscosity curve brans presented behavior by is typical of starch materials with high fiber content (NASCIMENTO *et al.*, 2022).

Correlation analysis provides the association between two or more quantitative variables. It is primarily based on the postulation that there exists a linear relationship between the quantitative variables. It evaluates the "strength" or "extent" of a relationship between variables as well as its direction in terms of the Correlation coefficient (R), which ranges from −1 to +1. Pearson correlation analysis was used in the study to determine the correlation between the variables (JOSHI T; SRINIVASA RAO, 2024).

For positive (in red) and negative (in blue) correlations, the stronger the association, the more intense the color. The r-value ranged from -1 to 0.99, demonstrating both positive and negative correlations, showing the possible impact of multicollinearity (ALIN, 2010). Recovery bran showed a strong positive relationship with total starch (0.96) and debranning (0.81). Moisture showed a strong positive relationship with $\Delta E*$ (0.95). Colorimetric analyses showed a strong positive and negative relationship with paste properties. $L*$ showed a strong positive relationship with minCV (0.91) and PV (0.87) and a strong negative relationship with PT (-0.92). This confirms that the higher the debranning, the higher the $L*$ and the higher the PV.

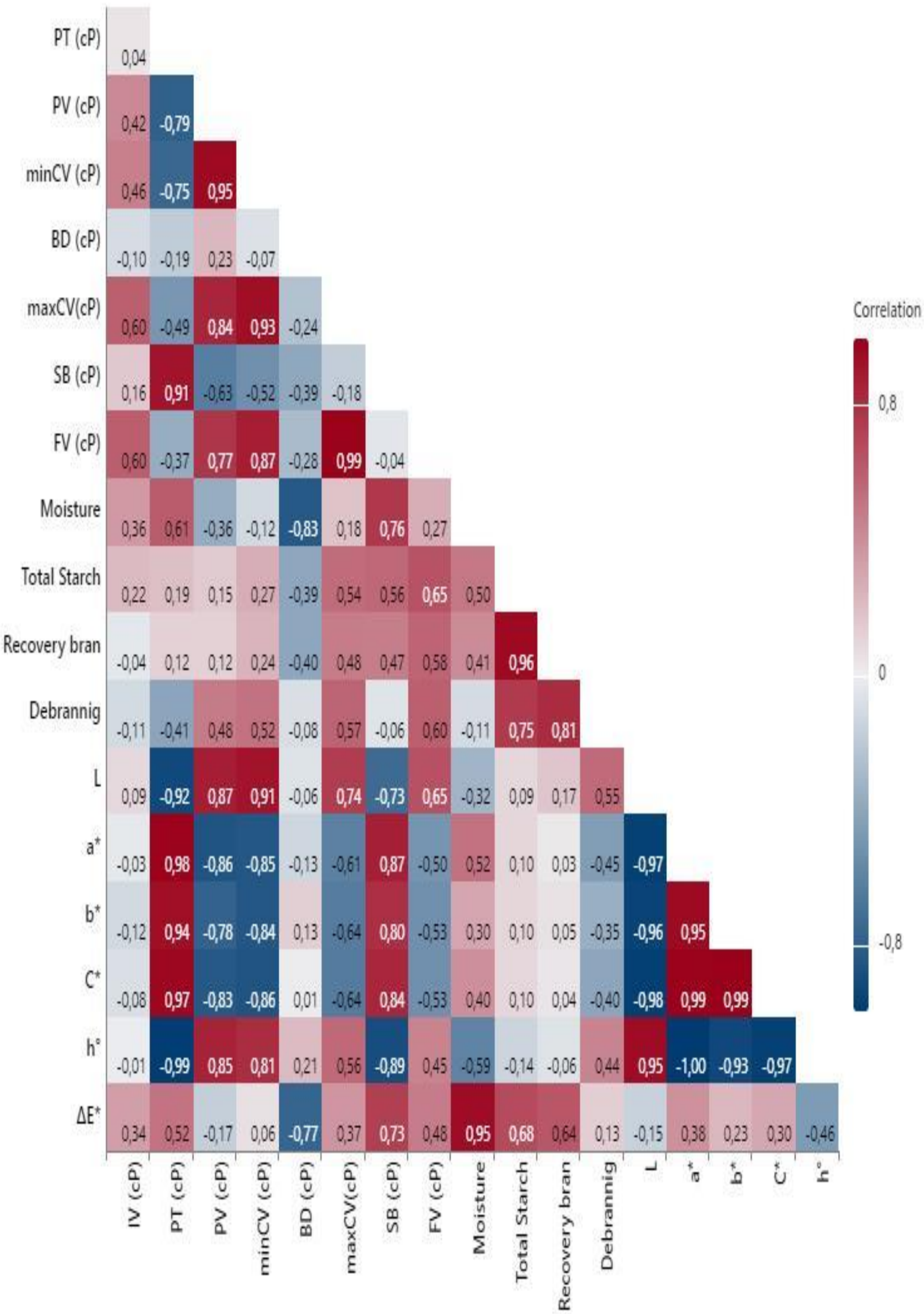

Figure 5: Correlation matrix plot depicting the correlations between different variables. PT, paste temperature; PV, peak viscosity at 95 °C; minCV, minimal cooling viscosity; maxCV, maximum cooling viscosity; FV, final viscosity; BD, breakdown and SB, setback

Multivariate analysis using PCA was carried out to study the similarities between sorghum and millet bran at different debranning times with paste properties, moisture, total starch, debranning, recovery bran and colorimetry to determine their grouping (Figure 6). The PC1 (52.22 %) and PC2 (26.60 %) components showed 81.22 % variability. In PCA, vectors in proximity are positively correlated and distant vectors are negatively correlated

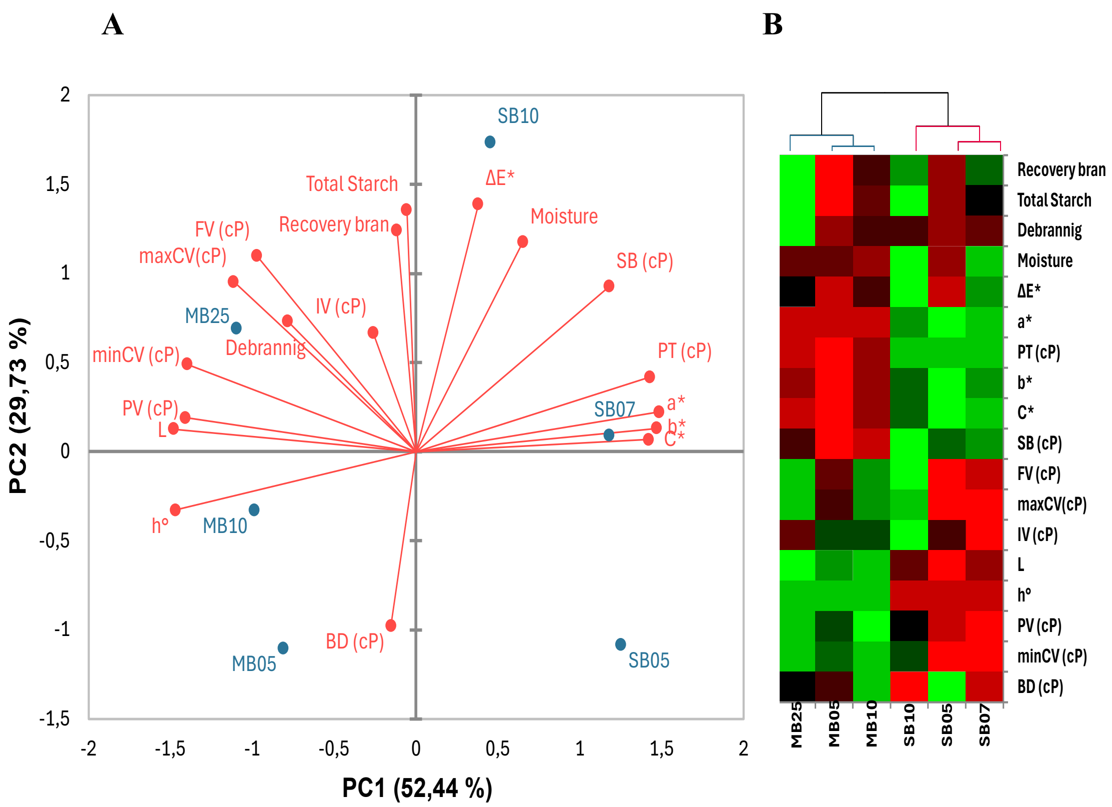

Figure 6: Multivariate analysis A) Biplot chart of principal component analysis (PCA) of samples, and physical and chemical properties; B) Heatmap grouping samples by Hierarchical clustering of principal components, the green color indicates the highest value and red color indicates the lowest value.

The length of each line (vector) is proportional to the variability of the parameters in relation to the two PCs being showcased, with parameters that are positively correlated appearing in proximity. The proximity of sample points to specific vectors and their distance from the origin in the biplots indicates the degree of influence these vectors exert on the samples (PRASHANTH *et al.*, 2024).

The graph shows that the samples with longer debranning time presented greater proximity with the analyses performed (Figure 6A). MB25 presents proximity with total starch, recovery bran, IV, FV, minCV, PV and L, being more closely linked to debranning, FV and maxCV which can be confirmed by the green color in the color map (Figure 6B). SB10 and

SB07 showed proximity to moisture, SB, PT, a^*, b^* and C*, with the last four being strongly linked to SB07. MB05, MB10 and SB05 were presented in the negative quadrants of the graph, h^o is close to MB10, MB05 shows low or no proximity to DP, and SB05 did not show any proximity relationship with any of the analyses The results presented in this study corroborate Munshi & Dashora, (2024) who observed high proximity of millet flour with paste properties, especially PV, BD and FV.

Applying the HCPC (groups of samples in Figure 6B), 2 groups of samples were suggested, MB25 and MB05-MB10 formed cluster 1 (blue) and SB10 and SB05-SB07 formed cluster 2 (red). In the heat map, the dark colors indicate the lowest values, allowing easy detection of the variables that characterized the samples and confirming the information from the PCA.

2.5 CONCLUSION

The results of this study showed a direct relationship between debranning time and bran yield. Pearl millet showed higher bran recovery compared to sorghum. In addition, the debranning time directly influenced the starch content, which has a direct impact on the properties of the paste, such as initial viscosity and peak viscosity. The study also allowed a better understanding of the anatomical structure of sorghum and pearl millet grains and how it can influence the processing of these grains, and the properties analyzed. These results suggest that sorghum and millet bran can be incorporated into food products that do not require high viscosity.

2.6 Acknowledgements

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3 CAPÍTULO II

EFFECT OF EXTRUSION ON THE PHENOLIC COMPOUND CONTENT, ANTIOXIDANT CAPACITY AND PHYSICAL PROPERTIES OF SORGHUM (S*orghum bicolor* (L.) Moench) AND PEARL MILLET (*Pennisetum glaucum* (L.) R.BR.) BRAN

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3.1 ABSTRACT

Pearl millet (*Pennisetum glaucum* (L) R.BR) and Sorghum (*Sorghum bicolor* (L.) MOENCH) are among the most widely produced cereals in the world. Both are gluten-free, high in fiber and polyphenols and they can reduce lipid disorders and cardiovascular diseases. Most health-beneficial compounds are found in the bran. Thermoplastic extrusion (TE) can be used to increase the availability of those compounds. This study investigated the effect of TE on the decorticated grains and brans of pearl millet and sorghum. The samples were processed in a single-screw laboratory extruder. For bran, extruded bran, and hulled grains, chemical analyses, total phenolic compounds (TPC), antioxidant capacity by ABTS (CA-ABTS) and physical analyses, particle size distribution (PZD), density and specific volume. The analysis showed that TPC ranged from 99.78 (SDG) to 1240.94 (MEB) (µg GAE/100 g) and AC from 4.55 (SDG) to 55.51(SEB) (µmol TE/ g). The lowest concentrations of TPC and AC were observed in DSG and DMG. Extrusion significantly increased TPC in ESB and raised AC in all samples, reaching 55.51 (µmol TE/ g) in SEB. (SB) had the highest D[4.3] (161.30 µm), and EMB was the lowest (81.31 µm). Extrusion reduced D[3,2] by 24.82 (%) in SB and 16.46 (%) in MB. PCA highlighted differences between dehulled grains and bran, extruded and non-extruded, and Pearson's correlation indicated strong positive relationships between TPC, AC-ABTS and SV, showing the effect of extrusion on the bioactivity of bran. This study showed that thermoplastic extrusion (TE) has a positive effect on the availability of bioactive compounds in millet and sorghum bran, significantly increasing the content of total phenolic compounds (TPC) and antioxidant capacity (AC-ABTS).

Keywords: thermoplastic extrusion, bioactive compounds; gluten-free cereals;

3.2 INTRODUCTION

Pearl millet, *Pennisetum glaucum* (L.) R. Br, is the sixth most produced cereal, with a production of 27.8 million tons (SRIVASTAVA; SAINI; SINGH, 2020). Sorghum (*Sorghum bicolor* (L.) Moench), is the fifth cereal crop in the world, with a production of 58,70 million tons in 2020 (FAOSTAT, 2022). Pearl millet and sorghum grains are a gluten-free cereal, rich in dietary fiber and polyphenols that can reduce the occurrence of lipid disorders, cardiovascular disease and hyperglycemia. Despite these benefits, it is still an underused cereal for human consumption in many countries, including Brazil (ANUNCIAÇÃO *et al.*, 2019; DIAS-MARTINS *et al.*, 2018b; OFOSU *et al.*, 2020; SRIVASTAVA; SAINI; SINGH, 2020).

The richness in dietary fiber, the absence of gluten and the interesting protein and fat profiles are other properties that make these cereals very balanced food options. (MAWOUMA *et al.*, 2022b). The most important anatomical parts of a cereal grain are endosperm, bran, and germ. The bran represents about 14-19% of the grain's weight. Despite being a rich source of vitamins, minerals, phytochemicals and dietary fiber, bran is usually discarded during primary processes such as peeling, grinding and polishing (PATEL, 2015).

Thermoplastic extrusion (TE) is a green technology that consists of a thermomechanical process that combines humidity, pressure, temperature and shear. Some properties are very important for the process, especially the moisture content, particle size distribution and material density (CARVALHO *et al.*, 2010) . Bioactive compounds such as phenols, anthocyanins, tannins and other flavonoids, are present in the bran fraction and recent studies show that the use of extrusion as a pretreatment positively affects the phenolic compounds and the antioxidant capacity of sorghum bran (ORTIZ-CRUZ et al., 2020).

Wu et al., (2020) reported positive effects of the use of thermoplastic extrusion in noodles produced with extruded rice bran, observing an increase in the levels of phenolic acids, such as vanillic, ferulic, coumaric and protocatechuic acid. Similarly, Campelo et al., (2020), when studying different genotypes of extruded sorghum, identified an increase in the content of fiber, phenolic compounds, and antioxidant activity.

To add bioactive compounds, dietary fiber and satisfy the demands of increasingly health-conscious consumers, many studies have been carried out with the aim of adding bran in food formulations. However, for this to be possible, it is necessary to study the physical properties of the materials, directing to the appropriate product and technology process..

3.3 OBJECTIVE

Investigate the effect of the extrusion process on the total phenolic compounds, antioxidant activity and physical properties on the pearl millet and sorghum bran.

3.4 MATERIALS AND METHODS

3.4.1 Samples preparation

The grains of pearl millet, hybrid ADRg9070, were kindly donated by Atto Sementes (Rondonópolis, Brazil) and sorghum by Embrapa Milho e Sorgo (Sete Lagoas, Brazil). The whole grains were cleaned to remove foreign materials, broken grains and other types of grain in a Clipper Office Tester 400/B (A.T. Ferrell Co., Bluffton, USA). After cleaning, the grains were debranned in a benchtop test rice mill model MT-97, nº 3788-5 (Suzuki, Santa Cruz do Rio Pardo, Brazil) using the specific compartment where the grain was abraded in order to remove the pericarp at a flow rate of 150 g for 10 min and 5 min, respectively for pearl millet and sorghum. The bran and decorticated grains were then ground in a hammer mill model 3100 (Perten Instruments AB, Huddinge, Sweden). At the end of this process, the following were obtained: sorghum bran (SB), pearl millet bran (MB), pearl millet dehulled grain (MDG) and, sorghum dehulled grain (SDG).

3.4.2 Extrusion conditions

The sorghum and pearl millet bran were packed at 14% moisture (wet basis) and stored in plastic bags overnight in a refrigerator to even out water absorption. The samples were processed in a 19/20 DN single-screw laboratory extruder (Brabender, Duisburg, Germany), coupled to a Plasti-Corder Lab Station torque rheometer (Brabender, Duisburg, Germany). The extruder was equipped with a 3:1 compression screw; 3 mm circular die; three heating zones from feed to outlet set at: 40, 80 and 120 °C, respectively; screw speed set at 200 rpm and volumetric vertical screw feeder (Brabender, Duisburg, Germany) at 25 rpm, providing ~ 4.0 kg / h (TOLEDO *et al.*, 2020). At the end of this process, the following were obtained: pearl millet extruded bran (MEB) e sorghum extruded bran (SEB)

Immediately after the extrusion process, the extrusion was collected and dried in a forced-air oven at 55 °C. They were then ground in a hammer mill. The dehulled grains, bran and extruded bran were vacuum-packed in polyethylene bags, protected from light and kept at freezing temperature until extraction and analysis.

3.4.3 Particle size distribution (PZD) and Density and specific volume

The PZD of the raw flours was determined in duplicates, using a S3500 series particle size analyzer (Microtrac Inc., Montgomeryville, USA) with deionized water, using three size ranges: <0.1 mm, from 0.1 to 0.5 mm and from >0.5 to 2 mm. The particulate material were determined by the gas displacement method in a pycnometer system AccuPyc II 1340 (Micromeritics, Norcross, USA), using helium as displacement medium.

3.4.4 Antioxidant capacity (AC-ABTS)

$ABTS^+$ antiradical capacity was determined according to Re et al., (1999) . The extract was obtained using methane (50% v/v) and acetone (70% v/v). $ABTS^+$ radical was prepared with the addition of 5 mL of an aqueous ABTS solution (7 mM) and 0.088 µL of K_2SO_5 solution (140 mM) in an amber flask for 16 h. After obtaining the radical, this solution was diluted in 95% ethanol to obtain a spectrophotometric reading of 0.7 ± 0.02 at 734 nm. The reaction was performed by mixing 30 µL of extracts with 3 mL µL of the radical solution, rested for 6 min. The absorbance of the reaction mixture and the control were read at 734 nm. A standard Trolox® (6-hydroxy-2,5,7,8-tetramethylchromo-2- carboxylic acid) curve (0–2.0 µM) was used and the results were expressed as Trolox equivalent antioxidant capacity (mmol Trolox / g).

3.4.5 Total phenolic compounds (TPC)

Total phenolic content was determined based on the methodology described by Singleton et al., (1965) using the Folin-Ciocalteu method. The extraction of phenolic compounds was performed using acetone (70% v/v). An aliquot of Folin-Ciocalteu reagent (1.25 mL at 10%) was added to 250 µL of the extract and reacted for 2 min at room temperature. Then, 1 mL of sodium carbonate (7.5% w/v) was added, taken to a water bath at 50 °C for 15 min, and cooled in an ice bath. The absorbance was measured at 760 nm in a UV–Vis spectrophotometer (Shimadzu 1800, Japan) and the blank was prepared by replacing the sample with distilled water. Gallic acid (5-130 mg/L) was used as standard and a calibration curve was constructed. Measurements were performed in triplicate and the results were expressed in equivalent micrograms of gallic acid/g of dry weight sample (µg GAE / g)

3.4.6 Statistical analysis

Analysis of variance (tow-way ANOVA), Tukey's test at 5 %, Principal Component Analysis (PCA), Pearson's coefficient (r) and Hierarchical Clustering on Principal Components (HCPC) were performed. All analyzes were performed using R software version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

3.4.7 RESULTS AND DISCUSSION

The volumetric mean D[4.3], arithmetic D[3.2] and span are shown in Table 1 and Figure 1(ab). The hierarchical classification (Figure 1b) formed 4 groups (SDG-SB, MDG, MEB-MB and SEB).

Significant differences were observed between M (Millet) and S (Sorghum) for D[4,3], d[3,2] and span, these results may be related to the chemical composition of the outer layers of the grains, which decrease the hardness, making the decortication process easier (EARP; MCDONOUGH; ROONEY, 2004).

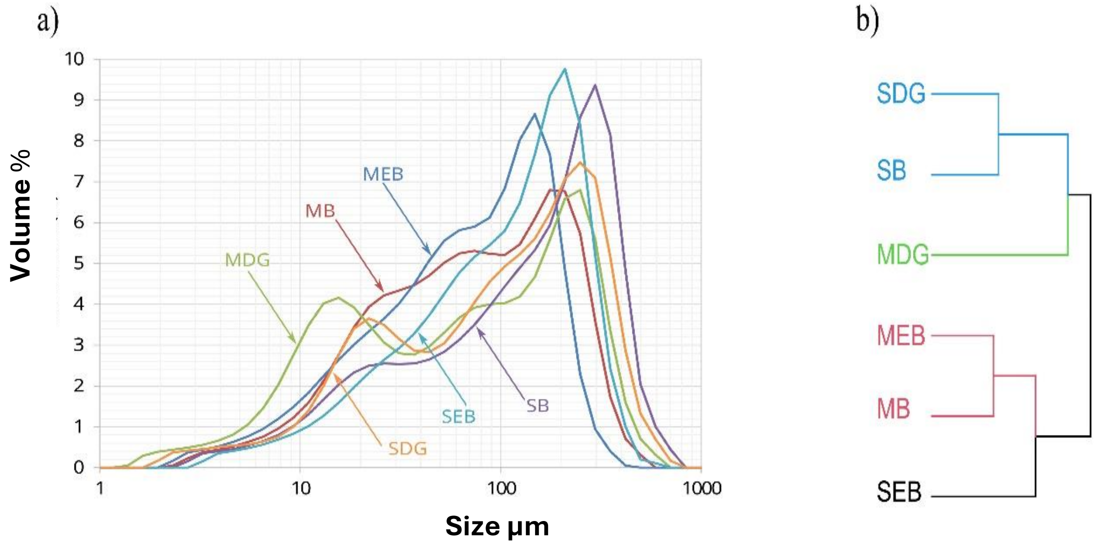

Figure 1: Particle sizes brans, extruded brans and decorticated grains. (a) Particle size distribution (b) Hierarchical classification. SB, Sorghum bran; MB, Pearl millet bran; MEB, Pearl millet extruded bran; SEB, Sorghum extruded bran; MDG, Pearl millet dehulled grain and SDG, Sorghum dehulled grain.

The highest D[4.3] was observed in SB (161.30 µm) and the lowest 81.3 µm was observed in EMB. After extrusion D[4,3] had a significant reduction of 24.82% in SB when compared to ESB and a reduction of 16.46% in MB. As for D[3,2], the DMG and DSG fractions showed the lowest values 21.3 and 32.1 µm, respectively. The span index in SB was reduced by 11.44% which indicates a greater homogeneity of the sample after extrusion. This same behavior, after extrusion, was reported by Cao et al., (2021) in their study with extruded rice bran, where the authors attributed these effects to the high temperature, pressure, and shear force present in the extrusion process.

The sorghum and millet fractions were subjected to density and specific volume analysis (Table 1). The density ranged from 1.39 to 1.45 (g / cm^3). The highest densities were observed in the DMG and DSG fractions. The specific volume was inversely proportional to density, where the lowest values were observed in the DMG and DSG fractions. The extrusion process caused a significant difference between treatments. A similar effect was reported by Vargas-Solórzano et al., (2014) in their study with extruded flours.

Table 1: Specific volume, Density, D[4,3], D[3,2], span, TPC, and ABTS for millet and sorghum, in the bran, extruded bran and decorticated grain fractions.

Grain	Fraction	SV (cm³ / g)	Density (g / m³)	D[4,3] (µm)	D[3,2] (µm)	Span (%)	TPC (µgGAE / 100 g)	AC-ABTS (µmol TE / g)
M	B	0,72±0.0^{Aa}	1.39±0.0^{Bb}	97.37±10.2^{Ab}	32.26±3.08^{Ab}	3.00 ± 0.1^{Ba}	1225.72±79.83^{Aa}	41.1±0.97^{Bb}
M	EB	0,72±0.0^{Aa}	1.39±0.0^{Bb}	81.31±4.2^{Bb}	29.2±2.19^{Ab}	2.81 ± 0.0^{Ba}	1240.90 ± 20.00^{Aa}	44.89±0.4^{Ab}
M	DG	0,70±0.0^{Bb}	1.43±0.0^{Aa}	105.84±6.6^{Ab}	21.32±1.64^{Bb}	3.81 ± 0.2^{Aa}	272.30 ± 27.71^{Ba}	9.57±0.7^{Ca}
S	B	0,71±0.1^{Bb}	1.40±0.0^{Bb}	161.3±5.8^{Aa}	41.98±2.62^{Aa}	2.36 ± 0.0^{Bb}	1023.86 ± 8.1^{Bb}	49.22±1.47^{Ba}
S	EB	0,72±0.1^{Ab}	1.39±0.0^{Bb}	121.25±12.0^{Ba}	44.8±4.03^{Aa}	2.09 ± 0.1^{Cb}	1106.87 ± 2.65^{Aa}	55.51±0.72^{Aa}
S	DG	0,69±0.0^{Cb}	1.45±0.0^{Aa}	132.9±2.4^{Ba}	32.10±2.0^{Ba}	2.82 ± 0.2^{Ab}	99.78 ± 2.65^{Cb}	4.55±0.35^{Cb}

Capital letters in the same column indicate differences between fractions within the same grain ($p<0.05$), by tukey test.
Lower case letters in the same column indicate difference between grains within each fraction ($p<0.05$), by tukey test. Values expressed as mean ±standard deviation. M: pearl millet. S: sorghum, SV: specific volume, D[4,3]: arithmetic average, D[3,2]: volumetric average, Span: particle polydispersity index, TPC: total phenolic compounds and AC – ABTS: antioxidant capacity.

The results of the analyzes are shown in Table 1. The values for TCPs were in the range of 99.78-1240.94 µgGAE / 100 g while AC ranged from 4.55 to 55.51 µmol TE / g. The lowest TCPs and ACs were observed in DSG and DMG, which was expected since decortification essentially removes the outer pericarp of the grain, mainly non-starch polysaccharides and phenolic compounds (BARBHAI; HYMAVATHI, 2022).

The extrusion process did not cause statistical difference between MB and EMB. However, significant increases were identified in TPCs levels in SEB when compared with SB, the same behavior reported by Ortiz-Cruz et al., (2020) in extruded sorghum bran with 30% moisture. This increase due to extrusion can be explained by the structural modification of cell walls under high temperature, pressure, and shear conditions, which promotes cell wall matrix disintegration and the breaking of covalent bonds in high molecular weight polyphenolic complexes, improving the accessibility of phenolic compounds (SALAZAR LOPEZ *et al.*, 2016).

In AC analyses, extrusion caused a significant increase for all treatments, the highest AC was observed in ESB (55.51 µmol TE / g). The AC in cereals has been mainly related to phenolic acids and non-starch polysaccharides present in the cell wall (TAKOUDJOU MIAFO *et al.*, 2022). The breakage of covalent bonds caused by the extrusion process reflects the increase in AC and TPCs (TAYLOR; DUODU, 2015).

The two principal components (PC) explain ~96.3% of the data variance (Dim1 67%, Dim2 29.3%). PCs analysis (Figure 2a) showed the differences between decorticated fractions (DMG and DSG) and bran with and without extrusion (MB/EMB and SB/ESB). The main variables that characterized the raw and extruded meals were the physical properties, SV and D [3,2] and the functional properties AC-ABTS and TPC, while the decorticated fractions showed higher D[4,3]. PC were used to apply HPCP (Figure 2b), resulting in 3 groups depending on the treatment applied, namely decorticated grain (MDG and SDG), millet bran and extruded bran (MB and MEB) and sorghum bran and extruded bran. (SB and SEB). Pearson's correlation coefficient (Figure 2c) showed a high positive correlation ($0.90 > r \leq 0.99$) between TPC - AC-ABTS, AC-ABTS - SV and TPC-SV and a high negative correlation ($0.70 > r \leq 0.90$) between span-D[3,2], Density -SV, TPC-Density and Density-AC-ABTS.

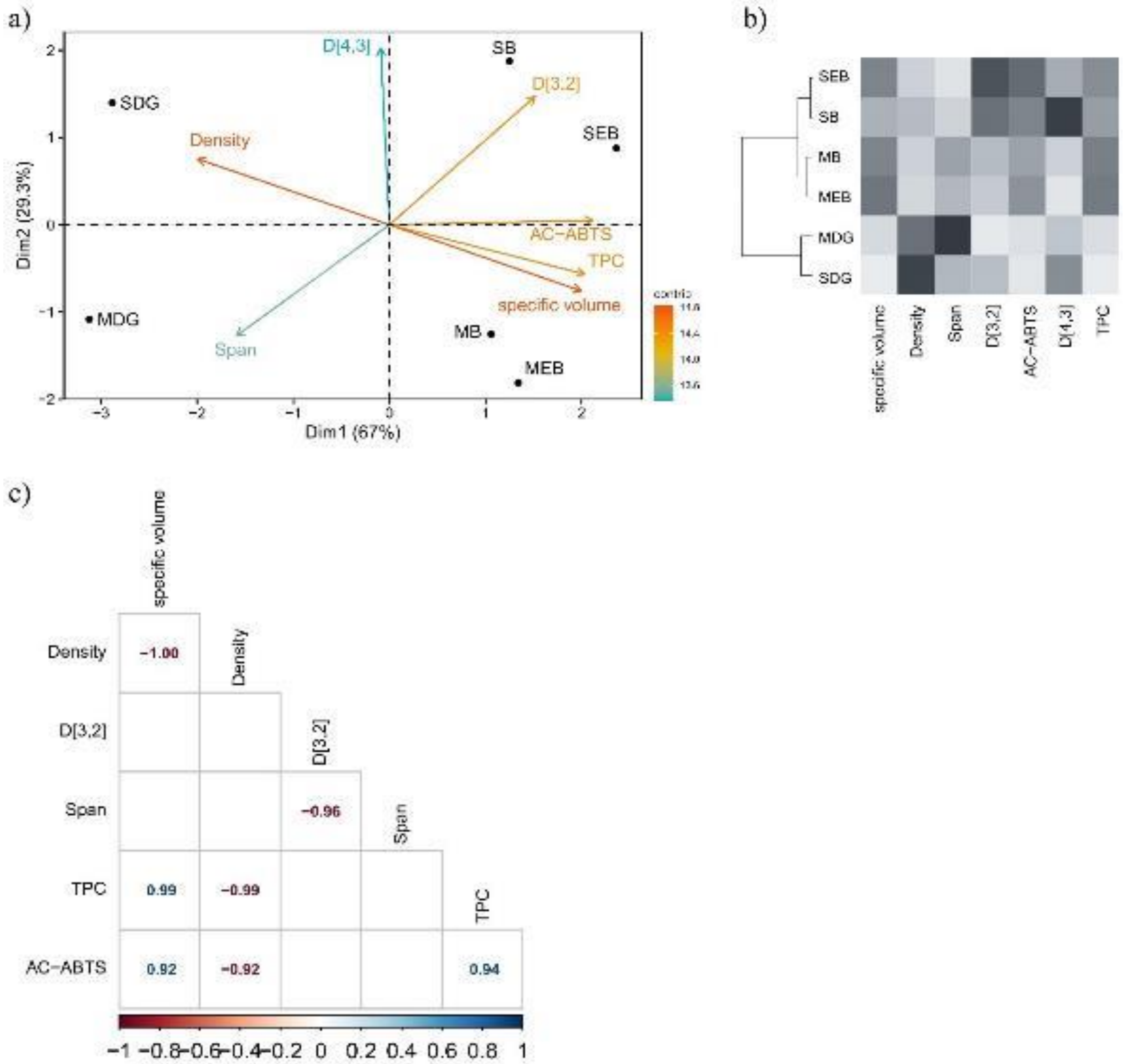

Figure 2: (a) Principal component analysis (PCs), (b) hierarchical clustering on principal components (HCPC) and (c) Pearson's correlation coefficient for bran, extruded bran and decorticated grain.

3.5 CONCLUSION

The study showed that thermoplastic extrusion significantly increased the total phenolic compounds (TPC) and antioxidant capacity (AC) in sorghum and millet brans. Principal component analysis and hierarchical classification showed that the bran fractions had more intense functional properties (TPC and CA) than the decorticated fractions. Pearson correlations indicated a strong relationship between TPC, CA and specific volume (SV). The results show that extrusion was effective in concentrating the bioactive compounds of these brans that can be applied in the food and nutraceutical industries.

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4 CAPÍTULO III

THERMOPLASTIC EXTRUSION AS A PRE-TREATMENT FOR SUPERCRITICAL EXTRACTION OF BIOACTIVE COMPOUNDS IN SORGHUM (S*orghum bicolor* (L.) Moench) AND PEARL MILLET (*Pennisetum glaucum* (L.) R.BR.) BRAN

THERMOPLASTIC EXTRUSION AS A PRE-TREATMENT FOR SUPERCRITICAL EXTRACTION OF BIOACTIVE COMPOUNDS IN SORGHUM (S*orghum bicolor* (L.) Moench) AND PEARL MILLET (*Pennisetum glaucum* (L.) R.BR.) BRAN

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4.1 ABSTRACT

Pearl millet (*Pennisetum glaucum* (L) R.Br.) and sorghum (*Sorghum bicolor* (L.) Moench) are cereals rich in bioactive compounds of considerable antioxidant capacity, that are mainly concentrated in the bran. However, bran is an underutilized resource as resulting from refined flour, despite of its health benefits. Green technologies such as supercritical fluid are known to extract bioactives. This study evaluated the impact of thermoplastic extrusion (TE) as pre-treatment of pearl millet and sorghum bran for supercritical extraction (SFE). The physical properties analyzed were particle size distribution (PZD), colorimetric analysis, pasting properties (PP), water absorption (WAI) and solubility indices (WSI). Chemical analyses such as proximal composition, total phenolic compounds (TPC), antioxidant capacity (AC) by FRAP and ABTS. Kinetic and yield studies were also performed. The extruded samples after milling showed greater homogeneity and apparent density (29.72 %), but lower porosity. WAI showed an increase, while WSI showed a reduction The colorimetric analysis showed ΔE of 8.49 for EMB and 9.78 for ESB. In SFE, the highest concentration of total phenolic compounds (TPC) was 21.34 (mg GAE/g extractc) obtained at 40 °C, 10 MPa and 15 % co-solvent. The highest antioxidant activity (CA) for ABTS was 4.47±0.01 (µM TE / g extract) and for FRAP was 157,93±0.61 (µM TE / g extract), obtained using 50 °C, 10 MPa and 15 % cosolvent and 50 °C, 20 MPa and 05% cosolvent respectively. The variables temperature (T), pressure (P) and co-solvent (C%) directly influenced the TPC and CA-ABTS/FRAP. In the kinetic study, the highest yield was 20 % for ESB and the fraction of extract recovered in the CER phase was higher in MB (79.71 %) with greater total recovery in the extruded sample. The results of the study demonstrate that TE significantly modified the physical and chemical properties of sorghum and millet brans. In the SFE process, the temperature, pressure and co-solvent conditions directly influenced the concentration of the analyzed compounds. SFE and TE are green and efficient technologies for obtaining extracts rich in bioactive compounds.

4.2 INTRODUCTION

Cereals are staple foods, widely grown around the world. They provide nutrition and several benefits to human and animal health (OFOSU *et al.*, 2021). Two non-traditional cereals have great potential source of bioactives, sorghum [*Sorghum bicolor* (L.) Moench] and pearl millet [*Pennisetum glaucum* (L.) R. Br. Both are originated in Africa, domesticated between 3,000 and 5,000 years ago. Sorghum is among the five most important cereals in the world, preceded by wheat, rice, corn and barley (DE MORAIS CARDOSO *et al.*, 2017). According to FAO (2023) the world production of sorghum was 61.36 million tons in 2021. Pearl millet is the sixth most produced cereal in the world. The world production of pearl millet is estimated at 30.89 million tons per year, of which 95.7% is produced in tropical and semi-arid environments of Asia and Africa (FAOSTAT, 2023). In Brazil, pearl millet covers an estimated area of more than 4 million hectares, whereas sorghum occupies an area of 1.04 million hectares. Particularly, pearl millet is essentially used as an intermediate crop between corn and soybeans, with the aim of improving soil conditions for these main crops (DIAS-MARTINS *et al.*, 2018b; FAOSTAT, 2023).

The cultivation of these cereals is extremely important culturally and economically for Asian, African and semi-arid regions, being widely used for human food. In the Americas, such as the USA and Brazil, sorghum is mostly used for animal feed whereas pearl millet for soil recovery and also for animal feed. Nowadays, there has been observed a growth interest of human consumption for functional foods as source of bioactive compounds and gluten-free (DE MORAIS CARDOSO *et al.*, 2017). Those cereals are interesting sources offering alternatives for diet diversification.

Cereals in general are usually debranned to improve the taste and biological value of porridges and flours intended for human consumption. In this process, about 35% of the total grain weight is lost, generating a substantial amount of bran (DE MORAIS CARDOSO *et al.*, 2017). However, bran remains an underutilized resource, despite containing a diverse range of compounds beneficial to health, including polyphenolic compounds such as phenolic acids, tannins, and flavonoids as well as unsaturated fatty acids such as linoleic and oleic acid (DONG *et al.*, 2019).

Despite their richness in bioactive compounds, cereal brans are usually destined for the production of animal feed or most of the time remain without adequate disposal, causing economic and environmental impacts (MORE; JAMBRAK; ARYA, 2022). The full use of food matrices is one of the United Nations (UN) Sustainable Development Goals (SDGs) that aims to overcome sustainability challenges through sustainable patterns of production and consumption (SDG 12). The latest UN report shows positive results, indicating a growing awareness of the need to prioritize sustainable practices in all sectors (UN, 2023).

The bioactive compounds present in the bran fraction have motivated researchers to study and improve extraction, concentration, purification, and application techniques (AFRIN; SIDHU, 2021; TUHANIOGLU; UBEYITOGULLARI, 2022; XIONG *et al.*, 2022). The extraction of bioactive compounds is of interest to produce functional foods, pharmaceutical products, and dietary supplements. The efficiency of extraction requires advanced pretreatment and extraction techniques. Supercritical fluid extraction (SFE) and thermoplastic extrusion (TE) clearly fall into this category (HERRERO; IBAÑEZ, 2018; VIGANÓ *et al.*, 2016).

Thermoplastic extrusion is a versatile process that combines operations such as mixing, cooking, compression, friction, expansion, texturing and molding (SINGH GUJRAL; SINGH, 2002; XU *et al.*, 2016). In this technique, the starch and protein material present in the matrix are modified under different combinations of process parameters, which provides a unique opportunity to prepare a new product (LIU *et al.*, 2011). Positive effects related to the use of thermoplastic extrusion were reported by Wu et al., (2020) in their study with noodles produced with extruded rice bran, where an increase in the content of phenolic acids was observed, such as vanillic acid, ferulic acid, coumaric acid and protocatechuic acid. Similarly, when studying different extruded sorghum genotypes, Campelo et al., (2020) e Vargas-Solórzano et al., (2014) evidenced an increase in fiber, phenolic compounds and antioxidant activity showing extrusion cooking can also be used as a pre-treatment for other extraction processes such as SFE. Similar findings of the outstanding antioxidant capacity and health benefit of whole grain extruded flour of pearl millet has been addressed (DIAS-MARTINS *et al.*, 2018b; DOS SANTOS *et al.*, 2024; THEODORO *et al.*, 2024)

SFE is a green technology with potential applications for the food industry and has been widely used for the extraction of bioactive compounds in different raw materials (CLÍMACO; VARDANEGA; FASOLIN, 2023; MOREIRA *et al.*, 2023; TEJEDOR-

CALVO *et al.*, 2021). The SFE technique uses properties of the supercritical fluids that can be altered by manipulating temperature and pressure, making it possible to modulate their density and consequently the solvation power and selectivity (FRATERRIGO GAROFALO; TOMMASI; FINO, 2020). Because of these physical properties, supercritical fluids act as excellent solvents, since they have a high capacity to penetrate a solid matrix and solubilize the solute even when it is strongly bound to the cell wall (L. ZABOT; N. MORAES; ANGELA A. MEIRELES, 2014). The use of supercritical carbon dioxide (sc-CO_2) as a solvent is also an excellent alternative to replace conventional organic solvents (PEREIRA; MEIRELES, 2010). However, a limiting factor of the use of CO_2 is its polarity being then more efficient to extract nonpolar compounds of low molecular weight. Nevertheless, this can be overcome with the use of cosolvents such as ethanol, which has the function of modifying the polarity and increasing the extraction yield of polar compounds (MORE; JAMBRAK; ARYA, 2022).

SFE technology has been used to extract bioactive compounds from cereal bran such as wheat, rye, rice, oats, sorghum, and millet (FRATERRIGO GAROFALO *et al.*, 2023; LIU *et al.*, 2020; POVILAITIS; VENSKUTONIS, 2015; SAHU; KUNDU; SETHI, 2021; SHI *et al.*, 2015; TUHANIOGLU; UBEYITOGULLARI, 2022). The literature does not report any studies using thermoplastic extrusion as a pre-treatment for supercritical extraction in sorghum and millet bran. However, LIU et al., (2020) used TE as a pre-treatment to obtain oil from oat bran (*Avena Sativa* L.) by SFE, while Uquiche, (2022) used the same technologies in combination to obtain extracts from red pepper (*Capsicum annuum* L.). The authors identified positive effects on antioxidant capacity and phenolic compound content for extracts obtained with extruded oat bran, while the carotenoid content in red pepper was lower in the extracts obtained using TE as a pre-treatment. The authors emphasized the benefits of the two technologies together and the need for further studies to gain a deeper understanding of the processes and their effects.

In this context, the objective of this study was to explore sorghum and pearl millet bran as a potential source of bioactive compounds evaluating the thermoplastic extrusion (TE) as a pretreatment for the SFE extraction. The supercritical extracts obtained were characterized for extraction yield, chemical composition, and antioxidant activity.

4.3 Materials and methods

4.3.1 Material

4.3.1.1 Sorghum and pearl millet grains.

The grains of millet hybrid ADRg9070 were kindly donated by Atto Sementes (Rondonópolis, Brazil) and sorghum by Embrapa Milho e Sorgo (Sete Lagoas, Brazil). The whole grains were cleaned to remove foreign materials, broken grains, and other types of grain in a Clipper Office Tester 400/B (A.T. Ferrell Co., Bluffton, USA). After cleaning, the grains were debraned in a benchtop test rice mill model MT-97, nº 3788-5 (Suzuki, Santa Cruz do Rio Pardo, Brazil) using the specific compartment where the grain was abraded to remove the pericarp at a flow rate of 150 g for 10 min and 5 min, respectively for pearl millet and sorghum. The bran was then ground in a hammer mill model 3100 (Perten Instruments AB, Huddinge, Sweden). At the end of this process, the following were obtained: sorghum bran (SB), pearl millet bran (MB).

4.4 Extrusion processing

The sorghum and millet bran were packed at 14% moisture (wet basis) and stored in plastic bags overnight in a refrigerator to even out water absorption. The samples were processed in a 19/20 DN single-screw laboratory extruder (Brabender, Duisburg, Germany), coupled to a Plasti-Corder Lab Station torque rheometer (Brabender, Duisburg, Germany). The extruder was equipped with a 3:1 compression screw; 3 mm circular die; three heating zones from feed to outlet set at: 40, 80 and 120 °C, respectively; screw speed set at 200 rpm and volumetric vertical screw feeder (Brabender, Duisburg, Germany) at 25 rpm, which equates to ~ 4.0 kg / h (TOLEDO *et al.*, 2020). At the end of this process, the following were obtained: pearl millet extruded bran (EMB) e sorghum extruded bran (ESB)

Immediately after the extrusion process, the extrusion was collected and dried in a forced-air oven at 55 °C. They were then ground in a hammer mill. Bran and extruded bran were vacuum-packed in polyethylene bags, protected from light and kept at freezing temperature until extraction and analysis.

4.5 Bran characterization

4.5.1 Proximal composition

The sorghum and pearl millet bran samples were analyzed for their centesimal composition: moisture, ash, lipids, protein and dietary fiber (soluble and insoluble) using the methods: 931.04, 923.03, 945.38, 2001.11, 985.29 (AOAC, 2010). The total carbohydrate content was calculated by difference and the energy value of the sample was calculated by applying the factors 4, 9 and 4 for each gram of protein, lipid and carbohydrate, respectively according to the method of (SHRESTHA; NOOMHORM, 2002).

4.5.2 Particle size distribution

The particle size distribution of the bran was determined using an S3500 series particle size analyzer (Microtrac Inc., Montgomeryville, USA) with deionized water, using three size ranges: <0.1 mm, from 0.1 to 0.5 mm and from >0.5 to 2 mm.

4.5.3 Absolute density, bulk density and porosity

Absolute density was determined using an AccuPyc II-1340 helium gas pycnometer (Micromeritics, Norcross, USA). The analysis consisted of 10 gas purges for each sample at a constant temperature of 24-26 ºC, pressure rate of 3.45×10^{-5} MPa with a maximum pressure of 0.13 MPa. The samples were placed in a 100 cm^3 aluminum container, weighed and placed in the pycnometer chamber. After the gas purge cycles, the readings were recorded in the Accupyc II 1340 software (Micromeritics, Norcross, USA) (BERNARDO *et al.*, 2018). The bulk density was determined in quadruplicate according to (ORTEGA-RIVAS, 2009). The masses of the samples were freely poured into a graduated cylinder leveled at 200 cm^3. The particle bed was obtained by placing the graduated cylinder under the outlet of a volumetric single screw feeder at 20 rpm (Brabender, Duisburg, Germany) dropping 50 g and then dropping 50 g of sample at 2 kg / h. The feed rate (kg / h) was calculated by interpolating the rotation speed curves (rpm) as a function of the particle mass, collected in 1 minute (VARGAS-SOLÓRZANO *et al.*, 2020). Porosity was calculated according to equation 3 a below.

$$\text{Porosity}\ \% = \left(1 - \frac{\text{Bulk density}}{\text{Absolute density}}\ \text{x}\ 100\right) \qquad (3)$$

4.5.4 Colorimetric analysis

The color analysis of the bran was carried out by transmittance on a Hunterlab Colorquest colorimeter, model XE (Reston, Virginia, USA). A CIELAB and CIELCh scale will be used to measure Hunter's color parameters ($L*$, $a*$, $b*$ $C*$ and $h°$). The parameters $L*$ = luminosity (0 = black and 100 = white); $a*$ = (80$a*$ = (80 to 0 = green, 0 to +100 = red) and $b*$ = (100 to 0 = blue, 0 to +70 = yellow) were measured and recorded as indicated by the International Commission on Illumination (CIE, 2004). The color variation ($\Delta E*$) was calculated using Equation (4). The delta values ($\Delta L*$, $\Delta a*$ and $\Delta b*$) indicate how much the bran and extruded bran differ in L, a. and b. Three replicates were carried out for each sample.

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (4)$$

Where: $\Delta a*$, $\Delta b*$ and $\Delta L*$ are the differences between the standard and the sample in $a*$, b* and $L*$, respectively.

4.5.5 Water absorption index (WAI) and water solubility indexes (WSI)

Water absorption index (WAI) and water solubility index (WSI) were determined by the methodology described by Anderson et al., (1970) with modifications. Approximately 1 g of sample was weighed in falcon tubes, added 10 mL of distilled water, and homogenized for 20 s in a vortex mixer. Then, the tubes were kept at 25 ± 5°C without agitation for 30 min. After, the tubes were centrifuged in a Universal 320R (Hettich) for 15 min at 7000 rpm. The supernatant was dried in a fan oven (Hauber Macanuda, Joinville, Brazil) at 105 °C until constant weight. The precipitated was weighed. WAI and WSI were calculated according to Equations (5) and (6)

$$\text{WAI(g/g)} = (\frac{\text{Weight of wet sediment}}{\text{Dry weight of sample}} \text{ x } 100) \quad (5)$$

$$\text{WSI (\%)} = (\frac{\text{Weight of dispersed solids in the supernatant}}{\text{Dry weight of sample}} \text{ x } 100) \quad (6)$$

4.6 Supercritical fluid extraction (SFE)

The sorghum and pearl millet extruded and non-extruded bran were transported to the Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Plant Extracts (LASEFI) at the State University of Campinas (UNICAMP), located in the city of Campinas (São Paulo, Brazil) for the sequencing of this study. The supercritical fluid extractions were carried out using the MV 10 ASFE System (Waters, Milford, MA, USA), equipped with a 25 cm^3 extraction cell. The extraction cell was filled with the sample (approximately 10 g) and the extraction bed had an apparent density of 0.464 g / cm^3. The tests were carried out in a randomized manner based on a complete factorial experimental design (3 × 3 × 2), using ethanol as the co-solvent. Different levels of pressure (10, 20 and 30 MPa), temperature (40, 50 and 60 °C) and percentage of cosolvent (5 and 15 % (m/m)) were evaluated, totaling 18 experiments. The ratio between the mass of solvent and the mass of raw material (S/F) was set at 25 and the flow parameters are shown in table 1.

Table 1: Parameters used to extract bioactive compounds from extruded and non-extruded sorghum and millet bran using supercritical CO_2.

Extractions	Co-solvent (% m / m)	Flow rate CO_2 (cm^3 / min)	Flow rate CO_2 (g / min)	Flow rate co-solvent (cm^3 / min)	Flow rate co-solvent (g / min)	S/F	Time (min)
CO_2 + EtOH	5	2.13	1.9	0.13	0.1	25	145
	15	1.9	1.7	0.38	0.3	25	145

EtOH: Ethanol; S/F: Ration between mass of solvent and mass of feedstock (m/m).

After optimizing the pressure, temperature and cosolvent percentage parameters, a kinetic study of the process was carried out to better understand and optimize the extraction steps and time. The kinetics was be carried out in the Spe-ed SFE supercritical fluid extraction unit (Applied Separations, USA), equipped with a 300 cm^3 extraction vessel. The extraction cell was filled with 50 g of sample and the final volume was filled with glass beads, so the bed had an apparent density of 0.541 g / cm^3. The ratio between the mass of solvent and the mass of raw material (S/F) was set at 25, the CO2 flow rate at 4048.06 cm^3 / min and the ethanol flow rate at 1.9 cm^3 / min. The extraction curve was be determined under the extraction condition with the highest yield of the compounds of interest. The extraction conditions of the optimized process were maintained, in relation to the apparent density of the bed. Throughout the extraction kinetics, 10 experimental points were taken. The extraction curves were fitted to the models proposed by (MEIRELES, 2008) (Equations 7, 8 and 9). Equation 7 represents the constant extraction rate (CER) stage. Equation 8 is related to the decreasing extraction rate (FER) stage. Finally, Equation 9 represents the adjustment to the diffusion period (DC). The fitted data defined the optimum condition for the solvent/raw material ratio.

$$y = b_0 + a_1 t \ ; \ t \leq t_{CER} \quad (7)$$
$$y = b_0 - a_2 t_{CER} + (a_1 + a_2)t \ ; \ t_{CER} < t \leq t_{FER} \quad (8)$$
$$y = b_0 - a_2 t_{CER} - a_3 t_{FER} + (a_1 + a_2 + a_3)t \ ; \ t_{FER} < t \quad (9)$$

Where: *y* is the extract mass (g), *b0* is the linear coefficient, *ai (i = 1, 2, 3)* is the angular coefficient, *t* is the time (me), t_{CER} is the time related to the increasing extraction rate, t_{FER} is the time related to the decreasing extraction rate.

4.7 Extracts characterization

4.7.1 Determining overall yield (X_0)

The ethanolic extracts were dried in a rotary evaporator MA 120 (Marconi, Piracicaba, Brazil) (T < 35 °C) to determine the extract yield. The overall extract yield was calculated according to Equation 10, based on the ratio between the mass of extract and the mass of sample.

$$X_0 = \frac{m_{extract}}{m_{sample}} \times 100 \quad (10)$$

4.7.2 Total phenolic compounds

The content of total phenolic compounds in the extracts was determined using the Folin-Ciocalteau method (SINGLETON; ORTHOFER; LAMUELA-RAVENTÓS, 1999). For analysis, 0.25 mL of the diluted extracts, 1.25 mL of 10% (v/v) Folin-Ciocalteau reagent and 1 mL of 7.5 % (m/v) sodium carbonate (Na_2CO_3) were added to cuvettes. The reaction was left to stand in a dark environment for 90 min at room temperature (25 ± 1°C). The absorbance was then read in a spectrophotometer 800 XI (Femto, São Paulo, Brazil) at 760 nm. The content of phenolic compounds was expressed as milligram equivalents of gallic acid (EAG) per gram of dry raw material (mg EAG / g extract) based on the equation of the standard curve of known concentrations of gallic acid (Figure 1S) .

4.7.3 Antioxidant capacity

The antioxidant capacity of the extracts obtained was determined by the extract's ability to neutralize the ABTS radical (2,2-azino-bis-(3 ethylbenzothiazoline-6-sulfonic acid) and by the ferric-tripyridyl-triazine complex reduction method (FRAP). The antioxidant activity using the ABTS radical was carried out according to the methodology proposed by Re and colleagues (1999). The ABTS solution was diluted in ethanol (C_2H_6O) until the absorbance reached 0.7 at 734 nm. 30 µL of extract and 2970 µL of

ABTS radical were added to a quartz cuvette. The cuvette was sealed with Parafilm M®, the mixture was homogenized, and after 6 min of reaction, it was read in a UV-vis spectrophotometer 800 XI (Femto, São Paulo, Brazil) at 734 nm. The antioxidant capacity of the extracts was determined using the Trolox standard curve and the results were expressed as μmol trolox equivalent (TE) per g de extract (μmol TE / g extract) (Figure 2S).

The FRAP method was performed according to an adaptation of the procedure described by Benzie; Strain (1996). An aliquot of 90 µL of extract was taken and added to a test tube. Next, 270 µL of distilled water and 2.7 mL of the FRAP reagent (0.3 M acetate buffer, 10 mM TPTZ (2,4,6-Tri-(2-Pyridyl)-1,3,5-Triazine) solution and 20 mM aqueous ferric chloride (FeC_{l3}) solution) were added. The tubes were kept in a heating bath at 37 °C for 30 min. The samples were read using a UV-vis spectrophotometer (Femto, 800 XI, São Paulo, Brazil) at 595 nm. The antioxidant capacity of the extracts was determined using the Trolox standard curve and the results were expressed as µmol Trolox equivalent (TE) per g de extract (µmol TE / g extract) (Figure 3S).

4.8 Statistical analysis

The experiments was carried out using a full factorial design to evaluate the effects of the following independent variables: bran pre-treatment (control or extruded bran), SC-CO2 extraction temperature (T: 40, 50 and 60 °C), SC-CO2 extraction pressure (P: 10, 20 and 30MPa) and cosolvent percentage (5 and 15%). The response variables (dependent) will be yield, total phenolic content, antioxidant capacity The effect of the parameters for both extraction methods was determined by analysis of variance (ANOVA) and Tukey's test, using Minitab 22 (Minitab Ltd., State College, PA, USA) and XLSTAT 24 (Addinsoft, Nova York, EUA) with a 95 % confidence level ($\alpha = 0.05$).

4.9 Results

4.9.1 Proximal composition

The proximal composition of the extruded millet and sorghum bran is shown in table 2. The analyses show that extrusion had a significant effect on all the treatments and gains evaluated. An increase was observed in ash, protein, dietary fiber and carbohydrates, while a decrease was observed in moisture, fat and calorific value.

The moisture content of the extruded and non-extruded bran varied between 5.33 and 8.14%. This is within the range recommended by the Codex-Alimentarius for Sorghum (14,5 %) and Pearl Millet (13 %) grain. The fat content ranged from 5.92 to 9.32 %. The extrusion process reduced the fat content by 19.53 % for sorghum bran and 20 % for millet bran.

Table 2: The centesimal composition for extruded and non-extruded brans pearl millet and sorghum.

Parameters	Pearl millet		Sorghum	
	Bran	**Extruded bran**	**Bran**	**Extruded bran**
Moisture (%)	8.03±0.1[b]	5.33±0.0[d]	8.14±0.0[a]	6.02±0.0[c]
Ash (%)	2.91±0.0[b]	2.98±0.0[a]	2.42±0.0[d]	2.51±0.0[c]
Protein (%)	15.12±0.0[b]	15.87±0.0[b]	10.81±0.0[d]	10.98±0.0[c]
Fat (%)	7.40±0.6[b]	5.92±0.3[c]	9.37±0.2[a]	7.54±0.5[b]
Dietary fiber (%)	44.68±0.0[d]	47.86±0.0[c]	48.94±0.0[b]	51.24±0.0[a]
Carbohydrate (%)	21.85±0.0[b]	22.04±0.0[a]	20.32±0.0[d]	21.7±0.0[c]
Calorific Value (kcal)	214.57±0.0[a]	204.92±0.0[c]	208.85±0.0[b]	198.67±0.0[d]

Different letters in the same row indicates significant differences between brans ($p < 0.05$)

This reduction shows that extrusion can be an alternative way of increase the shelf life of bran. Similar results for fat content in millet and sorghum bran were found by Barbhai et al., (2022) 7,85 (g / 100g) e Adebo et al., (2023) 5,71 (g / 100 g) respectively.

4.9.2 Particle size distribution

The volumetric mean D[4,3], arithmetic mean D[3,2] and amplitude are shown in table 3 and Figure 1(AB).

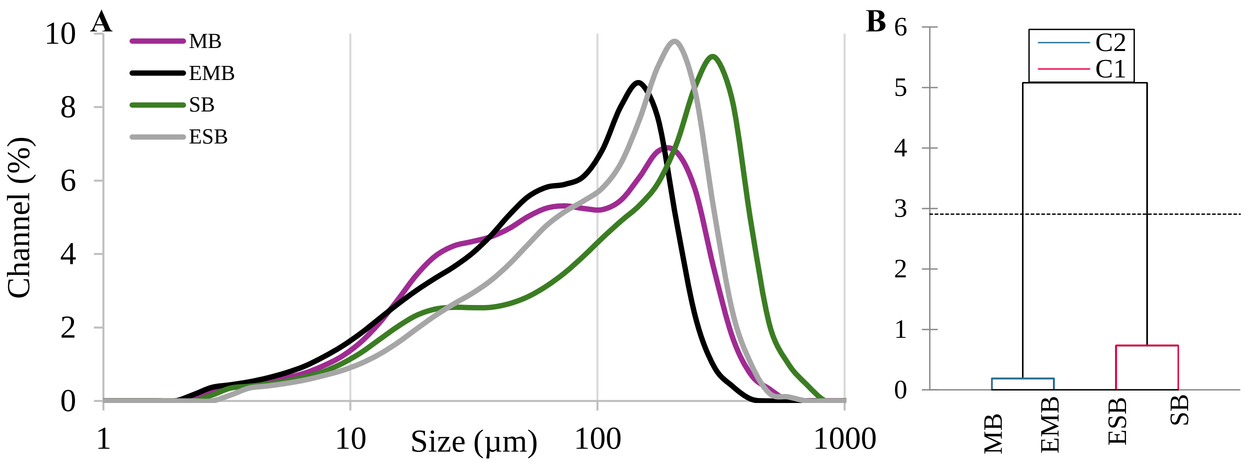

Fig. 1: A particle sizes for bran and extruded bran. (a) Particle size distribution (b) Hierarchical classification.

The hierarchical classification (Fig. 1B) formed two groups (MB-EMB and ESB-SB). Significant differences were observed between extruded and non-extruded sorghum and millet bran for D[4,3], d[3,2] and span, which may be related to the chemical composition of the outer layers of the grains (EARP; MCDONOUGH; ROONEY, 2004).

Table 3 Particle size averages for extruded and non-extruded sorghum and millet bran

Different letters in the same row indicates significant differences between brans ($p < 0.05$)

Parameters	Pearl millet		Sorghum	
	Bran	**Extruded bran**	**Bran**	**Extruded bran**
D[4,3] (%)	97.36±10.0[c]	81.31±4.2[d]	161.30±5.8[a]	121.25±12.0[b]
D[3,2] (%)	32.26±3.0[b]	29.20±2.1[b]	41.97±2.6[a]	44.80±4.0[a]
Span (%)	2.99±0.1[a]	2.80±0.0[b]	2.36±0.0[c]	2.09±0.1[d]

The highest D[4,3] was observed in SB (161.30μm) and the lowest 81.31μm was observed in EMB. Extrusion was significantly reduced by 24.82 % in SB compared to ESB and by 16.46 % in MB. The span index in SB and MB was reduced by 11.44 and 6 % respectively. This indicates greater homogeneity of the samples after extrusion. This same behavior after extrusion was reported by Cao et al., (2021). It is important to mention that both bran, non-extruded and extruded, were milled in the same mill type indicating that extrusion may have facilitated the reduction in size during the milling process, since it was a reduction in lipids and in moisture turning the bran less flexible thus prone to be cut into smaller particles

4.9.3 Physical properties

The sorghum and millet samples were analyzed for apparent density, absolute density, specific volume, porosity, WAI, WSI and color (table 4). For the specific volume analysis, the extruded millet bran sample (0.72 cm^3/g) was the only one to differ statistically from the other samples. Apparent density ranged from 0.26 g / cm^3 to 0.37 g / cm^3, with extruded sorghum and millet bran being significantly higher than non-extruded bran. The highest absolute density was observed in sorghum bran (1.41 g / cm^3), which was the only sample to differ significantly. As for porosity, significant differences were observed in all the samples studied, with millet bran showing the highest average of 81.07%. These results corroborate those obtained by Vargas-Solórzano et al. (2014) in their studies with extrudates obtained from different sorghum genotypes.

WAI measures the amount of water absorbed by the sample and can be used as an index too measure the interaction with an aqueous solution. WAI values ranged from 2.86 (millet bran) to 3.24 % (extruded sorghum bran). A slight increase can be seen in all extruded samples. However, when compared to the non-extruded samples, only millet samples showed a significant increase of 0.8 %. These results are close to those found by Barbhai e Hymavathi, (2022) (264.56±7.92 g / 100g) who studied Foxtail millet foxtail bran. Extrusion at low feed moisture can produce greater mechanical shear, resulting in more fragmented materials that have more exposed hydrophilic groups, leading to a lower apparent density and a higher WAI her WAI (HOU *et al.*, 2024; PHILIPP *et al.*, 2017).

Unlike WAI, WSI showed a significant reduction in all extruded samples, the lowest value being found in extruded sorghum bran (5.45 %). This same effect was reported by Cao et al., (2021), in his study with wheat bran, who observed a reduction in WSI in the extruded samples, which was associated with the formation of complexes such as lipid-starch complexes and Maillard reaction products during extrusion.

Table 4: Apparent density, absolute density and specific volume of raw and extruded bran of pearl millet and sorghum

Parameters	Pearl millet		Sorghum	
	Bran	Extruded bran	Bran	Extruded bran
Specific volume (cm^3 / g)	0.71±0.0^b	0.72±0.0^a	0.70±0.0^b	0.71±0.0^b
Apparent density (g / cm^3)	0.26±0.0^c	0.37±0.0^a	0.27±0.0^c	0.35±0.0^b
Absolute density (g / cm^3)	1.39±0.0^b	1.38±0.0^b	1.41±0.0^a	1.39±0.0^b
Porosity (%)	81.07±0.2^a	73.91±0.0^c	80.91±0.0^a	74.46±0.0^b
WAI (g / g)	2.86±0.0c	3.11±0.0b	3.22±0.0^a	3.24±0.0^a
WSI (%)	11.36±0.0a	9.03±0.0b	8.29±0.0c	5.45±0.0d
*L**	66.30±0.0^a	60.48±0.5^b	55.85±0.6^c	46.41±0.1^d
*a**	1.31±0.0^d	3.27±0.1^c	14.86±0.3^b	16.51±0.1^a
*b**	16.49±0.0^c	22.35±0.0^a	21.96±0.4^a	19.97±0.1^b
*C**	16.54±0.0^d	22.59±0.1^c	26.52±0.5^a	25.91±0.2^b
h°	85.44±0.0^a	81.67±0.2^b	55.91±0.1^c	50.42±0.1^d
*ΔE**		8.46		9.78

Different letters in the same row indicates significant differences between brans ($p < 0.05$

The color parameters (*L*, a* b*, C* and *h*°) of the extruded and non-extruded sorghum and millet bran are displayed in Table 4. The extrusion process significantly affected the color of the bran. The difference in color (*ΔE**) between millet bran and extruded millet bran was 8.46, and between sorghum bran and extruded sorghum bran this difference was even greater, 9.78. By increasing the temperature of the extruder the oxidation of pigments in cereals is enhanced, which affects color by forming brown pigments. The formation of brown pigments reduces the L^* value and increases the a^* and b^* values. A similar effect was reported by Jafari et al., (2017) in their study on extruded sorghum flour. Significant differences in ΔE^* were observed by Dias-Martins et al., (2024) when comparing pearl millet raw flour and extruded.

4.9.4 Pasting properties

The paste properties of extruded and non-extruded millet and sorghum bran are shown in Table 5 and Figure 2.

Table 5: Paste properties of extruded and non-extruded pearl millet and sorghum bran

Parameters	Pearl millet		Sorghum	
	Bran	**Extruded bran**	**Bran**	**Extruded bran**
Paste temperature (°C)	28.5±0.0^{b}	0.0±0.0^{d}	20.5±0.0^{c}	38.00±1.4^{a}
Initial viscosity (cP) at 25 °C	64.5±0.9^{b}	80.00±0.1^{a}	49.50±0.59^{c}	53.00±0.0^{c}
Peak viscosity (cP) at 95 ºC	71.50±6.3^{b}	80.00±1.4^{a}	49.50±4.9^{c}	53.00±5.6^{c}
Minimal cooling viscosity (cP)	59.00±2.8^{a}	35.00±1.4^{c}	33.59±4.9^{c}	42.50±0.7^{b}
Break down (cP)	12.50±9.1^{c}	45.00±2.8^{a}	16.00±0.0^{b}	10.50±0.2^{c}
Maximum cooling viscosity (cP)	87.00±1.4^{a}	48.00±1.0^{c}	63.00±1.4^{b}	65.00±1.4^{b}
Setback (cP)	28.00±4.2^{a}	13.00±0.0^{c}	29.50±3.5^{a}	22.50±2.1^{b}
Final viscosity (cP)	74.50±2.1^{a}	35.50±3.5^{d}	47.7±0.5^{c}	61.5±1.1^{b}

Different letters in the same row indicates significant differences between brans ($p < 0.05$

As expected, both brans presented very low paste profile as a clear indication of very low starch content when compared to the paste viscosity of whole grain flours. The paste properties of the extruded bran and non-extruded show that the results change depending on the source and applied treatment. It can be noticed that the shape and intensity of viscosity for millet was changed by the extrusion process, where peak viscosity was missed as an indication of the presence of native starch, as it swells during heating. Concerning sorghum there are a small difference than can be considered negligible.

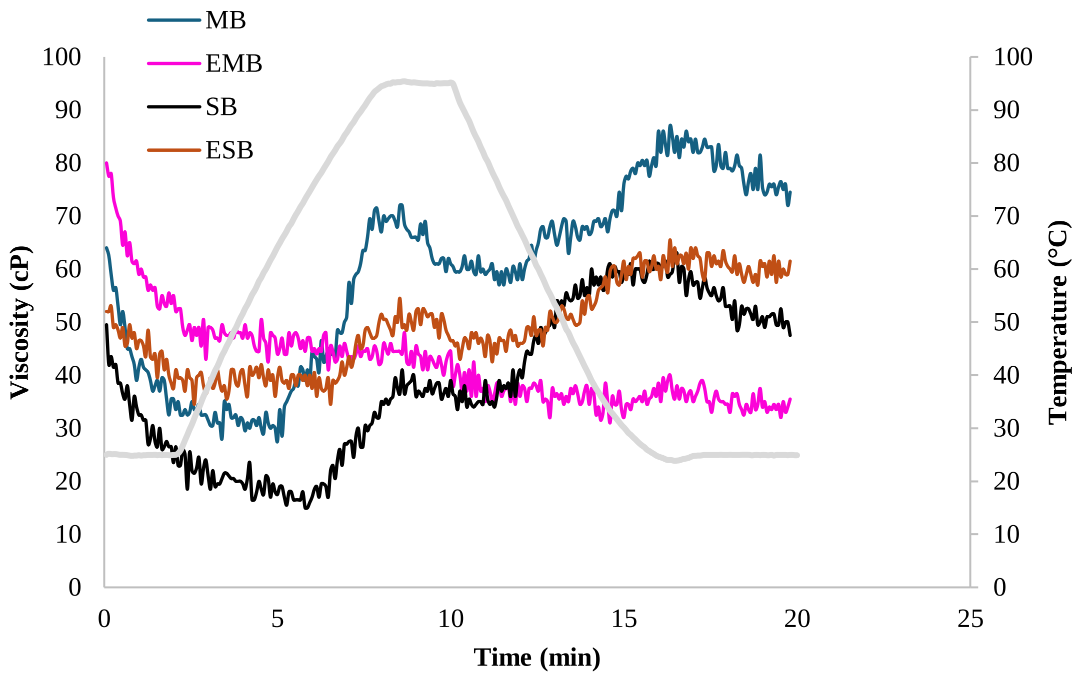

Fig. 2: Paste viscosity profile of extruded and non-extruded millet sorghum bran

Paste viscosity analysis was conducted to evaluate the functionality of the bran as a potential ingredient for various applications. It was also an indicator of the presence of starch granules that would affect the paste viscosity, if partial or totally intact starches granules would be present in the brans. For sorghum bran, the extrusion process did not cause significant changes in the maximum cold viscosity (cP), maximum hot viscosity (cP) and maximum cooling viscosity (cP), while the other properties of sorghum bran were altered. As for millet bran, all the properties changed significantly after the extrusion process. In the first 5 min of analysis (Fig. 2) MB, SB and ESB showed a certain degree of viscosity which increased during the heat cycle, and small shoulder-shaped bumps could be seen in this region. This may represent the presence of remnants of starch (intact fragments of amylopectin) capable of swelling even slightly (DOS SANTOS *et al.*, 2024). On the other hand, the EMB did not show any of these characteristics and in general, the samples did not show high viscosity peaks, since the bran has a low starch content and the combination of temperature and high shear (extrusion) caused irreversible damage to the starch in its original structure, converting it into broken starch, thus reducing the viscosity peaks, but presenting a slight increase in the cold viscosity (VARGAS-SOLÓRZANO et al., 2014).

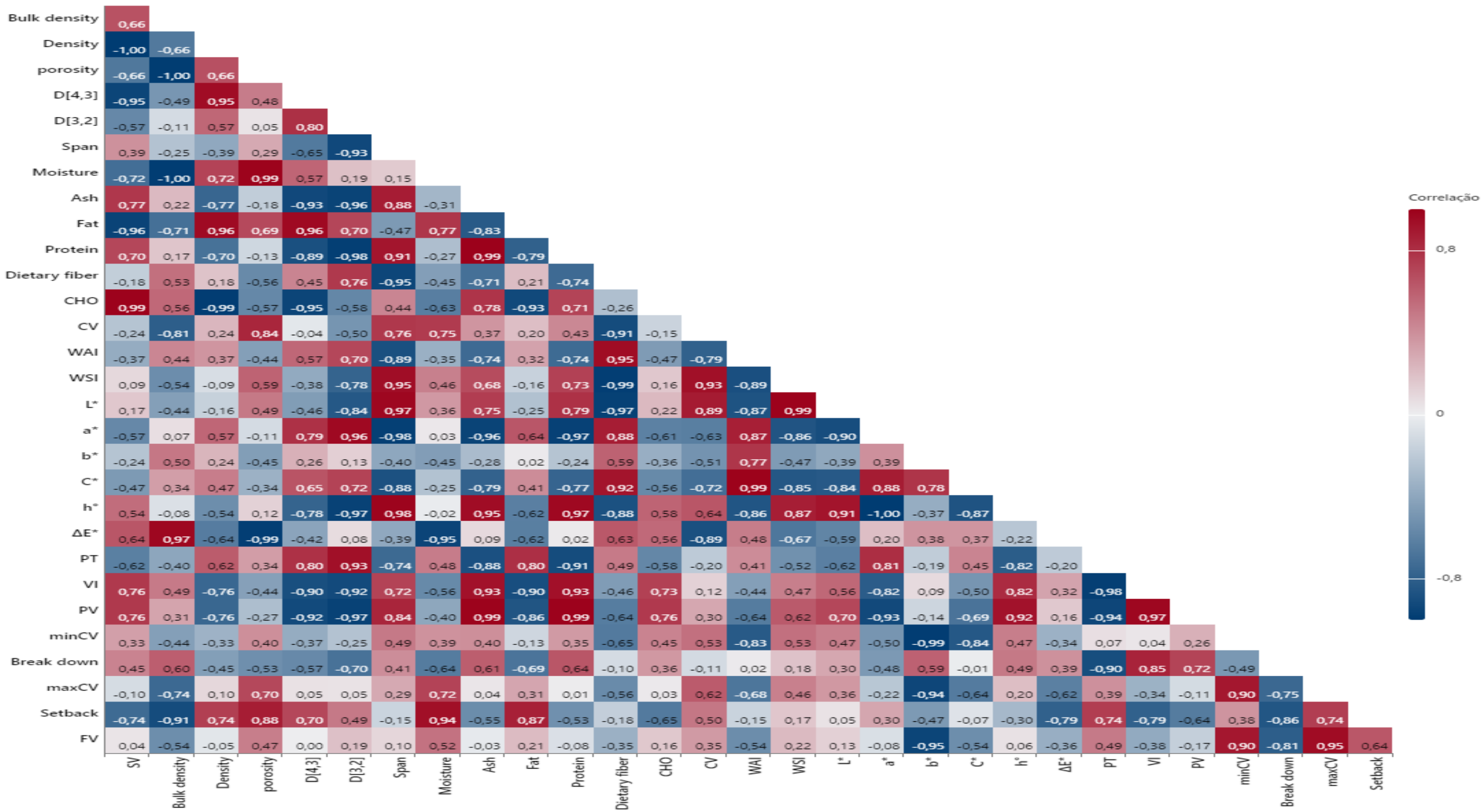

Fig. 3: Correlation matrix plot depicting the correlations between different variables. CHO, Carbohydrate; CV, calorific value; WAI, water absorption index; WSI, water solubility indexes; VI, initial viscosity; PV, peak viscosity at 95 °C; minCV, minimal cooling viscosity; maxCV, maximum cooling viscosity; FV, final viscosity.

4.9.5 Correlation analysis

Correlation analysis examines the relationship between two or more quantitative variables, assuming a linear connection. It measures the strength and direction of this relationship using the Correlation Coefficient (r), which ranges from -1 to +1. The study utilized Pearson correlation to analyze variable relationships, with results visualized in a correlation matrix (Fig. 3). Positive correlations are represented in red and negative in blue, with color intensity indicating the association's strength. The r-values, ranging from -1,00 to 0,99, revealed both positive and negative correlations, highlighting potential.

Strong positive and negative relationships were presented (Fig. 3) confirming the previous analyses. SV showed a strong positive relationship with CHO (0.99), bulk density a strong positive relationship with *ΔE** (0.99), density a strong positive relationship with fat (0.96), porosity a strong positive relationship with moisture and a moderate relationship with CV (0.84), D[3,2], D[4.3] and span showed a strong positive relationship with lipids (0.96), PT (0.93) and WSI (0.95), protein had a strong positive relationship with VI (0.93) and PV (0.99) and ash (0.99), dietary fiber showed a strong negative relationship with *L** and a strong positive relationship with WAI (0.95) moisture showed a strong relationship with delta and setback.

4.9.6 Multivariate analysis

PCA was performed to study the influence of thermoplastic extrusion (TE) and similarities between sorghum and pearl millet brans using physical, physicochemical, technological, and colorimetric analyses to determine their clustering (Fig. 4). Components PC1 (51.36 %) and PC2 (35.99%) showed 87.35 % variability. In PCA, vectors in proximity are positively correlated and vectors far apart are negatively correlated.

The length of each vector reflects the variability of parameters relative to the two principal components (PCs), with positively correlated parameters appearing close together. The proximity of sample points to vectors and their distance from the origin in biplots indicate the vectors' influence on the samples (PRASHANTH *et al.*, 2024).

The influence of TE on pearl millet and sorghum brans is displayed in Fig. 4A. EMB presents proximity to most of the analyses carried out, namely *ΔE**, bulk density, BD, SV, CHO, VI, PV, ash, and proteins, ESB present proximity to color properties (*a**,

b^* and C*) and is closely linked to WAI and dietary fiber, which can be confirmed by the green color in the heatmap (Fig. 4B).

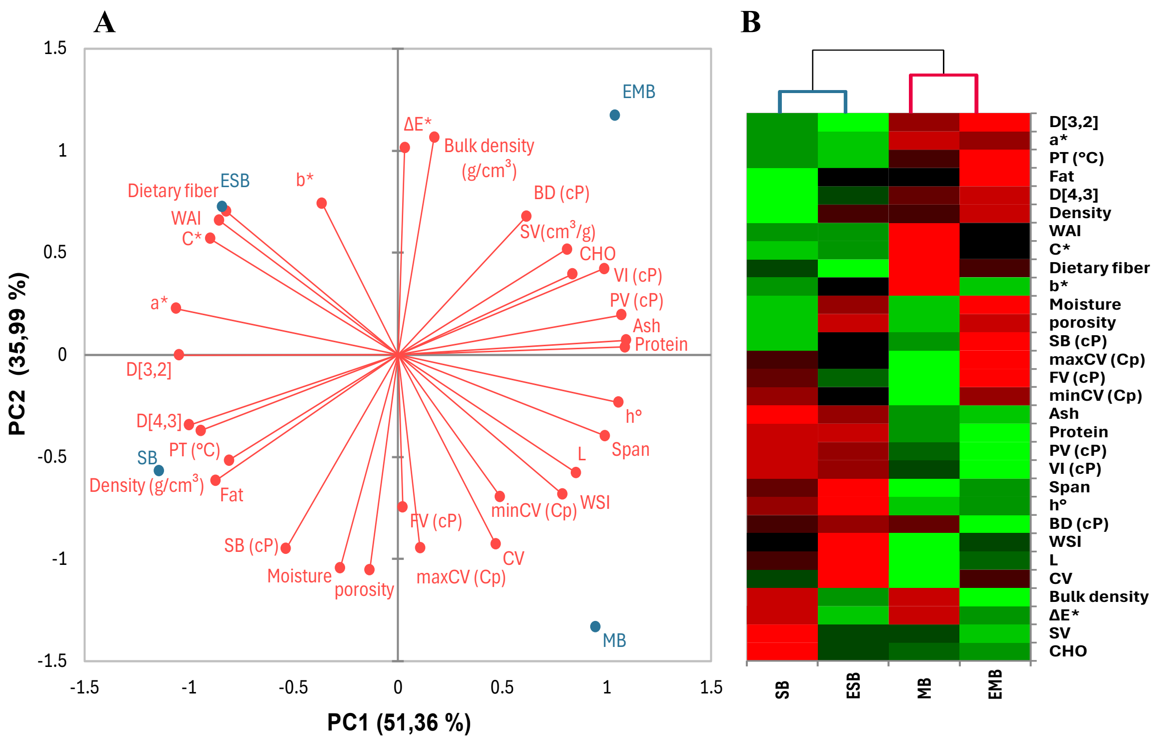

Fig. 4: Multivariate analysis. A) Biplot chart of principal component analysis (PCA) of brans, and physical and chemical properties; B) Heatmap grouping samples by Hierarchical clustering of principal components, the green color indicates the highest value and red color indicates the lowest value. CHO, Carbohydrate; CV, calorific value; WAI, water absorption index; WSI, water solubility indexes; VI, initial viscosity; PV, peak viscosity at 95 °C; minCV, minimal cooling viscosity; maxCV, maximum cooling viscosity; FV, final viscosity.

MB presents proximity to PV, maxCV, CV, minCV, L^*, span and h°. SB presents proximity to porosity, moisture, SB, D[3,2], and is closely linked to D[4,3], fat and density. Applying HCPC (sample groups in Fig. 4B), 2 sample groups were suggested, SB-ESB formed cluster 1 (blue) and MB-SB formed cluster 2 (red). The results presented in this study corroborate those of Comettant-Rabanal et al., (2021) where they observed the influence of thermoplastic extrusion on wheat and sorghum flours.

4.10 Supercritical fluid extraction (SFE)

4.10.1 Determining overall yield (X_0)

The yields corresponding to each extraction condition are shown in Figure 5. Samples extruded and extracted using 15% ethanol as cosolvent had the highest overall yields.

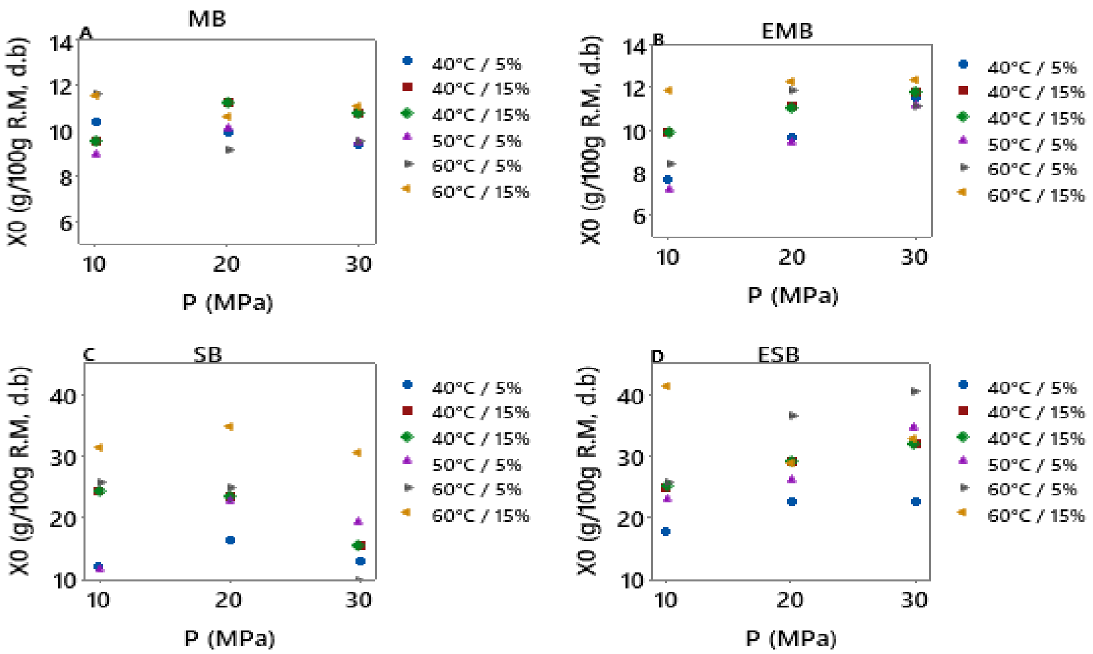

Fig. 5: Overall yield isotherms (X0) of the extracts obtained by SFE. A) Pearl millet bran. B) Extruded pearl millet bran. C) Sorghum bran. D) Extruded sorghum bran;

The extruded sorghum bran extracted at 60°C, 10 MPa and 15 % co-solvent showed the highest overall yield (41.52 %). For pearl millet, the highest yield was observed in extruded bran, which showed a 12.38 % yield at 60 ºC, 30 MPa and 15 % co-solvent. The overall yields found in this study were similar to those reported by (SHI *et al.*, 2015) in their study with foxtail millet (*Setaria italica* (L.) P.Beauv.) bran (19.65%) and seven fold higher than those found by (TUHANIOGLU; UBEYITOGULLARI, 2022) in Sorghum (*Sorghum bicolor* (L.) Moench) bran (6.0 %).

The extruded materials had the highest overall yield, which may be related to the combined effects shear stress, pressure and temperature during the extrusion process, even when applied for a short time. The extruded materials had a more uniform particle size distribution, which may have facilitated the interaction between the solvents (CO_2+Ethanol) and the sample, resulting in a higher overall yield (DURANTE *et al.*, 2012). The use of higher temperatures (50°-60°C) during extraction is known to increase the overall yield. This could be explained by the reduction of molecular mass, hence reducing the viscosity and surface tension of the solvents, which may increase their diffusivity into the matrix and consequently increases the mass transfer rate (VIGANÓ *et al.*, 2016) However, using very high temperatures (60-80 °C) would cause the compounds to degrade, reducing the quality of the obtained extract (GALLEGO; BUENO; HERRERO, 2019).

4.10.2 **Extract characterization**

The results of the analysis of total phenolic compounds and antioxidant capacity by FRAP and ABTS extracted under different conditions are shown in table 6, 7 and 8, respectively. Similarly to the overall yield, extruded sorghum and pearl millet bran samples extracted with 15% cosolvent had the highest total phenolic compound levels.

The highest values of total phenolic compounds (TPC) were obtained in pearl millet samples, being the highest when extruded 21.34 (mg GAE / g extratc). This yield was achieved at 40 MPa, 40 ºC and 15% co-solvent. As for sorghum, the highest TPC value was also found in extruded bran, 8.77 (mg GAE / g extratc) using 30 MPa, 50 ºC and 15 % co-solvent. There was an increase in TPC content when 15% ethanol was used as co-solvent. This effect can be explained by the increased density of the solvent caused by the higher pressure, which results in greater solubility of the compounds and consequently higher TPC content.

Table 6: Results for total phenolic of the extracts obtained by SFE.

Parameters	PHENOLICS (mg GAE / g extratc)			
	Pearl millet		**Sorghum**	
	MB	**EMB**	**SB**	**ESB**
40 (°C) 10(MPa) 05%	9.66±0.89^{eB}	17.38±0.38^{efA}	5.30±0.05^{dC}	3.29±0.02^{hiD}
40 (°C) 10(MPa) 15%	14.51±1.00^{abcB}	21.34±0.40^{aA}	5.18±0.01^{dD}	8.72±0.23^{abC}
40 (°C) 20(MPa) 05%	11.91±0.56^{deB}	20.46±0.03^{abcA}	4.90±0.00^{deD}	7.75±0.05^{bcdC}
40 (°C) 20(MPa) 15%	11.65±0.72^{deB}	20.87±0.26^{abA}	4.68±0.00^{defD}	7.67±0.04^{bcdC}
40 (°C) 30(MPa) 05%	11.62±0.00^{deB}	17.12±0.12^{fA}	7.17±0.00^{abC}	6.64±0.02^{deD}
40 (°C) 30(MPa) 15%	12.85±0.00^{bcdB}	20.96±0.19^{abA}	7.48±0.00^{aC}	6.96±0.13^{deC}
50 (°C) 10(MPa) 05%	4.70±0.73^{fB}	19.13±0.29^{dA}	5.96±0.25^{cB}	2.38±0.01^{iC}
50 (°C) 10(MPa) 15%	14.89±0.68^{abB}	19.39±0.08^{cdB}	6.57±0.21^{bcC}	7.37±0.28^{cdeC}
50 (°C) 20(MPa) 05%	12.44±0.83b^{cdB}	19.83±0.38^{bcdA}	3.95±0.38^{efgD}	7.01±0.03^{deC}
50 (°C) 20(MPa) 15%	15.49±0.22^{aB}	20.46±0.17^{abcA}	6.11±0.67^{cD}	8.48±0.07^{abcC}
50 (°C) 30(MPa) 05%	15.61±0.00^{aB}	18.67±0.53^{deA}	4.40±0.00^{defC}	6.10±0.16^{efC}
50 (°C) 30(MPa) 15%	15.41±0.11^{aB}	19.10±0.0^{8dA}	4.97±0.02^{deD}	8.97±0.04^{aC}
60 (°C) 10(MPa) 05%	13.56±0.06^{abcdB}	18.71±0.00^{deA}	4.11±0.15^{efgC}	0.72±0.01^{jD}
60 (°C) 10(MPa) 15%	14.84±0.51^{abB}	19.73±0.24^{bcdA}	4.57±0.06^{defC}	5.16±0.08^{fgC}
60 (°C) 20(MPa) 05%	12.20±0.17^{cdeB}	18.71±0.58^{deA}	4.43±0.01^{defD}	5.06±0.18^{fghC}
60 (°C) 20(MPa) 15%	15.46±0.40^{aB}	20.69±0.18^{abA}	3.34±0.00^{gD}	7.96±0.19^{abcdC}
60 (°C) 30(MPa) 05%	11.84±0.00^{deB}	16.99±0.54^{fA}	4.41±0.00^{defC}	3.66±0.00^{hiD}
60 (°C) 30(MPa) 15%	14.22±0.55^{abcdB}	18.45±0.36^{deA}	3.68±0.00^{fgC}	4.24±0.02^{ghC}

Capital letters in the same row indicate differences between brans ($p<0.05$), by Tukey's test. Lowercase letters in the same column indicate differences between SFE extraction conditions ($p<0.05$), by Tukey's test. Values expressed as mean ± standard deviation.

The use of a co-solvent can also cause changes in the matrix, which can make it possible to obtain higher yield, even at lower pressures (CADENA-CARRERA *et al.*, 2019). The content TPC was superior to those found by (TUHANIOGLU; UBEYITOGULLARI, 2022) and (ORTIZ-CRUZ *et al.*, 2020a).

The FRAP and ABTS antioxidant capacities (AC) of the extracts obtained from the extruded and non-extruded millet and sorghum matrices are shown in Table 7 and 8. The two methodologies applied had different optimum conditions. Extrusion had positive effects on pearl millet bran. ABTS increased by 28% and FRAP increased by 102%. Negative effect was observed in sorghum ABTS reduced by 38% and FRAP reduced by 48.88%.

Table 7: Results for CA-FRAP of the extracts obtained by SFE.

Parameters	FRAP (µM TE / g extract)			
	Pearl millet		Sorghum	
	MB	EMB	SB	ESB
40 (°C) 10(MPa) 05%	40.40±0.84^{fB}	148.78±0.75^{bA}	36.84±0.94^{cdC}	16.58±0.26^{fgD}
40 (°C) 10(MPa) 15%	78,01±1,07^{aB}	140,57±0,71^{bcdA}	26,67±0,87^{efC}	26.78±1.48^{bcC}
40 (°C) 20(MPa) 05%	62.2±2.35^{bcdB}	149.39±0.41^{bA}	30.21±0.57^{deC}	33.55±1.19^{aC}
40 (°C) 20(MPa) 15%	63.27±3.95^{bcdB}	120.66±0.43^{defA}	26.50±1.29^{efC}	24.66±1.34^{cdC}
40 (°C) 30(MPa) 05%	74.06±0.31^{abB}	129.08±0.30^{bcdefA}	41.52±1.10^{cC}	35.17±1.88^{aD}
40 (°C) 30(MPa) 15%	48.31±0.81^{defC}	124.92±0.30^{cdefA}	56.12±1.11^{bB}	22.61±0.86^{deD}
50 (°C) 10(MPa) 05%	46.21±0.49^{efB}	145.84±0.67^{bcA}	29.86±0.30^{deC}	14.57±0.44^{gD}
50 (°C) 10(MPa) 15%	59.14±1.41^{cdeB}	117.77±0.97^{defA}	37.20±1.25^{cdC}	21.33±0.78^{deD}
50 (°C) 20(MPa) 05%	57.04±1.30^{cdeB}	157.72±0.61^{aA}	19.99±0.69^{efgD}	33.23±1.98^{aC}
50 (°C) 20(MPa) 15%	56.72±0.84^{cdeB}	135.50±0.61^{bcdeA}	35.91±1.42^{cdC}	26.14±0.48^{bcD}
50 (°C) 30(MPa) 05%	64.93±2.09^{bcB}	148.01±0.63^{bA}	27.60±0.73^{efC}	23.35±1.78^{cdD}
50 (°C) 30(MPa) 15%	54.14±3.12^{cdefB}	133.82±0.54^{bcdefA}	23.98±0.75^{efgC}	28.99±0.83^{bC}
60 (°C) 10(MPa) 05%	54.40±1.74^{cdefB}	113.14±0.48^{efA}	19.74±1.06^{fgC}	4.00±0.05^{hD}
60 (°C) 10(MPa) 15%	55.55±0.76^{cdeB}	110.72±0.57^{fA}	24.87±1.40^{efgC}	19.79±0.40^{efD}
60 (°C) 20(MPa) 05%	54.45±0.32^{cdefB}	128.99±1.65^{bcdefA}	24.62±1.71^{efgC}	16.98±0.87^{fgD}
60 (°C) 20(MPa) 15%	66.18±1.24^{bcB}	123.75±1.17^{cdefA}	15.69±0.10^{gD}	26.32±0.42^{bcC}
60 (°C) 30(MPa) 05%	68.70±0.15^{abcB}	135.44±0.32^{bcdeA}	68.94±1.31^{aB}	21.97±0.56^{deC}
60 (°C) 30(MPa) 15%	56.88±0.26^{cdeB}	119.93±0.84^{defA}	25.65±1.41^{efC}	24.52±0.66^{cdC}

Capital letters in the same row indicate differences between brans ($p<0.05$), by Tukey's test. Lowercase letters in the same column indicate differences between SFE extraction conditions ($p<0.05$), by Tukey's test. Values expressed as mean ± standard deviation.

This increase can be explained by the structural modification of cell walls under conditions of high temperature, pressure and shear, which promotes the disintegration of the cell wall matrix and the breaking of covalent bonds into high molecular weight polyphenolic complexes, improving the accessibility of phenolic compounds (SALAZAR

LOPEZ *et al.*, 2016). It is important to note that these compounds can also be degraded with the effect of temperature since they are thermosensitive (YOUSEFI *et al.*, 2019)

Using the FRAP method, the extruded millet samples showed the highest AC values of 157,93±0,61 (µM TE / g extract), which was obtained using 50ºC, 20MPa and 5% solvent, while the lowest values were observed in extruded sorghum bran, which showed 4.00±0,05 (µM TE / g extract), obtained using 60º C, 10 MPa and 5 % co-solvent. For the ABTS method, the highest AC value was observed in extruded millet bran 4,47±0,01 (µM TE / g extract), obtained using 50 ºC, 20 MPa and 5% co-solvent and the lowest CA value was observed in extruded sorghum bran 0,46±0,00 (µM TE / g extract) obtained using 60 ºC, 10 MPa and 5 % co-solvent.

Table 8: Results for CA-ABTS of the extracts obtained by SFE.

Parameters	ABTS (µM TE / g extract)			
	Pearl millet		Sorghum	
	MB	EMB	SB	ESB
40 (°C) 10(MPa) 05%	2.84±0.01[bB]	4.45±0.33[aA]	2.88±0.00[cB]	2.03±0.01[aC]
40 (°C) 10(MPa) 15%	2.16±0.01[fA]	2.14±0.03[cdA]	1.22±0.01[iB]	1.22±0.01[cdB]
40 (°C) 20(MPa) 05%	2.53±0.01[cdeB]	2.66±0.05[bA]	2.03±0.01[eC]	1.46±0.01[bD]
40 (°C) 20(MPa) 15%	1.61±0.00[iB]	2.02±0.04[cdeA]	1.27±0.00[iC]	1.03±0.01[defgD]
40 (°C) 30(MPa) 05%	2.42±0.03[eB]	2.14±0.03[cdC]	2.49±0.02[dA]	1.44±0.02[bcD]
40 (°C) 30(MPa) 15%	1.77±0.02[ghiAB]	1.82±0.10[defA]	1.68±0.02[fB]	0.90±0.01[efgC]
50 (°C) 10(MPa) 05%	3.47±0.02[aB]	4.47±0.01[aA]	3.02±0.01[bC]	1.56±0.00[bD]
50 (°C) 10(MPa) 15%	1.64±0.02[iA]	1.64±0.04[efA]	1.56±0.01[gB]	0.96±0.01[efgC]
50 (°C) 20(MPa) 05%	2.63±0.02[cdA]	2.35±0.05[bcB]	1.42±0.01[hC]	1.12±0.01[defD]
50 (°C) 20(MPa) 15%	1.90±0.01[gA]	1.64±0.06[efC]	1.71±0.00[fB]	1.02±0.01[defgD]
50 (°C) 30(MPa) 05%	2.52±0.06[cdeA]	1.99±0.03[cdefB]	1.70±0.01[fC]	0.94±0.01[efgD]
50 (°C) 30(MPa) 15%	1.70±0.05[hiA]	1.56±0.03[fB]	1.01±0.01[jD]	1.13±0.01[deC]
60 (°C) 10(MPa) 05%	1.67±0.01[hiB]	2.74±0.01[bA]	1.23±0.06[iC]	0.46±0.00[iD]
60 (°C) 10(MPa) 15%	1.34±0.01[jB]	1.75±0.04[defA]	0.75±0.03[lC]	0.63±0.01[hiD]
60 (°C) 20(MPa) 05%	2.78±0.02[bcA]	2.03±0.03[cdeB]	1.35±0.00[hiC]	0.89±0.01[fgD]
60 (°C) 20(MPa) 15%	1.86±0.10[ghA]	1.80±0.02[defA]	0.90±0.01[kC]	1.02±0.00[defgB]
60 (°C) 30(MPa) 05%	2.47±0.02[deB]	2.31±0.03[bcC]	3.28±0.03[aA]	0.80±0.00[ghD]
60 (°C) 30(MPa) 15%	1.62±0.07[iA]	1.67±0.03[efA]	1.02±0.01[jB]	0.87±0.01[ghC]

Capital letters in the same row indicate differences between brans ($p<0.05$), by Tukey's test. Lowercase letters in the same column indicate differences between SFE extraction conditions ($p<0.05$), by Tukey's test. Values expressed as mean ± standard deviation.

The effect of temperature, pressure and % of co-solvent is also observed. In other words, in general, by increasing the temperature and pressure increased the antioxidant capacity of the extracts. The FRAP and ABTS yields suggest an interaction effect between temperature and pressure because the solvating power of the solvent mixture depends on temperature and pressure. This same behavior was reported by Tuhanioglu et al. (2022) in their studies on sorghum bran oil obtained by SFE.

Based on table 6, 7 and 8, an analysis of variance was carried out on the influence of the variables pressure, temperatures and percentages of cosolvent, as well as the interaction between the variables on the responses obtained in the planning, for phenolics, antioxidant capacity for the ABTS and FRAP methods.

The pressure variable had a positive effect on the phenolic, ABTS and FRAP content with an increase to 30 MPa. This behavior was already expected when increasing the pressure from 10 MPa to 30 MPa, which is justified by the increase in the density of the solvent and consequently the increase in the solvation power of the supercritical fluid (L. ZABOT; N. MORAES; ANGELA A. MEIRELES, 2012).

On the other hand, the temperature variable presented a negative effect on the concentrations of phenolics and antioxidant capacity (FRAP and ABTS), when the condition varies from the lowest to the highest level, except for the MB sample in phenolics. This behavior can be explained by the degradation of the compounds of interest, since these compounds are considered thermosensitive. The percentage of co-solvent had a positive effect on phenolic content. However, the effect was negative for CA and FRAP and ABTS. This same behavior was reported by (CLÍMACO; VARDANEGA; FASOLIN, 2023) in his study with *Hibiscus sabdariffa* (L.), the highest value found was 0.6 (mgTE / g extract) obtained at 60°C, 10 MPa and 5% co-solvent.

Considering the analysis of variance (ANOVA), all the variables and the interaction between them were significant ($p < 0.05$) for most of the responses evaluated. However, the variable T(°C)*C(%) had little or no influence on the phenolic compounds for ESB and SB. The variable T(°C)*C(%) and T(°C)*P(MPa) had no significant influence ($p<0.05$) on the FRAP content for MB (attached Figures S4-S9)

4.10.3 Multivariate analysis

A PCA was performed to study the similarities between pearl millet and sorghum bran extracts obtained under different conditions of temperature , pressure and co-solvent percentage and the response variables, phenolics, antioxidant capacity (FRAP and ABTS) and extract yield to determine their clustering (Fig. 6).

Components PC1 (38.51 %) and PC2 (18.69 %) showed 57.22 % variability. In PCA, vectors in proximity are positively correlated and vectors distant are negatively correlated.

A

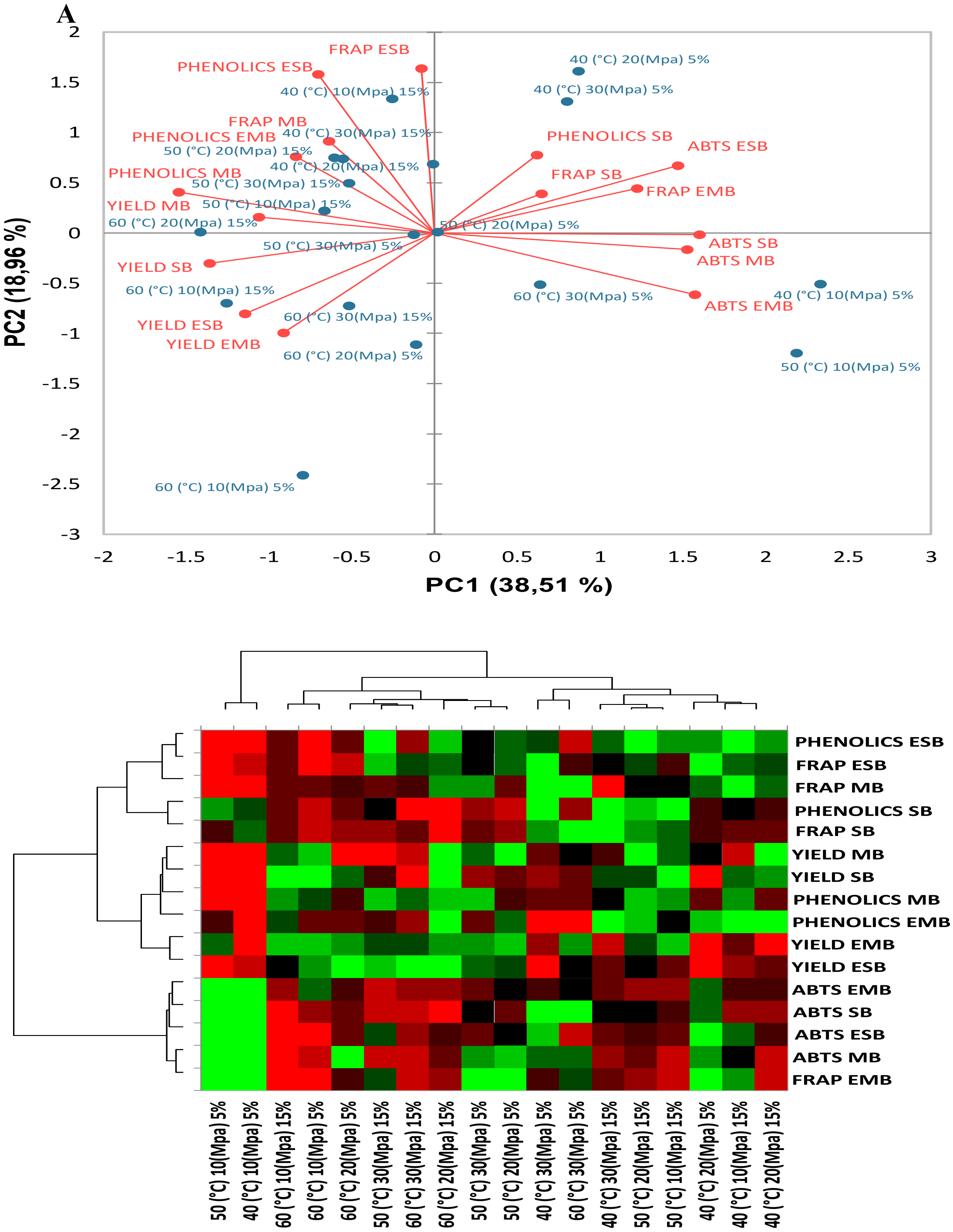

Fig. 6: Multivariate analysis. A) Biplot chart of principal component analysis (PCA) for pearl millet and sorghum bran extracts obtained under different conditions of temperature (T ºC), pressure (MPa) and co-solvent percentage (%); B) Heatmap grouping samples by Hierarchical clustering of principal components, the green color indicates the highest value and red color indicates the lowest value.

The extruded pearl millet and sorghum bran showed strong vectors in all quadrants of the PCA biplot comprising all the analyses carried out; this can be seen in YIELD ESB, ABTS EMB, FRAP EMB and PHENOLICS ESB (Fig. 6A). The extraction conditions proved to be specific for each analysis carried out, for PHENOLICS (top left quadrant) it has a strong proximity with pressures of 10 to 30 MPa and temperatures of 40 to 60 ºC, being predominantly influenced by the percentage of cosolvent (15%), ABST behaves in the opposite way being strongly influenced by 05 % of cosolvent which can be confirmed by the green color in the heatmap (Fig. 6B).

FRAP is very similar at temperatures of 40 and 50 ºC, pressures of 20 and 30 MPa and is strongly influenced by 5% ethanol. FRAP is closely related to temperatures of 40 and 50 ºC, pressures of 20 and 30 MPa and is strongly influenced by 5% ethanol. In the negative quadrant of the graph, YIELD shows a strong proximity with 60 ºC. YIELD has a weak relationship with most of the analyses carried out, especially CA, and this can be seen in the distance between the YIELD ESB and ABTS ESB vectors.

Notably, the treatments show a clear separation in the clusters (Fig. 6B). This behavior indicates the prominent impact of TE as a pre-treatment for SFE (Fig. 6AB). Pretreatment is an alternative to preserve and improve the quality of extracts obtained by SFE, (PRASHANTH *et al.*, 2024) use hydrothermal treatment on pearl millet, (PINTO *et al.*, 2023) use vacuum drying at different temperatures to extract oil from hop (*Humulus lupulus L.*) and both evaluated pretreatments positively.

4.10.4 Extraction curves

Based on the content obtained for the classes of phenolic compounds and antioxidant capacity, it was possible to select the SFE operating conditions for the kinetic study of the extract yield. The SFE condition selected was for ESB 50 ºC, 10 MPa and 15% of co-solvent, for SB, MB and EMB condition was 40 ºC, 10 MPa and 15 % of co-solvent (figure 7, 8 and 9). The extraction curves showed the three distinct regions: constant extraction rate (CER), falling extraction rate (FER), and diffusion-controlled (DC) (MEIRELES, 2008)

For MB and SB, an extraction yield of approximately 8 g extract/100 g dry raw material (R.M.) was obtained in 100 min with S/F = 25 and for EMB and ESB, approximately 10 (g extract / 100 g R.M.) was obtained under the same conditions. Thus, the SFE process allowed a recovery of 0.31 g extract / solvent in MB and 0.35 g extract /

solvent in SB, while in the extruded samples the recovery was 0.39 g extract / solvent for EMB and ESB. This shows that extrusion led to a 21 % increase in extraction yield.

The slope of the first line represents the mass transfer rate (M_{CER}) of the CER stape. The time that dictates the intersection of the first two lines is t_{CER} (25, 25, 25 and 30 for MB, EMB, SB and ESB respectively), which represents the end of the CER step; the intersection time between the second and third lines is t_{FER} (40 for all samples). Finally, R_{CER} is the extraction yield obtained in the R_{CER} step (6.25, 7.68, 666, 649 for MB, EMB, SB and ESB respectively).

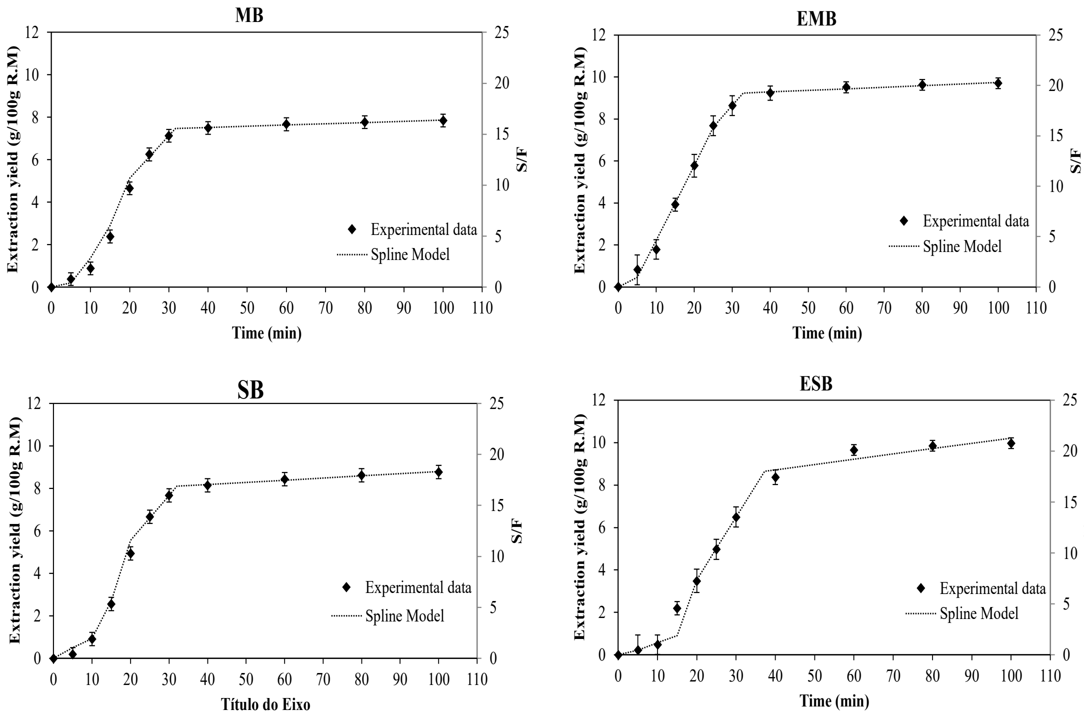

Fig. 7: Extraction curves for yield. MB, Pearl millet bran; EMB, Pearl millet extruded bran; SB, Sorghum bran and ESB, Sorghum extruded bran

For the MB and EMB samples, the fraction of extract recovered in the CER period referring to the total extracted was 79.71 and 79.17 %, respectively, and for the SB and ESB samples it was 75 and 68.09 %, respectively; as a consequence, lower yields were obtained for the FER phase, at 31.9%. Clímaco et al., (2023) reported similar behavior in their study with hibiscus (*Hibiscus sabdariffa* L.), which represents an extract recovered with low resistance to mass transfer, which may be related to the availability of the compounds and the high solubility of the extract in the solvent, since in this phase, the solvent does not need to diffuse completely into the matrix to perform the extraction.

It was observed that the duration of the CER phase for MB, EMB and SB was 25 min, about 3 times less than the total extraction time (100 min) with an S/F of less than 16.0, while for ESB it corresponds to 30 min of the total extraction time (100 min) with an S/F = 16.54

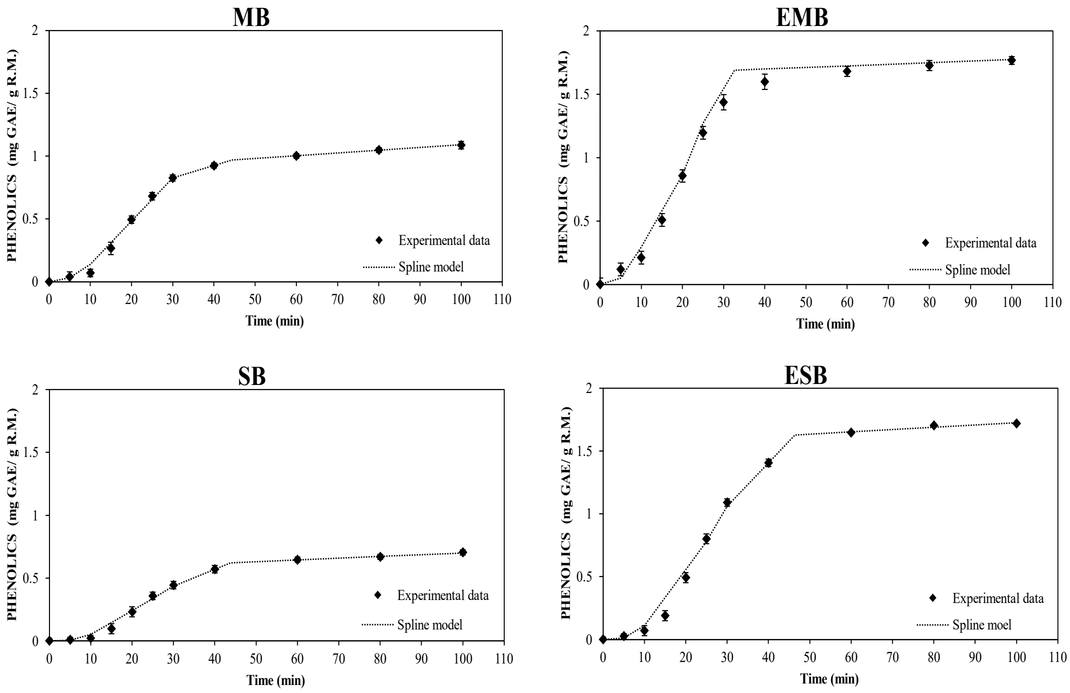

Fig. 8: Extraction curves for Phenolics. MB, Pearl millet bran; EMB, Pearl millet extruded bran; SB, Sorghum bran and ESB, Sorghum extruded bran

From the extracts obtained, total phenolic and antioxidant capacity analyses were carried out (FRAP-ABTS) and are shown in Figures 8-10. The yield of phenolics ranged from 0.70 (SB) to 1.76 mg GAE / g R.M. (EMB), the fraction of phenolics extracted in the tCER step, which lasted 30 min, represented 75.97(MB), 81.29(EMB), 59.62(SB), 63.01 (ESB) % in relation to the total extraction time (100 min). The curves showed a diffusion controlled (DC) period after 40 min, indicating that maximum extraction of the bran had been achieved.

Analysis of CA (FRAP) showed that MB presented the lowest value of 5.79 µM TE / g R.M. and EMB presented the highest value of 9.39 µM TE / g R.M., the tCER stage lasted 30 min for all pearl millet and sorghum bran, in which values of 4.49, 6.70, 5.19, and 5.61 µM TE / g R.M. were found for MB, EMB, SB and ESB, respectively. tCER represented 77.57 (MB) 71.57 (EMB), 76.84 (SB), 62.25 (ESB) % of the total extraction time. The period (DC) was observed after 50min of extraction.

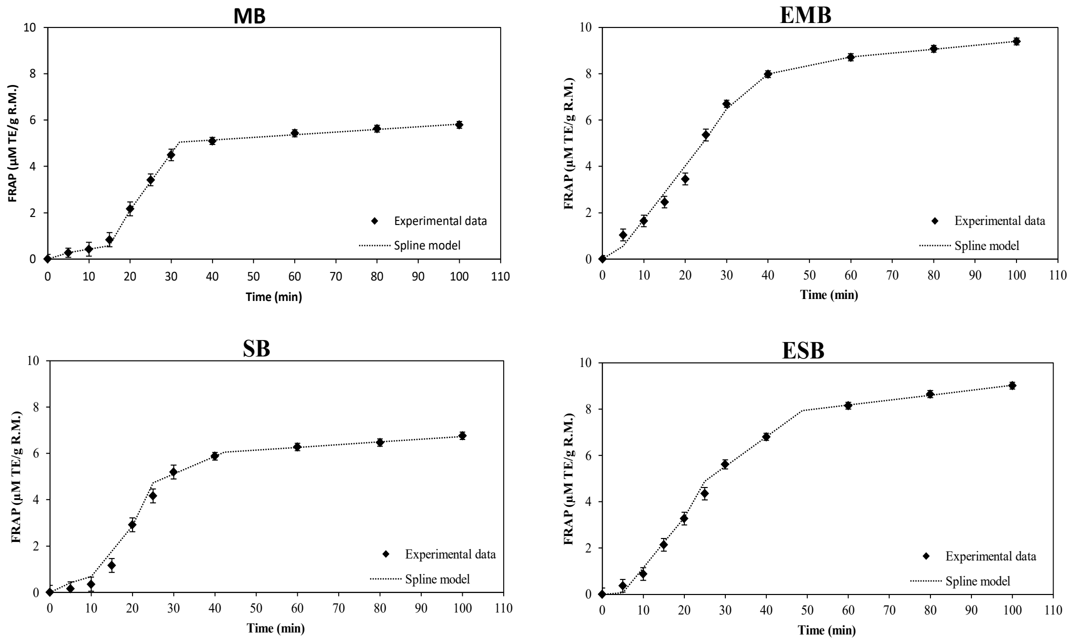

Fig. 9: Extraction curves for FRAP. MB, Pearl millet bran; EMB, Pearl millet extruded bran; SB, Sorghum bran and ESB, Sorghum extruded bran

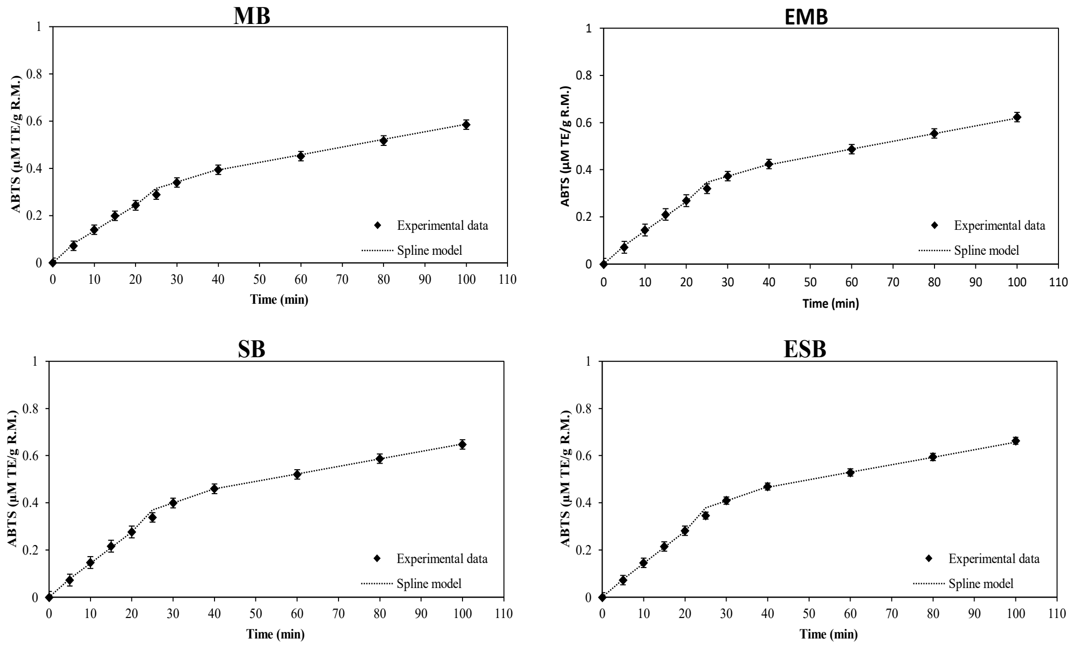

Fig. 10: Extraction curves for ABTS. MB, Pearl millet bran; EMB, Pearl millet extruded bran; SB, Sorghum bran and ESB, Sorghum extruded bran

The antioxidant capacity measured by ABST showed values between 0.58 (MB) and 0.66 (ESB) µM TE / g R.M., similarly to the previous analyses, the CER stage lasted 30 min and showed the highest recovery in relation to the total extraction time, this stage represented 58.21, 59.92, 61.64 and 61.82 % for MB, EMB, SB and ESB, respectively. A trend towards CD was observed after 50 minutes of extraction.

4.11 CONCLUSION

Extrusion caused changes in the chemical composition of the bran. There was an increase in ash, protein, dietary fiber and carbohydrates. Moisture and lipids were reduced. Particle size analysis showed that the extruded samples were more homogeneous. The apparent density of the extruded samples increased by around 29.72 % compared to the non-extruded samples, while the opposite effect was observed in the porosity of the extruded samples, which may have affected the results of the technological analyses. WAI increased significantly and WSI decreased in all extruded samples. The results of the colorimetric analysis show that EMB and ESB obtained a ΔE^* of 8.49 and 9.78 respectively. The paste properties were altered, albeit to a small extent, by the extrusion process. MB showed the greatest changes. The extracts obtained by SFE were positively affected by TE, increasing the extract yield. The variables T (°C), P (MPa) and C (%) also influenced the results of the analyses of phenolic compounds and AC. The best TPC was obtained using 40 °C, 10 MPa and 15 % co-solvent. CA, the best ABTS condition was at 50 °C, 10 MPa and 15 % co-solvent; for FRAP, the best condition was 50 °C, 20 MPa and 05 % co-solvent. In the kinetic study, the highest yield was 10 g for ESB. The fraction of extract recovered in the CER phase was 79.71 % for MB and 79.17 % for EMB, the total recovery was higher in the extruded samples (0.41 g in EMB and 0.43 g in ESB).

The results of this study demonstrate that sorghum and millet brans are a potential source of bioactive compounds, presenting yields comparable to or superior to those of other cereals. TE proved to be efficient as a pre-treatment; both are green technologies and were efficient in obtaining extracts rich in phenolic compounds and antioxidant capacity.

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5 CONSIDERAÇÕES FINAIS

Esse estudo destacou o potencial dos processos de extrusão termoplástica (ET) e extração por fluido supercrítico (EFS) aplicados aos grãos e farelos de sorgo e milheto pérola. Embora a EFS seja eficaz na extração de compostos bioativos, seu alto custo inicial pode limitar a viabilidade comercial, sendo a ET uma alternativa promissora como pré-tratamento para otimizar a extração.

Os resultados mostraram que o tempo de brunimento influencia diretamente o rendimento do farelo e o teor de amido, impactando as propriedades de pasta e umidade. O farelo de milheto pérola apresentou maior recuperação comparado ao de sorgo, reforçando seu potencial de aplicação em alimentos de baixa viscosidade. Além disso, a Extrusão Termoplástica (ET) aumentou significativamente os teores de compostos fenólicos totais (TPC) e a capacidade antioxidante (CA), com análises multivariadas evidenciando propriedades funcionais mais intensas nas frações de farelo em comparação aos grãos decorticados.

A forte correlação entre TPC, CA e volume específico (VE) reforça a eficiência da ET em concentrar compostos bioativos, indicando que os farelos de sorgo e milheto pérola podem ser utilizados como ingredientes funcionais nas indústrias alimentícia e nutracêutica, com potencial para atender às demandas de produtos mais saudáveis e sustentáveis.

Foi possível avaliar o impacto da ET nas características físicas, químicas e tecnológicas dos farelos de sorgo e milheto pérola, além de investigar a ET como pré-tratamento para a EFS utilizando CO_2 e etanol. A pesquisa também examinou a influência de variáveis como temperatura, pressão e porcentagem de cossolvente na extração dos farelos.

Os resultados mostraram que a extrusão alterou a composição centesimal dos farelos, com aumento nos teores de cinzas, proteínas, fibras dietéticas e carboidratos, enquanto a umidade e os lipídios foram reduzidos. A análise de partículas indicou maior homogeneidade nas amostras extrudadas, com aumento de 29,72% na densidade aparente e redução da porosidade, o que pode ter afetado as análises tecnológicas. As propriedades de absorção de água (IAA) aumentaram e o índice de solubilidade em água (ISA) diminuiu em todas as amostras extrudadas. Além disso, a análise colorimétrica revelou valores de ΔE^* de 8,49 para o farelo extrudado de milheto (EMB) e 9,78 para o farelo extrudado de sorgo (ESB), com pequenas alterações nas propriedades da pasta, sendo o farelo de milheto pérola (MB) o mais impactado.

A extrusão também afetou positivamente a obtenção de extratos pela EFS, aumentando o rendimento de extratos. As variáveis temperatura (T), pressão (P) e concentração de cossolvente (C) influenciaram os compostos fenólicos e a capacidade antioxidante (CA), com as melhores condições de extração de compostos fenólicos totais (TPC) sendo 40 °C, 10 MPa e 15% de cossolvente, e para a CA, 50 °C, 10 MPa e 15% de cossolvente. Para o FRAP, as melhores condições foram 50 °C, 20 MPa e 5% de cossolvente. O estudo cinético indicou que o maior rendimento foi de 10 g para o extrato do farelo extrudado de milheto pérola (ESB). A fração recuperada na fase CER foi de 79,71% para o farelo de sorgo (MB) e 79,17% para o farelo extrudado de milheto pérola (EMB), com maior recuperação nas amostras extrudadas.

O estudo evidenciou que os farelos de sorgo e milheto pérola podem ser considerados fontes promissoras de compostos bioativos, com rendimentos semelhantes ou superiores a outros cereais. As tecnologias de EFS e ET mostraram-se eficientes na obtenção de extratos ricos em compostos fenólicos e capacidade antioxidante, com grande potencial para aplicação nas indústrias alimentícia e farmacêutica.

6 SUGESTÕES PARA TRABALHOS FUTUROS

- Modelagem de extração dos farelos; Tempo, e diferentes porosidades (Pedras)
- MEV desses grãos em diferentes Tempo*Pedras
- Textura / Dureza
- Perfil fenólico desses farelos - HPLC
- Acoplar fluido supercrítico na extrusora
    - Extrusão Supercrítica.

7 ANEXOS

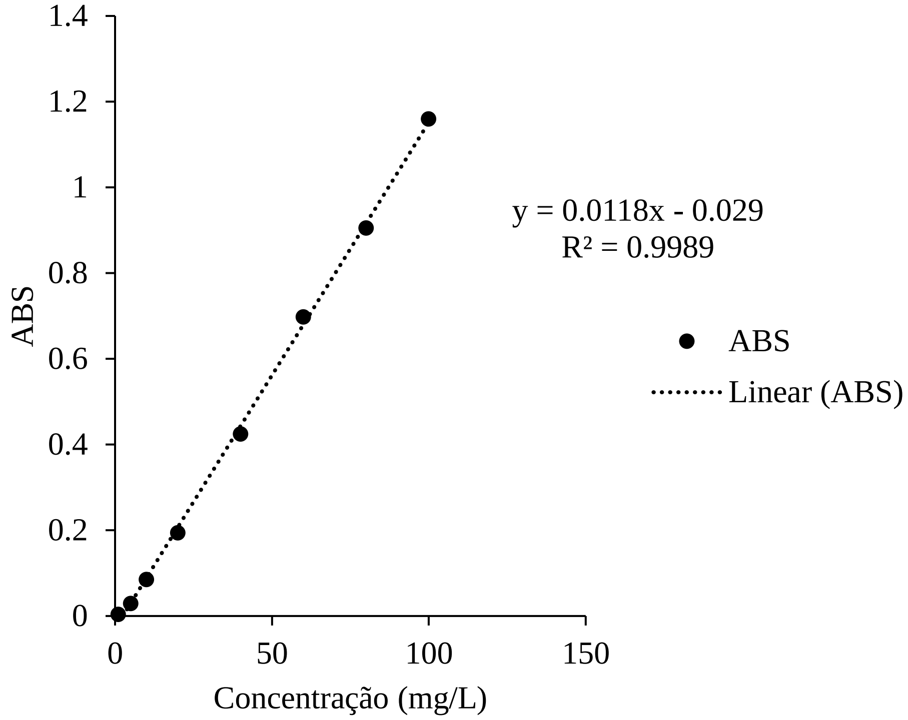

Figure S 1: Curva padrão de Ácido Gálico

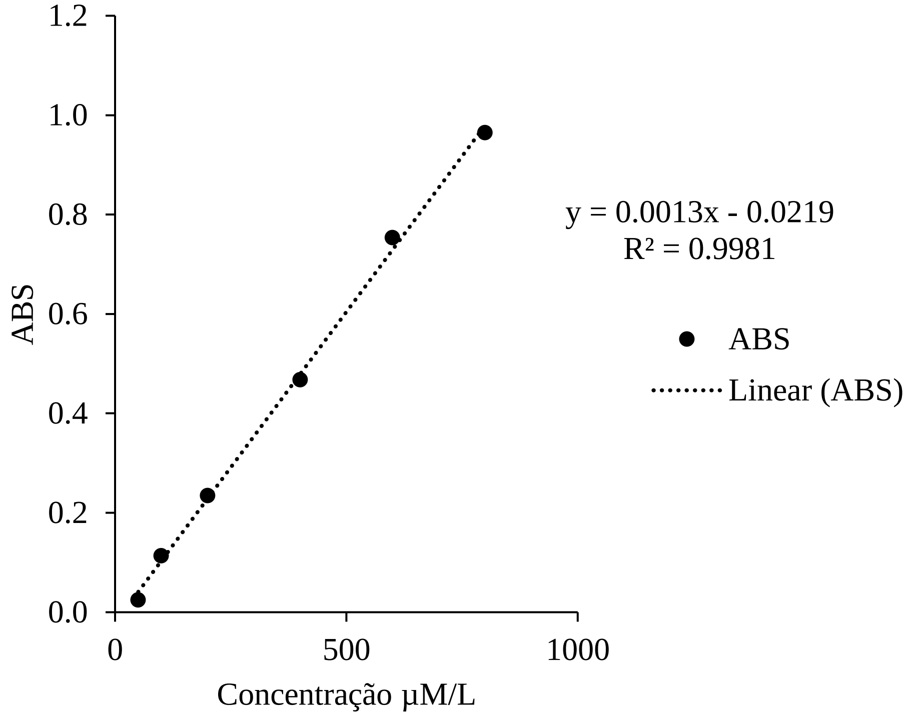

Figure S 2:Curva padrão de Trolox para determinação da atividade antioxidante pelo método ABTS.

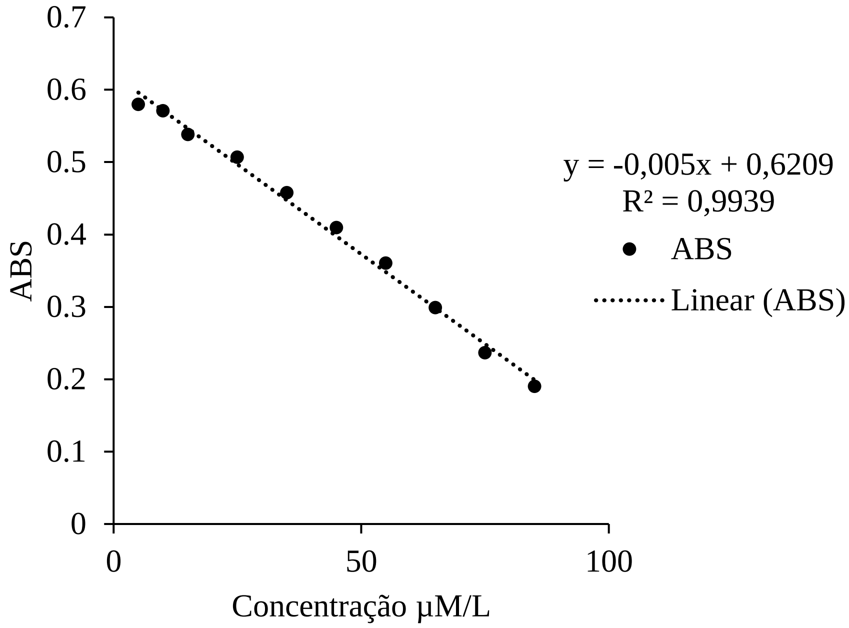

Figure S 3: Curva padrão de Trolox para determinação da atividade antioxidante pelo método FRAP.

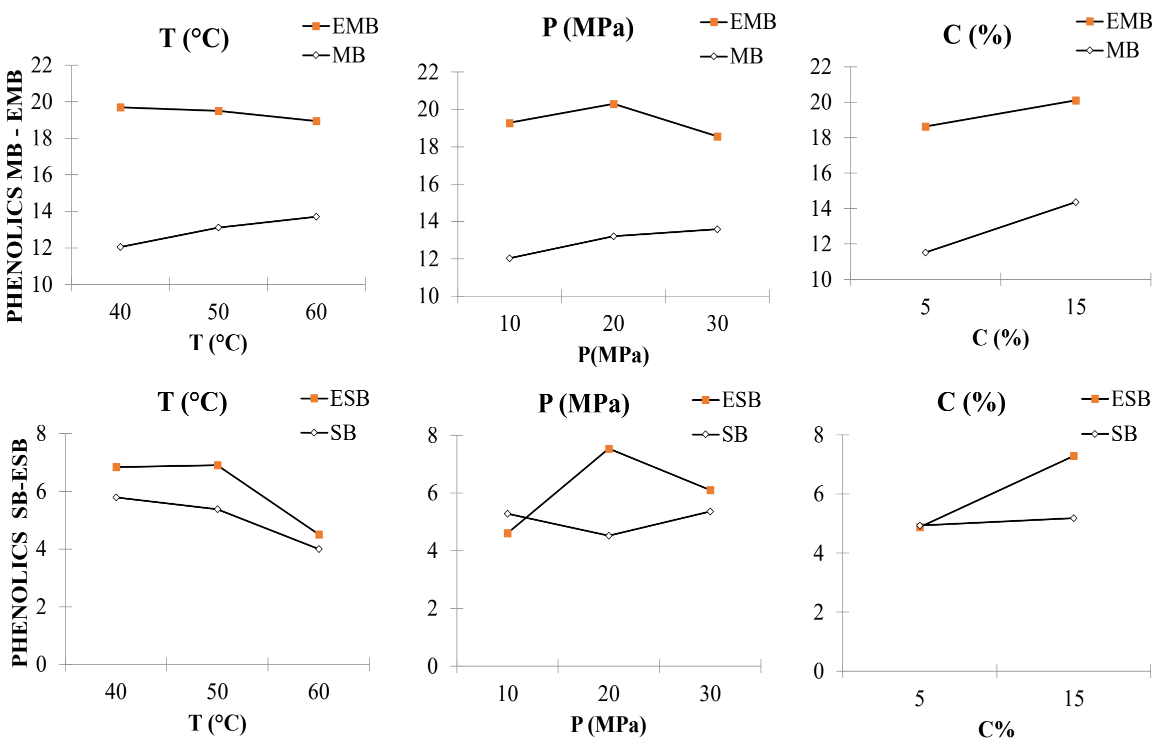

Figure S 4: Influence of the isolated variables Temperature (TºC), Pressure (P MPa) and co-solvent (C%) in PHENOLICS. MB: Millet bran, EMB: Extruded Millet bran, SB: Sorghum bran and ESB: Extruded sorghum bran

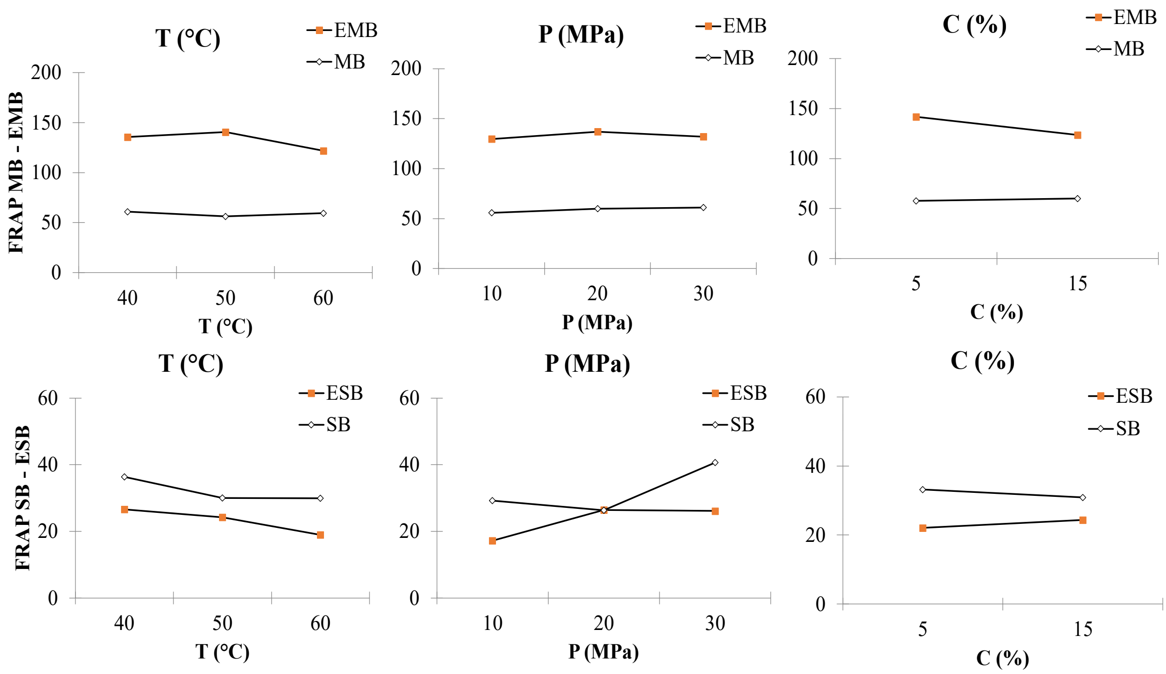

Figure S 5: Influence of the isolated variables Temperature (TºC), Pressure (P MPa) and co-solvent (C%) in FRAP. MB: Millet bran, EMB: Extruded Millet bran, SB: Sorghum bran and ESB: Extruded sorghum bran.

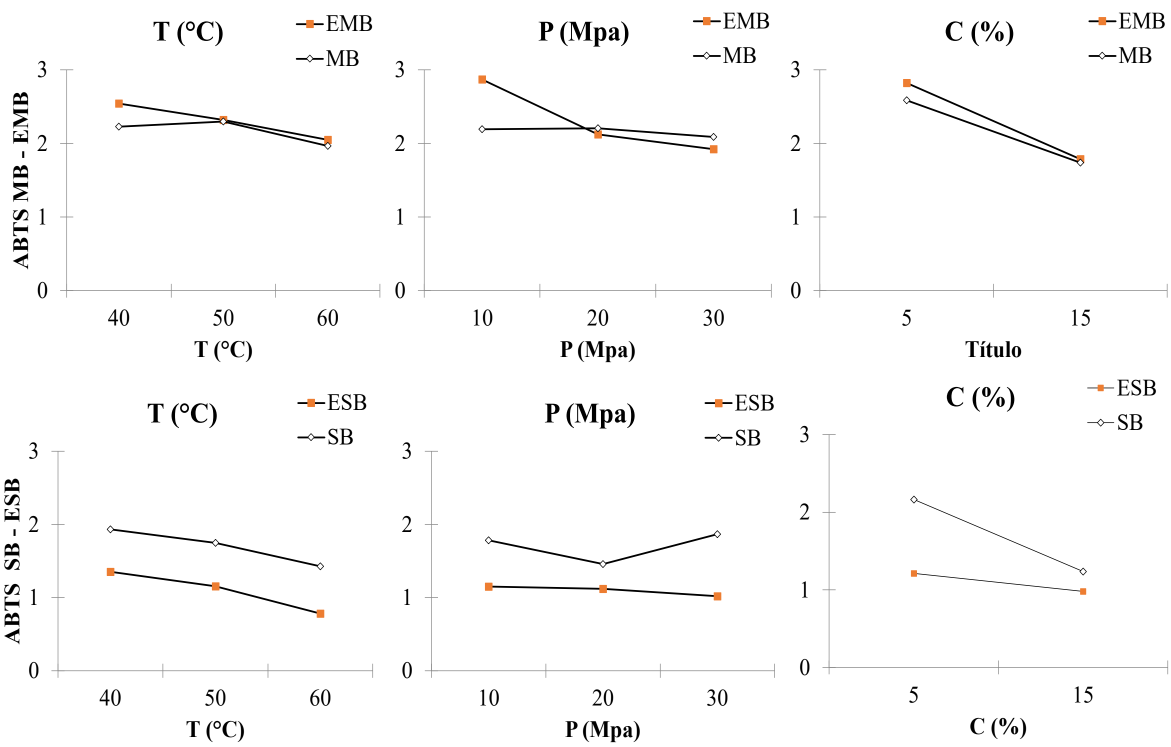

Figure S 6: Influence of the isolated variables Temperature (TºC), Pressure (P MPa) and co-solvent (C%) in ABTS. MB: Millet bran, EMB: Extruded Millet bran, SB: Sorghum bran and ESB: Extruded sorghum bran..

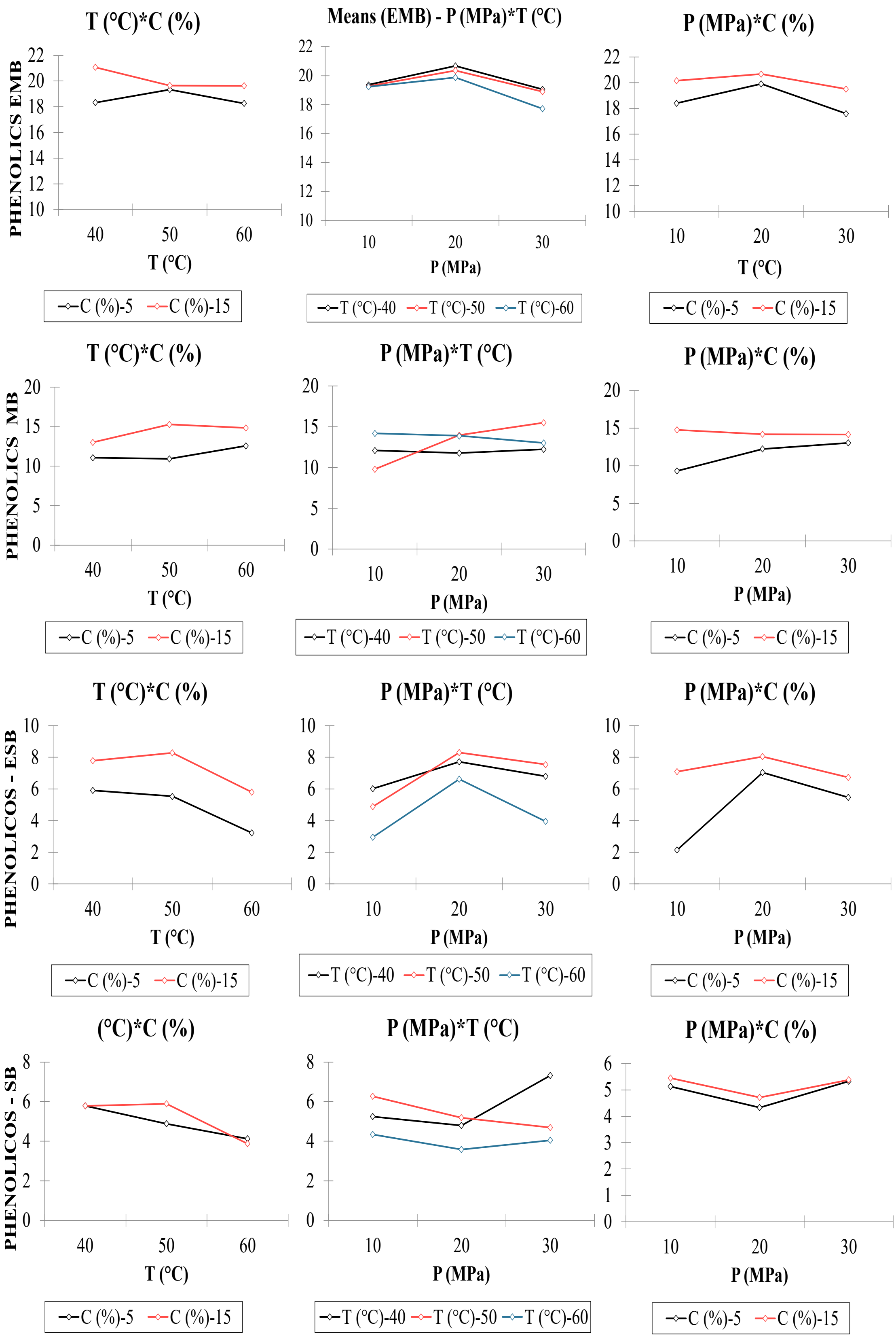

Figure S 7: Influence of interactions between the variables Temperature (Tº C), Pressure (P MPa) and co-solvent (C %) in PHENOLICS. MB: Millet bran, EMB: Extruded Millet bran, SB: Sorghum bran and ESB: Extruded sorghum bran.

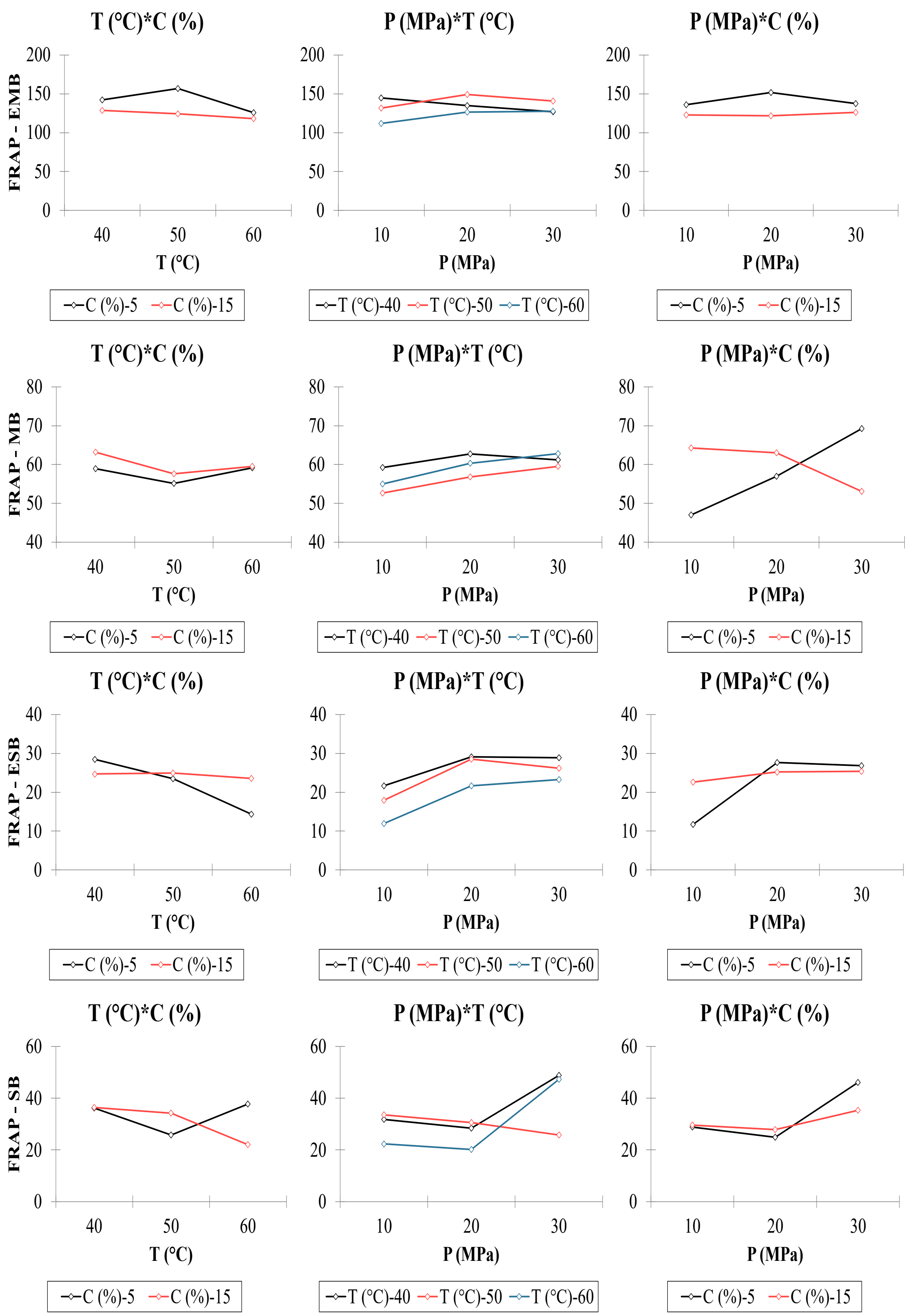

Figure S 8: Influence of interactions between the variables Temperature (T° C), Pressure (P MPa) and co-solvent (C %) in FRAP. MB: Millet bran, EMB: Extruded Millet bran, SB: Sorghum bran and ESB: Extruded sorghum bran..

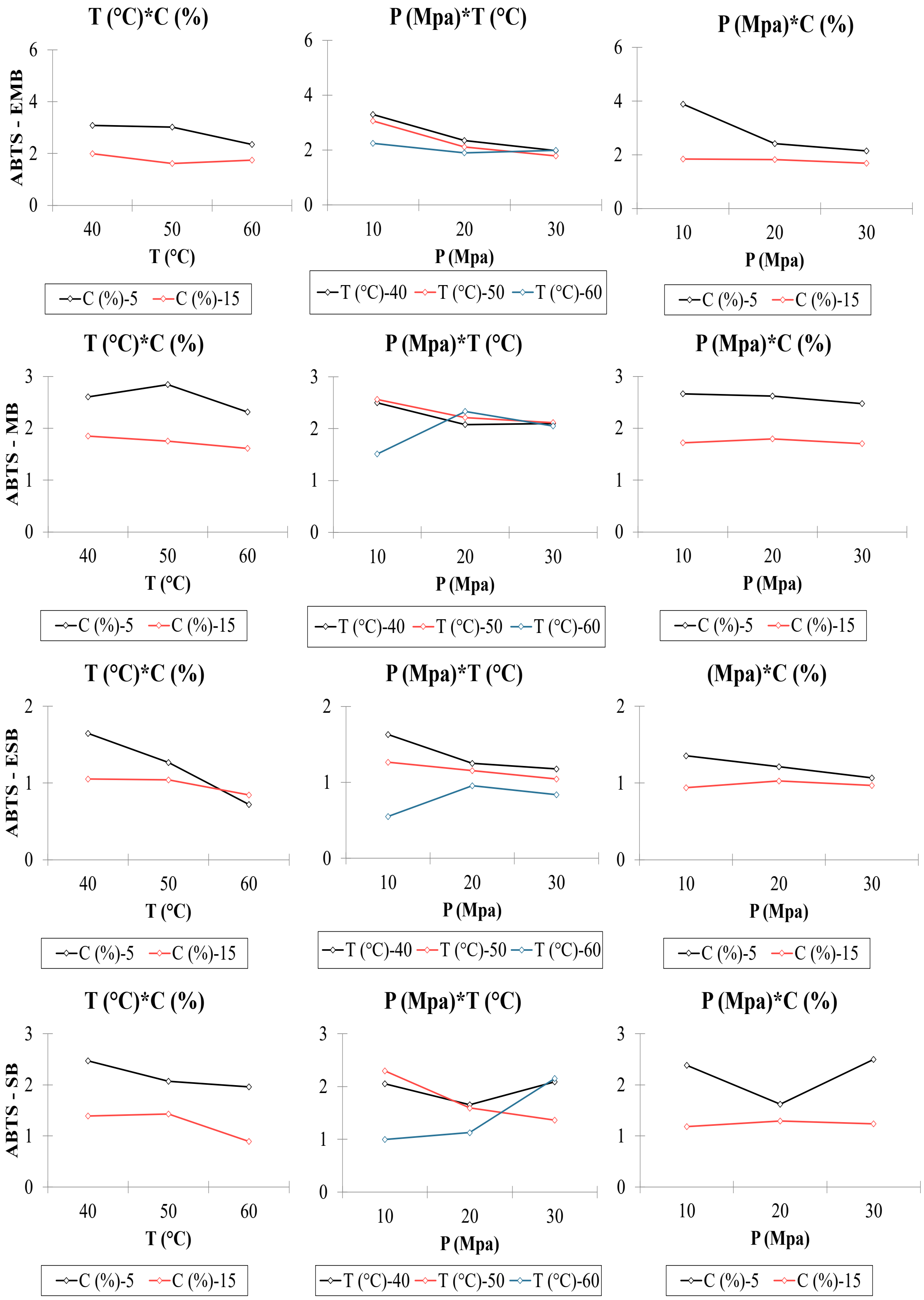

Figure S 9: Influence of interactions between the variables Temperature (T° C), Pressure (P MPa) and co-solvent (C %) in ABTS. MB: Millet bran, EMB: Extruded Millet bran, SB: Sorghum bran and ESB: Extruded sorghum bran..