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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

TESE

Extração de Amido, Processamento e Caracterização Físico-Química, Nutricional e Estudo de Estabilidade em Farinhas (Integrais, Decorticadas e Germinadas) Cruas e Pré-Cozidas por Extrusão de Milheto, Destinadas ao Consumo Humano

Thaís Barbosa dos Santos

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INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

EXTRAÇÃO DE AMIDO, PROCESSAMENTO E CARACTERIZAÇÃO FÍSICO-QUÍMICA, NUTRICIONAL E ESTUDO DE ESTABILIDADE EM FARINHAS (INTEGRAIS, DECORTICADAS E GERMINADAS) CRUAS E PRÉ-COZIDAS POR EXTRUSÃO DE MILHETO, DESTINADAS AO CONSUMO HUMANO

THAÍS BARBOSA DOS SANTOS

Sob a Orientação do Professor Doutor
Carlos Wanderlei Piler de Carvalho

e Co-orientação do Professor Doutor
Davy Willian Hidalgo Chávez

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

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CARLOS WANDERLEI PILER DE CARVALHO, (Dr) EMBRAPA (orientador)
EVELINE LOPES ALMEIDA, (DRa) UFRJ
MARIANA SIMÕES LARRAZ FERREIRA, (DRa) UNIRIO
ELISA HELENA DA ROCHA FERREIRA, (DRa) UFRRJ
JOSE LUIS RAMIREZ ASCHERI, (Dr) EMBRAPA

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ELISA HELENA DA ROCHA FERREIRA
PROFESSOR DO MAGISTERIO SUPERIOR
DTA (12.28.01.00.00.00.00.46)
Matrícula: ###069#6

(Assinado digitalmente em 15/03/2023 14:27)
JOSE LUIS RAMIREZ ASCHERI
ASSINANTE EXTERNO
CPF: ###.###.788-##

(Assinado digitalmente em 15/03/2023 09:01)
MARIANA SIMÕES LARRAZ FERREIRA
ASSINANTE EXTERNO
CPF: ###.###.998-##

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CARLOS WANDERLEI PILER DE CARVALHO
ASSINANTE EXTERNO
CPF: ###.###.587-##

(Assinado digitalmente em 14/03/2023 16:37)
EVELINE LOPES ALMEIDA
ASSINANTE EXTERNO
CPF: ###.###.796-##

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RESUMO GERAL

SANTOS, Thaís Barbosa dos. **Extração de amido, processamento e caracterização físico-química, nutricional e estudo de estabilidade em farinhas (integrais, decorticadas e germinadas) cruas e pré-cozidas por extrusão de milheto, destinadas ao consumo humano**. 2023. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2023.

A Organização das Nações Unidas para Alimentação e Agricultura declarou 2023 como o ano internacional dos milhetos, como uma oportunidade para divulgação deste cereal, contribuindo para o desenvolvimento sustentável e melhoria nutricional. O milheto [*Pennisetum glaucum* (L.) R. Br.], originário do continente africano, é um cereal sem glúten, amplamente cultivado e consumido na África e Ásia, para a alimentação animal e humana, capaz de crescer em condições adversas de seca. No Brasil, seu potencial é subutilizado como cultura forrageira e alimentação animal, apesar de seus grãos conterem muitos componentes promotores da saúde humana. Destaca-se nos grãos de milheto, altos teores de fibras e proteínas. No entanto, um dos fatores que levam ao baixo consumo é o alto teor de lipídio, contribuindo para o desenvolvimento de reações oxidativas. O processamento por extrusão, pode ser uma alternativa na estabilidade das farinhas de milheto. Desta forma, este estudo teve por objetivo avaliar o impacto do processamento nos extrudados de milheto (híbrido ADRg 9070) e suas farinhas extrudadas, em relação às propriedades físico-químicas e nutricionais, digestibilidade *in vitro* de carboidratos, assim como extrair e avaliar o amido de milheto e avaliar a estabilidade físico-química de farinhas extrudadas. Os resultados mostraram que, apesar de apresentarem menor expansão radial, os extrudados de milheto e suas farinhas extrudadas tiveram até 20% mais proteína, 32% mais fibra alimentar e 20% menos digestibilidade de carboidrato quando comparados aos mesmos produtos de milho, evidenciando a superioridade nutricional do milheto; o amido de milheto apresentou uma resistência na formação de pasta associada à baixa viscosidade no processamento a quente (abaixo de 80 °C); podendo estar relacionado à menor expansão encontrada nos extrudados de milheto, por fim, no estudo de estabilidade de farinhas extrudadas, o processamento por extrusão foi capaz de aumentar a estabilidade destas farinhas em até 3 meses, em comparação às farinhas cruas. Portanto, o processamento por extrusão nas farinhas de milheto contribuiu para o aumento da estabilidade físico-química, assim como maior valor nutricional quando comparado aos mesmos produtos de milho.

Palavras-chaves: Cozimento por extrusão, Extrudados sem glúten, Nutri-cereais.

GENERAL ABSTRACT

SANTOS, Thaís Barbosa dos. **Starch extraction, processing and physical-chemical, nutritional characterization and stability study in raw and pre-cooked flours (whole, decorticated and germinated) by millet extrusion, intended for human consumption**. 2023. Thesis (Doctorate in Food Science and Technology). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2023.

The Food and Agriculture Organization of the United Nations declared 2023 as the International Year of Millets, as an opportunity to promote this cereal, contributing to sustainable development and nutritional improvement. Pearl millet [*Pennisetum glaucum* (L.) R. Br.], originally from the African continent, is a gluten-free cereal, widely cultivated and consumed in Africa and Asia, for animal and human consumption, capable of growing in adverse drought conditions. In Brazil, its potential is underused as a forage crop and animal feed, although its grains contain many components that promote human health. It stands out in millet grains, high fiber and protein contents. However, one of the factors that lead to low consumption is the high lipid content, contributing to the development of oxidative reactions. Extrusion processing can be an alternative for the stability of millet flours. Thus, this study aimed to evaluate the impact of processing on extruded millet (hybrid ADRg 9070) and its extruded flours, in relation to physicochemical and nutritional properties, *in vitro* digestibility of carbohydrates, as well as to extract and evaluate the starch of millet and to evaluate the physical-chemical stability of extruded flours. The results showed that, despite having less radial expansion, the millet extrudates and their extruded flours had up to 20% more protein, 32% more dietary fiber and 20% less carbohydrate digestibility when compared to the same corn products, evidencing the superiority millet nutrition; millet starch showed resistance to paste formation associated with low viscosity in hot processing (below 80 °C); which may be related to the lower expansion found in millet extrudates, finally, in the stability study of extruded flours, extrusion processing was able to increase the stability of these flours in up to 3 months, compared to raw flours. Therefore, extrusion processing on millet flours contributed to an increase in physicochemical stability, as well as greater nutritional value when compared to the same corn products.

Keywords: Extrusion cooking, Gluten-free extrudates, Nutri-cereals.

SUMÁRIO

1 INTRODUÇÃO GERAL

A Assembleia Geral das Nações Unidas declarou 2023 como o Ano Internacional dos Milhetos (International Year of Millets 2023 - IYM 2023). IYM 2023 é uma oportunidade para a conscientização e direcionamento de atenção política para os benefícios de cultivo e nutricionais deste cereal (FAO, 2022). Nativo da África, o milheto é um dos cereais mais resistentes à seca, tendo a 6ª maior produção agrícola mundial. Além disso, possui crescimento rápido e produtividade em condições adversas, onde os cereais mais consumidos, como o arroz, trigo e milho, não desenvolveriam. Portanto, o interesse por essa cultura cresce simultaneamente às perspectivas de aquecimento global (SALEH *et al*., 2013; HOUISSA *et al*., 2019).

De acordo com os objetivos de desenvolvimento global sustentável, previstos na Agenda de 2030 pelas Nações Unidas, o Acordo de Paris e o Green Deal determinados pela União Europeia, possíveis soluções foram estipuladas e a principal solução é: o uso racional dos recursos naturais (CVETKOVIĆ *et al*., 2022; FAO, 2022). Nesse sentido, o milheto [*Pennisetum glaucum* (L.) R. Br] é um cereal que vem ganhando destaque devido à excelente tolerância às variações climáticas, podendo ser referência para estudos de resistência à seca pela sua rápida resposta ao estresse hídrico, devido a alguns genes encontrados no milheto como CCCH, ADH1 e FtsH responsáveis por esta tolerância (ZHANG *et al*., 2021).

O milheto também tem recebido destaque nutricionalmente devido à baixa digestibilidade do amido de milheto e o baixo índice glicêmico, provavelmente relacionados ao maior número de cadeias longas de amilose com segmentos de cadeia longa entre os pontos de ramificação. Como consequência, a digestão e absorção do amido tornam-se mais lentas, resultando em menos glicose liberada na corrente sanguínea e uma resposta glicêmica mais baixa (PUNIA *et al*., 2021).

Atualmente, o *Pennisetum glaucum* torna-se cada vez mais atraente devido aos seus benefícios à saúde, por fornecer alta qualidade nutricional, alto teor de proteínas, ácidos graxos, vitaminas e minerais, além de ser um cereal sem glúten (SORATTO *et al*., 2012; DIAS-MARTINS *et al*., 2018). No entanto, apesar de possuir vantagens em relação aos macronutrientes e micronutrientes, o consumo de produtos à base de milheto para humanos ainda se encontra subaproveitado, devido à vida útil para farinha crua limitada à 1 semana (YADAV *et al*., 2012; REDDY, VISWANATH, 2019).

Outro fator a ser avaliado, para melhorar a utilização do milheto, é a escolha de técnicas de processamento adequadas. O milheto, para consumo e preparação de alimentos é geralmente processado por técnicas tradicionais como a decorticação dos grãos, branqueamento, germinação, fermentação e moagem, visando melhorar suas propriedades nutricionais e sensoriais. No entanto, alterações negativas nessas propriedades durante o processamento não são evitáveis, pois estes métodos industriais de processamento não são tão desenvolvidos quanto os métodos usados para o processamento de arroz e trigo, sendo necessário o estudo em pesquisa e desenvolvimento de tecnologias de processamento (FAO, 2012).

A extrusão termoplástica apresenta-se como uma das mais vantajosas alternativas, para a substituição de processos convencionais, como por exemplo, a secagem e a atomização, na produção de farinhas pré-cozidas, pois apresenta controle mais rigoroso do grau de cozimento do amido, além disso, fatores antinutricionais podem ser inativados durante o processo de extrusão (CLERICI, SILVA, EL-DASH, 2008; BALASUBRAMANIAN, KAUR, SINGH, 2011).

Portanto, através de estratégias de valor agregado como a seleção de grãos, germinação e o uso de tecnologias adequadas de processamento, os grãos de milheto são potencialmente promissores na preparação de novos produtos, por serem promotores de saúde, podendo resultar na alta demanda pela população, principalmente em usuários não tradicionais de trigo e milho (SALEH *et al.*, 2013).

Este estudo teve por objetivo inicial realizar a revisão bibliográfica, como introdução geral, abordando aspectos teóricos relacionados ao grão de milheto, composição, germinação, produção de farinha, o estudo inicial do processamento por extrusão e vida útil, atrelada às legislações vigentes, que serviram de base para os capítulos I à III que foram organizados na forma de artigo (Capítulo I – propriedades físico-químicas e digestibilidade de carboidratos *in vitro* dos extrudados expandidos de milheto integral; Capítulo II - propriedades físico-químicas do amido de milheto como potencial ingrediente alimentar com resistência ao calor; Capítulo III – avaliação físico-química em produtos extrudados de milheto, para previsão de vida útil e determinação do potencial tecno-funcional) e por fim, foram descritas as considerações finais deste estudo.

2 JUSTIFICATIVA

Segundo a Organização das Nações Unidas, as projeções de crescimento populacional mundial atingirão os valores de 9.6 bilhões em 2050 e 10.9 bilhões em 2100 (ONU, 2013). Além disso, cenários de mudanças climáticas e estresse hídrico são prejudiciais às principais culturas, como milho e trigo, mas contribuem para a produção de culturas resistentes, como por exemplo o milheto.

Outro fator considerado é o baixo custo do milheto, quando comparado, por exemplo, ao milho, principalmente em períodos de entressafra. Por esses motivos, a importância do milheto não deve ser subestimada (DIAS MARTINS *et al.*, 2018; VILA-REAL *et al.*, 2017). A partir destas qualidades, o ano de 2023 visa divulgar o potencial do milheto, que atualmente representa menos de 3% do comércio global de grãos, como cultura nutricionalmente superior, economicamente rentável e sustentável (FAO, 2023).

O milheto é isento de glúten contribuindo para a oferta de produtos, em especial às pessoas com intolerância ao glúten e doença celíaca (BASTOS *et al.*, 2004). Embora o grão de milheto seja uma excelente fonte de nutrientes, sua vida útil e de sua farinha é baixa, devido ao seu alto teor de lipídio e enzimas.

Essas características indesejadas podem ser minimizadas por tratamento térmico, como por exemplo, o processamento por extrusão (JAIN, BAL, 1997; BALASUBRAMANIAN *et al.*, 2011; KOTAPATI *et al.* 2016; REDDY, VISWANATH, 2019). Neste sentido, para uma melhor utilização do milheto, quanto ao valor nutricional e estabilidade físico-química, torna-se necessária a investigação de processamentos e interações entre os macronutrientes. Portanto, novos conhecimentos em relação ao *Pennisetum glaucum* mostram-se extremamente valiosos, para a sua valorização.

3 REVISÃO BIBLIOGRÁFICA

3.1 MILHETO

O milheto é o termo genérico que descreve grãos pequenos de cereais. Entre as diversas espécies de milhetos produzidos, há o finger millet (*Eleusine coracana*) (Figura 1A), proso millet (*Panicum miliaceum*, conhecido como painço no Brasil) (Figura 1B), foxtail millet (*Setaria itálica*) (Figura 1C), barnyard millet japanese (*Echinochloa utilis*) (Figura 1D), little millet (*Panicum sumatrense*) (Figura 1E), pearl millet (*Pennisetum glaucum*, com sinônimos de *Pennisetum americanum*, *Pennisetum typhoides*, *Pennisetum typhoideum; Pennisetum spicatum*), (Figura 1F) kodo millet (*Paspalum scrobiculatum*), browntop millet (*Brachiaria ramosum*); shama (*Echinocloa colona*) e sorghum (*Sorghum bicolor*) (DIAS-MARTINS *et al*., 2018; FAO, 1995).

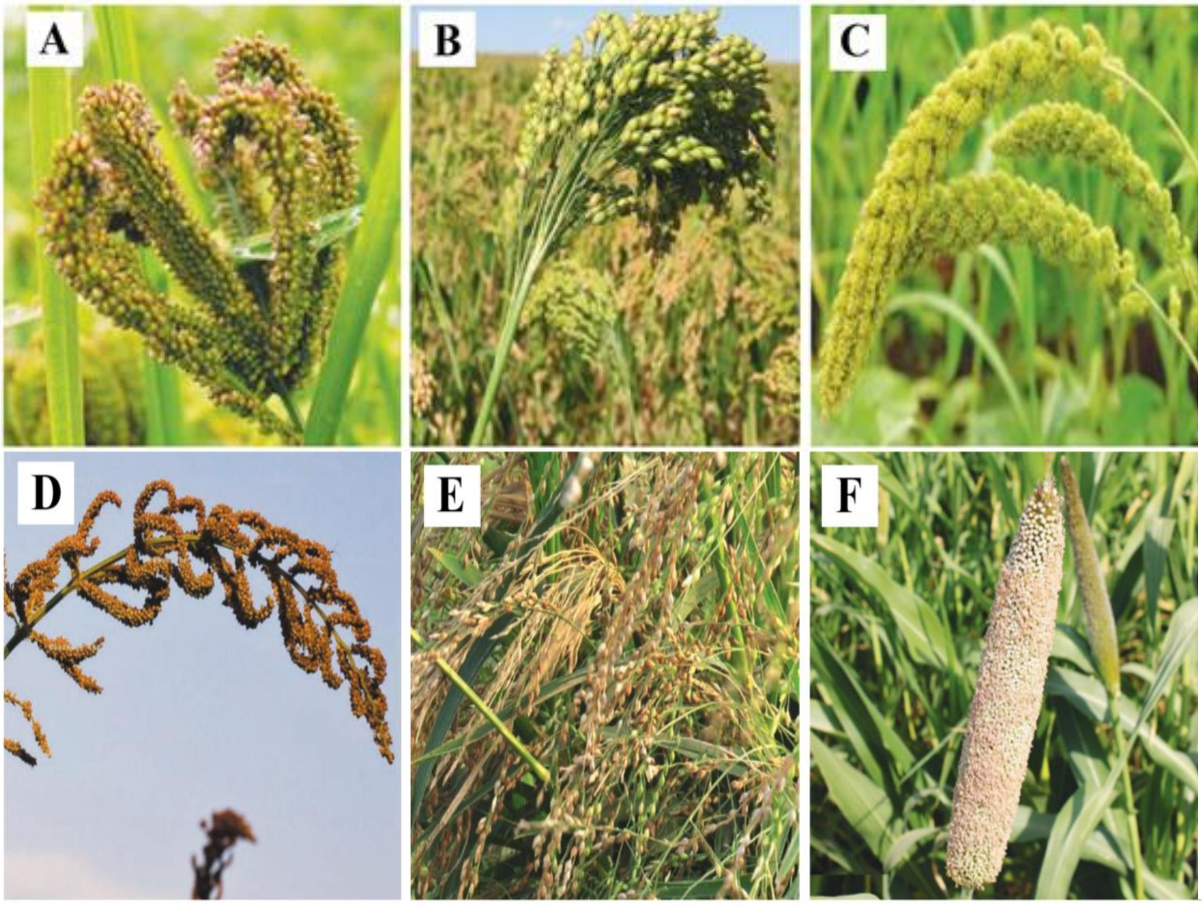

Figura 1. A) *Eleusine coracana*; B) *Panicum miliaceum*; B) *Setaria itálica*; D) *Echinochloa utilis*; E) *Panicum sumatrense*; F) *Pennisetum glaucum* (DAYAKAR *et al*, 2017).

Originário da África, o milheto é um dos cereais mais cultivados no mundo. Possui rápido crescimento e alto nível de tolerância ao calor comparado ao milho, podendo ser cultivado em solo arenoso e pobre em nutrientes. Estas características se devem à presença de um sistema radicular extenso da planta, que permite a eficaz extração de água e nutrientes das camadas mais profundas do solo, produzindo altos rendimentos econômicos (BARYEH, 2002; TAYLOR, 2016).

No Brasil, os primeiros relatos do cultivo são a partir de 1920 no Rio Grande do Sul. Desde então, a cultura do milheto expandiu-se pelo país, principalmente nos cerrados, sendo utilizado como cultura forrageira e alimentação animal. Em países da África e Ásia, é utilizado para alimentação humana, cujos grãos são um componente importante na dieta alimentar de habitantes dessas regiões (BRASIL, 2019).

Entre as diversas espécies de milheto produzidas no mundo para diversos fins, a estrutura básica do grão é semelhante, sendo constituído principalmente de farelo, endosperma e gérmen (Figura 2).

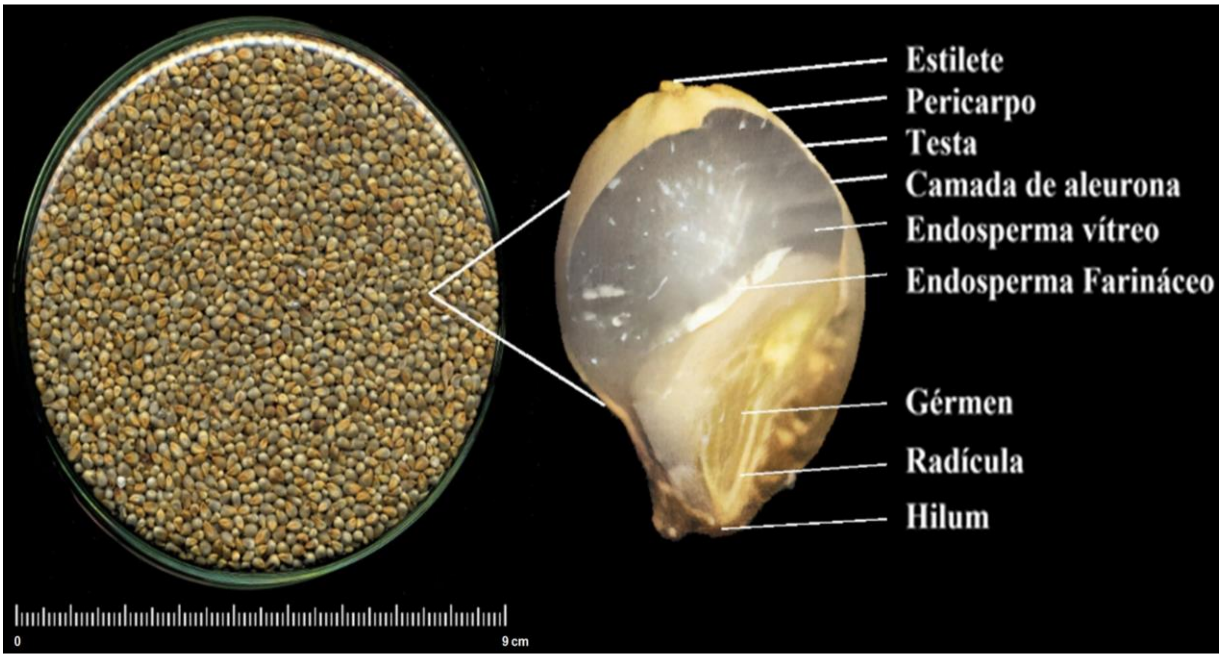

Figura 2. Estrutura do grão de milheto, *Pennisetum glaucum* (Por: Thaís B. Santos).

O farelo é a camada mais externa e protetora que engloba o endosperma e o gérmen da semente. O endosperma é composto por endosperma amiláceo e camada de aleurona que contém proteínas, lipídios, vitaminas e minerais. O gérmen do grão inclui o embrião e o escutelo, rico em lipídios, proteínas e minerais. Contudo, nota-se que a espessura do pericarpo e a relação gérmen-endosperma variam significativamente entre as espécies, estando bem estabelecido que a maioria dos bioativos no milheto está localizada no revestimento das sementes (AKANBI, TIMILSENA, DHITAL, 2019).

No entanto, em países desenvolvidos, há uma demanda crescente por alimentos e bebidas sem glúten, por pessoas com doença celíaca. Uma dieta sem glúten afeta principalmente o consumo de grupos à base de trigo, cevada e centeio. E como substituição de alimentos que contenham glúten, há uma adequação de dieta incluindo arroz, milho, sorgo, quinoa e o milheto, destacando-se o *Pennisetum glaucum* como espécie de milheto com alto valor nutricional, em ascensão (SALEH *et al*., 2013; REDDY, VISWANATH, 2019).

3.1.1 *Pennisetum glaucum*

O *Pennisetum glaucum* é a espécie de milheto mais cultivada, principalmente em países em desenvolvimento, com produção de 30 milhões de hectares em todo o mundo (REDDY, VISWANATH, 2019), sendo a Índia, atualmente, a maior produtora desta espécie (AKANBI, TIMILSENA, DHITAL, 2019). Possui, assim como as demais espécies de milheto, estação de crescimento anual e resistência à seca, salinidade e estresse por temperatura. Além disso, é facilmente adaptável a solos arenosos e de baixa fertilidade (HOUISSA *et al*., 2019).

A qualidade nutricional dos alimentos é um dos fatores essenciais para o funcionamento do organismo e manutenção da saúde, neste sentido, quanto aos problemas de insegurança alimentar e desnutrição, a qualidade da dieta deve ser levada em consideração (SINGH, RAGHUVANSHI, 2012). A média da composição de macro nutrientes dos grãos de milheto *Pennisetum glaucum*, comparada a outros grãos, está resumida na Tabela 1.

Tabela 1. Composição do milheto *Pennisetum glaucum* comparado a outros grãos.

Composição (g / 100 g)	***Pennisetum glaucum***	**Milho**	**Trigo**	**Sorgo**	**Centeio**	**Arroz**	**Aveia**
Carboidrato	69.4–76.1 (72.2)	69.4-85.1 (78.1)	67.7-69.3 (68.8)	72.2-77.1 (74.6)	64.7-67.7 (66.4)	81.9-88.2 (84.9)	60.6-62.9 (61.4)
Proteína	9.7-14.5 (11.8)	7.7-12.1 (9.2)	13.2-14.8 (14.0)	9.4-11.8 (10.7)	11.6-14.0 (13.0)	6.7-11.0 (8.6)	4.9-17.1 (16.7)
Lipídio	5.1-9.5 (6.4)	1.9-4.3 (3.3)	1.5-2.8 (2.2)	3.2-3.7 (3.4)	1.8-2.0 (1.9)	1.6-2.2 (2.1)	6.4-8.6 (7.6)
Fibra alimentar	7.0-8.5 (7.8)	4.5-8.2 (8.1)	11.9-15.1 (13.1)	7.3-11.8 (9.6)	16.1-17.4 (16.8)	2.7-4.7 (3.5)	1.2-12.5 (11.8)
Cinzas	0.8-2.5 (1.8)	0.7-1.8 (1.3)	1.8-2.0 (1.9)	1.5-1.8 (1.7)	1.7-2.0 (1.9)	0.6-1.4 (0.9)	1.4-3.6 (2.5)

*Todos os valores são expressos com base na matéria seca. O número superior refere-se ao intervalo de valores e o inferior indica o valor médio da composição (DIAS-MARTINS *et al*., 2018).

Devido à sua importante contribuição para a segurança alimentar e os benefícios à saúde, principalmente na África e Ásia, o grão de milheto está recebendo crescente interesse de cientistas de alimentos, tecnólogos e nutricionistas mundialmente em relação à sua composição (SALEH *et al*., 2013).

Os lipídios presentes no *Pennisetum glaucum* possuem altos teores de ácidos graxos insaturados (77%), com maiores concentrações dos ácidos oleico (27%), linoleico (25 a 46%) (NAMBIAR *et al*., 2011; MARMOUZI *et al*., 2016; SLAMA *et al*., 2020). Apesar da alta concentração lipídica ocasionar problemas tecnológicos como o aumento de oxidação lipídica, devido ao aumento de reações oxidativas, por outro lado, os ácidos graxos auxiliam na redução de triglicerídeos e efeitos negativos relacionados ao colesterol, previne doenças cardíacas (KOTAPATI *et al*., 2016).

Segundo a FAO (1995), a qualidade da proteína em grãos de milheto satisfaz os requisitos nutricionais de um adulto, mas não atende às necessidades proteicas de lactentes e crianças, devido à quantidade de aminoácidos, especialmente lisina, que geralmente é baixa em cereais. Entretanto, o teor de lisina do milheto é superior ao milho, centeio, trigo e sorgo, sendo significativamente mais ricos em aminoácidos essenciais como a isoleucina.

A média da composição de aminoácidos em 100 g de proteína do milheto *Pennisetum glaucum*, foi comparada a outros grãos (Tabela 2).

Tabela 2. Composição de aminoácidos em grãos de cereais.

Aminoácidos (g /100 g proteína)	**Milheto**	**Milho**	**Trigo**	**Sorgo**	**Centeio**	**Arroz**	**Aveia**
Leucina	10.7	12.3	6.8	12.9	5.4	8.2	7.6
Isoleucina	4.4	3.6	3.3	3.7	2.0	4.1	4.1
Valina	4.9	5.1	4.3	4.6	3.0	5.8	5.5
Treonina	4.0	3.8	2.8	3.7	2.8	3.5	3.4
Arginina	4.6	4.9	4.9	3.9	4.4	8.7	7.1
Lisina	3.1	2.8	2.7	2.1	2.8	3.5	4.1
Metionina	1.1	2.1	1.7	1.7	1.5	2.4	1.8
Cisteína	1.5	1.8	2.0	1.9	na	1.8	2.4
Triptofano	1.4	0.7	1.3	1.2	1.0	1.2	1.4
Alanina	8.7	7.5	3.7	8.9	3.9	5.6	5.2
Prolina	5.8	8.7	15.7	7.7	7.8	4.7	5.5
Fenilalanina	4.4	4.9	5.2	5.2	4.2	5.3	5.3
Tirosina	3.0	4.1	2.1	2.7	1.9	5.3	3.4
Histidina	2.3	3.0	2.7	1.9	1.8	2.5	2.4
Glicina	2.7	4.1	4.3	3.7	4.0	4.5	4.9
Serina	5.2	4.7	4.7	4.9	4.4	5.2	4.4

De acordo com Dias-Martins *et al.* (2018).

De acordo com Dias-Martins *et al.* (2018) e Vila-Real *et al.* (2017), o milheto se destaca pelo alto valor nutricional, devido ao maior teor de fibra, comparado por exemplo com o arroz, maior teor de aminoácidos essenciais (destacando-se a isoleucina, comparado a outros cereais tradicionais, como trigo e centeio) e excelente teor de micronutrientes, especialmente os minerais, sendo uma importante fonte de cálcio e ferro. Embora o milheto tenha um alto potencial para a alimentação humana, produtos derivados de milheto, ainda não são comercializados no Brasil para consumo humano.

No continente africano e asiático, o processamento do milheto e os diversos produtos consumidos, oferecem segurança nutricional também para humanos. Serve como um ingrediente importante para produção de alimentos e bebidas tradicionais, como pão, mingau e biscoitos; em outras partes do mundo é utilizado para o preparo de macarrão. É, também, uma importante mercadoria para norte-americanos e europeus, sendo destacada sua importância como ingrediente em produtos multigrãos e sem glúten (CHANDRASEKARA, SHAHIDI, 2012; SALEH *et al*., 2013; BUENO *et al*., 2015).

Neste sentido, os grãos de milheto possuem grande potencial no desenvolvimento de produtos, destacando-se a germinação e extrusão como forma de agregar valor aos produtos, por apresentarem baixo custo de produção, alto valor nutricional, além de contribuírem com a redução de fatores antinutricionais, no entanto, poucos estudos são realizados no milheto (BADAU, NKAMA, JIDEANI, 2005; BALASUBRAMANIAN *et al*., 2014).

3.1.2 Processo de germinação e extrusão do milheto

Para o processamento dos cereais, incluindo os de milheto, a maioria dos grãos podem ser moídos, decorticados, germinados, fermentados, cozidos e extrudados, para obter produtos como farinhas, biscoitos, massas e bebidas não lácteas. Sabe-se que a funcionalidade de grãos está diretamente ligada ao processo aplicado (DIAS-MARTINS *et al*., 2018).

A germinação é um processo biológico que pode ser utilizado industrialmente, fazendo-se uso a partir do grão integral de controle de temperatura e umidade adequados específico para cada vegetal, para a obtenção de um grão germinado. Este processamento é classificado em três etapas: imersão em água, germinação e secagem, promovendo alterações físico-químicas nos grãos (TAYLOR, 2016).

Para uma melhor germinação, é fundamental o controle das condições de estocagem através do armazenamento em câmara fria e seca, sendo observados resultados superiores de germinação, em relação às sementes sem armazenamento ou armazenadas em ambiente não-controlado (GASPAR, NAKAGAWA, 2002). Na Figura 3, são demonstrados os grãos de *Pennisetum glaucum* germinados.

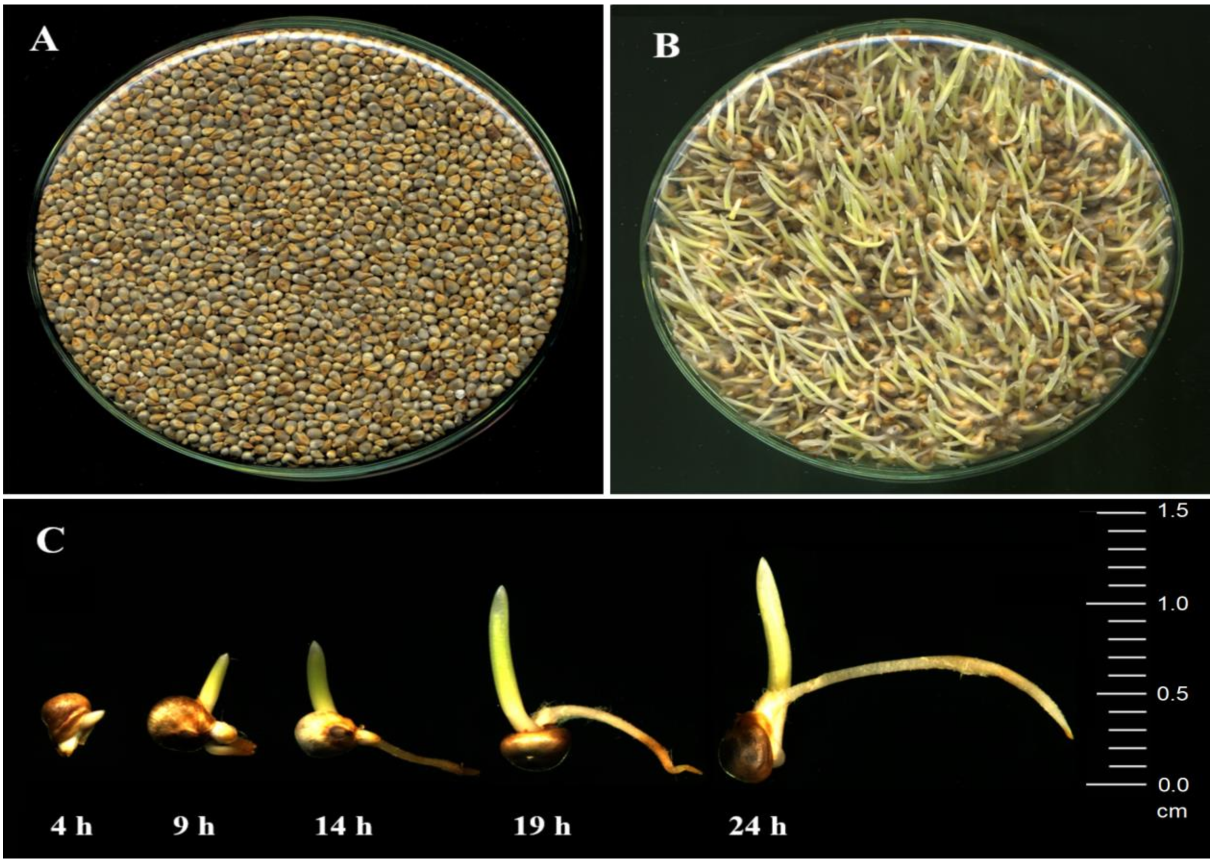

Figura 3. *Pennisetum glaucum*: A) Grãos integrais; B) Grãos germinados; C) Grão acompanhados durante 24 h, à 30 °C e 90% de umidade relativa (Por: Thaís B. Santos).

Além disso, o tamanho da semente é, também, um dos fatores que podem influenciar a germinação. As sementes de maior tamanho, ou as que apresentam maior densidade, são melhores nutridas durante seu desenvolvimento e normalmente possuem embriões bem formados, sendo potencialmente, as mais vigorosas, bem como, com maior potencial de sobrevivência (GASPAR, NAKAGAWA, 2002).

A germinação tem fundamental importância no aumento de conteúdo antioxidantes presente no grão sendo descrito por Xu, Lei *et al*. (2017), em milheto, que os grãos germinados tiveram um aumento de 29.7 até 102.07 mg/100 g no final da germinação, no conteúdo de ácido γ-aminobutírico (GABA), um aminoácido não proteico que age como neurotransmissor no cérebro. Neste sentido, utiliza-se as sementes germinadas, principalmente nos países africanos e asiáticos, possuem fundamental importância em hábitos saudáveis (TAYLOR, 2016). Entretanto, estudos limitados foram realizados para verificar as alterações físico-químicas causadas pelo processamento, sendo necessários mais estudos para melhor entender os efeitos da germinação em milheto (REDDY, VISWANATH, 2019).

Em relação ao processamento por extrusão, Yadav, Dalbhagat e Mishra (2022) em revisão sobre extrusão de milheto, destaca que poucos estudos relataram os efeitos nas características de cozimento, microestruturas e propriedades físicas e térmicas. O processamento por extrusão termoplástica pode ser definido como uma forma de cocção rápida, contínua e homogênea pelo uso de altas temperaturas e curto tempo, utilizando várias operações unitárias e modificações físico-químicas, frequentemente em combinação, como: mistura, moldagem, gelatinização e secagem; podendo ser produzidos diferentes produtos como cereais matinais, *snacks* e farinhas instantâneas (ONYANGO *et al*., 2004).

A extrusora, equipamento utilizado na extrusão, possui uma alimentação inicial onde são inseridas as matérias-primas e ingredientes, uma rosca sem fim, podendo ser simples (Figura 4) ou dupla rosca visando criar forças de cisalhamento na mistura de alimentos e forçar a passagem do material. Deste modo, após as respectivas matérias-primas sofrerem um aquecimento que conduz ao seu amolecimento ou até à fusão, o produto é moldado por um orifício de saída denominado matriz (KAMAU, NKHATA, AYUA, 2020).

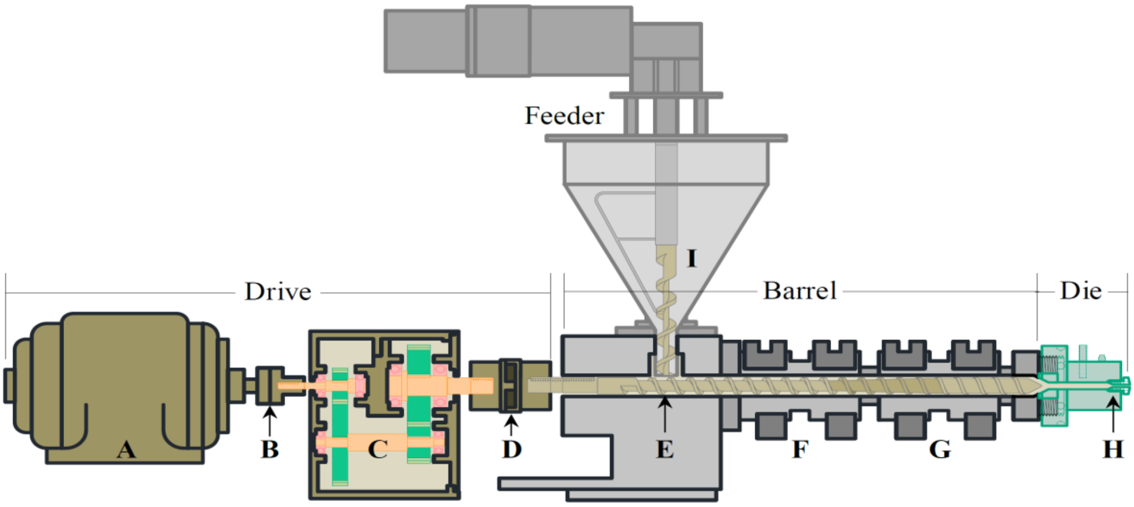

Figura 4. Esquema de uma extrusora monorosca: (A) motor, (B) Reômetro de torque acoplado, (C) caixa de engrenagens, (D) acoplamento; (E) parafuso extrusor, (F) primeira zona de aquecimento/resfriamento, (G) segunda zona de aquecimento/resfriamento; (H) matriz circular; (I) parafuso alimentador (VARGAS-SOLÓRZANO, 2019).

O processamento por extrusão reduz fatores antinutricionais, além de promover o aumento da digestibilidade, aumento da viscosidade e solubilidade do material, sendo o perfil físico-químico de um extrudado dependente diretamente das variáveis do processo como, a extrusora utilizada, as temperaturas empregadas, a rotação dos parafusos, entre muitos outros parâmetros, como também a matéria-prima utilizada (LOPES, SANTOS, CHOUPINA, 2015; ONYANGO *et al*., 2004).

Destaca-se também a ação do processamento térmico, o aumento da estabilidade lipídica no armazenamento, através da inativação de enzimas. Estas enzimas comprometem sensorialmente a estabilidade físico-química do milheto através do processo degradativo que consiste na fase ácida ou hidrolítica, onde a enzima lipase atua sobre os lipídios, causando rancidez oxidativa, tendo como resultado deste processo, alterações na cor, aroma, sabor e consistência, além de reduzir a qualidade dos produtos (SALEH *et al.*, 2013; ANTUNES, 2016; REDDY; VISWANATH, 2019). Neste sentido, torna-se evidente a importância do processamento para melhoria da estabilidade físico-química do milheto.

3.2 PROCESSAMENTO PARA PRODUÇÃO DE FARINHA

A farinha é definida pela Agência Nacional de Vigilância Sanitária sendo o "produto obtido de partes comestíveis de uma ou mais espécies de cereais, leguminosas, frutos, sementes, tubérculos e rizomas por moagem ou outros processos tecnológicos considerados seguros para a produção de alimentos" e as farinhas integrais definidas como "produto resultante da trituração ou moagem de cariopses intactas de alpiste, amaranto, arroz, arroz selvagem, aveia, centeio, cevada, lágrimas-de-Jó, milheto, milho, painço, quinoa, sorgo, trigo, trigo sarraceno e triticale, onde os componentes anatômicos – endosperma amiláceo, farelo e gérmen – estão presentes na proporção típica que ocorre no grão intacto, sendo permitidas perdas de até 2% do grão ou 10% do farelo" (BRASIL, 2022).

As farinhas representam uma grande variedade de alimentos em pó, os quais se diferenciam segundo a sua composição química e suas características. Na indústria de alimentos participam do processo de produção não só como matérias primárias e intermediárias, como também de produtos finais (MOREIRA, CARVALHO, VASCONCELOS, 2006). A retomada dos hábitos de alimentação saudável por parte da população tem colocado os grãos integrais em destaque, ou seja, sendo mantidas as fibras, resultando em uma maior saciedade durante as refeições (ANTUNES, 2016).

As etapas para obtenção da farinha crua consistem em limpeza (determinação do teor de impurezas), classificação dos grãos, estabilização da umidade e moagem, obtendo-se a farinha integral crua, podendo ser realizados outros processos e posteriormente embalada (MOREIRA, CARVALHO, VASCONCELOS, 2006). Contudo, ainda não foram realizados estudos para a produção de farinha de milheto germinada e suas propriedades.

Para a obtenção da farinha é realizada, inicialmente, uma limpeza nos grãos, para que seja determinado o teor de impurezas no cereal. Assim como descrito para o trigo, por Germani, Benassi e Carvalho (1997), o cereal possui diversas impurezas originárias do campo, da estocagem e do transporte; ou seja, devem ser retirados os grãos quebrados, não desenvolvidos ou danificados, todos os componentes que diferenciam do cereal sadio e normal, devendo ser também expressos como a porcentagem de impureza da amostra. Quanto mais impurezas apresente um determinado lote de cereal, menor será o aproveitamento após a limpeza, podendo deteriorar mais facilmente e consequentemente gerar menor rendimento econômico.

Já as matérias estranhas, retiradas durante a etapa de limpeza, podem ser de diversas origens e tipos, como de origem vegetal (grãos de outros cereais e palha), origem animal (pelos e excrementos de ratos, insetos ou mesmo fragmentos de insetos), minerais (pó, barro, pedras, objetos ou fragmentos de metal) e de outras origens; (GERMANI, BENASSI, CARVALHO, 1997).

Quanto ao processamento, para a obtenção da farinha, estabiliza-se a umidade do grão de cereal, através da secagem ou adição de água obtendo-se uma umidade desejada, observando-se também as condições de armazenamento (temperatura e umidade relativa) (SALEH *et al.*, 2013; DIAS-MARTINS *et al.*, 2019). Além disso, pode ser moído e sua farinha, crua ou pré-cozida, pode ser usada para produção de uma ampla variedade de alimentos (AKINOLA *et al.*, 2017).

A moagem para grãos integrais é o procedimento onde um alimento é levado a uma trituração com o objetivo da redução do tamanho de suas partículas. Após a realização da moagem podem passar ou não por um sistema de peneiramento para padronizar a granulometria da farinha obtida, sendo posteriormente embalados, em embalagem de polietileno. Neste sentido, produtos com alto teor de fibra como o arroz e o trigo, e óleo como o milho e a aveia, são geralmente mais difíceis de ser realizada a moagem do que em produtos com alto teor de amido e secos (GERMANI, BENASSI, CARVALHO, 1997; MOREIRA, CARVALHO, VASCONCELOS, 2006).

Ao serem obtidas as farinhas cruas, estas precisam de tratamentos ou condições convenientes para melhorar a estabilidade, devido ao impacto do aumento da exposição dos nutrientes, enzimas, alto teor lipídico e lixiviação de materiais intracelulares na produção de farinha (SALEH *et al.*, 2013; DIAS-MARTINS *et al.*, 2019). YADAV *et al.* (2012) mencionam que o consumo da farinha integral de milheto crua é comprometido devido a sua reduzida vida útil (5 a 6 dias) associada ao alto teor de lipídio e alterações enzimáticas, inviabilizando o armazenamento por períodos mais extensos, sendo fundamental o processamento da farinha antes da estocagem.

No Brasil, ainda não há limites estabelecidos na legislação brasileira para as farinhas de milheto na determinação da vida útil, sendo também poucas as atualizações relacionadas às farinhas sem glúten. Já a RDC 263 de 2005 da ANVISA que dispõe sobre produtos de cereais, amidos, farinhas e farelos, estipula a umidade máxima de 15%, sendo este limite aplicado de maneira geral às farinhas de cereais.

O estabelecimento da estabilidade físico-química e microbiológica é fundamental para a segurança do alimento; o período fora da vida útil caracteriza-se pelo início de reações de deterioração dos lipídeos encontrados nos cereais resultando em rancidez oxidativas e sabor descrito como amargo e adstringente, em consequência principalmente da ação das lipoxigenases, limitando o consumo e causando o gosto desagradável, principalmente nos alimentos não processados, assim como o aumento de riscos microbiológicos (ANTUNES, 2016). Portanto, a aplicação de métodos de processamentos adequados, como por exemplo o cozimento por extrusão pode ser uma alternativa para o aumento da estabilidade físico-química e vida útil.

4 CONCLUSÃO

A partir desta revisão, foi possível o entendimento de etapas importantes para o processamento como seleção/limpeza dos grãos de milheto e extrusão, sendo etapas potencialmente efetivas para aumentar a estabilidade físico-química dos extrudados e suas farinhas processadas, através da inativação ou redução de enzimas, ocasionada principalmente pelo processamento por extrusão.

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CAPÍTULO I

PROPRIEDADES FÍSICO-QUÍMICAS E DIGESTIBILIDADE DE CARBOIDRATOS *IN VITRO* DOS EXTRUDADOS EXPANDIDOS DE MILHETO INTEGRAL

PHYSICOCHEMICAL PROPERTIES AND *IN VITRO* CARBOHYDRATE DIGESTIBILITY OF WHOLE PEARL MILLET EXPANDED EXTRUDATES

Thaís Barbosa dos Santos[1], Davy William Hidalgo Chávez[1], Caroline Grassi Mellinger[2], Carlos Wanderlei Piler de Carvalho[2]

[1] Universidade Federal Rural do Rio de Janeiro, Rodovia Br 465, km 7, CEP 23890-000 Seropédica, RJ, Brazil.
[2] Embrapa Agroindústria de Alimentos, Avenida das Américas 29501, Guaratiba, CEP 23020-470 Rio de Janeiro, RJ, Brazil.

ABSTRACT

In order to encourage the use of wholemeal pearl millet, processed by extrusion, for the development of extruded products and whole flours with health-promoting values, and rational use of natural resources; whole grain pearl millet (ADRg 9070 hybrid) and commercial whole grain corn flours were individually single screw extruded at varied moisture content with the aim to compare the effect of the extrusion process on physicochemical properties, as well as *in vitro* carbohydrate digestibility. The physical properties analyzed were expansion properties and density, instrumental texture, morphology, relative crystallinity, thermal properties, pasting properties, water absorption and solubility indices; Chemical analysis such as centesimal composition and total starch content were also carried out. By increasing water content (20%), whole pearl millet puffed surprisingly presented an increase in radial expansion and water solubility index contrasting to whole corn puffed extrudates that presented a reduction in radial expansion. The presence of starch birefringence and enthalpy in the whole pearl extrudates at 14% demonstrated that an unknown mechanism of reducing shearing in the extruder preserved the starch integrity. In addition, compared to whole corn, whole millet extrudates were nutritionally superior with up to 32% more fiber and 20% more protein, and about 20% less digestible in carbohydrates. Proving the nutritional viability of whole millet extrudates and providing nutritional aspects required by consumers.

Keywords: starch conversion, extrusion cooking, whole meal puffed extrudates, multivariate analysis.

Practical Application

Whole pearl millet extrudates showed distinct properties compared to corn extrudates exhibiting lower expansion and 20% less *in vitro* carbohydrate digestibility for different extruded flours, higher protein, and up to 32% higher in dietary fiber content; which may contribute as a source for providing healthier gluten-free products that can be adopted by the food industry. By comparing whole grain, pearl millet with corn was able to show distinguished differences, although both are cereals. Also, the idea of using whole grain pearl millet processed by extrusion is to demonstrate the viability of using the cereal as a whole imputing interesting nutritional aspects, as high content of protein, fiber, and less carbohydrate digestibility, lately required by the consumers as a choice of ready-to-eat product.

1 INTRODUCTION

Millets often called “Nutri-Cereals” are ancient crops with small seeds belonging to the Poaceae family. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is traditionally cultivated and consumed mainly by developing countries and it has been receiving special attention from the Food and Agriculture Organization because it is resistant to pests and diseases and it has the ability to grow in dry and less fertile land and being sources of bioactive compounds of great health interest, joining health needs with nutrition (CEASAR, MAHARAJAN, 2022; FAO, 2021; SHEETHAL *et al.*, 2022).

Pearl millet is a gluten-free cereal, highly nutritious (9.7–14.5% protein and fiber 7.0-8.5%) and has higher concentrations of iron and zinc than rice and corn (DIAS-MARTINS *et al.*, 2018; ZHANG *et al.*, 2021). Pearl millet germ is, proportionally, the largest of all cereals, representing about 16.6% of the grain, being composed of oils, proteins (albumins and globulins), soluble sugars and also minerals and vitamins. The endosperm, comprises proteins (prolamins) and mainly starch (60-70% in raw grain) and represents 75.1% of the grain anatomy (EMBASHU, NANTANGA, 2019).

Cereals and starches are the main raw materials to prepare instant breakfast cereals, snack foods, flours, pastas, bakery products, infant foods, and other products due to their functional properties, availability, less cost and versatility (KAUR *et al.*, 2022). The starch is responsible to 30–40% of daily energy intakes, and is broken down in the gastrointestinal tract, releasing glucose into blood circulation, having as a consequence the increase of blood sugar concentration. The starch hydrolysis is dependent on rate hydrolysis, classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (YAN *et al.*, 2019).

Extrusion cooking is one of the processes utilized for starch-based food production, usually showing starch hydrolysis and modifications in different ways. However, the precisely thermomechanical control of the extrusion process is still very challenging. The quality of extrudates can be affected by the raw material (proximate composition, particle size distribution, moisture), by the extruder type (single or twin-screw, screw length, die design), and the extrusion parameters (temperature zones in extrude, feed rate and screw speed). As a consequence of the extrusion irreversible changes result in macroscopic (shape, texture and color) and microscopic changes (proximate composition, complex conformational and starch changes, etc.) (YANG *et al.*, 2020; ZHANG *et al.*, 2019).

The development of pearl millet extruded products can be promising due to grains that can be produced at low cost and have high nutritional value (BALASUBRAMANIAN, KAUR, SINGH, 2014). However, there are few studies observing the effects of the extrusion process on the physicochemical properties of the whole grain pearl millet expanded extrudates, while corn is currently the main gluten-free cereal that is source for producing commercially ready-to-eat puffed snacks (GRASSO, 2020; LEONARD, 2020; ROMERO RODRÍGUEZ *et al.*, 2021).

In a recent study with the aim to increase the nutritional quality of puffed extrudates, a mix (1:1) of refined corn grits and pearl millet flour was processed in a single screw extruder at varied temperature and moisture content to obtain healthier puffed snacks. The sensory approved puffed extrudates were obtained at 90 °C at 11% of moisture content with 9.5% of protein and 5.9% of dietary fiber (OLIVEIRA *et al.*, 2021). However, the authors did not study the extrusions of whole corn and whole millet processed separately.

In this sense, the aim of this study was to evaluate the single screw extrusion on whole grain pearl millet and whole grain corn flours on the production of extrudates, as well as to understand correlations through principal component analysis (PCA), hierarchical clustering on principal components (HCPC) and Pearson’s correlation, in order to evaluate the physicochemical properties and *in vitro* digestibility of pearl millet compared to corn.

2 MATERIALS AND METHODS

2.1 SAMPLE PREPARATION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] grains of hybrid ADRg 9070 kindly donated by ATTO Sementes located in Rondonópolis – Brazil harvested in July of 2021 at geographical coordinates: 16°29' S latitude and 54°38' W longitude were cleaned in a seed air cleaner separator (Clipper Office Tester, AT Ferrell Company Inc., Bluffton IN, USA). Commercial yellow dent hybrid corn grains were kindly donated by Indústrias Granfino (Nova Iguaçu, Brazil). Whole millet and corn grains (moisture content of 4 ± 0.5%) were ground in a hammer mill LM3100 (Perten Instruments AB, Huddingen, Sweden) equipped with a 0.8 mm sieve into whole grain fine flours that were used in the extrusion process.

2.2 EXTRUSION PROCESS

Raw whole grain millet and corn flours, preconditioned at varied moistures of 11, 14, 17, 20 and 23% (to obtain different expanded extrudates) were processed in a single-screw extruder 19/20 DN (Brabender, Duisburg, Germany) attached to a torque rheometer PlastCorder LabStation (Brabender, Duisburg, Germany).

The extruder was fitted with a 3:1 (compression rate) screw, a 3 mm round die running at constant speed of 250 rpm and temperature heating zones adjusted to 40, 80 and 120 °C from feeding to the outlet die in order to allow the formation of internal air bubbles inside the extrudates (puffed snacks). The raw whole grain flours were fed using a volumetric vertical feeder running at variable speed to provide a constant feed rate of 4 kg/h. Die temperature (°C) and motor torque (Nm) shown on the extruder control panel were recorded. The experiment was carried out seven times using constant operating parameters in order to be sure of the reproducibility.

The extrudates were dried at 50 °C (up to 4 ± 0.5% moisture content) in a fan oven and stored in plastic bags at room temperature for further analyses. Part of the extrudates were milled in a hammer mill Lab Mill 3100 (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm sieve size to obtain extruded whole grain flours that were also packed and stored at room temperature.

2.3 EXPANSION PROPERTIES AND APPARENT DENSITY

The radial expansion index (REI), sectional expansion index (SEI), the apparent density of extruded pieces (ρ_e), the longitudinal expansion index (LEI) and the volumetric expansion index (VEI) were then calculated according to Alvarez-Martinez; Kondury and Harper (1988). Extrudates were randomly selected from each extrusion trial. The diameter and length of specimens taken from each extrudates were measured using a digital caliper (ZAAS Precision, Curitiba, Brazil) in triplicate.

2.4 INSTRUMENTAL TEXTURE

Previously dried (50 °C up to 4% ± 0.5 moisture content) and kept in a desiccator at room temperature, the extrudates (20 mm long cut) were used to determine the maximum compression force at 1 mm/s using a BSK (blade set knife) probe in a Texture Analyzer TA-XT Plus (Stable Micro Systems, Surrey, UK) equipped with a 30 kg load cell. The force-time curve was analyzed by the Exponent software program version 6.1.11.0 (Stable Micro Systems, Surrey, UK).

2.5 PROXIMATE COMPOSITION

Moisture and ash were determined using the thermogravimetric analyzer TGA- 2000 (Navas Instruments, Conway, USA), according to method n° 925.09 and 923.03 respectively, total dietary fiber following the method 991.43 (enzymatic-gravimetric method), and protein by Kjeldahl method 2001.11 (AOAC, 2005). Lipid by official method Am 5-04 (AOCS, 2005). Carbohydrate content was estimated by difference. The proximate composition was carried out in duplicate.

2.6 TOTAL STARCH AND AMYLOSE CONTENT

Total starch content of raw and extruded flours was determined in duplicate by method 76-11 using the total starch Megazyme assay kit procedure (AA/AMG) (Megazyme International Ireland Ltd, Bray, Ireland) (AACC, 1976).

Amylose content was determined according to ISO 66470 using the Megazyme assay kit (Megazyme International Ireland Ltd, Bray, Ireland) (ISO, 1987). The results were carried out in duplicate.

2.7 MORPHOLOGY OF THE FLOURS

Raw and extruded flours were placed on a histological slide covered with a thin glass plate and observed under an optical microscope Leitz Laborlux S (Leica, Famalicão, Portugal). A polarized filter was employed in order to observe the birefringence of the starch granules at magnification of 400x. The collected images were recorded by a digital camera (MShot, Guangzhou, China) attached to one of the optical microscope.

2.8 X-RAY DIFFRACTION AND RELATIVE CRYSTALLINITY

Diffraction pattern of raw and extruded flours with 7 ± 1% water content was performed in an X-ray diffraction D2 Phaser (Bruker, Rheinfelden, Germany), operating at target voltage and current of 30 kV and 10 mA, respectively, using a copper Kα X-ray source emitting radiations of 0.15406 nm wavelength. Samples were analyzed from 2 to 32° (2θ) at a rate of 4.11°/min, with step size of 0.05°, fixed diverge slit width of 1 mm, 1 mm anti-scatter-screen and using a detector 1D Lynxeye. The relative crystallinity (RC) was calculated considering the ratio of the crystalline area and total area using the Diffrac.Suite Eva version 3 software (Bruker AXS, Rheinfelden, Germany).

2.9 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The thermal properties were carried out in duplicate using a DSC Q200 (TA Instruments, New Castle, USA). The gelatinization temperatures: onset (To), peak (Tp.) and conclusion (Tc), as well as the values of calorimetric enthalpy (ΔH) were calculated from the thermograms using the Advantage software version 5 (TA Instruments, New Castle, USA). Approximately 2 mg of raw and extruded whole grain flours added of deionized water at a ratio of 2:1 were placed in a hermetic aluminum pan. The pans were sealed and remained at rest overnight at room temperature. An empty pan was used as reference. Scan occurred in the range from 5 to 110 °C at a rate of 10 °C/min.

2.10 PASTING PROPERTIES

The pasting viscosity profiles were determined in duplicate using a Rapid Viscosity Analyzer - RVA Series 4 (Newport Scientific, Warriewood, Australia), running at 160 rpm and starting heating with a temperature of 25 °C (COMETTANT-RABANAL *et al.*, 2021). 3 g of raw and extruded flours with adjusted moisture content at 14% wet basis were weighed and added to 25 mL of distilled water. The data were analyzed using Thermocline software for Windows and the respective readings of pasting curve were recorded: paste temperature (PT), peak viscosity at 95 °C (PV), minimum viscosity after heating (mV) and final viscosity (FV); and calculated: breakdown viscosity (BD = PV-mV) and setback viscosity (SB = FV-mV).

2.11 WATER ABSORPTION (WAI) AND WATER SOLUBILITY INDICES (WSI)

Raw and extruded flours (2.5 g with 7 ± 1% moisture content) were placed in Universal 320R centrifuge (Hettich, Tuttlingen, Germany) in 50 mL tube with 30 mL distilled water at 30 °C running at 3.000 *g* for 15 min according method described by Chávez *et al.* (2017), with modification. The samples were then shaken for 30 min. The supernatant was removed and dried at 105 °C (overnight), to determine WSI. The sediment was weighed and used to determine WAI. The experiment was carried out in quadruplicate.

2.12 *IN VITRO* DIGESTIBILITY OF CARBOHYDRATES

Raw and extruded flours were analyzed in triplicate in three phases of digestion: mouth, stomach and small intestine, using the respectively simulated digestion fluids prepared according to INFOGEST consensual method (MINEKUS *et al.*, 2014), with some adaptations. In the first step the oral phase was performed by diluting 1 g of flour into a total of 5 mL containing simulated salivary fluid and amylase with concentration of 75 U/mL (α-amylase Type IX-A, 1000-3000 U/mg protein, Sigma).

The simulated salivary fluid with flour was incubated while mixing for 2 min, pH 7.0, at 37 °C. Afterwards, for the gastric phase, the mix oral bolus was added into simulated gastric fluid (1:1 (vol/vol)) containing pepsin with concentration of 2000 U/mL (TS 1:10000 U/mL, Bela Vista$^{®}$, Brazil) and remained under agitation for 2 h, pH 3.0, 37 °C. Sequentially, small intestine phase started by adjusting the chime pH to 7.0 with a simulated intestinal fluid (1:1 (vol/vol)) added pancreatin solution 800 U/mL (Porcine pancreas 8 × USP, Sigma®, USA) was kept while mixing for 2 h, pH 7.0, 37 °C.

Afterwards the samples were centrifuged (4.000 *x g*, 10 min) and the supernatant were frozen until analyses of reducing sugar to evaluate the *in vitro* digestibility of total carbohydrates. The reducing sugar was analyzed in triplicate by using DNS (3,5-dinitrosalicylic acid) methodology with the digested supernatant samples (1:1 (vol/vol)), according to the method described by Miller (1959).

2.13 STATISTICAL ANALYSIS

Analysis of variance (one-way ANOVA) and Tukey’s test for mean comparison were used to analyze the extrusion process effect. Hierarchical clustering on principal components (HCPC) was conducted using Euclidean distances and Ward's method, and heatmap. Principal component analysis (PCA) was performed after variable standardization to avoid the influence of different magnitude orders. Pearson's correlation was used to analyze the interactions of variables. These statistical analyses were performed by using R free statistical software, version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria). Analyses were carried out at a significant level of $p \leq 0.05$.

3 RESULTS AND DISCUSSIONS

3.1 EXPANSION PROPERTIES AND INSTRUMENTAL TEXTURE

Pearl millet expanded extrudates (Figure 1A) mainly at low moistures (11 and 14%) presented lower expansion than those extrudates processed at higher moisture. Yadav, Dalbhagat and Mishra (2022) reported in their review study that the feed moisture in pearl millet flours presented a negative effect on radial expansion index (REI); In this sense, a direct (unexpected) behavior between moisture and diameter of specimens related with radial expansion index was not described.

A possible factor for reduced expansion is the reduction in starch conversion generating fewer modifications between starch molecules (CARVALHO, MITCHELL, 2000). In our view, a possible reason for the lower expansion of millet starch compared to corn, is related to the composition, as already described in this study, in millet, the high lipid and protein content may have reduced starch access to water and with that , possibly there was greater difficulty in modifying the millet starch during expansion, such as poorly gelatinized starches, due to the lesser access of starch to water, generating smaller networks, making it difficult to form air cells, and consequently generating less extrudates expanded.

On the other hand, whole corn extrudates showed an expected reduction in sectional expansion as water content was increased (Figure 1B), highlighting that lower moisture content led to higher sectional expansion. According to Romero Rodríguez *et al.* (2021) the highest expansion in corn also occurred under lower moisture and corn expanded are usually explained by the effect of high temperatures and shear on starch that contribute to evaporation at the exit of the extruder and increase REI, generating thin-walled air cells.

Furthermore, the polynomial function of whole pearl millet extrudates (Figure 1C, green color) showed that, the point 11% was discarded in the straight line equation for this cereal, it demonstrates that the increment of moisture from 11 until 14% had no effect on REI; and finally, the increase of pearl millet flour moisture up to 20 and 23% caused the highest value and a reduction in REI, respectively. This function confirms the highest REI correlated to 14% of moisture content for whole corn extruded.

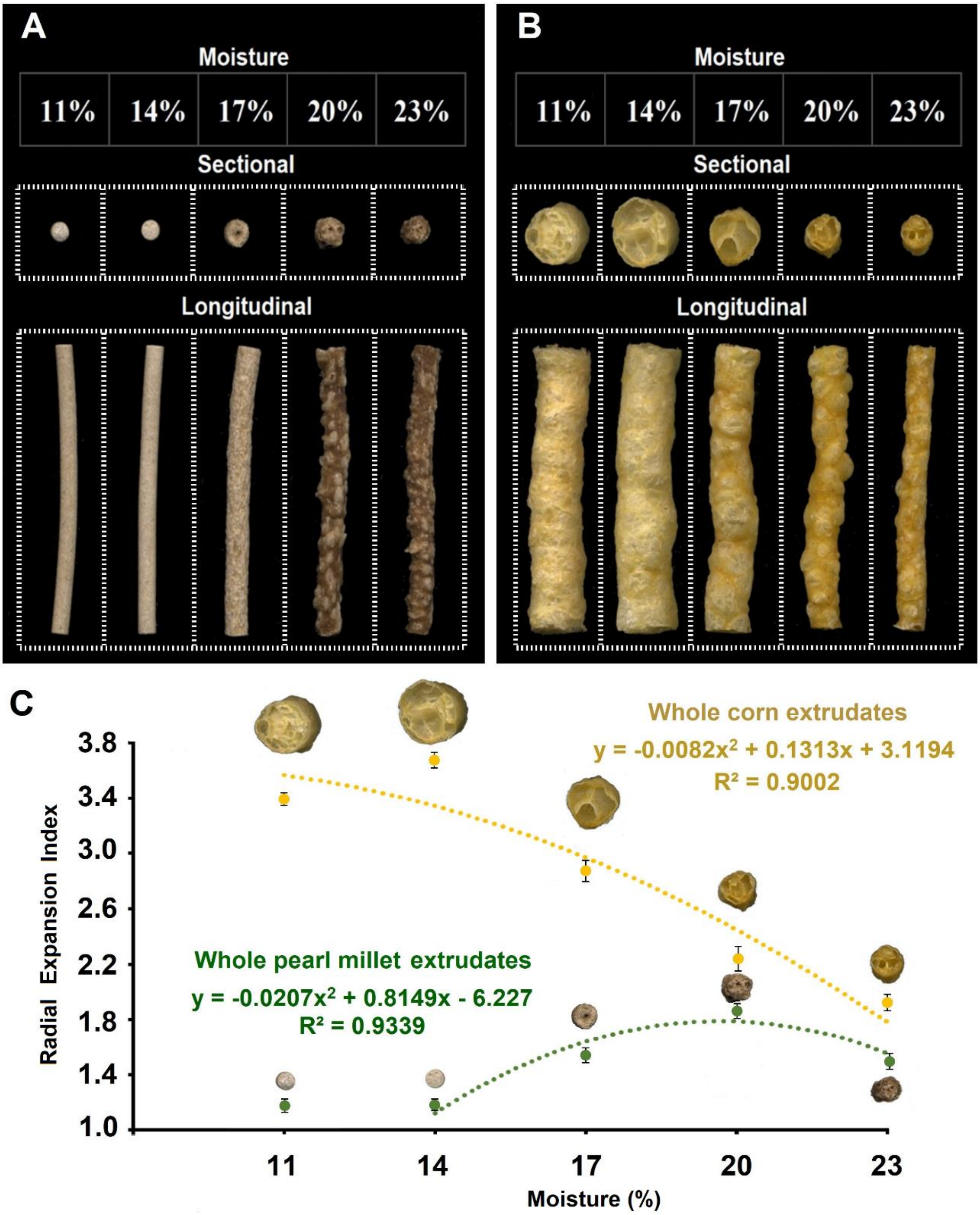

Figure 1. Effect of varied moisture content (11, 14, 17, 20 and 23%) on the extrusion of pearl millet and corn flours*: A) Sectional and longitudinal images of whole pearl millet extrudates**; B) Sectional and longitudinal images of whole corn extrudates**; C) Polynomial functions for Radial Expansion Index and initial moisture content of the flour in the extruder feed, located at the bottom (green color) for pearl millet and at top (yellow color) for corn, as well as the respective section images of the extruded products for each moisture content (11, 14, 17, 20, 23%). *The image capture of the extrudates was achieved in a single image to reduce the influence of color or distance. ** Scale in mm, at the edge of each image.

In this sense, whole pearl millet and corn extrudates had greater REI when produced at 20 and 14% moisture content, respectively. Therefore, these levels were chosen for the next analyses; because greater REI tends to increase the consumers' acceptability (YADAV, DALBHAGAT, MISHRA, 2022). In addition, these moistures levels (14 and 20%) were analyzed in each cereal and the samples were called as MW14 (whole millet extruded at 14% moisture), MW20 (whole millet extruded at 20% moisture), CW14 (whole corn extruded at 14% moisture), and CW20 (whole corn extruded at 20% moisture), as well as MW (raw whole grain millet flour) and CW (raw whole grain corn flour) were analyzed.

Expansion properties (Table 1) showed that, when compared the same moisture level, MW14 presented the lowest values of SEI (1.33), torque (5.84 Nm) and die temperature (115.78 °C) with temperature below the stipulated die temperature (120 ºC), as well as the highest ρ_e (1.14 g/cm^3) than CW14. In relation to LEI, MW14 results did not differ significantly (p>0.05) to CW14, although there was a difference in VEI. In addition, MW20 and CW20 did not differ significantly (p>0.05) for ρ_e, VEI, die temperature and compression force.

Table 1. Expansion properties and instrumental texture of millet and corn extrudates processed at 14 and 20% moisture.

Variables	MW14	MW20	CW14	CW20
SEI	$^{B;b}$1.33±0.01^{d}	$^{A;b}$3.52±0.07^{c}	$^{A;a}$13.58±0.36^{a}	$^{B;a}$4.83 ±0.30^{b}
ρ_e (g/cm^3)	$^{A;a}$1.14±0.01^{a}	$^{B;a}$0.50±0.01^{b}	$^{B;b}$0.12±0.01^{c}	$^{A;a}$0.46 ±0.04^{b}
LEI	$^{A;a}$0.89±0.00^{a}	$^{B;a}$0.75±0.00^{b}	$^{A;a}$0.84±0.03^{a}	$^{B;b}$0.59 ±0.01^{c}
VEI	$^{B;b}$1.02±0.00^{c}	$^{A;a}$1.40±0.01^{b}	$^{A;a}$3.09±0.16^{a}	$^{B;a}$1.29 ±0.07^{b}
Torque (Nm)	^{B,b}5.84±0.39^{d}	^{A,a}13.93±0.44^{b}	$^{A;a}$16.07±3.83^{a}	$^{A;b}$11.05±1.70^{c}
Die temperature (°C)	$^{B;b}$115.78±2.09^{c}	$^{A;a}$131.32±3.00^{b}	$^{A;a}$153.33±2.47^{a}	$^{B;a}$130.41±1.37^{b}
Compression force (N)	$^{A;a}$1.02±0.36^{a}	$^{A;a}$0.96±0.54^{a}	$^{B;b}$0.39±0.17^{b}	$^{A;a}$1.05±0.54^{a}

SEI, sectional expansion index; ρ_e, apparent density; LEI, longitudinal expansion index; VEI, volumetric expansion index. Codes displayed: MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; CW14, whole corn extruded at 14% moisture; CW20, whole corn extruded at 20% moisture. Statistical analysis: Left side - Different capital letters, in the same column, indicate statistical difference (p≤0.05), between moisture levels (14%, 20% and raw), within the same type of grain; Different lowercase letters, in the same column, indicate statistical difference (p≤0.05), between grain types, within the same moisture level (14%, 20% and raw). Right side - Completely Random Design: Values with different letters, in the same column, indicate statistical difference (p≤0.05).

3.2 PROXIMATE COMPOSITION, TOTAL STARCH AND AMYLOSE CONTENT

Protein content (Table 2) was statistically different (p≤0.05) among all samples, moreover, when compared the raw flours, MW presented 10.25 g/100 that had approximately 20% more protein than CW (8.59 g/100 g). Yadav, Dalbhagat and Mishra (2022) report that a greater presence of protein affects the radial expansion (and consequently SEI), increasing the hardness of the extrudates making the extrusion structure more resistant with that there is a greater impediment to expansion. In this sense, the highest protein content naturally present in the pearl millet grain may have hindered the expansion. In addition, all millet extrudates remained with higher levels of protein (~10 g/100 g) compared to corn extrudates (~8 g/100 g).

Table 2. Proximate composition and total starch of raw and extruded millet and corn flours in dry basis (g/100 g).

Analyze	**MW**	**MW14**	**MW20**	**CW**	**CW14**	**CW20**
Ash	$^{A;a}1.62\pm0.01^{a}$	$^{A;a}1.59\pm0.02^{ab}$	$^{A;a}1.43\pm0.08^{b}$	$^{A;b}1.22\pm0.07^{c}$	$^{A;b}1.19\pm0.01^{c}$	$^{A;b}1.20\pm0.01^{c}$
Protein	$^{A;a}10.25\pm0.02^{a}$	$^{A;a}9.92\pm0.13^{b}$	$^{A;a}9.83\pm0.14^{c}$	$^{A;b}8.59\pm0.22^{d}$	$^{B;b}7.93\pm0.05^{f}$	$^{AB;b}8.10\pm0.05^{b}$
Lipid	$^{A;a}6.16\pm0.10^{a}$	$^{A;a}5.76\pm0.09^{b}$	$^{B;a}3.98\pm0.17^{d}$	$^{A;b}4.78\pm0.00^{c}$	$^{B;b}2.32\pm0.46^{e}$	$^{B;b}2.07\pm0.08^{e}$
Fiber	$^{A;b}10.92\pm0.02^{b}$	$^{C;a}5.58\pm0.00^{d}$	$^{B;a}6.59\pm0.00^{c}$	$^{A;a}15.04\pm0.01^{a}$	$^{B;b}4.90\pm0.00^{e}$	$^{C;b}4.50\pm0.00^{f}$
Carbohydrate	$^{B;a}71.05\pm0.17^{d}$	$^{A;b}77.15\pm0.25^{c}$	$^{A;b}78.17\pm0.39^{c}$	$^{C;a}70.37\pm0.29^{d}$	$^{B;a}83.66\pm0.53^{b}$	$^{A;a}84.13\pm0.13^{a**}$
Total starch	$^{A;b}63.61\pm0.95^{b}$	$^{A;b}63.33\pm0.65^{b}$	$^{A;a}58.92\pm3.76^{bc}$	$^{A;a}69.13\pm2.36^{ab}$	$^{A;a}68.14\pm1.10^{b}$	$^{A;a}60.54\pm3.46^{b}$

Codes displayed: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; CW, raw whole grain corn flour; CW14, whole corn extruded at 14% moisture; CW20, whole corn extruded at 20% moisture. Statistical analysis: Left side - Different capital letters, in the same column, indicate statistical difference ($p \leq 0.05$), between moisture levels (14%, 20% and raw), within the same type of grain; Different lowercase letters, in the same column, indicate statistical difference ($p \leq 0.05$), between grain types, within the same moisture level (14%, 20% and raw). Right side - Completely Random Design: Values with different letters, in the same column, indicate statistical difference ($p \leq 0.05$).

The lipid content of MW (6.16 g/100 g) was superior to CW flour (4.78 g/100 g) by approximately 20%. Furthermore, different moisture levels in the extrusion processing caused a reduction in the lipid content for all extruded samples, compared to all flours, however, comparing the flours of each cereal, separately, only MW14 did not differ significantly ($p>0.05$) compared to the respectively raw flour (Table 2).

The decrease in lipid levels resulting from extrusion processing is related to the lower content of extractable lipids, and it has been reported that only 40-55% of the lipid can be extracted after extrusion due to the complexation of monoglycerides and free fatty acids with amylose and protein (KAMAU, NKHATA, AYUA, 2020). The lipid content of 5.76 g/100 g in MW14 (Table 2) can also be an indication of the contribution of these lipids as lubricants during extrusion.

Regarding dietary fiber content, all samples showed statistical differences ($p\leq 0.05$). Comparing the fiber content of the raw and extruded, corn extrudates showed a reduction of approximately 70% in the fiber content; while the millet extrudates had the lowest reduction, of 45 and 40% for the MW14 and MW20, respectively. By comparing the extrudates produced at the same moisture content, MW14 had 12% more fiber than CW14, and MW20 had 32% more fiber compared to CW20.

A reasonable explanation for the reduction in dietary fiber content in the extruded samples is due to the breakdown of carbohydrates caused by the combining effect of shearing and heat on the non-starch molecules. The effect of extrusion was also studied by Roye *et al.* (2020) on the β-glucan depolymerization caused by single-pass extrusion processing potentiated by high shear, generating less fiber content.

Concerning the nutritional aspect of pearl millet compared to corn, according to Oliveira *et al.* (2021) pearl millet is considered nutritionally superior to corn, not only because it has higher protein content, but it is also superior in protein digestibility. In addition, pearl millet is composed of lipids containing higher polyunsaturated acid content, such as linolenic acid (ω3) which is recognizable as a healthy nutrient along with higher dietary fiber content than corn.

Total starch content was similar in almost all flours except for CW with 69.13 g/100 g, in contrast to MW20 with 58.92 g/100 g of total starch content (Table 2), when compared all samples indicating that corn has higher content than pearl millet. However, comparing the total starch content of each cereal, separately, extrudates did not show statistical difference compared to the respective raw flour of each cereal. When analyzing the amylose content of starches, corn showed slightly higher content, 29.24% than millet with 28.26%, and considering the close values, both cereals showed similar potential of forming amylose lipid complexes during the extrusion process.

Though, due to the higher lipid content found in millet it would be reasonable to consider greater formation of amylose-lipid complex in millet extrudates.Panyoo and Emmambux (2017) confirm this explanation, mentioning that the degree of formation of amylose-lipid complexes depends on the amylose content, and furthermore, when processing in a single screw extruder, using corn with amylose contents close to 35%, the greatest formation of complexes was observed at high lipid content (~7% of lipid, dry basis); similar to the values found for millet.

3.3 MORPHOLOGY OF STARCH GRANULES

Micrographic images using polarized light microscopy were performed to evaluate the morphology on the starch granules of the extruded flours (Figure 2).

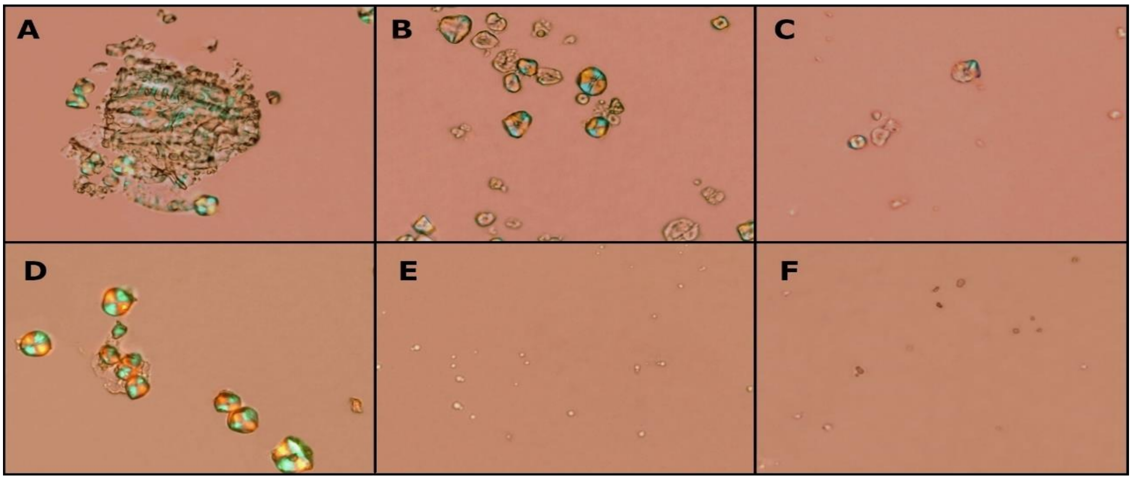

Figure 2. Light microscopy of raw and extruded flours visualized at 400x. A) MW, raw whole grain millet flour; B) MW14, whole millet extruded at 14% moisture; C) MW20, whole millet extruded at 20% moisture; D) CW, raw whole grain corn flour; E) CW14, whole corn extruded at 14% moisture; F) CW20, whole corn extruded at 20% moisture.

It is clear the presence of irregular-polyhedral starch granules in MW (Figure 2A) with their maltese cross showing integrity of the original structure. It is also clear the presence of fibrous structure representing chunks of the pericarp characteristic of a whole grain flour. Starch birefringence is clearly seen in the extrudates MW14 (Figure 2B), which indicates that the single screw extrusion did not affect the starch structural integrity, whereas CW14 extrudates showed non-starch structure, evidencing fully corn starch breaking down, in contrast of whole grain corn flour CW. The presence of native millet starch in MW14, certainly shows non changes caused by the extrusion process, which may explain the low expansion of the millet extrudates.

In contrast, the image of millet extrudates MW20 (Figure 2C) showed partial and total loss of birefringence, indicating that starch damage occurred to a certain extent in by the complete loss of birefringence of the starch granules. When comparing to corn extrudates CW20 as for CW14, it was not possible to easily identify starch granule integrity seen by the completely loss of birefringence indication that at both extrusions processed moisture content, corn starch granules were entirely damaged by the combination of heat and shearing in the single screw extruder.

Image of native corn starch (CW), it can be seen the typical polyhedral shaped granules (Figure 2D), similar in shape to MW. As mentioned, single screw extrusion caused loss of birefringence in the starch of CW14 and CW20 (Figure 2E and 2F, respectively). Li *et al.* (2014) described that before extrusion processing it is possible to observe the "maltese cross" by the presence of the semi-crystalline structure in the starch granule, and after the possessing with consequently starch degradation, is not possible to observe the presence of birefringence.

3.4 X-RAY DIFFRACTION AND RELATIVE CRYSTALLINITY

The X-ray diffraction patterns (Figure 3A) of MW and CW were similar and presented peaks at 15, 17, 18 and 23º angle 2 theta, approximately, typical of A-Type crystallinity pattern, as reported by da Cruz *et al.* (2015).

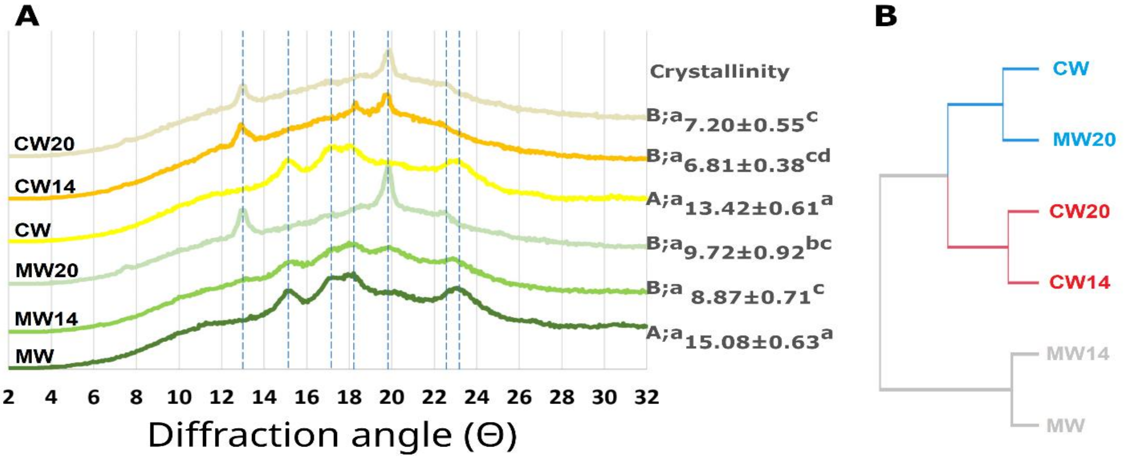

Figure 3. A) X-ray pattern and relative crystallinity of raw and extruded millet and corn flours at 14 and 20% moisture (dotted vertical bars highlight the peaks found for the respective angle). Statistical analysis: Left side - Different capital letters, indicate statistical difference ($p \leq 0.05$), between moisture levels (14%, 20% and raw), within the same type of grain; Different lowercase letters, indicate statistical difference ($p \leq 0.05$), between grain types, within the same moisture level (14%, 20% and raw). Right side - Completely Random Design: Values with different letters indicate statistical difference ($p \leq 0.05$); B) Hierarchical clustering of principal components of DR-X profiles. Codes displayed: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; CW, raw whole grain corn flour; CW14, whole corn extruded at 14% moisture; CW20, whole corn extruded at 20% moisture.

In addition, the crystallinity did not differ significantly ($p > 0.05$) between the raw flours. In Figure 3B, hierarchical clustering on principal components (HCPC) showed the grouping of the samples according to the similarity of the X-ray profiles. In HCPC, CW belonged to the same cluster (blue color) to MW20, and differed to MW, whereas MW showed the same X-ray profile that MW14 (grey color cluster); despite MW and CW had similar 2θ angle and did not differ significantly ($p > 0.05$) in relation to crystallinity.

MW20 and CW20 extrudates showed the same diffraction angles near 13 and 20° (2θ), as well as the angle approximately at 23° (2θ), and did not differ significantly ($p > 0.05$). Starch crystalline structures can be varied from A-type to V-type; as starch conversion occurs, due to a combination of heat and shearing, it allows the formation of amylose lipid complexes leading to peaks at 13 and 20° (2θ) (CAI *et al.*, 2022). It is possible to see that MW20 extrudates presented the highest amylose-lipid complex formation represented by the peaks at 13 and 20º (2θ).

MW14 showed A-type crystallinity pattern, similar to the raw flours, demonstrating that the extrusion process did not change the crystal conformation of starch granules as confirmed by the micrograph images. He *et al.* (2021) mentioned that during extrusion, a phase transition occurs in the starch at low moisture content, causing starch breaking down of the crystalline structure. However, virtually non-loss of crystallinity was observed in MW14.

3.5 FLOUR CHARACTERIZATION

3.5.1 Thermal properties

The calorimetric DSC readings are displayed in Table 3. Raw millet flour, MW showed readings higher than extruded MW14. By looking at the DSC data, it is possible to see that the single screw extrusion processing slight changed the calorimetric properties of the raw pearl millet flour, however the changes can be considered very low if compared to the changes caused in the extruded readings of MW20. Particularly considering the enthalpy reading (ΔH), it decreased from 6 to 4 J/g, which represented a loss of 42% of starch integrity. It is worth noting that it was not possible to see the starch damage by looking at the X-ray crystallinity reading.

Sahu, Patel and Tripathi (2022) reported that physicochemical transformations during the extrusion process include loss of birefringence and thermal degradation. Nonetheless, the starch granules found in MW14 did not show a clear loss of birefringence, but it was possible to verify a loss of starch integrity through thermal degradation as shown earlier (Table 3).

Zhao *et al.* (2022) mentioned that the reduction in ΔH can be caused by the effect of shearing forces as an important factor causing starch degradation, physically modifying the granules, resulting in reduction of the enthalpy. The authors also mention that there are samples that is not possible to detect the enthalpy as the signal is low indicating completing loss of starch granule integrity, which occurred in the corn extrudates CW14 and CW20 and in the millet extrudates processed at 20% (MW20).

3.5.2 Pasting properties

The pasting readings of raw and extruded millet and corn are displayed in Table 3. As expected, PV, FV, BD and SB values of raw millet (MW) were higher than millet extrudates (MW14 and MW20). The pasting readings of MW14 were close to the MW readings, indicating low starch conversion which corroborates with the results of micrograph, x-ray and calorimetry. In contrast, CW14 and CW20 showed great modification in the pasting readings when compared to raw corn flour.

Paste temperature (PT) of the raw samples MW and CW (Table 3) were similar (83.0 °C), while the extruded samples, CW14 and CW20, had reduced PT (25.0 °C) when compared to the raw samples, indicating that during processing by extrusion had a great modification of the starch present in the corn extrudates. On the other hand, PT of MW14 and MW20 presented a higher temperature demand (85.6 and 75.0 °C, respectively), indicating less modification in relation to MW. Tomar *et al.* (2022) explains that high values for the beginning of starch cooking indicate greater resistance to disintegration and may be associated with the formation of a hydrophobic insulating layer of lipids around the starch granule, due to the formation of complexes with amylose making it difficult to absorb water.

Table 3. Thermal and physical properties of raw and extruded millet and corn flours.

Analyze	**Variable**	**MW**	**MW14**	**MW20**	**CW**	**CW14**	**CW20**
Thermal properties	To (°C)	69.03±0.35^{a}	64.17±0.11^{c}	NQ	66.10±0.45^{b}	NQ	NQ
	Tc (°C)	85.89±0.23^{a}	83.64±0.75^{b}	NQ	87.44±0.36^{a}	NQ	NQ
	Tp (°C)	74.43±0.03^{a}	70.31±3.73^{a}	NQ	73.76±0.06^{a}	NQ	NQ
	ΔH (J/g)	6.00±0.12^{a}	4.22±0.15^{b}	NQ	5.95±0.59^{a}	NQ	NQ
Pasting profile	PT (°C)	$^{B;a}$83.00±0.57^{b}	$^{A;a}$85.60±0.00^{a}	$^{C;a}$75.00±0.00^{c}	$^{A;a}$83.00±0.57^{b}	$^{B;b}$25.00±0.00^{d}	$^{B;b}$25.00±0.00^{d}
	PV (cP)	$^{A;a}$923.00±4.24^{a}	$^{B;a}$742.50±3.54^{b}	$^{C;b}$192.00±12.73^{f}	$^{A;b}$608.50±9.19^{c}	$^{B;b}$414.50±6.36^{d}	$^{C;a}$377.50±4.95^{e}
	mV (cP)	$^{A;b}$482.00±2.83^{b}	$^{A;a}$498.50±2.12^{b}	$^{B;b}$118.50±7.78^{e}	$^{A;a}$587.00±0.00^{a}	$^{C;b}$170.50±2.12^{d}	$^{B;a}$209.50±9.19^{c}
	FV (cP)	$^{A;b}$2151.50±10.61^{b}	$^{B;a}$1817.50±7.78^{c}	$^{C;b}$205.50±10.61^{f}	$^{A;a}$2327.00±5.66^{a}	$^{C;b}$277.00±5.66^{e}	$^{B;a}$417.50±4.95^{d}
	BD (cP)	$^{A;a}$441.00±1.41^{a}	$^{B;a}$244.00±1.41^{b}	$^{C;b}$73.50±4.95^{d}	$^{C;b}$21.50±9.19^{e}	$^{A;a}$244.00±8.49^{b}	$^{B;a}$168.00±4.24^{c}
	SB (cP)	$^{A;b}$1669.50±7.78^{b}	$^{B;a}$1319.00±5.66^{c}	$^{C;b}$87.00±2.83^{e}	$^{A;a}$1740.00±5.66^{a}	$^{C;b}$106.50±3.54^{e}	$^{B;a}$208.00±4.24^{d}
Water absorption and solubility indices	WAI (g/g)	$^{C;b}$2.20±0.03^{f}	$^{B;b}$2.67±0.06^{d}	$^{A;b}$4.12±0.03^{c}	$^{C;a}$2.49±0.04^{e}	$^{A;a}$5.96±0.05^{b}	$^{B;a}$5.80±0.07^{a}
	WSI (%)	$^{A;a}$8.50±0.91^{c}	$^{C;b}$5.79±0.13^{e}	$^{B;b}$6.99±0.18^{d}	$^{C;b}$5.29±0.07^{e}	$^{A;a}$17.76±0.52^{a}	$^{B;a}$12.13±0.31^{b}

Temperatures properties: To, onset; Tp, peak; Tc, conclusion; ΔH, calorimetric enthalpy; NQ, values not quantified. Pasting profile: PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity; BD = PV-mV, breakdown viscosity; SB = FV-mV, setback viscosity. Water absorption and solubility indices: WAI, water absorption index; WSI, water solubility index. Codes displayed: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; CW, raw whole grain corn flour; CW14, whole corn extruded at 14% moisture; CW20, whole corn extruded at 20% moisture. Statistical analysis: Left side - Different capital letters, in the same column, indicate statistical difference ($p \leq 0.05$), between moisture levels (14%, 20% and raw), within the same type of grain; Different lowercase letters, in the same column, indicate statistical difference ($p \leq 0.05$), between grain types, within the same moisture level (14%, 20% and raw). Right side - Completely Random Design: Values with different letters, in the same column, indicate statistical difference ($p \leq 0.05$).

Regarding the minimum viscosity after heating (mV) higher values occurred in the raw flours (Table 3): MW (482.00 cP) and CW (587.00 cP), mV reduced in all extruded flours, except for MW14 (498.50 cP) which was not significantly different ($p>0.05$) to MW, indicating that part of its native starches were preserved or little modified in MW14 as shown by the small thermal degradation (DSC reading). Concerning the slight millet starch modification observed in MW14, in relation to native millet starch present in sample MW, a possible explanation for less starch conversion could be attributed to greater formation of amylose-lipid complex difficulty the complete starch modification.

The retrogradation viscosity (SB) in MW14 showed the highest value (1319.00 cP) when compared to other extruded samples (87.00 to 208.00 cP) and relatively close to MW (1669.50 cP) and CW (1740.00 cP). These results increase the theory that for this sample (MW14), despite heat and shear applied during extrusion processing, for an initial moisture of 14%, linked to the other processing conditions were not enough to cause greater modifications in the starch. Part of these starches were less modified by the protective effect of other macronutrients, such as fiber, protein and lipids, which difficulty the water to enter in starch granules.

3.5.3 Water absorption (WAI) and water solubility indices (WAI)

The water absorption and solubility indices are shown in Table 3. MW had the lowest WAI (2.20 g/g) along with CW, as expected values for raw flours. The extrusion process caused a slight increase of WAI in MW14 (2.67 g/g) whereas greater increase was observed for MW20 in almost twice higher (4.12 g/g).

Extruded corn (CW14 and CW20) showed higher values (5.96 and 5.80 g/g, respectively) than extruded millet. According to Carvalho *et al*. (2010) it is expected the shear combined with heat cause starch granules breakdown during extrusion leading to an increase of WAI values, as disrupted starch has a higher water holding capacity at room temperature, which did not happen to pearl millet single extruded at 14% MW14.

Percentage of WSI showed that in relation to MW (8.50%) the extrusion process generated low solubility in MW14 and MW20 (5.79 and 6.99%, respectively), and this reduction was not observed in CW (5.29%) compared to extruded corn flours (CW14 and CW20, 17.76 and 12.13%, respectively).

The reduction of solubility in extruded millet flours was probably caused by insoluble or poorly soluble non-starch compounds such as lipids and proteins and may be also attributed to the formation of amylose-lipid complexes (YADAV, DALBHAGAT, MISHRA, 2022). According to Wang *et al.* (2020) amylose is the main starch molecule that forms complexes leading to structures of binary complexes (starch-lipid and/or starch-protein) and ternary complexes (starch-lipid-protein) reducing starch solubility because these starch-lipid complexes form an insoluble film on the starch granules surface. It can be assumed that high lipid, protein and fiber content found in pearl millet (Table 2) may have contributed to the increase insoluble on the surface of millet starch through the formation of binary or ternary complexes.

3.6 *IN VITRO* DIGESTIBILITY OF CARBOHYDRATES

Undigested samples presented a concentration of reducing sugars between 0.49 and 1.26 µmol/mL for almost all samples, except for MW20 which presented the highest concentration of 4.25 µmol/mL (Figure 4), probably generated in the extrusion processing.

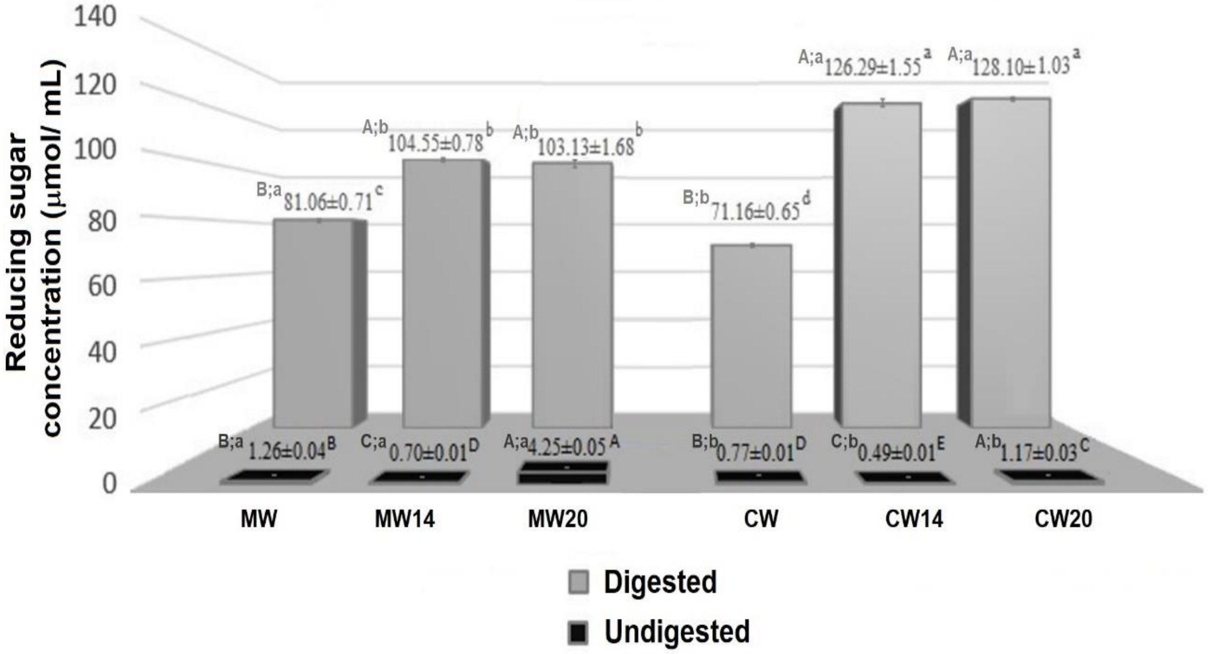

Figure 4. Reducing sugar concentration in digested (simulated *in vitro* gastrointestinal digestion with enzymatic action) and undigested (without enzymes) raw and extruded millet and corn flours. Statistical analysis: Left side - Different capital letters, indicate statistical difference ($p \leq 0.05$), between moisture levels (raw, 14% and 20%), within the same type of grain; different lowercase letters, indicate statistical difference ($p \leq 0.05$), between grain types, within the same moisture level (raw,14% and 20%). Right side - Completely Random Design: Values with different letters for undigested (capital letter) or digested (lower-case) indicate statistical difference ($p \leq 0.05$). Sample codes displayed: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; CW, raw whole grain corn flour; CW14, whole corn extruded at 14% moisture; CW20, whole corn extruded at 20% moisture.

MW20 presented the highest concentration of reducing sugar in the undigested sample and possibly contributed to the darkening of this sample (Figure 1 A); these darker pigments can be associated with a possible Maillard reaction caused by the interaction between amino acids and reducing sugars generated due to heat (YADAV, DALBHAGAT, MISHRA, 2022).

The digested samples had higher concentration of reducing sugars compared to undigested one, as a result of enzymatic action on simulated *in vitro* gastrointestinal digestion (COLOSIMO *et al.*, 2020). MW and CW were responsible for the lowest digestibility of carbohydrates (reducing sugars concentration of 81.06 and 71.16 µmol/mL, respectively), however they were control samples and not used directly for human consumption as raw, while all extruded flours, presented higher concentration of reducing sugar after digestion, and according to Krishnan *et al.* (2021) the structural integrity that implies high crystallinity of the starch polymorph cause limited digestion by the enzymes. Digested extruded flours of the same cereal (MW14 compared with MW20, and CW14 compared with CW20) did not present difference in carbohydrate digestibility ($p > 0.05$), demonstrating, that processing at different moisture content (14 and 20%) did not affect the digestion. However, when comparing extruded samples of millet and corn, MW14 with CW14, and MW20 with CW20, the extruded millet samples showed almost 20% less digestibility than corn.

This data suggests that extruded millet flours may have a lower glycemic response due to a possible reduction in glucose and insulin rates, as they form more digestion-resistant carbohydrates and more complexes formed by amylose and lipids compared to corn flour with the same treatment, which was shown by the X-ray diffraction pattern of millet, particularly, MW20 (Figure 3). According to Cai *et al.* (2022), complexes formed between amylose and lipids in rice starch can decrease glycemic responses and might be related to the reducing sugar of risk of the development of Type II diabetes, as unstable and amorphous amylose forms with lipids an ordered lamellar crystal structure that is more amylase resistant compared to free amylose.

The more ordered structure possibly present in millet starch, in addition to contributing to the lower digestibility of the starch in the extruded sample, may be one of the responses to the lower leaching of amylose and consequent gelatinization of the starch contributing to the lower expansion. Further studies on extruded millet flours need to be addressed to ensure this hypothesis.

3.7 MULTIVARIATE ANALYSIS

The two first PCAs explained 77.6% (60.2 plus 17.4%) of the total variability (Figure 5A), in addition, PCA (Biplot) was realized for all samples, highlighted black dots, as well as physical and chemical responses were demonstrated as vectors.

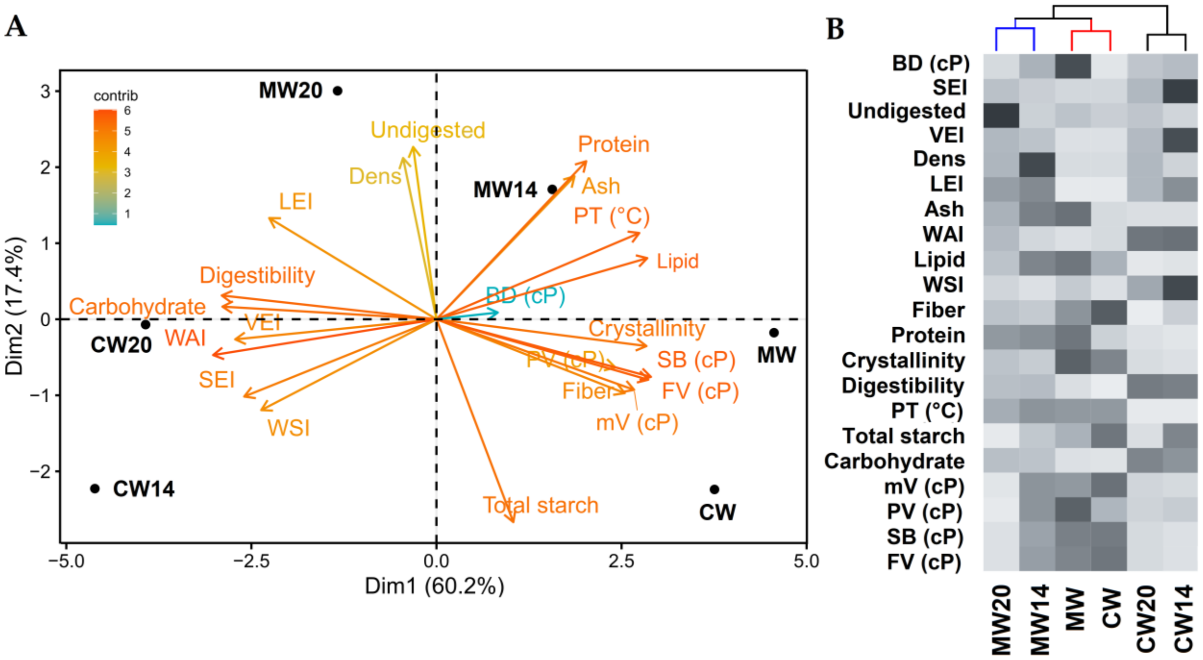

Figure 5. Multivariate analysis of raw and extruded flours using physical and chemical responses (SEI, sectional expansion index; Dens, apparent density; LEI, longitudinal expansion index; VEI, volumetric expansion index); proximate composition (ash, protein, lipid, fiber, carbohydrate); total starch; crystallinity; pasting properties (PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity, BD: breakdown and SB: setback); WAI (water absorption index); WSI (water solubility index); Digestibility (digestibility of carbohydrate) and Undigested (digestion control, without enzymes)): 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 darkest color indicates the highest value and light color indicates the lowest value. Sample codes displayed: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; CW, raw whole grain corn flour; CW14, whole corn extruded at 14% moisture; CW20, whole corn extruded at 20% moisture.

CW20 and CW14 presented higher values of SEI and WAI vectors (as mentioned in 3.1 and 3.5 sections), these were corroborated by the heatmap (Figure 5B, for presenting more intense colors). It was reported by Chou and Hsu (2021), that the increase in gelatinization of mechanical destruction of the starch molecules in the extrudates with high expansion capacity had the water solubility too. Corn extrudates were also influenced by the vectors of Digestibility, Carbohydrate and Total starch. Consequently, the higher starch content concentration contributes to greater enzymatic digestion (ZHANG, *et al.*, 2022).

On the other hand, raw samples (MW and CW) were characterized by highest values of FV, mV, SB and crystallinity (Figure 5A and B), corroborating to previous findings (3.4 and 3.5 sections), low values of digestibility (3.6 section), and without influence of the vectors related to the expansion properties of the extruded products, due to the MW and CW being raw samples.

Finally, MW14 and MW20 showed high values of Undigested and Density, and the lowest values of carbohydrates as well as high protein values (Table 2), PT (Table 3) and low WAI (Table 3), for example. While MW20 presented the highest value of reduction sugar in an undigested sample (Figure 4).

Applying HCPC (sample groups in Figure 5B), it was suggested three sample groups (based on their physical and chemical similarities), MW20 and MW14 formed cluster 1 (blue). MW and CW formed cluster 2, in red. These two clusters presented the greatest similar properties. Finally, CW20 and CW14, in black, formed a group (cluster 3), due to its characteristics with extreme values. In the heatmap, darkness colors indicate highest variable values, which allowed easily the detection of the variables which characterized the samples and as was previously discussed, it corroborated the information of PCA.

3.7.1 Pearson’s correlation between physical and chemical properties

According to the scale of strengthening from Pearson’s correlation coefficient, values close to 1, have very high correlation (TELES *et al.*, 2019). In this regard, there were found very strong and positive correlations (blue numbers, Figure 6) mainly between SB and FV (r=1); mV and FV (r=0.99); mV and SB (r=0.98); carbohydrate and digestibility, and ash and protein (r=0.97); and SEI and VEI (r=0.96). When evaluating the negative correlation (red numbers, Figure 6), lipid and WAI had strong negative correlations (r=-0.97), as well as PT and WAI (r=-0.95), indicating that higher values of lipid and PT reducing the water solubility index.

From the Pearson’s correlation, fiber and digestibility also showed a strong negative correlation (r=-0.95), and it is possible to explain e.g. that the digestibility and dietary fiber, were respectively, directly (r=0.97) and negatively (r=-0.95) correlated with the carbohydrate contents. Higher content of dietary fiber is constituted primarily by non-starch polysaccharides, as hemicelluloses and non-digestible carbohydrate that are resistant to digestion, that pass through the small intestine intact and they are fermented only in the large intestine (MUDGIL, BARAK, 2013). In this sense, the higher fiber content negatively correlated with digestibility may be related to the high content of non-starch polysaccharides non-digestible or slowly digestible.

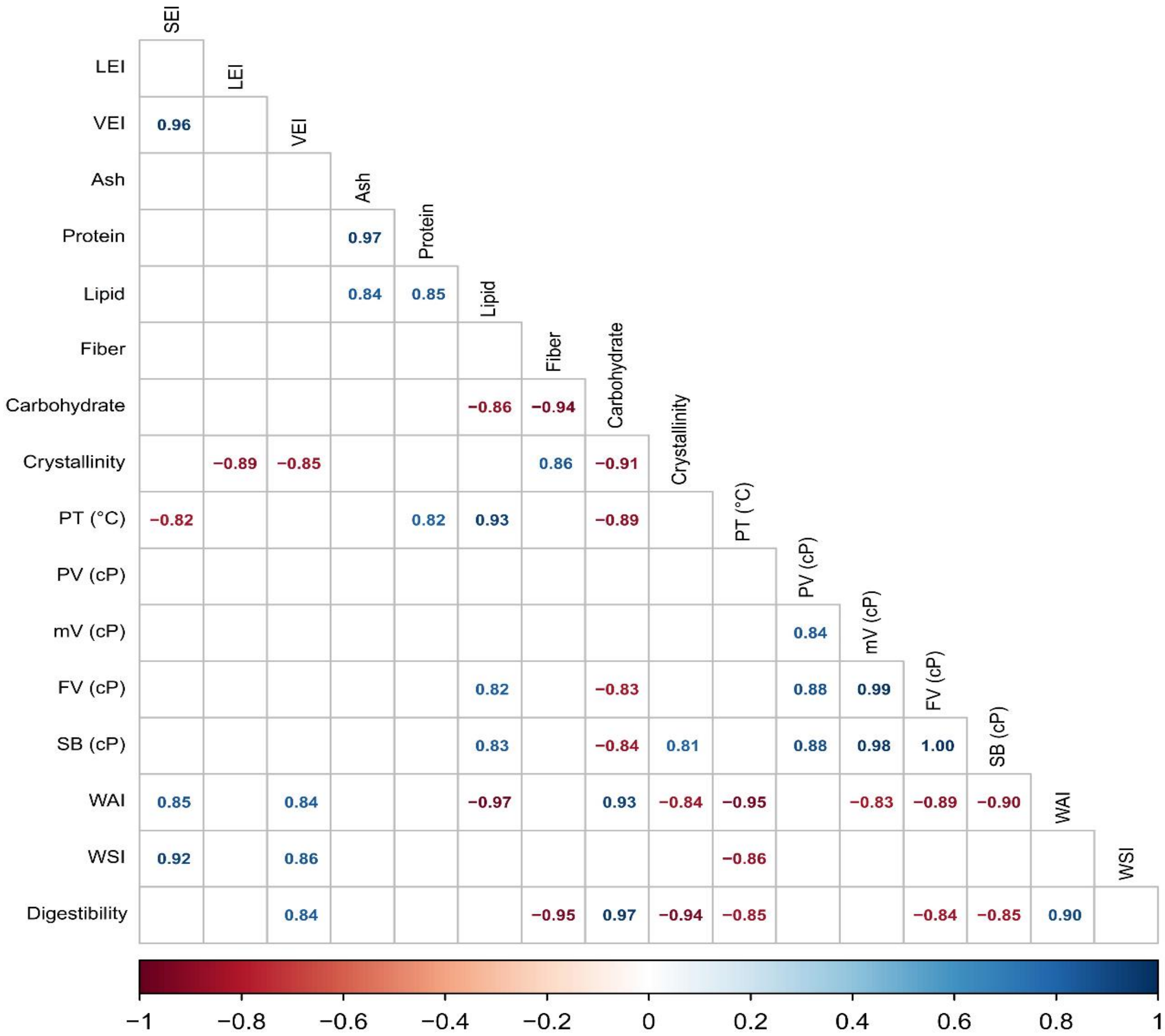

Figure 6. Correlogram for the physical and chemical parameters (SEI, sectional expansion index; Dens, apparent density; LEI, longitudinal expansion index; VEI, volumetric expansion index); proximate composition (ash, protein, lipid, fiber, carbohydrate); total starch; crystallinity; pasting properties (PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity, BD: breakdown and SB: setback); WAI (water absorption index); WSI (water solubility index); Digestibility (digestibility of carbohydrate) and Undigested (digestion control, without enzymes)). Red and blue numbers indicate negative and positive correlations, respectively.

4 CONCLUSIONS

Pearl millet extrudates processed at low water content of 14% showed unusual non-expansion highly differing from corn extrudates processed at same extrusion conditions. The small expansion and very low starch conversion demonstrated that pearl millet, contrary to corn, needs higher water content when processed in a single screw extruder. The *in vitro* digestibility of carbohydrates showed that extruded millet is less susceptible to digestive enzymes than corn. Therefore, for the production of whole extruded millet, these changes are technologically and nutritionally favorable for its use in whole flour-based food products and gluten-free food markets. More studies would be recommended to be carried out in a twin screw extruder in order to evaluate higher heat and shearing conditions to produce whole meal pearl millet puffed extrudates.

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CAPÍTULO II

PROPRIEDADES FÍSICO-QUÍMICAS DO AMIDO DE MILHETO COMO POTENCIAL INGREDIENTE ALIMENTAR

PHYSICOCHEMICAL PROPERTIES OF PEARL MILLET STARCH AS POTENTIAL FOOD INGREDIENT

Thaís Barbosa dos Santos[1], Davy William Hidalgo Chávez[1], Carlos Wanderlei Piler de Carvalho[2]

[1] Universidade Federal Rural do Rio de Janeiro, Rodovia Br 465, km 7, CEP 23890-000 Seropédica, RJ, Brazil.
[2] Embrapa Agroindústria de Alimentos, Avenida das Américas 29501, Guaratiba, CEP 23020-470 Rio de Janeiro, RJ, Brazil.

ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is cultivated in vast areas of Brazil, used in no-tilling practices for soil preservation. Physicochemical properties of pearl millet hybrid ADRg9070 isolated starch were compared to commercial corn starch by proximate composition, total starch, morphology, particle size distribution, pasting properties, solubility index and swelling power. Morphology showed that starches were similar in format, however pearl millet had a higher amount (42.90%) of small granules (<10 µm), when compared to corn (14.04%). The pearl millet starch pasting presented greater resistance in a hot process (below 83.4 °C), differing from corn starch (72.7 °C); furthermore, the retrogradation and final viscosity of millet starch were very higher, highlighting the gelling potential found in millet starch as food ingredient.

Keywords: millets; native starch; non-conventional starch; paste viscosity; starch isolation.

1 INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is cultivated in vast areas of Brazilian savannas mainly used as a forage, in no-tilling practices for soil preservation. As differential, it has ability to grow in adverse conditions such as dry and less fertile land, where other cereals would not grow (DE ASSIS, DE FREITAS, MASON, 2018; SHEETHAL *et al.*, 2022). Compared with other cereals, pearl millet also possesses abundant polysaccharide. Its raw grain has the range of 60 to 70% starch, being a potential sustainable and cheaper starch source (EMBASHU, NANTANGA, 2019; PUNIA *et al.*, 2021).

Corn is the main commercial source of starch, being widely used with chemical modifications to meet the different properties demanded by the food industries. Whereas the demand for starch grows continuously to meet the food sector, these modifications increase the value of starch almost 3x greater than native starch, due to production costs such as energy for example. Therefore, new sources that do not need chemical modification become a trend in the global market (ADEWALE, YANCHESHMEH, LAM, 2022).

Added to the decrease in water availability in the world, the decrease in corn cultivation areas and mainly the scarcity of information regarding the physicochemical properties of pearl millet starch, this unconventional source of starch needs to be better explored, to investigate the starch behavior and its applications (SANDHU, SIROHA, 2017).

In this sense, the non-conventional pearl millet starch may be a potential substitute for corn. That encourages its use to meet the growing demand for non-chemically modified starches, especially as an ingredient in industrial food products (SANDHU, SHARMA, KAUR, KAUR, 2020). Thus, this study aimed to perform the isolation and physicochemical analysis of pearl millet starch compared to commercial corn starch, investigating its properties.

2 MATERIALS AND METHODS

2.1 MATERIALS

The pearl millet [*Pennisetum glaucum* (L.) R. Br.] grains of ADRg 9070 hybrid were donated in July of 2021 by ATTO Sementes located in Rondonópolis – Brazil (geographical coordinates: 16°29' S latitude and 54°38' W longitude). The grains were cleaned in a seed air cleaner separator (Clipper Office Tester, AT Ferrell Company Inc., Bluffton IN, USA). The weight of 0.5 kg of pearl millet was decorticated in a rice milling machine TM – 97 (Suzuki S/A, São Paulo, Brazil) and stored in plastic (low-density polyethylene – PE-LD 4) bags kept at room temperature, for subsequent starch extraction.

After extraction, pearl millet starch was compared with commercial corn starch, acquired from the local market of Rio de Janeiro (Brazil). The samples were called: millet starch (MS) and corn starch (CS).

2.2 METHODS

2.2.1 Pearl millet starch isolation

Pearl millet starch isolation was accomplished by wet milling processing following the methodology described by Santos *et al.* (2022), with modification. The grain to water ratio was increased because it was found that greater water volume facilitated the separation of starch during sieving, according steps bellow: 1) Decorticated grain was submitted to fragmentation in water (1:4 grain:water) in an industrial blender model SPL-049 (SPOLU, Itajobi, Brazil) for 5 min for suspension; 2) Sieving the suspension through a 0.180 and after 0.106 mm sieve; 3) Using a Universal 320R centrifuge (Hettich, Tuttlingen, Germany) at 7500 x *g* for 5 min, approximately 40 mL of sifted on centrifuge tubes, discarding the supernatant; 4) Centrifuge process was repeated using the same tubes (without discarding the precipitate) completed until 40 mL with the sifted, discarding the supernatant after each centrifugation; 5) Centrifuge process was repeated using the same tubes with precipitate but completed with 70% alcohol solution (for the removal of prolamins from millet), and the samples were vortexed using a Vortex Genie 2 (Scientific Industries, Bohemia, NY, USA) for 1 min before centrifugation. After, the supernatant was discarded, followed by manual scraping of the upper lipid and protein layers; 6) The same process was realized with 90% alcohol solution (for the removal of prolamins); 7) Dry starches at 45 °C overnight, in glass beakers (50 mL), until approximately 10% moisture, followed by scraping of upper ash, lipid and protein layers and steeping (Figure 1A); 8) Ultimately was done a steeping to obtain MS (Figure 1B).

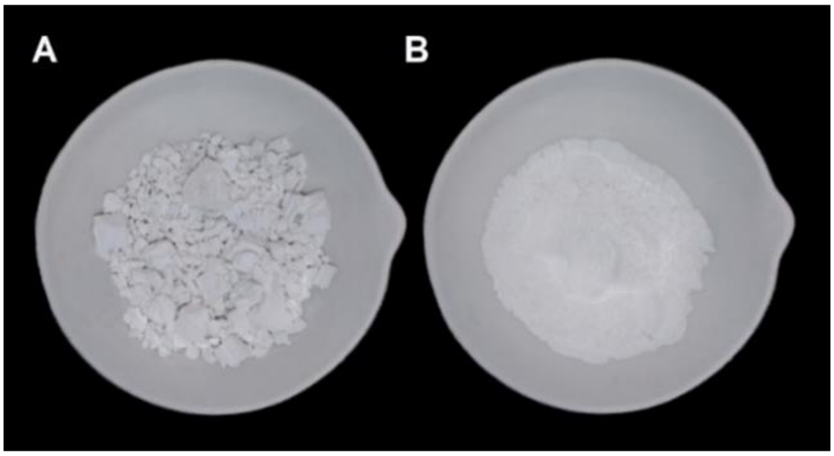

Figure 1. MS isolation: (A) Before steeping; (B) After steeping.

2.2.2 Proximate composition

Proximate composition was determined in duplicate. Moisture and ash content were determined using the same equipment, a thermogravimetric analyzer TGA- 2000 (Navas Instruments, Conway, United States) according to method n° 925.09 and 923.03, respectively, and protein (6.25 x N for corn; 5.75 x N for pearl millet) was determined by Kjeldahl method n° 978.04 (AOAC, 2005). Lipid according to the official method Am 5-04 of the American Oil Chemists Society (2005). Carbohydrate content was calculated by difference.

2.2.3 Total starch content

Total starch content was determined in duplicate by method 76-11 (AACC) using the total starch Megazyme assay kit procedure (AA/AMG) (Megazyme International Ireland Ltd, Bray, Ireland) (AACC, 1976).

2.2.4 Morphology of the starch granules

The morphology was observed under an optical microscope Leitz Laborlux S (Leica, Famalicão, Portugal), with a polarized filter. Starches were photographed at a total magnification of 400X. The collected images were recorded by a digital camera (MShot, Guangzhou, China) attached to the optical microscope.

2.2.5 Particle size distribution

The particle size distribution of starches was determined by analyzing each sample 6 times, according to the method 55–40.01 of the American Association of Cereal Chemists (1999) . The starch solution was placed on a S3500 laser particle size analyzer (Microtrac Inc., Montgomeryville, USA).

2.2.6 Pasting properties

The pasting viscosity profiles were determined in duplicate using a Rapid Viscosity Analyzer - RVA Series 4 (Newport Scientific, Warriewood, NSW, Australia) with Thermocline software for Windows. following the methodology described in Comettant-Rabanal *et al.* (2021). The data were analyzed using Thermocline software for Windows and the respective readings of pasting curve were recorded: paste temperature (PT), peak viscosity at 95 °C (PV), minimum viscosity after heating (mV) and final viscosity (FV); and calculated: breakdown viscosity (BD = PV-mV) and setback viscosity (SB = FV-mV).

*2.2.*7 Solubility index (SI) and Swelling power (SP)

The estimation of solubility index (SI) and swelling power (SP) at varied temperatures (55-95 °C) were determined in quadruplicate, following with some modifications, the methodology described by Tsai, Li and Lii (1997), heated in water bath, at varied temperatures (55, 65, 75, 85, 95 °C, respectively).

SI was expressed as the ratio of dry matter supernatant (m_s) to the initial mass (m_i) of starch (Equation 1).

$SI = (m_s \div m_i)$ (Equation 1).

SP was described as the ratio of the sedimented starch (m_a) to the initial starch weight (m_i) multiplied by (1–SI) (Equation 2) as below:

$SP = (m_a) \div [m_i (1\text{-}SI)]$ (Equation 2).

2.2.8 Statistical analyses

Analysis of variance (ANOVA) was developed to determine the statistical differences ($p \leq 0.05$) and, when differences were found a multiple mean Tukey test was carried out at a significant level of 5%. The outcomes between the starches were evaluated with a T-test. Statistical difference ($p \leq 0.05$) is shown by different letters. These statistical analyses were performed by using R free statistical software, version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

3 RESULTS AND DISCUSSIONS

3.1 PROXIMATE COMPOSITION AND TOTAL STARCH

The proximate composition of MS compared to CS is displayed on a dry basis in Table 1; the starches did not show statistical difference ($p > 0.05$) for all macro nutrients, which may be interesting when pure starch is desirable.

Table 1 Proximate composition and total starch of MS and CS in dry basis (g/100 g).

Composition	MS	CS
Ash	00.00±0.00^a	00.00±0.00^a
Protein	00.00±0.00^a	00.00±0.00^a
Lipid	00.21±0.01^a	00.15±0.02^a
Carbohydrate	99.79±0.01^a	99.85±0.02^a
Total starch	92.15±2.77^a	93.15±2.85^a

Results were expressed in (g/100 g). Values with different letters, in the same line, indicate statistical difference ($p \leq 0.05$).

Proximate composition analysis, in dry basis (Table 1), revealed for MS and CS that ashes and protein were not quantitated (0 g/100 g), indicating that the starches were successfully isolated. According to Cardoso, Samios and Silveira (2006), the increasing use of starches by the food industry has grown the interest of removing protein from binding starch granules, since the higher the purity of the granule, the better its performance as an additive.

Lipid also presented low content for MS (0.21 g/100 g) and CS (0.15 g/100 g) starch. Concerning carbohydrate content, MS obtained 99.79 g/100 g while CS presented 99.85 g/100 g, this difference in values was not enough to cause a statistical difference ($P > 0.05$). Punia *et al.* (2021) reported in revision about pearl millet starch that values finding (dry basis) of 0.11 g/100 g ash, 0.61 g/100 g protein, 0.43 g/100 g lipid, as well as the carbohydrate content of 98.85 g/100 g lower than the found in this search.

Total starch (Table1) was analyzed in order to determine how pure the starches were. The proposed extraction method led to a small decrease in total starch content for MS (92.15 g/100 g) and the decrease was also demonstrated by CS (93.15 g/100 g). These variations, in total starch content among samples were not enough to cause a statistical difference, the same small reduction was verified in relation to the carbohydrate content, therefore, the lower total starch content may be related to the lower accuracy of this method. Wang *et al.* (2022) reported for normal maize starch analyzed the total starch content was 92.2 g/100 g, and also mentioned that decrease in the total starch content, compared to carbohydrate content is related with an increase in the damaged starch content by others physical treatments generating damage to the internal structure of starch.

3.2 STARCH CHARACTERIZATION

3.2.1 Morphology of the starch granules

MS granules (Figure 2A) exhibited polygonal, irregular, and spherical shapes and showed typical birefringence under polarized light, with slightly modified the starch with physic isolation, the same observations can be applied to MS (Figure 2B). Consequently, as a result of the presence of the ‘Maltese cross’ patterns identified using polarized microscopy with observation of high contrast the birefringence after the physic isolation was preserved (GOVINDARAJU *et al.*, 2021). MS showed the largest apparent amount of small granules while CS presented the biggest granules with a low amount of small granules, and this distribution was possible to be confirmed through particle size analysis.

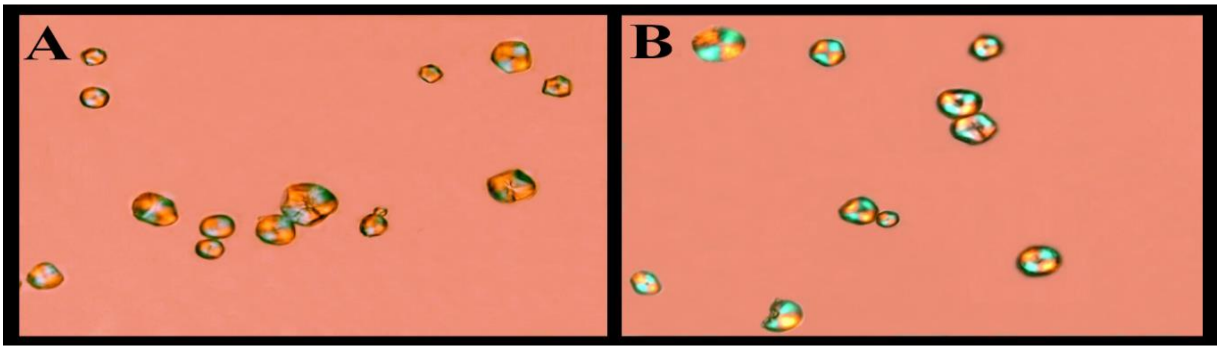

Figure 2. Light microscopy of starches (400x): (A) MS (millet starch); (B) CS (corn starch).

3.2.2 Particle size distribution

The particle size distribution of the starch granules is displayed in Figure 3. A large difference ($p \leq 0.05$) was found between MS that presented the highest amount (42.90%) of small starch granules (<10 µm), whereas CS showed the lowest amount (14.04%). Siroha *et al.* (2020) analyzed the particle size of millet starch granules and identified that starches smaller than 10 µm ranged from 36.23 to 48.34%, similar to the results found in this study. Lindeboom, Chang and Tyler (2004) mentioned that in relation to small granules, a common problem in the isolation of starch by centrifugation, is that generally a dark layer, formed by non-carbonyl compounds such as protein, when scraped off, severe losses of small granules occur, this severe loss was not observed for MS.

Concerning the particle size distribution (Figure 3) in the range 10 – 20 µm had the major volume for all starches with 50.68% for MS and 62.92% for CS, however indicate statistical difference ($p \leq 0.05$). In addition, other ranges: 20 – 30 µm and >30 µm, CS showed the higher percent values (17.67 and 5.37%, respectively) compared to MS (4.30 and 2.12%, respectively), confirming that the starches had a significant difference ($p \leq 0.05$) in particle size distribution.

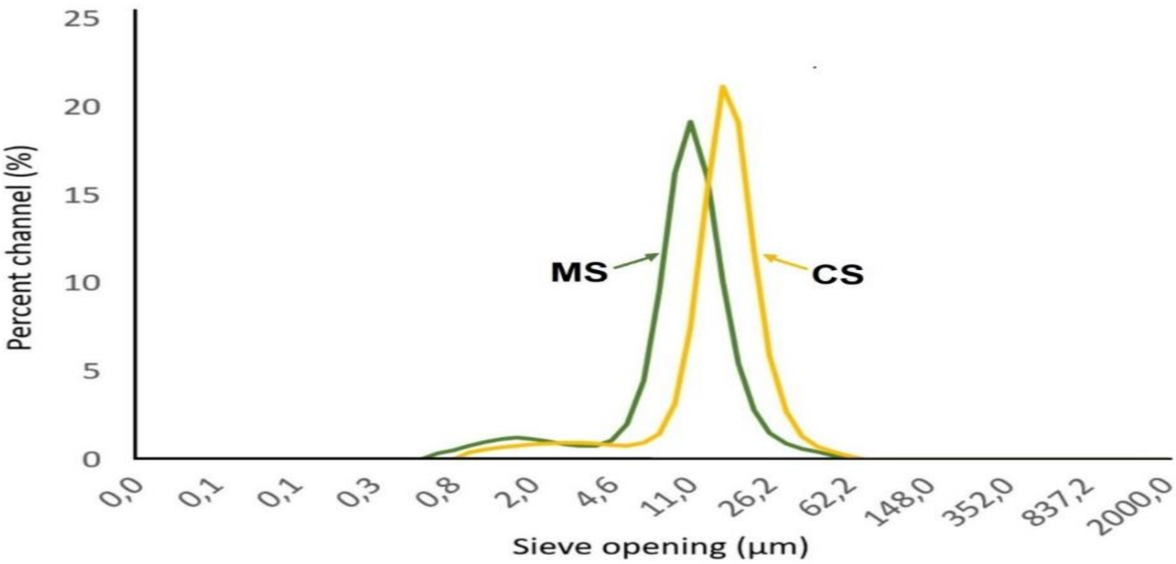

Figure 3. Particle size distribution of MS (millet starch) and CS (corn starch).

These distributions allow analyzing that the largest volume in particle size distribution of MS was in the range between 0-20 µm (93.58%) confirming the smallest granules and with greater uniformity, while CS presented the highest percentage between 10-30 µm (80.59%) with medium granules. In food products smaller and uniform granules contribute to a better creamy mouthfeel, being a desirable quality in frozen desserts, pies, cookies, for example (LINDEBOOM, CHANG, TYLER, 2004).

3.2.3 Pasting properties

The pasting properties of MS and CS were displayed in Figure 4A. MS had higher PT (83.4 °C) compared to CS (72.7 °C), indicating greater resistance in pearl millet pasting and low viscosity (100 cP), in a hot process (below 80.0 °C), differing ($P < 0.05$) from CS with very high viscosity (1844.4 cP, around 80 °C). Native starch generally has limitations such as the inability to withstand high temperatures, which extremely restricts its applications in the food industry, making it important to search for chemically unmodified starches to understand their behavior and find these specific properties without the need for modifications (HAN; SHI; SUN, 2020). In relation to withstand high temperatures, MS meets this specific property.

Peak viscosity had a significant difference ($p \leq 0.05$) between starches, while MS (with sharp peak viscosity at 2724.5 cP) presented the highest, CS had the least peak viscosity (2300.5 cP) (Figure 4A), indicating more swelling capacity for MS at higher temperatures. Final viscosity of MS (4316.0 cP) was statistically different ($p \leq 0.05$) compared to CS (3767.5 cP) and MS starches presented a disturbance of viscosity flow after 15 min run, which may indicate an intermittent formation of entanglements of amylose molecules due to constant shear. According to Santos *et al*. (2022) the gel formation was probably hampered by the formation of complexes between amylose and other residual macromolecules such as lipids and proteins. In addition, the retrogradation or setback viscosity of MS was very high (2888.50 cP) which was interesting in conditions where high gel strength associated with high viscosity is desired, while CS presented retrogradation viscosity of 2351.0 cP).

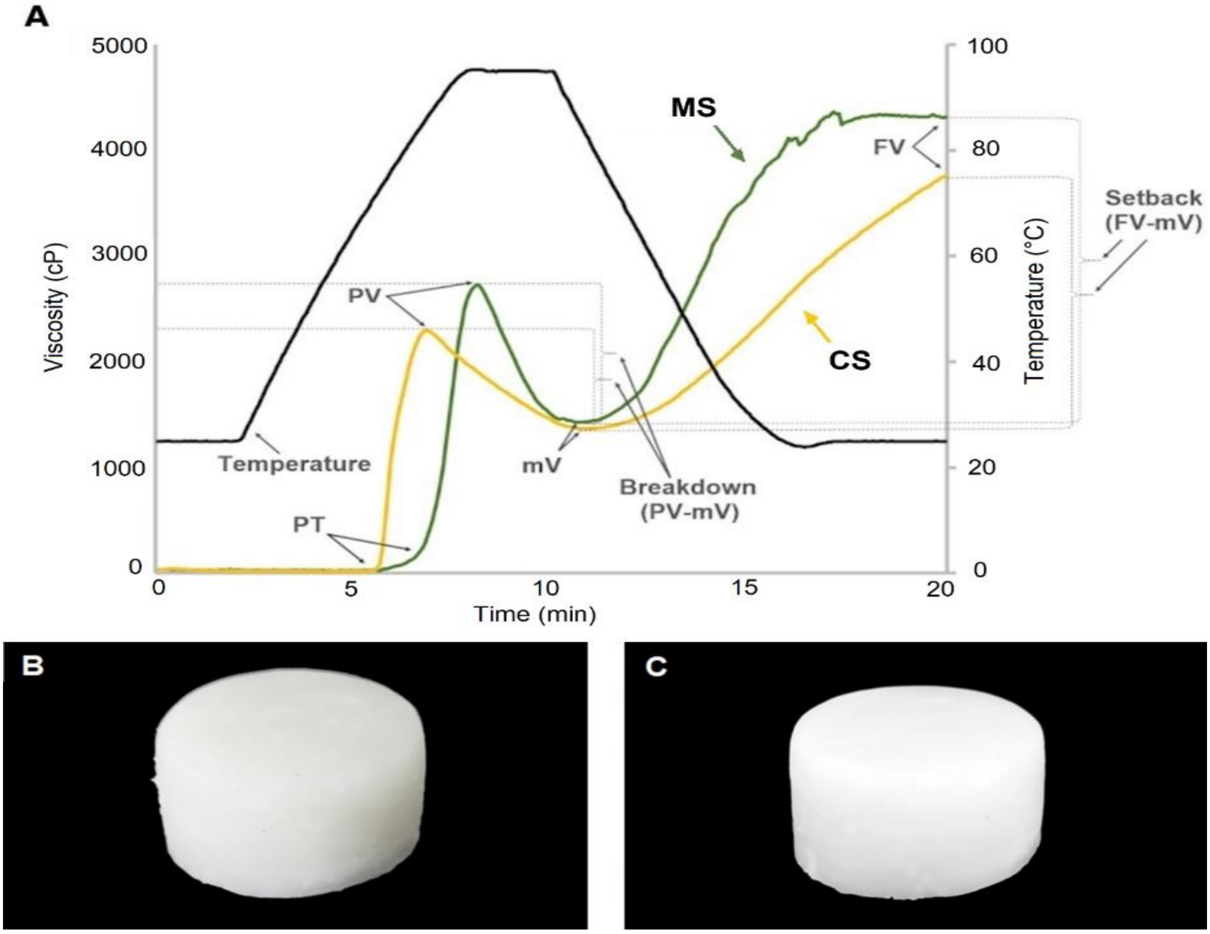

Figure 4. (A) Pasting properties of MS (millet starch) and CS (corn starch): PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity; Breakdown = PV-mV, breakdown viscosity; Setback = FV-MV, setback viscosity; Visual aspects of starch gels formed after 24 freezing: (B) MS (millet starch); (C) CS (corn starch).

In addition, after 24h freezing the gel of MS and CS are shown in Figure 4B and 4C, respectively. Although the visual appearance of the gels is similar, the particle size and the lower initial viscosity (below 80 °C) can be a differential for the use of MS in food products.

3.2.4 Solubility index and swelling power

MS showed the lowest SI (Figure 5A) with significant difference ($p \leq 0.05$) when compared to CS, in all temperatures analyzed. MS showed an initial SI of 0.02 (55 °C), which remained statistically unchanged up to 85 °C, showing greater stability in these ranges, while the increase in temperature from 85 to 95 °C showed a significant difference between the solubility indices found (0.06 to 0.09, respectively).

As for CS, there was no statistical difference when subjected to temperatures lower than 55 and 65 °C (SI of 0.06 and 0.09, respectively), while the increase up to 75 and 85 °C (SI of 0.13 and 0.19, respectively) generated the highest solubility, when compared to the previous temperature range analyzed, finally SI at 85 (0.13) and 95 °C (0.22) showed no statistical difference, indicating stability in solubility in these ranges. According to Punia (2020), during heat in excess of water, the starch granules swell, the crystalline structure is broken, and consequently the hydroxyl groups of the amylopectin molecules and mainly amylose bind to water by hydrogen bonds, resulting in increased solubility.

This could be an indication that the amylopectin molecules in SI generate greater steric blockage, preventing the formation of hydrogen bonds, resulting in lower solubility for the MS starch.

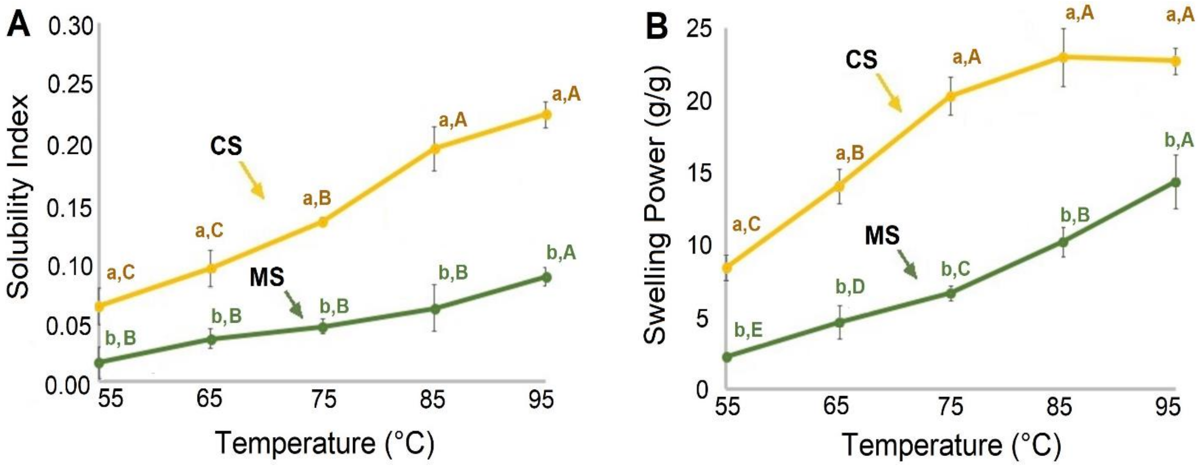

Figure 5. MS (millet starch) and CS (corn starch): (A) Solubility index and (B) Swelling power of. The capital letter means significant differences among different temperatures in the same sample ($p \leq 0.05$). Small letters indicate significant differences among samples at the same temperature.

The SP (Figure 5B) profile of all starch granules (Figure 5B) increased from 55 to 95 °C and CS had the highest SP compared to MS ($p \leq 0.05$). Punia (2020) reports that the swelling behavior of starch cereals is mainly related to the presence of amylopectin, hence higher values of swelling power suggest higher amylopectin content with less rigid granular structure. In comparison with CS, MS showed to be less susceptible to breakage under extended heating. However, for MS it is possible to observe an increasing SP between temperatures from 55 to 95 °C, while for CS between 75 to 95 °C it is possible to observe stability in SP.

According to Liu *et al.* (2016) and Olayinka, Adebowale and Olu-Owolabi (2013), low SP and SI may indicate more stronger interactions among amylose-amylopectin. In addition, in review of Zhu (2014), starch can be indicated for the production of noodles, having required properties such as lower cooking loss (low SI) and elastic property correlated with high pasting viscosity; and these properties being found in MS.

4 CONCLUSIONS

It was possible to isolate native pearl millet starch without chemical treatment, being possible getting high carbohydrate levels. Millet starch presents the same proximate composition compared to commercial corn starch, but difference in particle size was observed. Millet starch showed the lowest granules and a greater resistance to form pasting associated with low viscosity in a hot process (below 80.0 °C), these differences represent distinct functional properties such as gelling agent due to RVA profile compared with commercial corn starch. In addition, millet starch presented lower SI and SP compared to CS. The results among high PV and low SI contributing to MS being used in starch noodles production. Furthermore, these results encourage advances in the study of pearl millet starch and application of new starches not yet commercially established like millet starch.

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CAPÍTULO III

PRODUTOS EXTRUDADOS DE MILHETO (INTEGRAIS, DECORTICADOS E GERMINADOS): AVALIAÇÃO FISICO-QUÍMICA PARA PREVISÃO DE POTENCIAL TECNOFUNCIONAL E ESTABILIDADE

PEARL MILLET EXTRUDED PRODUCTS (WHOLE, DECORTICATED AND GERMINATED): PHYSICOCHEMICAL EVALUATION FOR PREDICTION OF TECHNO-FUNCTIONAL POTENTIAL AND PHYSICOCHEMICAL STABILITY

Thaís Barbosa dos Santos[1*], Raul Comenttant-Rabanal[2], Davy William Hidalgo Chávez[1], Carlos Wanderlei Piler de Carvalho[3]

[1] Universidade Federal Rural do Rio de Janeiro, Rodovia Br 465, km 7, CEP 23890-000 Seropédica, RJ, Brazil.
[2] Escuela Profesional de Ingeniería Agroindustrial, Facultad de Ingenierías, Universidad Privada San Juan Bautista, Ica, Perú.
[3] Embrapa Agroindústria de Alimentos, Avenida das Américas 29501, Guaratiba, CEP 23020-470 Rio de Janeiro, RJ, Brazil.

ABSTRACT

In this study, extrusions were carried out with constant parameters (3 mm round die running at 250 rpm; heating zone: 40, 80 and 120 °C; constant feed: 4 kg/h; and feed moisture: 14 or 20%) in order to produce healthy extrudates and flours from whole, decorticated and germinated grains, carrying out the physical-chemical characterization and multivariate analysis (expansion indexes, instrumental texture, pasting properties, proximate composition, total and resistant starch) to determine the techno-functionality potential (farinograph measurement) and physicochemical stability. The results indicate that the pearl millet extrudates obtained from wholemeal flour presented medium expansion, compared to the twinned and decorticated extruded ones, in this sense, the sprouted extruded ones presented the lowest expansion values, possibly related to the higher fiber content (7.32%) and lower total starch content (75.34%) of the flour used in the feed, while the decorticated flour produced the most expanded extrudates, possibly related to the lower fiber content (12.34%) and higher total starch content (62.52%). Through multivariate analysis, whole and germinated extruded flours were chosen for monitoring of physicochemical stability, identifying the effectiveness of the extrusion process to increase the physicochemical stability in these extruded millet flours up to 3 months.

Keywords: gluten-free products, extrusion cooking, deterioration kinetics, farinography.

1 INTRODUCTION

The ready-to-eat food market is a growing segment of the food industry, often linked to growing consumer awareness related to the impact of food on metabolic disorders. This new awareness also stimulates the growth of the healthy food market, such as gluten-free foods, which are a special part of the diets of consumers with irritable bowel syndrome and non-celiac wheat sensitivity. In this sense, developing gluten-free products with superior nutritional values and great technological properties is a challenge (RADOŠ *et al.*, 2023).

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a gluten-free cereal that presents high content of proteins with balanced amino acids, minerals, unsaturated fatty acids, carbohydrates (approximately 70%) and bioactive compounds (TOMAR *et al.*, 2022). These qualities make pearl millet a potential candidate to increase new foods for human consumption and also be used as an ingredient in diversified foods, especially in gluten-free food products (DIAS-MARTINS *et al.*, 2018). In addition, to the nutritional value, the importance of millet was highlighted by the Food and Agriculture Organization of the United Nations (FAO), when it named 2023 as the year of millet (IYM 2023) because it has nutritional qualities superior to other gluten-free cereals, such as wheat and rice, which is a reason of worldwide attention to give visibility to this cereal with the potential to strengthen the food security, agriculture, and economy, contributing to sustainability, and stimulating the consumption of crops not yet been commercially established (FAO, 2022).

In Brazil, an important step towards the consumption of millet in human food was taken in 2022. Through Resolution N° 711 of July, 2022 of the National Health Surveillance Agency. The use of millet was approved as cereal to be used in human food in starches, cookies, whole grains, processed cereals, bran, flour, whole grain flour, noodles and bread (BRASIL, 2022). Nonetheless, it is important to point out that millet has a high content of lipids and enzymatic activity that promote changes in aroma and undesirable flavor in unprocessed products. These alterations occur by the incorporation of oxygen molecules into polyunsaturated fatty acids (commonly found in millet as linoleic and linolenic acids) susceptible to enzymatic action, which are decomposed into other molecules of acids, aldehydes and short-chain ketones, contributing to the faster deterioration in non-heat treated products (SILVA, BORGES, FERREIRA, 1999; REDDY, VISWANATH, 2019).

For the development of healthier food products with a greater physicochemical stability, as ready-to-eat snack, extrusion cooking can be an alternative, with greater nutritional values, however, few studies have investigated the use of pearl millet and the physicochemical stability using extrusion cooking (REDDY, VISWANATH, 2019; OLIVEIRA, *et al.*, 2021; YADAV, DALBHAGAT, MISHRA, 2022). Physicochemical stability in processed foods can be defined as the final period in which the product maintains a level of required, well-defined qualities during storage. Preliminary steps for identifying the most critical chemical, physical or biological changes that lead to the end of product quality must be defined, followed by definitions of the relevant acceptability limit (CALLIGARIS *et al.*, 2016).

For pearl millet, definitions of these limits in legislation and other research are scarce. In this study, millet extrudates and extruded flours were produced in single screw extruder by 3 different treatments (whole, decorticated and germinated pearl millet grains) with the aim of physical-chemical characterization, and through multivariate analyses to choose 2 treatments to evaluate techno-functional (farinography) potential and stability.

2 MATERIALS AND METHODS

2.1 SAMPLE PREPARATION

Pearl millet grains of ADRg 9070 hybrid were donated by ATTO Sementes located in Rondonópolis – Brazil. The grains were cleaned in a seed air cleaner separator (Clipper Office Tester, AT Ferrell Company Inc., Bluffton IN, USA). Commercial yellow dent hybrid corn grains were kindly donated by Indústrias Granfino (Nova Iguaçu, Brazil). Whole grains (moisture content of 4 ± 0.5%) were stored in plastic bags at room temperature. In addition, pearl millet starch (MS) was isolated following the methodology described by Santos *et al.* (2022) and compared with other samples (obtained in section 2.2).

2.2 FLOURS PREPARATION

Pearl millet grains were separated for 3 different treatments (whole, decorticated, and germinated) (Figure 1), obtaining extrudates with 2 different moisture content (extrudates processed at 14 and 20% moisture content of raw flours).

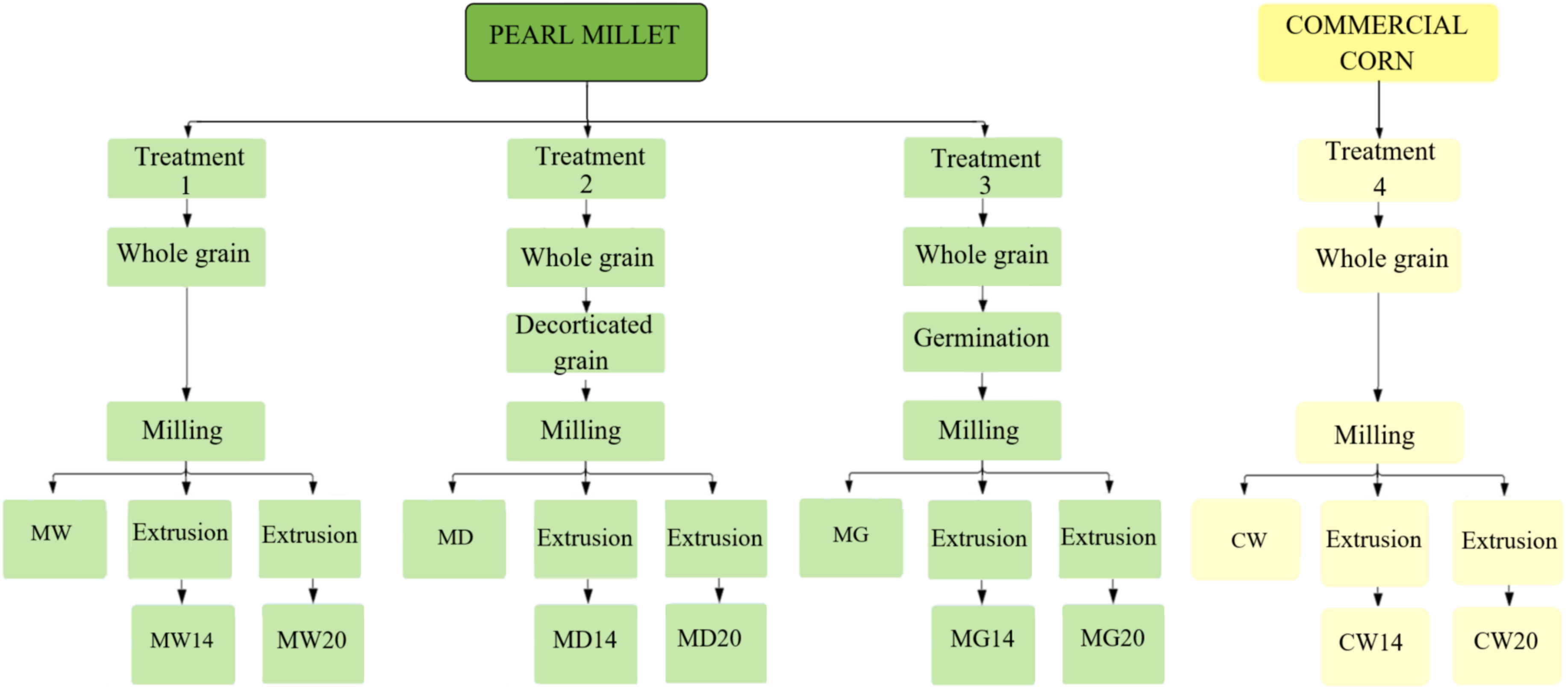


Figure 1. Flowchart referring to extrudates preparation. Codes displayed in samples: MW, whole millet flour; MW14 and MW20: extruded whole millet flour at 14 and 20% moisture, respectively; MD, decorticated millet flour; MD14 and MD20: extruded decorticated millet flour at 14 and 20% moisture, respectively; MG, germinated millet flour; MG14 and MG20: extruded germinated millet flour at 14 and 20% moisture, respectively; CW, whole corn; CW14 and CW20: extruded whole corn flour at 14 and 20% moisture, respectively.

2.2.1 Whole millet flour

To produce the different whole flours, in treatment 1, 3 kg of whole pearl millet grains were ground in a hammer mill LM3100 (Perten Instruments AB, Huddingen, Sweden) equipped with a 0.8 mm sieve. 1 kg were separated, obtaining raw whole grain millet flour (control 1, called as MW), 1 kg were intended for extrusion process to obtain whole millet extruded at 14% moisture (MW14), and 1 kg to produce whole millet extruded at 20% moisture (MW20).

2.2.2 Decorticated millet flour

For obtaining the different decorticated flours, in treatment 2, 3.3 kg of grains were partially decorticated in a rice milling machine TM – 97 (Suzuki S/A, São Paulo, Brazil). 3 kg of partially decorticated grains were ground using the same procedure as mentioned above for whole flour. 1 kg were separated, obtaining decorticated grain millet flour (control 2, called as MD), 1 kg were reserved for extrusion process to obtain decorticated millet extruded at 14% moisture (MD14), and 1 kg to produce decorticated millet extruded at 20% moisture (MD20).

2.2.3 Germinated millet flour

Finally, in production the different germinated flours, in treatment 3, pearl millet grains were soaked in water, ratio1:3 (grain:water) for 4 h, at a controlled temperature of 30 °C and then drained. After that, the grains were placed in a fermentation cabinet (National Mfg. Co., Lincoln, USA) at 30 °C, 90% relative humidity, for 24 h (obtaining 99% of the germinated grains), and then, in a fan oven at 30 °C the grains were dried until reaching a final moisture content of 4 ± 0.5%, according to method described by Comettant-Rabanal *et al*., (2021) that applied this process to obtain germinated flour and used in a gluten-free bread. Therefore, these same parameters were used to evaluate the germinated flour under the same conditions used previously. Then, 3 kg of germinated grains were ground under the same conditions as the whole. 1 kg were separated, obtaining germinated grain millet flour (control 3, called as MG), 1 kg were reserved for extrusion process to obtain germinated millet extruded at 14% moisture (MG14), and 1 kg to produce germinated millet extruded at 20% moisture (MG20).

2.2.4 Whole corn flour

For comparison with millet, in treatment 4, 3 kg of whole commercial corn grains (donated by Indústrias Granfino, Nova Iguaçu, Brazil) were milled using the same milling process as millet. 1 kg were separated, obtaining whole grain corn flour (control 4, called as CW), 1 kg were separated for extrusion process to obtain whole grain corn extruded at 14% moisture (CW14), and 1 kg to produce whole grain corn extruded at 20% moisture (CW20).

2.3 EXTRUSION PROCESS

The whole, decorticated, and germinated flours were preconditioned (separately) at moistures of 14 and 20% (using 1 kg for each treatment, in triplicate), and processed in a single-screw extruder 19/20 DN (Brabender, Duisburg, Germany) attached to a torque rheometer PlastCorder LabStation (Brabender, Duisburg, Germany). The extruder was fitted with a 3:1 (length/diameter ratio) screw coupled to the extruder, 3 mm round die running at constant 250 rpm and temperature heating zones adjusted to 40, 80 and 120 °C from feeding to the outlet. 1 kg of each flour (whole, decorticated, and germinated at 14 and 20% moisture content) was fed using a volumetric vertical feeder running at variable speed to provide a constant feed of 4 kg/h. Motor torque (Nm) shown on the extruder control panel was recorded.

2.4 EXPANSION PROPERTIES AND APPARENT DENSITY

The sectional expansion index (SEI), the apparent density of extruded pieces (ρ_e), the longitudinal expansion index (LEI) and the volumetric expansion index (VEI) were then calculated according to Alvarez-Martinez, Kondury and Harper (1988). Extrudates were randomly selected from each extrusion trial. The diameter and length of specimens taken from extrudates were measured using a digital caliper (ZAAS Precision, Curitiba, Brazil) in triplicate.

2.5 INSTRUMENTAL TEXTURE

Previously dried (50 °C up to 4% ± 0.5 moisture content) and kept in a desiccator at room temperature, the extrudates (20 mm long) were used to determine the maximum compression force at 1 mm/s using a BSK (Blade set knife) probe in a Texture Analyzer TA-XT Plus (Stable Micro Systems, Surrey, UK) equipped with a 30 kg load cell. The force-time curve was analyzed by the Exponent software program version 6.1.11.0 (Stable Micro Systems, Surrey, UK).

2.6 PASTING PROPERTIES

The pasting viscosity profiles were determined in duplicate using a Rapid Viscosity Analyzer - RVA Series 4 (Newport Scientific, Warriewood, Australia), running at 160 rpm and starting heating with a temperature of 25 °C (COMETTANT-RABANAL *et al.*, 2021). 3 g of raw and extruded flours with adjusted moisture content at 14% wet basis were weighed and added to 25 mL of distilled water. The data were analyzed using Thermocline software for Windows and the respective readings of pasting curve were recorded: paste temperature (PT), peak viscosity at 95 °C (PV), minimum viscosity after heating (mV) and final viscosity (FV); and calculated: breakdown viscosity (BD = PV-mV) and setback viscosity (SB = FV-mV).

2.7 PROXIMATE COMPOSITION

Moisture and ash were determined using the thermogravimetric analyzer TGA-2000 (Navas Instruments, Conway, USA), according to method n° 925.09 and 923.03 respectively, total dietary fiber following the method 991.43, and protein (6.25 x N for corn; 5.75 x N for pearl millet) was determined by Kjeldahl method 2001.11 (AOAC, 2005). Lipid by official method Am 5-04 (AOCS, 2005). Carbohydrate content was estimated by difference. Proximate composition was carried out in duplicate.

2.8 TOTAL AND RESISTANT STARCH

Total starch content of raw and extruded flours was determined in duplicate by method 76-11 (AACC) using the total starch Megazyme assay kit procedure (AA/AMG) (Megazyme International Ireland Ltd, Bray, Ireland) (AACC, 1976).

Resistant starch was determined according to method 2002.02 using the Megazyme assay kit (Megazyme International Ireland Ltd, Bray, Ireland) (AOAC, 2002). The results were expressed in duplicate.

2.9 FARINOGRAPH MEASUREMENT

Through multivariate analysis, 2 treatments were chosen based on physicochemical results, for techno-functional (farinography) evaluation and physicochemical stability.

To check the resistance of the dough to mixing, the farinography was measured using different nutritionally superior extruded and raw millet flours. The Farinograph© model FD0234H (Brabender, Duisburg, Germany) was used, according to modified method 54–21.01 AACC of AACC (2000), following adjustments proposed by Comettant-Rabanal *et al.* (2021) for treated-extrusion gluten-free flours. Farinography was used as an empirical rheological technique to evaluate raw (45 g) and extruded (33 g) gluten-free flours, with added water absorption levels of 68 and 100%, respectively. For standardization at 14% moisture content, and after flour weight and water volume were calculated, according to Equation 7 and 8:

$$W_{calculated} = (W_{empirical} \text{ x } 86)/(100\text{-moisture}) \qquad \text{Equation (7)}$$

$$V_{calculated} = (WA_{empirical} \text{ x } m_{calculated})/100 \qquad \text{Equation (8)}$$

Where:
$W_{calculated}$ is the calculated weight (g) used experimentally;
$W_{empirical}$ is the empirical weight (g), being equal to 45 g when analyzing raw samples and 33 when analyzing extruded samples;
moisture is the moisture content of gluten-free flour (%).
$WA_{empirical}$ is the water absorption (%), being equal to 68% when analyzing raw samples and 100% when analyzing extruded samples;
$V_{calculated}$ is the calculated volume (mL) of water used experimentally.

In the determination of farinographic properties for gluten-free flours, the following readings were considered: water absorption (WA, %), arrival time (T_a, min), departure time (T_d, min), dough stability time (DST, min = T_d-T_a), dough development time (DDT, min = 0.5 min after peak) and mixing tolerance index (MTI, BU), determined 5 minutes after the peak. To adapt the methodology to the profile of gluten-free flours, 500 Brabender Units (BU) were not considered as a reference, because the analyzed samples did not contain gluten. For the farinograms of the gluten-free flours, the maximum peaks were considered, since at this peak the flour absorbs all the water and the dough reaches its optimum dough consistency. Measurements were performed in triplicate.

2.10 PHYSICOCHEMICAL STABILITY

After techno-functional potential evaluation, these 2 treatments chosen were analyzed, for physicochemical stability prediction (though moisture content, acidity and colorimetry).

New extrusions (carried out according to items 2.2 and 2.3) were performed 7 times, using constant operational parameters, monitored through the torque of each processing (Annex A). The extrusions were realized during 6 months, including time zero, with an interval of ~30 days. In the first extrusion, the extruded flours and raw flour were packaged (in a transparent plastic bag, stand up pouch model, with zipper; at room temperature 25 ± 8°C), separately, and evaluated after 6 months (t=6); the second extrusion, followed the same procedure, being evaluated after 5 months (t=5); the other flours were extruded and sequentially evaluated; being the last extrusion (t=0), evaluated as a control. Moisture content, acidity and colorimetry, in physicochemical stability determination were evaluates as described below:

Moisture content

The moisture parameter is established in legislation, and cereal flours must have moisture content up to 15% (BRASIL, 2022). The moisture content of the flours was standardized at 7±1%, being evaluated over time in each flour, according to section 2.7.

Acidity

Total titratable acidity analysis was performed for determination of acidity, as an indirect parameter for determination of oxidative reactions in physicochemical stability determination, following analytical norms of the Adolf Lutz institute (2008). To carry out the analysis was used a phenolphthalein indicator and 0.1M NaOH solution for titration until 8.3 pH. The phenolphthalein turns were verified with a benchtop pH meter (PG1800; Gehaka, São Paulo, Brazil). The experiment was carried out in duplicate and results were expressed by mEq NaOH/100 g.

Colorimetry

Instrumental colors of samples were determined by direct reading assessed through a digital colorimeter Minolta Chrama CR400 (Konica Minolta, New Jersey, USA), to evaluate color variation (Annex B), as an indirect parameter of oxidative reactions. The instrument was calibrated with a standard white plate. Each flour (10 g) was weighed in a Petri plate and the parameters L* (lightness), a* (parameter from green to red) and b* (parameter from blue to yellow) were measured. Also, the chromaticity (C*) was calculated using values of a* and b*, as Equation 1 below:

$C^* = [(a^*)^2 + (b^*)^2]^{1/2}$ (Equation 1)

2.9.1 Modeling of physicochemical stability

Experimental data on moisture content, acidity and colorimetry parameters (analyzed once a month for 7 months, time: 0 to 6) were subsequently used for mathematical modeling, applied for physicochemical stability.

According to method described by Borguini *et al.* (2020), the mathematical models were derived from the fundamental equation of the deterioration kinetics (Equation 2) resulting in 4 models (n=0, n=1, n=2, and quadratic), following equations 3 to 6, respectively, as below:

$\partial C/\partial t = \pm kC^n$ Equation (2)

$C=C_0-k(t)$ Equation (3)

$C=C_o.\ e^{k.(t)}$ Equation (4)

$C=C_o/[1+C_o.(k).(t)]$ Equation (5)

$C=Co+a.(t)+b.(t)^2$ Equation (6)

Where,
a: concavity of the parabola (positive or negative);
b: direction of growth of the tangent line in (0, Co);
C: attribute of interest (moisture, acidity, or colorimetry parameters);
Co: Intersection with y axis;
k: rate constant;
t: time (0 to 6, within storage period);
n: reaction order;
±: indicates whether the attribute increases or decreases over time, respectively.

The coefficient of determination (R^2) was the indicative for evaluating the ability of each model to adjust to the experimental data, with the highest R^2 between the 4 mathematical models the criterion for choosing, for each sample in each analysis. The chosen mathematical model was applied and compared with the experimental values to determine the physicochemical stability according to the limit: up to 15% moisture content (BRASIL, 2022); or the acidity parameter, considering that there is no specific legislation for acidity limits in millet flour, the value of 3 mEq NaOH/100 g was determined (BRASIL, 1978; BRASIL, 2011), and the colorimetry parameter was used to evaluate a possible change over time.

2.11 STATISTICAL ANALYSIS

Analysis of variance (one-way ANOVA) was developed to determine the statistical differences among samples and, when differences were found a multiple mean Tukey test was carried out at a significant level of $p \leq 0.05$. Multivariate analyses were performed: Principal component analysis (PCA); Hierarchical clustering on principal component (HCPC), and Pearson's correlation. PCA was performed after variable standardization (data normalization) to avoid the influence of different magnitude orders. HCPC was conducted using Euclidean distances and Ward's method, and heatmap. Pearson's correlation was used to analyze the interactions of variables. The statistical analyses were developed by using R free statistical software, version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

3 RESULTS AND DISCUSSIONS

3.1 EXPANSION PROPERTIES AND INSTRUMENTAL TEXTURE

From whole, decorticated and germinated millet grains it was possible to produce flours (14 and 20% corrected moisture content) and obtain different extrudates, represented through its sectional and longitudinal images (Figure 2).

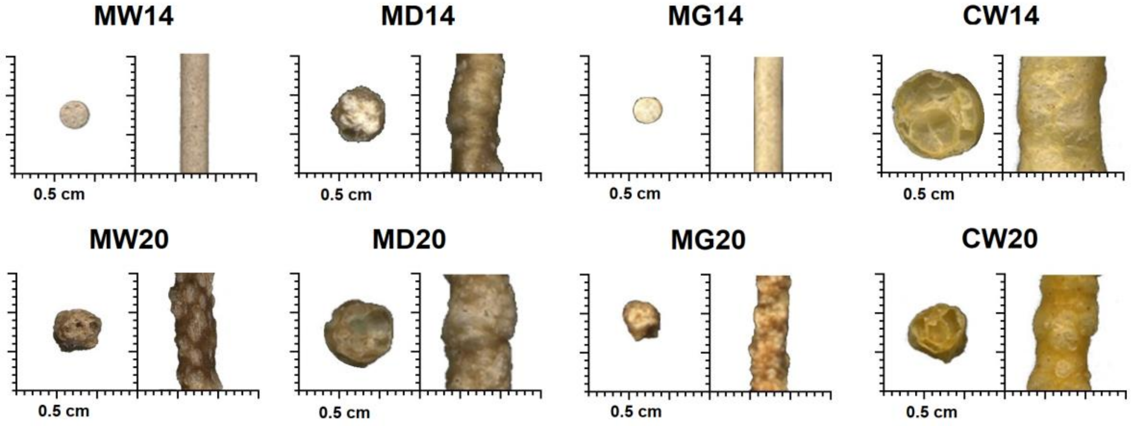


Figure 2. Visual aspect (sectional and longitudinal) of the extrudates, and in codes displayed: MW14 and MW20: extruded whole millet flour at 14 and 20% moisture, respectively; MD14 and MD20: extruded decorticated millet flour at 14 and 20% moisture, respectively; MG14 and MG20: extruded germinated millet flour at 14 and 20% moisture, respectively; CW14 and CW20: extruded whole corn flour at 14 and 20% moisture, respectively.

In expansion indices evaluating (Figure 2 and Table 1), all millet extrudates showed a directly proportional relationship between the moisture content at extrusion process and SEI. Whole, germinated and decorticated extrudates, processed at the lowest moisture content (14%) showed the lowest SEI values (1.33, 1.37 and 4.32, respectively), while those processed at the highest moisture (20%) showed the highest SEI values (3.52, 2.28, 7.17, respectively). This direct relationship is intriguing, as it is maintained in different millet extrudates (whole, decorticated and germinated); due to be unanimously described that an increase in the moisture content at feed, would alter the structure of the amylopectin, reducing elasticity and expansion, and increasing the density of the extrudates (MEHRAJ *et al.*, 2018).

Table 1. Expansion properties and instrumental texture of extrudates processed at 14 and 20% moisture.

Sample	SEI	ρ_e (g/cm^3)	Compression force (N)
MW14	1.33±0.01 $^{B;c}$	1.14±0.01 $^{A;a}$	1.02±0.36 $^{A;b}$
MW20	3.52±0.07 $^{A;c}$	0.50±0.01 $^{B;b}$	0.96±0.54 $^{A;a}$
MD14	4.32±0.05 $^{B;b}$	0.61±0.02 $^{A;c}$	2.26±1.10 $^{A;a}$
MD20	7.17±0.27 $^{A;a}$	0.30±0.01 $^{B;c}$	0.73±0.45 $^{B;a}$
MG14	1.37±0.02 $^{B;c}$	1.08±0.01 $^{A;b}$	1.23±0.37 $^{A;b}$
MG20	2.28±0.12 $^{A;d}$	0.65±0.03 $^{B;a}$	0.72±0.32 $^{B;a}$
CW14	13.58±0.36 $^{A;a}$	0.12±0.01 $^{B;d}$	0.39±0.17 $^{B;c}$
CW20	4.83±0.30 $^{B;b}$	0.46±0.04 $^{A;b}$	1.05±0.54 $^{A;a}$

Codes displayed in the column Sample: MW14 and MW20: extruded whole millet flour at 14 and 20% moisture, respectively; MD14 and MD20: extruded decorticated millet flour at 14 and 20% moisture, respectively; MG14 and MG20: extruded germinated millet flour at 14 and 20% moisture, respectively; CW14 and CW20: extruded whole corn flour at 14 and 20% moisture, respectively. SEI, sectional expansion index; ρ_e, apparent density. Values with the same capital letters in the same column indicate for the same treatment that percentages of moisture in the extrusion (14 and 20%) are not significantly different ($p \geq 0.05$), according to Tukey test. Values with the same lowercase letters in the same column indicate that different treatments compared in the same percentage of moisture (14 or 20%) are not significantly different ($p \geq 0.05$), according to Tukey test.

The most applicable explanation in the extrusion of millet in lower water content is that: fibers may have retained water in the flour and during extrusion cooking, hindered the generation of steam. Furthermore, fibers tend to be rigid compared to starch-based polymers, which may inhibit expansion (AUSSANASUWANNAKUL *et al.*, 2022); in this case, for lower moisture contents, competition between insoluble fibers and starch for water retention would be evident. As for the processing of whole corn, the corn extrudates showed an inverse relationship (expected) with the highest SEI (13.58) for the low moisture content (14%); and lower SEI (4.83) at higher moisture content (20%) in processing.

Analyzing the different densities and compressive strength (Table 1), at 14% moisture content, samples MW14 and MG14 showed the highest values (1.14 and 1.08 g/cm^3, respectively), while MD14 was characterized by the lowest value (0.61 g/cm^3).

Regarding density, an increase in stiffness of MD14 was noticeable with the highest force required for compression (2.26N), which was approximately 2 times greater than the other millet extrudates for the same moisture content analyzed. The most common extruded parameters in defining the quality of these products by consumers are radial expansion (directly related to sectional expansion) and low compression force, a characteristic of prime importance (PANDISELVAM *et al*., 2019).

It was possible to observe in MD14 (Figure 2) a difference in color between the internal (light, apparently with less alteration) and external (dark) region of this extrudates, these differences in regions may have influenced the greater external rigidity of this extrudates, possibly contributing to lower consumer interest. CW14, on the other hand, had the lowest density values (0.12 g/cm^3) among all extrudates, generating the lowest compression force (0.39 N).

The millet extrudates processed at 20% moisture showed lower apparent density when compared to the 14% extrudates. The MD20 sample also showed the lowest density (0.30 g/cm^3) and compression force (0.73 N) associated with the highest expansion, indicating that partial honing contributed to the texture of the extrudates. Greater expansion for extruded snacks presented larger air cells and thinner walls, generally resulting in better crispness, a sensorially desired property for these products (OLIVEIRA *et al.*, 2021).

3.2 PASTING PROFILE

In the evaluation of pasting properties, shown in Table 2, MW and MD presented the closest values among all raw samples (MW, MD, MG, CW), the highest viscosity values, in addition to close pasting properties than MS. The comparison between MG and MS allowed to identify a great change in the viscosity profile for MG, presenting the lowest values of PT, BD, mV, FV, PV and SB, being evident that the germination modified the starch and generated values below 150 cP.

Azeez *et al.* (2022) justified the pasting viscosity reduction, due to starch degradation, mainly due to the high activity of α-amylase in germination. Extruded samples, in general, showed that low pasting viscosities were obtained in all millet extruded processed at 20% moisture, as well as, in MD14 and corn extrudates, indicating that the extrusion processing in mainly in corn and decorticated samples, caused the highest modifications in the starch. In addition, it can be seen that all extruded samples had changes in their viscosity profile, to different degrees (greater and lesser intensity), and can be called pre-cooked flours.

High values in the pasting profile were found in samples MW14 and MG14, indicating less severity in the processing, due to the high viscosity presented through the highest values in BD, mV, FV, PV and SB in the extruded samples, even after thermal processing. It is important to highlight, in relation to extruded samples, that MW14 presented a small reduction in relation to its control sample (MW), while MG14 presented a great increase in viscosity properties after extrusion, in relation to MG.

A possible hypothesis for the increase in viscosity is that from the germinated raw flour (which had starch degradation due to germination), and during the extrusion to form the MG14 sample, the amylose may have undergone changes, in order to reorganize the structure of the hydrolyzed starch molecules, resulting in an increase in the viscosity of the pasting formed after extrusion at 14% moisture content.

Similar behavior of increased viscosity after extrusion was observed and mentioned by Comettant-Rabanal *et al.* (2021), that possibly, through the increase of the rigidity of the starch granule, generated an increase of the crystalline region, also increasing the interaction between the amorphous and crystalline regions, resulting in an increase of the PV in relation to the raw samples, due to the resistance to heat and shear, present in this modified starch.

Table 2. Pasting properties of raw and extruded millet and corn flours, and millet starch.

Sample	PT (°C)	PV (cP)	mV (cP)	FV (cP)	BD (cP)	SB (cP)
MW	83.00±0.57	923.00±4.24	482.00±2.83	2151.50±10.61	441.00±1.41	1669.50±7.78
MW14	85.60±0.00 $^{A;b}$	742.50±3.54 $^{A;a}$	498.50±2.12 $^{A;a}$	1817.50±7.78 $^{A;a}$	244.00±1.41 $^{A;a}$	1319.00±5.66 $^{A;a}$
MW20	75.00±0.00 $^{B;b}$	192.00±12.73 $^{B;c}$	118.50±7.78 $^{B;b}$	205.50±10.61 $^{B;d}$	73.50±4.95 $^{B;b}$	87.00±2.83 $^{B;d}$
MD	86.20±0.00	1143.00±2.83	611.00±1.41	2704.50±4.95	532.00±4.24	2093.50±6.36
MD14	25.15±0.21 $^{A;c}$	290.50±4.95 $^{A;d}$	165.50±6.37 $^{A;c}$	324.50±12.02 $^{A;c}$	125.00±1.41 $^{A;b}$	159.00±18.39 $^{A;c}$
MD20	24.95±0.07 $^{A;c}$	152.50±3.54 $^{B;d}$	103.00±2.83 $^{B;b}$	283.50±2.12 $^{B;c}$	49.50±0.71 $^{B;c}$	180.50±4.95 $^{A;c}$
MG	53.33±0.60	141.00±2.83	30.00±1.41	39.00±1.41	111.00±1.41	9.00±2.83
MG14	88.00±0.50 $^{A;a}$	472.50±0.71 $^{A;b}$	357.00±4.24 $^{A;b}$	991.50±4.95 $^{A;b}$	115.50±3.54 $^{A;b}$	634.50±9.19 $^{A;b}$
MG20	91.00±1.98 $^{A;a}$	264.50±9.19 $^{B;b}$	220.50±7.78 $^{B;a}$	504.50±12.0 2 $^{B;a}$	44.00±1.41 $^{B;c}$	284.00±4.24 $^{B;a}$
CW	83.00±0.57	608.50±9.19	587.00±0.00	2327.00±5.66	21.50±9.19	1740.00±5.66
CW14	25.00±0.00 $^{A;c}$	414.50±6.36 $^{A;c}$	170.50±2.12 $^{B;c}$	277.00±5.66 $^{B;d}$	244.00±8.49 $^{A;a}$	106.50±3.54 $^{B;d}$
CW20	25.00±0.00 $^{A;c}$	377.50±4.95 $^{B;a}$	209.50±9.19 $^{A;a}$	417.50±4.95 $^{A;b}$	168.00±4.24 $^{B;a}$	208.00±4.24 $^{A;b}$
MS	83.40±0.00	2724.50±31.82	1427.50±21.92	4316.00±506.29	1297.00±53.74	2888.50±528.21

Codes displayed in the column Sample: MW, whole millet flour; MW14 and MW20: extruded whole millet flour at 14 and 20% moisture, respectively; MD, decorticated millet flour; MD14 and MD20: extruded decorticated millet flour at 14 and 20% moisture, respectively; MG, germinated millet flour; MG14 and MG20: extruded germinated millet flour at 14 and 20% moisture, respectively; CW, whole corn; CW14 and CW20: extruded whole corn flour at 14 and 20% moisture, respectively; MS, Millet starch. Pasting profile: PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity; BD = PV-mV, breakdown viscosity; SB = FV-mV, setback viscosity. Values with the same capital letters in the same column indicate for the same treatment that percentages of moisture in the extrusion (14 and 20%) are not significantly different ($p \geq 0.05$), according to Tukey test. Values with the same lowercase letters in the same column indicate that different treatments compared in the same percentage of moisture (14 or 20%) are not significantly different ($p \geq 0.05$), according to Tukey test.

3.3 PROXIMATE COMPOSITION, TOTAL AND RESISTANT STARCH

Proximate composition of the extrudates is shown, on a dry basis, in Table 3. Analyzing the protein content in the extruded whole millet (MW14 and MW20) and germinated (MG14 and MG20), these did not show significant difference in each treatment, with values close to 10 g/100 g of protein; indicating that the germination did not generate difference between the control samples and also between the extruded ones, in addition, the moisture content of 14 and 20% in the processing also did not influence the reduction of the protein content for these samples.

Protein content among MD14 and MD20 showed statistical differences (8.81 and 9.21 g/100 g, respectively). Only MD14 reduced the protein content in relation to control sample (MD), however, compared to MW, decorticated extrudates had up to 14% protein content reduction. While the lowest protein values (approximately 8 g/100 g) were obtained in corn extrudates, CW14 and CW20.

Evaluating lipid content (Table 3) in samples processes at 14% moisture, only MW14 (5.76 g/100 g) did not differ ($p \geq 0.05$) from its control sample MW (6.16 g/100 g), in addition, MW14 showed no difference in relation to MG14 (4.87 g/100), while MD14 (2.52 g/100 g) had the highest reduction in the lipid content compared to the control sample (MD, containing 5.35 g/100 g lipid).

Evaluating the extrudates at 20% moisture, the greatest reduction of 59.4% in lipid content was observed in the MG20 (2.46 g/ 100 g) in relation to its control sample MG (5.35 g/ 100 g). CW14 and CW20 also showed low levels of lipids (2.32 and 2.07 g/100 g, respectively) and high reductions, when compared to CW (4.78 g/100 g).

The fiber content of the MW, MD, and MG controls (10.92, 7.73, 12.34 g/100 g, respectively) were lower than CW (15.04 g/100 g). Decortication in MD and germination in MG caused changes in the whole millet grain; generating a reduction of 33% and an increase of 13% in fiber content, respectively, compared to MW. Azeez *et al*. (2022) also identified an increase of 10.10% in the fiber content after germination compared to control, and this increase is associated with the formation of new primary cells during this bioprocessing.

Changes in the decorticated sample (MD), consequently caused an increase in the carbohydrate and total starch content, in addition, lower resistant starch values. These modifications caused greater expansion when compared to other millet extrudates (Figure 2, MD14 and MD20).

In germinated samples, the increase in fiber content in MG compared to MW (Table 3) produced the extrudates with the highest content of resistant starch, and less expanded millet extrudates (Figure 2, MG14 and MG20), indicating that mainly the decrease in carbohydrate content associated with greater values of other macronutrients contribute to smaller expansion.

Table 3. Proximate composition, total and resistant starch of raw and extruded millet and corn flours, and millet starch in dry basis (g/100 g).

Sample	Ash	Protein	Lipid	Fiber	Carbohydrate	Total starch	Resistant starch
MW	1.62±0.01	10.25±0.02	6.16±0.10	10.92±0.02	71.05±0.17	63.61±0.95	7.31±0.37
MW14	1.59±0.02 $^{A;a}$	9.92±0.13 $^{A;a}$	5.76±0.09 $^{A;a}$	5.58±0.00 $^{B;b}$	77.15±0.25 $^{A;b}$	63.33±0.65 $^{A;c}$	0.42±0.02 $^{A;b}$
MW20	1.43±0.08 $^{A;a}$	9.83±0.14 $^{A;a}$	3.98±0.17 $^{B;a}$	6.59±0.00 $^{A;b}$	78.17±0.39 $^{A;c}$	58.92±3.76 $^{A;b}$	0.24±0.03 $^{B;b}$
MD	1.07±0.02	9.27±0.09	5.35±0.20	7.32±0.00	76.99±0.30	75.34±1.12	0.93±0.01
MD14	1.13±0.08 $^{A;c}$	8.81±0.09 $^{B;b}$	2.52±0.04 $^{A;b}$	4.95±0.0 $^{B;c}$ 0	82.59±0.18 $^{B;a}$	75.03±0.74 $^{A;a}$	0.28±0.03 $^{A;b}$
MD20	1.18±0.01 $^{A;c}$	9.21±0.00 $^{A;b}$	2.46±0.12 $^{A;b}$	5.14±0.00 $^{A;c}$	82.01±0.16 $^{A;b}$	75.05±2.48 $^{A;a}$	0.15±0.03 $^{B;b}$
MG	1.69±0.05	9.94±0.13	5.62±0.02	12.34±0.05	70.41±0.20	62.52±2.86	7.75±0.04
MG14	1.34±0.06 $^{A;b}$	10.30±0.26 $^{A;a}$	4.87±0.01 $^{A;a}$	7.11±0.00 $^{B;a}$	76.38±0.33 $^{B;b}$	65.44±1.13 $^{A;bc}$	0.89±0.09 $^{A;a}$
MG20	1.34±0.04 $^{A;ab}$	10.06±0.09 $^{A;a}$	2.28±0.2 $^{B;b}$	7.33±0.01 $^{A;a}$	78.99±0.33 $^{A;c}$	63.43±1.53 $^{A;ab}$	0.77±0.01 $^{A;a}$
CW	1.22±0.07	8.59±0.22	4.78±0.00	15.04±0.01	70.37±0.29	69.13±2.36	2.00±0.01
CW14	1.19±0.01 $^{A;c}$	7.93±0.05 $^{A;c}$	2.32±0.4 $^{A;b}$ 6	4.90±0.00 $^{A;d}$	83.66±0.53 $^{B;a}$	68.14±1.10 $^{A;b}$	0.22±0.03 $^{A;b}$
CW20	1.20±0.01 $^{A;bc}$	8.10±0.05 $^{A;c}$	2.07±0.08 $^{A;b}$	4.50±0.00 $^{B;d}$	84.13±0.13 $^{A;a}$	60.54±3.46 $^{A;b}$	0.18±0.04 $^{A;b}$
MS	0.00±0.00	0.00±0.00	0.21±0.01	0.00±0.00	99.79±0.01	92.15±2.77	0.66±0.10

Codes displayed in the column Sample: MW, whole millet flour; MW14 and MW20: extruded whole millet flour at 14 and 20% moisture, respectively; MD, decorticated millet flour; MD14 and MD20: extruded decorticated millet flour at 14 and 20% moisture, respectively; MG, germinated millet flour; MG14 and MG20: extruded germinated millet flour at 14 and 20% moisture, respectively; CW, whole corn; CW14 and CW20: extruded whole corn flour at 14 and 20% moisture, respectively; MS, Millet starch. Values with the same capital letters in the same column indicate for the same treatment that percentages of moisture in the extrusion (14 and 20%) are not significantly different ($p \geq 0.05$), according to Tukey test. Values with the same lowercase letters in the same column indicate that different treatments compared in the same percentage of moisture (14 or 20%) are not significantly different ($p \geq 0.05$), according to Tukey test.

Based on the norms for proteins and fibers attributes (BRASIL, 2020), in 100 g of the food product. On wet basis, the protein values of samples CW14 and CW20 were equal to 7.37 g/100 g; MD14 and MD20, 8.17 and 8.51 g/100 g, respectively; MW14 and MW20, 9.23 and 9.11 g/100 g, respectively; MG14 and MG20, 9.60 and 9.26 g/100 g, respectively; providing 15 to 19% of the 50 g of protein recommended daily. Therefore, all extruded flours can be classified as "source of protein", highlighting the whole and germinated extruded flours with the highest protein content.

On wet basis, the fiber values of samples CW14 and CW20 were equal to 4.57 and 4.12 g/100 g; MD14 and MD20, 4.59 and 4.75 g/100 g, respectively; MW14 and MW20, 5.19 and 6.11 g/100 g, respectively; MG14 and MG20, 6.63 and 6.74 g/100 g, respectively; providing 16 to 27% of the 25 g of fiber recommended daily (BRASIL, 2020). Regarding fiber content, MG14 and MG20 receive the best classification of "increased fiber content", while MW14 and MW20, are classified as a “high fiber content”, lastly, corn and decorticated extruded flours are classified as a “source of fiber”.

3.4 MULTIVARIATE ANALYSIS

The two first PCs explained 83.5% (69.1 plus 14.4%) of the total variability (Figure 3A). The PCA was performed using the samples: MW, MG, MD, CW and MS, and was developed using the physical and chemical responses: proximate composition; total starch; resistance starch and pasting properties.

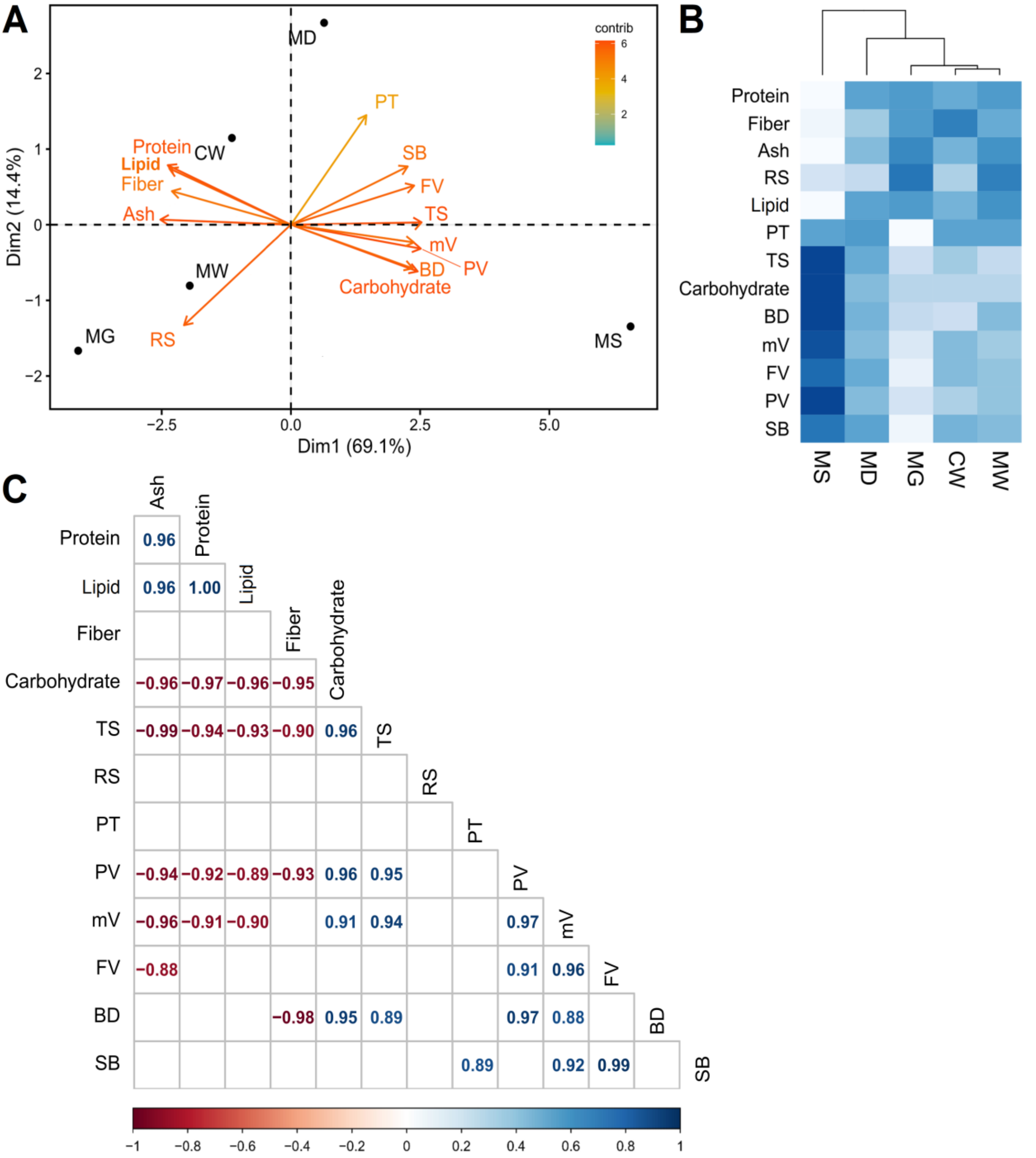
According to Santos *et al*. (2022) the color, size and direction show the influence of the vectors on the samples. Very distant (the highest and the lowest) values in each variable showed large vectors with greater influence on PCA, they were graphically illustrated by orange hue; the intermediate contribution to the PCA is shown by yellow hue vectors, as well as similar values in each parameter had a low contribution and generated small vectors, graphically illustrated by blue color.

The values that characterize the vectors shown in the PCA (Figure 3A) can be better understood when compared to the heatmap grouping samples by hierarchical clustering of principal components (Figure 3B), where the darkest and lightest tones indicate the highest and lowest values, respectively.

In relation to the PCA of the samples, it is possible to mention through the vector sum, that the raw MW and MG flours (Figure 3A, 3rd quadrant) have a strong influence of ash and lipid vectors (Table 3, as they present the highest values close to 1.6 and 6.0 g/ 100 g, respectively) and were mainly influenced by the resistant starch vector (Table 3, with the highest values close to 7.5%).

MD (Figure 3A, 1st quadrant) had more distance from the other millet samples (MW and MG), and due to ash, protein and lipid values (Table 3) close to CW (Figure 3A, 2nd quadrant) was also located closer to this sample, being also influenced by the higher PT value, indicating great resistance in pasting formation (Table 2, 86.2 °C), in addition to the strong influence of the others RVA vector’s, justifying the proximity of MS (Figure 3A, 4th quadrant).

By correlating the physical and chemical properties of all raw samples, using Pearson's correlation (Figure 3C), it was possible to identify that the strongest correlation was between lipid and protein content (r=1.00); where, in scale of strengthens from Pearson’s correlation coefficient, values close to +1 or -1 had very strong positive or negative correlation, respectively (TELES *et a*l., 2019).



	Ash	Protein	Lipid	Fiber	Carbohydrate	TS	RS	PT	PV	mV	FV	BD	SB
Protein	0.96												
Lipid	0.96	1.00											
Fiber													
Carbohydrate	−0.96	−0.97	−0.96	−0.95									
TS	−0.99	−0.94	−0.93	−0.90	0.96								
RS													
PT													
PV	−0.94	−0.92	−0.89	−0.93	0.96	0.95							
mV	−0.96	−0.91	−0.90		0.91	0.94			0.97				
FV	−0.88								0.91	0.96			
BD				−0.98	0.95	0.89			0.97	0.88			
SB								0.89		0.92	0.99		

Figure 3. Multivariate analysis of raw samples (raw flours and millet starch) using physical and chemical responses: proximate composition (ash, protein, lipid, fiber, carbohydrate); total starch; resistant starch, pasting properties (PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity, BD: breakdown and SB: setback); A) Biplot chart of principal component analysis (PCA) of raw samples and physical and chemical properties; B) Heatmap grouping raw samples by Hierarchical clustering of principal components, the darkest color indicates the highest value and light color indicates the lowest value. Codes displayed in figure 3A and 3B: the first letter M and C stand for millet and corn samples, respectively; the second letter W, D, G and S stand for whole, decorticated, germinated and starch, respectively; C) Correlogram for the physical and chemical parameters of raw samples. Red and blue numbers indicate negative and positive correlations, respectively.

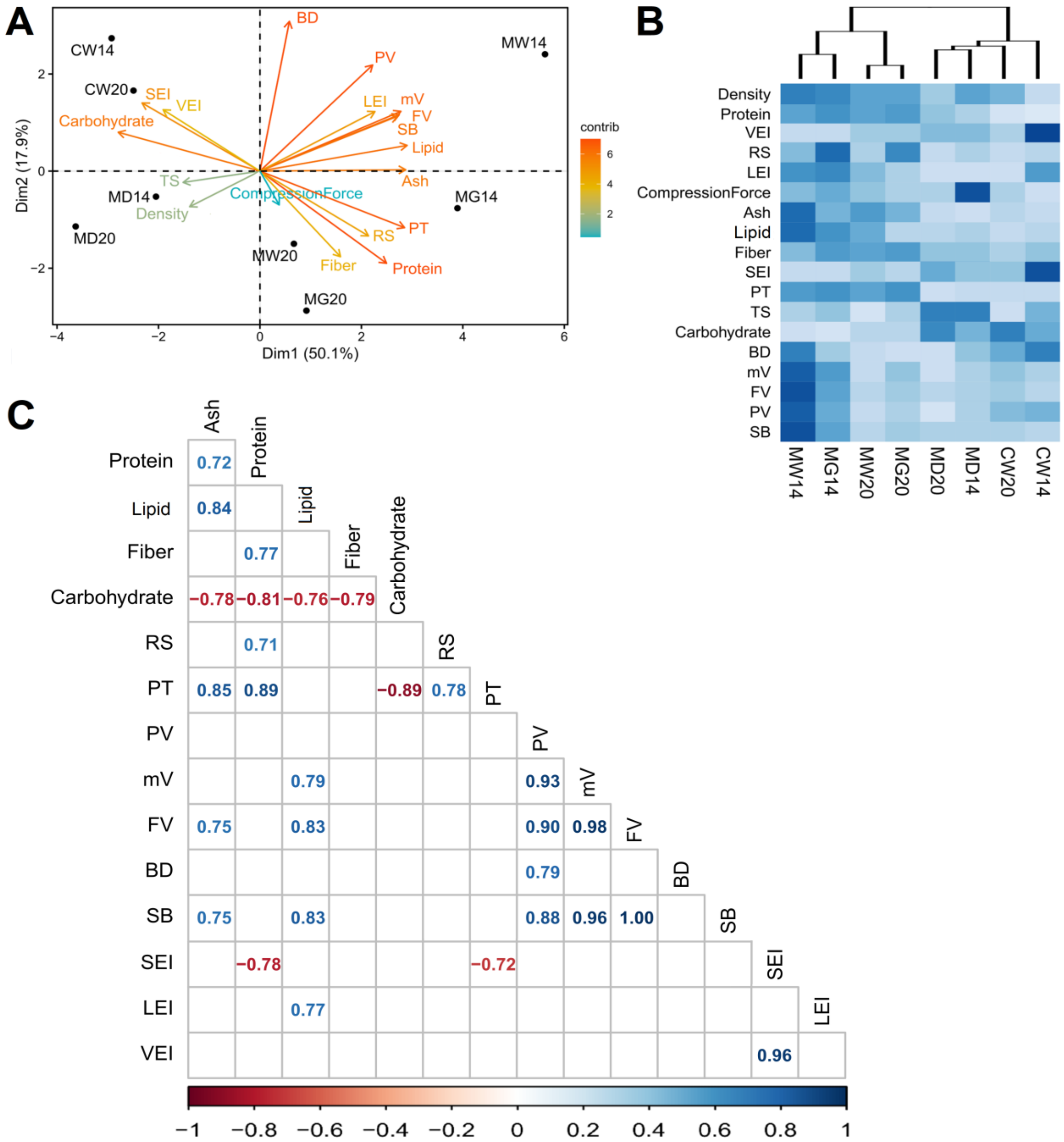
Carbohydrate values were negatively correlated with ash, protein, lipid, and fiber; (r=-0.97 to r=-0.95), indicating an inversely proportional relationship between carbohydrate and other macronutrients, as well as the carbohydrate values were strongly correlated with the total starch content (r=96) and pasting properties (PV, mV, and FV, with r=0.96, 0.91, and 0.95 respectively). This positive correlation (directly proportional) can be explained by the smaller impediment performed by the macronutrients in the starch, also decreasing the formation of complexes between amylose-lipid and amylose- protein, contributing to the reduction of swelling and complete gelatinization (YADAV; DALBHAGAT, MISHRA, 2022).

In the multivariate evaluation between different millet (MW14, MW20, MD14, MD20, MG14, and MG20) and corn (CW14, and CW20) extrudates, performed using different physicochemical parameters (proximate composition, total and resistant starch, pasting and extrusion properties, and compression force), in Figure 4A, the PCA explained 68% (50.1 plus 17.9%) of the total variability. Indicating that some analyzed components, as for example, total starch, compression force, and density, did not have a strong influence on the explanation.

In the analysis of the heatmap of HCPC (Figure 4B) it is possible to interpret, through very close tones, that many physical-chemical values were close, contributing to the smaller differentiation in the PCA. The PCA and HCPC showed that MW14 (Figure 4A, 1st quadrant) and MG14 (Figure 4A, 4th quadrant) were positioned/grouped closer (Figure 4B) because they are characterized by similar vector sums, with emphasis on the intermediate and strong influence of the opposite vectors: total starch and carbohydrate, respectively (Table 3); strong influence of the SEI vector, opposite positioned due to the low sectional expansion values observed in the Heatmap for these samples; as well as a strong influence of the pasting properties vectors (such as for example mV, FV, PV and SB; Table 2) and vectors that contributes nutritionally (resistant starch, protein and fibers, Table 3).

The vectors generated by the pasting property and that contributes nutritionally, which influenced MW14 and MG14, also had a strong influence on the grouping of the MW20 and MG20 samples (Figure 4A, fourth quadrant and Figure 4B). On the other hand, CW14 and CW20 (Figure 4A, 2nd quadrant) were characterized by the greater influence of carbohydrate vectors and expansion properties, generating the most expanded extrudates (Table 1). These vectors also influenced the MD14 and MD20 extrudates (Figure 4A, 3rd quadrant), producing the millet extrudates with greater expansion, however they showed opposite influence of the vectors that represent pasting properties (such as BD, mV, FV, PV and SB) and opposite influence of mentioned vectors that contributes nutritionally.

Just as the PCA had a lower explanation (68%), demonstrated by a greater number of vectors with a low degree of contribution (Figure 3A, blue, green and yellow vectors), the correlogram for the physical and chemical parameters of extruded samples (Figure 3C), also showed a smaller amount of strong correlations. The strongest positive correlation (r=1.00) between SB and FV stands out, as well as a greater number of correlations between the parameters found in the RVA, Similar results were found by Comettant-Rabanal *et al*. (2021), with a close correlation between SB and FV (r=0.99), when analyzing different extruded cereal flours, with the highest number of very high positive correlations (r>0.90) also being found between the variables obtained in analysis in the RVA, making it possible to differentiate these extruded products mainly by the pasting properties.



	Ash	Protein	Lipid	Fiber	Carbohydrate	RS	PT	PV	mV	FV	BD	SB	SEI	LEI
Protein	0.72													
Lipid	0.84													
Fiber		0.77												
Carbohydrate	−0.78	−0.81	−0.76	−0.79										
RS		0.71												
PT	0.85	0.89			−0.89	0.78								
PV														
mV			0.79					0.93						
FV	0.75		0.83					0.90	0.98					
BD								0.79						
SB	0.75		0.83					0.88	0.96	1.00				
SEI		−0.78					−0.72							
LEI			0.77											
VEI													0.96	

Figure 4. Multivariate analysis of extruded samples using physical and chemical responses: proximate composition (ash, protein, lipid, fiber, carbohydrate); total starch; resistant starch; pasting properties (PT, paste temperature; PV, peak viscosity at 95 °C; mV, minimum viscosity after heating; FV, final viscosity, BD: breakdown and SB: setback); expansion properties(SEI, sectional expansion index; Density, apparent density; LEI, longitudinal expansion index; VEI, volumetric expansion index); A) Biplot chart of principal component analysis (PCA) of extruded samples and physical and chemical properties; B) Heatmap grouping extruded samples by Hierarchical clustering of principal components, the darkest color indicates the highest value and light color indicates the lowest value. Codes displayed in figure 3A and 3B: the first letter M and C stand for millet and corn samples, respectively; the second letter W, D and G stand for whole, decorticated, and germinated, respectively; the numbers 14 and 20 stand for extrudates processed at 14 and 20% moisture content, respectively; (C) Correlogram for the physical and chemical parameters of extruded samples. Red and blue numbers indicate negative and positive correlations, respectively.

Based on the analysis of HCPC (Figure 4A and 4B), MW14, MW20, MG14, and MG20 were chosen (as well as, MW and MG as control) in order to promote the evaluation of techno-functional potential and physicochemical stability in millet extruded flours different from corn.

3.5 FARINOGRAPH MEASUREMENT

The techno-functional potential in extruded flours (MW14, MW20, MG14, and MG20) were analyzed, which showed water absorption (WA) levels of 100%, as well as 68% WA in raw flours, used as control (Figure 5A and Table 4).

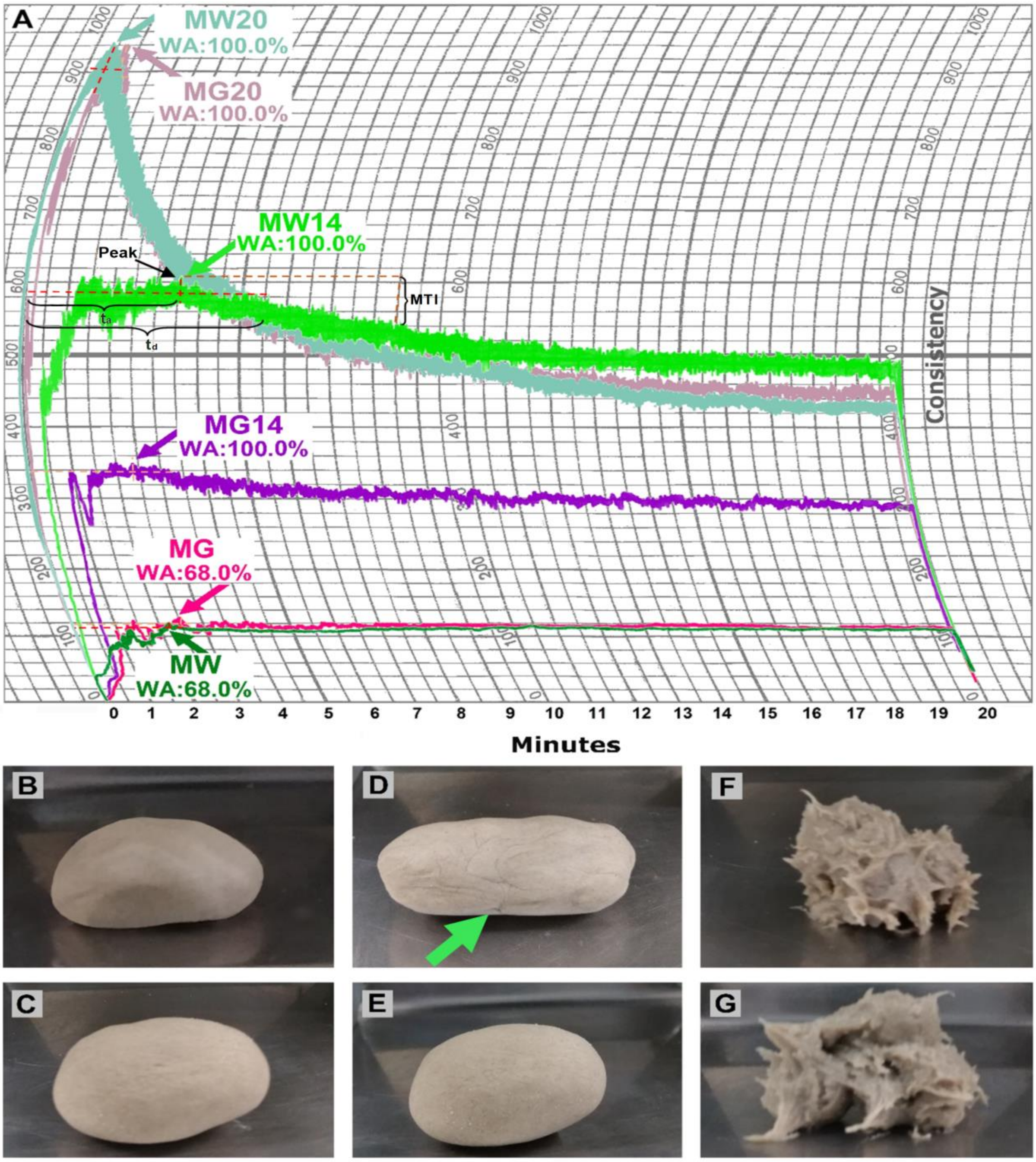


Figure 5. Techno-functional potential of pearl millet flours: (A) Farinograms with empirical water absorption (WA) of samples (MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; MG, germinated grain millet flour; MG14, germinated millet extruded at 14% moisture; and MG20, germinated millet extruded at 20% moisture); and dough obtained after 20 min of farinographic analysis: B) MW, C) MG, D) MW14, E) MG14, F) MW20, and G) MG20.

Table 4. Farinographic properties applied to raw and extruded millet gluten-free flours.

Sample	**Moisture content (%)**	**Empirical farinography**			**Calculated farinography**		**Farinographic properties**				
		Weight flour (g)	**Volume (mL)**	**WA (%)**	**Weight flour (g)**	**Volume (mL)**	**DDT (min)**	**T_a (min)**	**T_d (min)**	**DST (min)**	**MTI (BU)**
MW	7.06±0.04	45.0	30.6	68.0	41.6	28.3	2.7 ± 0.2^{b}	2.2 ± 0.2^{b}	2.4 ± 0.3^{b}	0.3 ± 0.1^{c}	15.0 ± 7.1^{c}
MW14	6.77±0.02	33.0	33.0	100.0	30.4	30.4	4.0 ± 0.3^{a}	3.5 ± 0.3^{a}	5.5 ± 0.4^{a}	2.0 ± 0.1^{a}	70.0 ± 14.1^{b}
MW20	7.44±0.01	33.0	33.0	100.0	30.7	30.7	1.0 ± 0.1^{c}	0.5 ± 0.1^{c}	0.9 ± 0.2^{c}	0.4 ± 0.1^{c}	367.5 ± 10.6^{a}
MG	7.54±0.06	45.0	30.6	68.0	41.9	28.5	2.9 ± 0.2^{b}	2.4 ± 0.1^{b}	2.7 ± 0.3^{b}	0.3 ± 0.1^{c}	17.5 ± 3.5^{c}
MG14	6.69±0.01	33.0	33.0	100.0	30.4	30.4	2.9 ± 0.2^{b}	2.3 ± 0.1^{b}	3.2 ± 0.4^{b}	0.9 ± 0.2^{b}	45.0 ± 10.6^{b}
MG20	6.93±0.03	33.0	33.0	100.0	30.5	30.5	1.1 ± 0.1^{c}	0.6 ± 0.1^{c}	1.0 ± 0.1^{c}	0.4 ± 0.1^{c}	400.0 ± 14.1^{a}

Samples: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; MG, germinated grain millet flour; MG14, germinated millet extruded at 14% moisture; MG20, germinated millet extruded at 20% moisture. Empirical farinography: values empirically established by the authors for carrying out the farinographic analysis of gluten-free flours. Calculated: values calculated for standardization of gluten-free flour at 14% moisture content. WA, water absorption (unique value without variation); T_a, arrival time; T_d, departure time; DST, dough stability time (T_d-T_a); DDT, dough development time (0.5 min after peak); MTI, mixing tolerance index (5 min after peak; BU, Brabender units). Values with different letter, in the same column, indicate statistical difference ($p \leq 0.05$).

The raw samples (MW and MG) showed weak farinographic resistance signals and had short DDT (about 3 min) and MTI (15 and 17, 5 BU, respectively) with linear readings and maximum consistency close to 100 BU, indicating absence of dough consistency and techno-functionality, so they were used as controls compared to the extrusion-treated flours. The visual appearance of the MW and MG doughs is shown in figures 5B and 5C, respectively.

The extrusion process at 14 and 20% in combination with germination caused variable and favorable modifications in the components of whole millet (mainly starch) associated with the increase in the ability of flours to interact with water molecules due to the increase in hydroxyl groups in the fragmented starch molecule, which formed hydrogen bonds causing higher WA, maximum peak of viscosity (500 to 940 BU) of the flours germinated and/or treated by extrusion, the strains showed evident functionality, which was reflected in the profiles and farinographic parameters (Table 4 and Fig. 5A).

WA indicates the ability to associate with water and flour, in limited conditions of water, and in farinography, water absorption is influenced mainly by starch. The WA increase can be associated with the increased presence of hydroxyl groups, a structure that helps to increase water retention by hydrogen bonds (BENKADRI *et al*., 2018). MW14, for example, obtained the highest mass stability expressed by DST of 2 min, and presented the highest DDT of 4 min, resulting in a cohesive mass conformation, evidenced by its mass lines, highlighted in Figure 5D.

While MG14 showed an intermediate DST (0.9 min) between the other extruded samples and raw samples, having the lowest consistency variation, evidenced by more stable mechanical efforts from the beginning to the end of the farinograms, and less difficulty in absorbing water compared to MW14. Bourekoua *et al*. (2016) and Comettant-Rabanal *et al*. (2021) also observed that extruded whole grain rice, corn, and sorghum flours had water absorption capacity similar, however the dough consistency and the farinographic profile were not the same between different extruded flours. On the other hand, MG14 showed similar DDT ($p<0.05$) to raw samples (MW and MG). The mass formed by MG14 appeared to be less cohesive than MW14, as evidenced by the absence of lines (Figure 5E).

Flours extruded at 20% moisture in processing (Table 4 and Figure 5A), MW20 and MG20 did not show statistical differences in all farinographic parameters analyzed, demonstrating the fastest DDT (close to 1 min), highest consistency achieved (close to at 920 BU) and greater consistency variation (Figure 5A) generating doughs with greater viscosity (Figure 5F and 5G, respectively), these being the flours that most differed from the MW and MG control samples. According to Comettant-Rabanal *et al.* (2021) these major changes in flour functionalities may be associated with greater modification of parameters related to paste and composition properties (Table 2 and 3), in addition, through fast DDT they may also reduce cost and time of processing.

3.6 PHYSICOCHEMICAL STABILITY

From the fundamental equation of deterioration kinetics and the experimental data performed (moisture content, acidity and colorimetry) on each sample (MW14, MG14, MW14, MW20, as well as, MW and MG as control) it was possible to choose the model that best explains each experiment. The real-time physicochemical stability test is a procedure that can theoretically be determined in all food products. For dry foods, such as flour and breakfast cereals, the loss of quality during storage is in most cases attributable to oxidation reactions, the physicochemical stability of which is limited by the development of oxidative reactions (CALLIGARIS *et al*., 2016).

In order to validate the physicochemical stability estimates in relation to moisture, acidity and colorimetry, the experimental values corresponding in real-time storage (monitored from 0 to 6 months) were plotted with predicted data by the mathematical modeling (Figure 6). In determining the physicochemical stability, moisture content (Figure 6) showed a small increase over time. The low initial moisture (7±1%) and the packaging used may have contributed to this lower variation and therefore, within the 6 months evaluated, it was well below the limit stipulated by Brazilian legislation; up to 15% moisture content (BRASIL, 2022). Nantanga *et al*. (2008), when evaluating the physicochemical stability of heat-treated millet flours, the moisture contents of all millet flours, despite increasing during the 3 months, were below the maximum recommended moisture limit.

The modeling also showed, through the proposed equations, that within 15 months the moisture content would not be the limiting factor for determining the physicochemical stability. The standardized initial moisture content and the packaging used were effective in controlling the moisture content. Jain and Bal (1997) and Reddy and Viswanath (2019) mention that, the moisture content of flour has a vital role in maintaining its quality and extending its physicochemical stability. In cereals, the high moisture content can compromise the maintenance of physicochemical stability and contribute to spoilage reactions. For the whole millet, a moisture control of 7 to 9% can contribute to better stability. However, even with low moisture content, the physicochemical stability of millet flour is short due to its high content of enzymes, mainly lipoxygenases and lipases, and the high content of lipids, contributing to the occurrence of oxidative reactions.

The acidity was the determinant analysis in the evaluation of the physicochemical stability; the limit being estimated at up to 3 mEq NaOH/100 g; value used as a basis for other gluten-free flours (BRASIL, 1978; BRASIL, 2011). Using the mathematical model, to eliminate possible experimental errors; the raw flours: MW (t=0, 2.55 mEq NaOH/100 g) would have a physicochemical stability of 7 days while MG (t=0, 2.13 mEq NaOH/100 g) up to 13 days. Being evidenced by the necessity of heat treatment to increase the physicochemical stability, thus facilitating the commercialization. Compared to raw whole sorghum flour, acidity value of 1.93 mEq NaOH/100 g was obtained and also compared to a limit of 3 mEq NaOH/100 g (CELIA *et al*., 2022); similar to the acidity found at time zero of the millet flours in this study. The low physicochemical stability can be explained by increases in deesterified fatty acids in the untreated sample caused by lipolysis after milling (NANTANGA, *et al*., 2008). In addition to enzymatic changes, a large number of factors can affect the speed of the oxidation reaction, occurring simultaneously or consecutively. For example, the evolution of the peroxide value over time, through the typical bell-shaped curve, where in the induction period, very low variations in the peroxide value are expected, subsequently there is a progressive increase until reaching a maximum, indicating that oxidative reactions approach the termination step (CALLIGARIS *et al*., 2016).

The samples obtained by extrusion cooking, on the other hand, had greater calculated physicochemical stability of 2 months for MW14 and MG14, and 3 months for MW20 and MG20. Possibly, the thermally processed samples with higher humidity generated the highest specific enthalpy of humid heat in the processing, contributing to a greater stability of acidity and enzymes (NANTANGA *et al*., 2008).

The colorimetry analysis, being the last parameter analyzed, evaluated chromaticity (C*) and lightness (L*). The C* value showed a tendency towards yellow, with the samples being more stable in relation to the MW and MG color; this can possibly be justified due to the fact that raw flour suffers a greater action of lipid oxidation, producing free fatty acids by the action of lipase and its subsequent oxidation contributing to greater acidity (NANTANGA, *et al*., 2008); Extruded Samples possibly suffered greater influence of other reactions, justifying the greater color variation over time in the extruded samples.

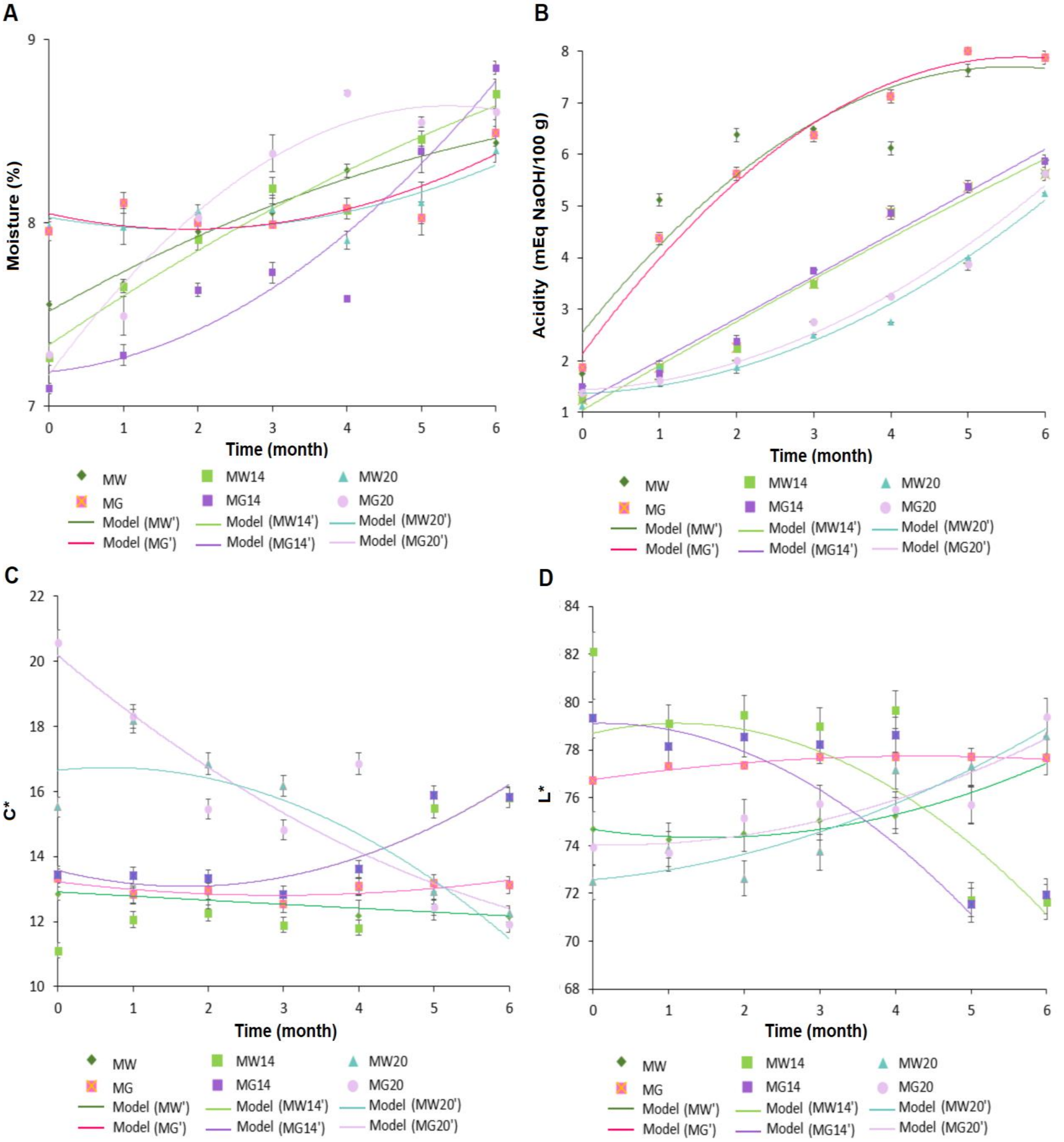


Figure 6. Experimental and predicted data (using mathematical models) in determining physicochemical stability (analyzed for 6 months, including time zero) through (A) moisture content; (B) acidity; and colorimetry parameters (C) C*, chromaticity and (D) L*, lightness; analyses. Samples: MW, raw whole grain millet flour; MW14, whole millet extruded at 14% moisture; MW20, whole millet extruded at 20% moisture; MG, germinated grain millet flour; MG14, germinated millet extruded at 14% moisture; MG20, germinated millet extruded at 20% moisture.

The value of L* in MW14 and MG14 showed a decrease (Figure 6) close to 1 and 2 months, a period that coincides with the end of the calculated physicochemical stability of 2 months, indicating oxidation of other compounds; according to Nantanga *et al*. (2008), assuming that the thermal treatments were effective in the inactivation of enzymes, this variation may be a consequence, for example, of the partial leaching of phenolic compounds, which can sequester radicals necessary for the autoxidation initiation step, contributing to an extension of the physicochemical stability and possibly greater color variation.

It is important to point out that mandatory validity indicators are scarce, mainly related to extruded millet. Therefore, the validity periods of this work were estimated, through physical parameters, being necessary microbiologic and sensory analysis. Calligaris *et al*. (2016) suggest, when not available, the acceptability limit can be determined through quality criteria of the industry itself, previous experiences, literature and competition data. There may be risks of critical overestimation or disadvantageous underestimation of the physicochemical stability. This danger is much more likely in the case of novel foods, for which there is no prior experience.

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4 CONCLUSIONS

It was possible to produce millet extrudates with different characteristics from whole, decorticated and germinated millet flour, in which the partial decortication was effective to obtain extrudates with the highest sectional expansion. This greater expansion in decorticated extrudates was possibly a consequence of processed flour with a higher carbohydrate and total starch content, as well as a lower fiber content, generating a possible lower influence on water retention and difficulty in starch gelatinization. Through the application of multivariate analyses, the extrudates of with the highest nutritional values (protein, fiber and resistant starch) and different pasting properties than corn extrudates, were identified (obtained from the whole grain, MW14 and MW20, as well as germinated millet, MG14 and MG20), allowing the evaluation of techno-functionality and physicochemical stability to these gluten-free extruded flours. The techno-functionality of millet in farinography showed different dough consistency in each treatment, with emphasis on the MW14 sample, which presented greater dough stability. The evaluation of the millet extruded flours physicochemical stability allowed to conclude that the extrusion processing positively influenced the stability of the flour when evaluating the acidity. Monitoring in storage, showed that the extrusion process contributed to an increase of the physicochemical stability up to 3 months in processed flours, contrasting with less than 1-month physicochemical stability for raw flours. It should be noted that other analyzes are needed to determine the physicochemical stability, as sensory and microbiologic analysis for flours, as well as the creation of specific parameters for pearl millet in Brazilian legislation, aiming at the quality of flour and millet-based products, that will be commercialized.

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CONSIDERAÇÕES FINAIS

Neste estudo de produção e obtenção de diferentes farinhas de milheto (híbrido ADRg 9070) cruas e extrudadas a partir do grão integral, decorticado e germinado, destacou-se a obtenção de diferentes farinhas extrudadas/pré-cozidas, possuindo com uma ampla variedade de viscosidade (associada à cada processamento específico) diferindo das amostras cruas, podendo ser potencialmente utilizada em diferentes produtos alimentícios que demandam uma viscosidade específica.

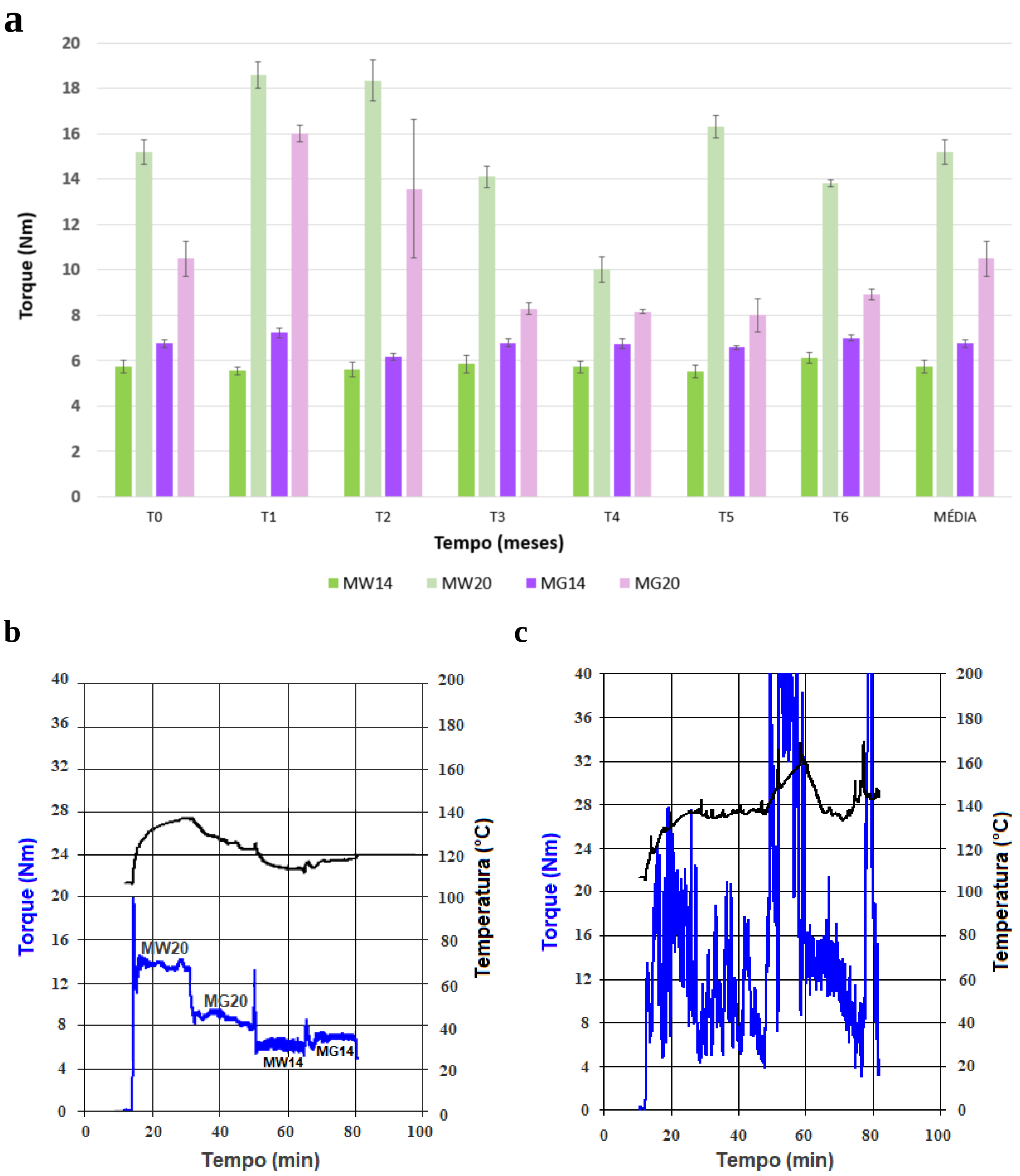
As 7 extrusões utilizando o milheto (Anexo A) permitiram a observação de processamentos estáveis, através da avaliação do torque e das propriedades dos extrudados, demonstram a contribuição deste cereal para obtenção de produtos com um mesmo perfil de qualidade, quando utilizado os mesmos parâmetros no processamento. O processamento por extrusão foi responsável pelo aumento da estabilidade físico-química das farinhas extrudadas de milheto, quando comparadas às respectivas farinhas cruas, estimulando a comercialização das farinhas extrudadas, assim como seus extrudados expandidos, aliando também alto valor nutricional quando comparados aos mesmos produtos de milho integral (apresentando teor até 32% maior de fibra alimentar, 20% maior de proteína e 20% menos digestibilidade de carboidratos).

Foi verificado um comportamento de expansão nos extrudados de milheto, obtidos a partir do grão integral, decorticado e germinado, diferente dos resultados reportados em literatura, tonando necessária a realização de análises complementarem em futuros projetos, como o estudo cromatográfico, em relação as frações de fibras, proteínas, lipídeos, carboidratos, para o melhor entendimento deste cereal no processamento, assim como, a realização de análises complementares na determinação da estabilidade da farinha, como por exemplo análises microbiológicas e sensoriais.

Portanto, novos conhecimentos em relação ao milheto ainda são necessários, como também se torna necessária a aplicação das farinhas extrudadas obtidas, para difundir o milheto na alimentação humana, principalmente dos brasileiros que conhecem pouco seu potencial. E de maneira indireta, espera-se estimular o agronegócio brasileiro, contribuindo na redução da dependência de grãos de cereais importados e por fim, contribuir para o desenvolvimento sustentável, redução da subnutrição, fortalecendo consequentemente, o rico potencial do *Pennisetum glaucum*.

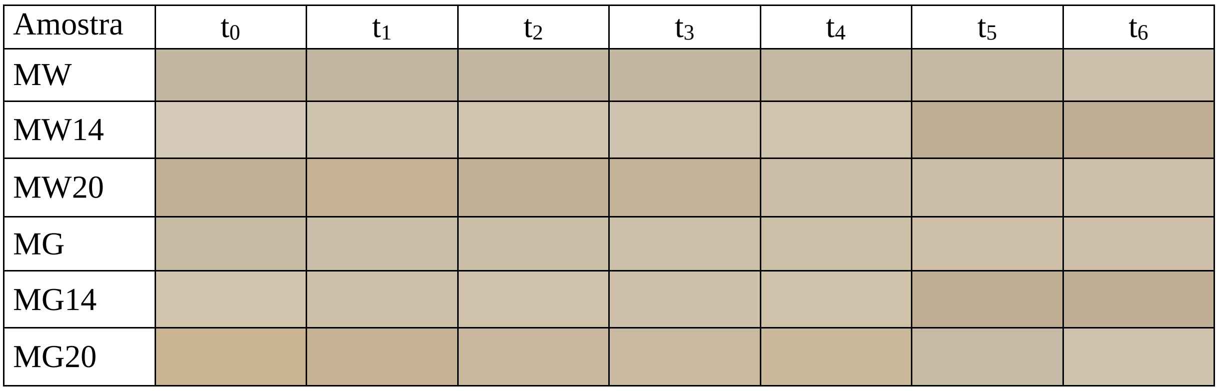
ANEXOS

Anexo A. Controle de torque no processamento por extrusão, utilizando extrusora monorosca (Brabender, Duisburg, Germany).



Anexo A. a) Controle de torque ao longo de 7 processamentos de farinhas de milheto (ADRg 9070) obtidas através da moagem do grão integral e germinado, inseridas na alimentação da extrusora em ordem aleatória; b) Exemplo de estabilidade obtida no processamento do milheto; c) Exemplo de variação de torque em outros cereais, como por exemplo o milho. Amostras: MW14, milheto integral extrudado à 14% de umidade; MW20: milheto integral extrudado à 20% de umidade; MG14, milheto germinado e extrudado à 14% de umidade; MG20: milheto germinado e extrudado à 20% de umidade.

Anexo B. Aspecto visual da interação dos valores de cromaticidade e luminosidade, ao longo de 7 processamentos de diferentes farinhas de milheto (ADRg 9070).



Amostra	t_0	t_1	t_2	t_3	t_4	t_5	t_6
MW	🟫	🟫	🟫	🟫	🟫	🟫	🟫
MW14	🟫	🟫	🟫	🟫	🟫	🟫	🟫
MW20	🟫	🟫	🟫	🟫	🟫	🟫	🟫
MG	🟫	🟫	🟫	🟫	🟫	🟫	🟫
MG14	🟫	🟫	🟫	🟫	🟫	🟫	🟫
MG20	🟫	🟫	🟫	🟫	🟫	🟫	🟫

Farinhas: MW, milheto integral cru; MW14, milheto integral extrudado à 14% de umidade; MW20: milheto integral extrudado à 20% de umidade; MG, milheto germinado; MG14, milheto germinado e extrudado à 14% de umidade; MG20: milheto germinado e extrudado à 20% de umidade. t_0 à t_6: tempo de processamento das farinhas de zero mês até 6 meses de armazenamento, respectivamente. Cores obtidas utilizando um conversor online: http://colormine.org.