UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO - UNIRIO
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE - CCBS
PROGRAMA DE PÓS-GRADUAÇÃO EM ALIMENTOS E NUTRIÇÃO - PPGAN

Lorrany Miranda Marinho

Efeito combinado da germinação, extrusão termoplástica e da panificação sobre as propriedades tecnológicas, químicas e atividade anti-hiperglicemiante *in vitro* de pães sem glúten à base de milheto (*Pennisetum glaucum* (L.) R. Br.)

Rio de Janeiro
2023

Lorrany Miranda Marinho

Efeito combinado da germinação, extrusão termoplástica e da panificação sobre as propriedades tecnológicas, químicas e atividade anti-hiperglicemiante *in vitro* de pães sem glúten à base de milheto (*Pennisetum glaucum* (L.) R. Br.)

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição, da Universidade Federal do Estado do Rio de Janeiro como requisito parcial para obtenção do título de Mestre em Alimentos e Nutrição.

Orientadora: Profª. Drª. Cristina Yoshie Takeiti
Coorientador: Prof. Dr. Carlos Wanderlei Piler de Carvalho

Rio de Janeiro
2023

FICHA CATALOGRÁFICA

Catalogação informatizada pelo(a) autor(a)

M337

Marinho, Lorrany Miranda
Efeito combinado da germinação, extrusão termoplástica e da panificação sobre as propriedades tecnológicas, químicas e atividade anti-hiperglicemiante in vitro de pães sem glúten à base de milheto (Pennisetum glaucum (L.) R. Br.) / Lorrany Miranda Marinho. -- Rio de Janeiro, 2023.
89

Orientadora: Cristina Yoshie Takeiti.
Coorientador: Carlos Wanderlei Piler de Carvalho.
Dissertação (Mestrado) - Universidade Federal do Estado do Rio de Janeiro, Programa de Pós-Graduação em Alimentos e Nutrição, 2023.

1. ciência dos alimentos. 2. pães sem glúten. 3. capacidade antioxidante. I. Yoshie Takeiti, Cristina, orient. II. Wanderlei Piler de Carvalho, Carlos, coorient. III. Título.

Lorrany Miranda Marinho

Efeito combinado da germinação, extrusão termoplástica e da panificação sobre as propriedades tecnológicas, químicas e atividade anti-hiperglicemiante *in vitro* de pães sem glúten à base de milheto (*Pennisetum glaucum* (L.) R. Br.)

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição, da Universidade Federal do Estado do Rio de Janeiro como requisito parcial para obtenção do título de Mestre em Alimentos e Nutrição.

Aprovada em: 30/10/2023

BANCA EXAMINADORA

Prof^a. Dr^a. Cristina Yoshie Takeiti
Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA
Programa de Pós-Graduação em Alimentos e Nutrição – PPGAN
Universidade Federal do Estado do Rio de Janeiro – UNIRIO
Presidente

Prof. Dr. Ricardo Felipe Alves Moreira
Programa de Pós-Graduação em Alimentos e Nutrição – PPGAN
Universidade Federal do Estado do Rio de Janeiro – UNIRIO
Membro interno ao PPGAN

Profª. Drª. Vanessa Dias Capriles
Programa de Pós-Graduação em Alimentos, Nutrição e Saúde
Universidade Federal de São Paulo – UNIFESP
Membro externo ao PPGAN

ASTEN ASSINATURA

Datas e horários baseados em Brasília, Brasil
Sincronizado com o NTP.br e Observatório Nacional (ON) em 30/10/2023 às 16:55:02

Ata_Defesa_Dissertação_Nº.87_Lorrany Miranda Marinho

Data e Hora de Criação: 30/10/2023 às 12:31:03

Documentos que originaram esse envelope:
- Ata_Defesa_Dissertac¸a~o_Nº.87_Lorrany Miranda Marinho_Aprovada.docx (Documento Microsoft Word) - 1 página(s)

Hashs únicas referente à esse envelope de documentos

[SHA256]: 8553cf9658ce3d11dc67249a535938361e36a1f3668fbeb879ba164f24852d3b
[SHA512]: 5b20cb04d425917e1df0faed2d65398f1395353d7d9dd86a7c76e56d4f61984194d250d9b10fefe6a7e6fb03d3964e629e54330a99cb0235851a37c7fdc48846

Lista de assinaturas solicitadas e associadas à esse envelope

ASSINADO - Cristina Yoshie Takeiti (cristina.takeiti@embrapa.br)
Data/Hora: 30/10/2023 - 12:48:12, IP: 191.32.155.133
[SHA256]: d0c4c8375f494b3e6f2156d7a79182b4ed684a8430de37f3455a819de038e491

ASSINADO - Ricardo Felipe Alves Moreira (ricardo.moreira@unirio.br)
Data/Hora: 30/10/2023 - 16:55:02, IP: 200.156.27.216
[SHA256]: e8d6181cfb38784bf212c4276476a9b07c69f3782f8cf1ebffd8e548701ce7fc

Ricardo Felipe Alves Moreira

ASSINADO - Vanessa Dias Capriles (vanessa.capriles@unifesp.br)
Data/Hora: 30/10/2023 - 14:16:11, IP: 201.69.93.74
[SHA256]: d6780fc954f48c770fdca45b99ffcd6c1617e017c494e2a8dfb465d4b0f870c8

Histórico de eventos registrados neste envelope

30/10/2023 16:55:02 - Envelope finalizado por ricardo.moreira@unirio.br, IP 200.156.27.216
30/10/2023 16:55:02 - Assinatura realizada por ricardo.moreira@unirio.br, IP 200.156.27.216
30/10/2023 16:54:23 - Envelope visualizado por ricardo.moreira@unirio.br, IP 200.156.27.216
30/10/2023 14:16:11 - Assinatura realizada por vanessa.capriles@unifesp.br, IP 201.69.93.74
30/10/2023 12:48:12 - Assinatura realizada por cristina.takeiti@embrapa.br, IP 191.32.155.133
30/10/2023 12:36:22 - Envelope registrado na Blockchain por ppgan.secretaria@unirio.br, IP 177.192.32.235
30/10/2023 12:36:21 - Envelope encaminhado para assinaturas por ppgan.secretaria@unirio.br, IP 177.192.32.235
30/10/2023 12:31:07 - Envelope criado por ppgan.secretaria@unirio.br, IP 177.192.32.235

AGRADECIMENTOS

Agradeço primeiramente a Deus, por ter me concedido saúde para desempenhar esse trabalho junto a essa equipe tão talentosa.

Agradeço de uma forma muito especial e com verdadeiro carinho à minha orientadora Profª. Drª. Cristina Takeiti. Nossa, é até difícil enumerar os motivos pelo qual sou grata. Seu coração grandioso, sua sensibilidade e principalmente sua humanidade são admiráveis. Inicio agradecendo pela pessoa que você é, pois eu verdadeiramente me senti abraçada desde o início de tudo. Sempre acreditei que a vida nos presenteia com anjos, e sem dúvida você foi um deles durante essa minha jornada acadêmica. Ao longo do tempo eu só pude admirar mais. Você é uma profissional e mestre admirável. Meu aprendizado foi sem igual, principalmente pela interdisciplinaridade que em todos os momentos trouxe para nossos debates. Obrigada por sempre ter segurado a minha mão (bem forte, rs). Parabéns por tudo que você é!!

Agradeço à minha família, que são fortaleza, amor e muita parceria. Sempre me encorajaram a conquistar meus sonhos e voar o mais longe que eu quisesse e pudesse (mesmo que fosse pra tão longe e sem comida paraense, rs). Eu sempre fui mais forte por que nós temos uns aos outros. Não há amor maior nessa vida do que esse (Pai, mãe, manos, cunhados e meus maiores presentes: Davi, Lucas e Luan).

Ao Jefferson, que por inúmeras vezes, do seu jeitinho, me trouxe conforto e bons momentos pra amenizar dias difíceis, escutou muito choro pelo cansaço da dupla jornada, e por se alegrar com as minhas pequenas conquistas (leia-se: testes que davam certo). Sem dúvida você muito contribuiu para que eu pudesse ter chegado até aqui. Obrigada!

Minha eterna gratidão à Monique Barreto, primeiro pela sua inteligência e em segundo lugar por dividir muito dela com todos nós (rsrs). Obrigada pela dedicação em tudo que faz e principalmente pela enorme contribuição e colaboração ao longo desse período que estivemos juntas.

À Maria Eugênia, pela ajuda, pela troca, pelo conhecimento compartilhado (que não foi pouco) e também pelas boas risadas que me proporcionou. Você é muito especial!

Meu muito obrigada ao Prof. Dr. Carlos Wanderlei Piler, por todo auxílio e contribuição com o projeto. À equipe da Embrapa, em especial à equipe da Planta IV, Neuri (o que seria de mim sem suas caronas e nossos bate-papos pra passar o tempo?!), Mari e Adriana por toda troca e auxílio durante as análises.

Não posso deixar de agradecer a toda rede de apoio: minhas queridas amigas de longa data, amigos do trabalho, inclusive minha chefinha que tanto me ajudou com trocas de plantão muitas vezes emergenciais. À Tia Simone e Tia Cristina, vocês são um presente lindo! Muitíssimo obrigada por tudo, principalmente nessa reta final. Concluir essa etapa com todo esse suporte fez toda a diferença. Obrigada!!!

“Eu sou parte de uma equipe.

Então, quando eu venço, não sou eu apenas quem vence.

De certa forma termino o trabalho de um grupo enorme de pessoas!”

Ayrton Senna

RESUMO

O pão é um alimento que possui alta aceitação e um dos mais importantes da história. No entanto, pode apresentar alto valor energético e índice glicêmico, além de baixo conteúdo nutricional. O atual cenário demonstra uma demanda crescente por produtos sem glúten, principalmente por parte de indivíduos adeptos de hábitos saudáveis e intolerantes ao glúten. Nesse contexto, o milheto pérola (*Pennisetum glaucum*) apresenta-se como uma matéria prima alternativa com alto valor nutricional em termos do perfil de amido e de compostos fenólicos. Neste trabalho avaliou-se o efeito do processo combinado de extrusão, da germinação e de dois diferentes processos de panificação (massa direta e massa esponja) sobre as propriedades tecnológicas, químicas e atividade anti-hiperglicemiante *in vitro* de pães isentos de glúten elaborados exclusivamente com milheto. As amostras de pães de milheto apresentaram valor superior de fibra (10,83g/100g) em relação às amostras controle à base de farinha de trigo (9,94g/100g e 5,44g/100g) e significativo aumento quando comparados aos dois métodos de panificação (6,89 g/100g: método massa direta – 8,83g/100g: método massa esponja). Os teores de fenólicos foram determinados por Folin-Ciocalteu (TPC) e a capacidade antioxidante pelos métodos de FRAP e DPPH nos extratos livres e ligados. O TPC variou de 287,3 a 4131,8 mg GAE/100g. Os valores dos extratos ligados (2572,93 mg GAE/100g) de pães de milheto elaborados por massa esponja a partir de uma farinha mista composta por farinha crua, extrudada e germinada de milheto (FMCEG) foram superiores aos extratos livres seja pelo método da massa direta (1134,73 mg GAE/100g) como pela massa esponja (1558,94 mg GAE/100g). A amostra FMCEG pelo método direto também apresentou maior capacidade antioxidante por DPPH, enquanto a amostra obtida pela farinha crua e extrudada (FMCE) mostrou maior capacidade pelo método de FRAP. A atividade anti-hiperglicêmica dos pães de milheto foi demonstrada pela concentração onde foi possível inibir pelo menos 50% da enzima α-amilase (IC_{50}). A amostra FMCE elaborada por massa esponja apresentou a menor concentração necessária para inibir a enzima (1,36 mg/mL), ficando abaixo apenas do extrato livre de pão de trigo (controle: 1,27 mg/mL). Conclui-se que a apesar dos pães isentos de glúten não demonstrarem semelhança com pães de trigo quanto ao volume específico, foram nutricionalmente mais adequados do que pães comerciais demonstrando que a combinação dos processos analisados foi benéfica para o desenvolvimento de pães isentos de glúten.

Palavras-chaves: pães sem glúten, capacidade antioxidante, capacidade anti-hiperglicêmica

ABSTRACT

Bread is a food product that has high acceptance, representing one of the most important foods in history. However, depending on formulation it has a high-energy value and glycemic index, as well as low nutritional content. The current scenario demonstrates a growing demand for gluten-free products, especially by individuals that follow healthy habits and/or are gluten intolerant. In this context, pearl millet (*Pennisetum glaucum*) presents as an alternative raw material with high nutritional value. In this work, the effect of the combined process of extrusion, germination and two different baking processes (straight-dough and sponge-dough) on the technological, chemical and anti-hyperglycemic properties *in vitro* of gluten-free millet-based breads was evaluated. The millet bread samples showed a higher fiber value (10.83g/100g) compared to the control samples (9.94g/100g e 5.44g/100g), and a significant increase if compararred the two breadmaking methods. Phenolic contents were determined by Folin-Ciocalteu (TPC) and antioxidant capacity by FRAP and DPPH methods in both free and bound extracts. The TPC ranged from 287.3 to 4131.8 mg GAE/100g. The values of the bound extracts (2572,93 mg GAE/100g) of millet bread prepared using a mixture of raw, germinated and extruded millet flour (RGEMF) by sponge-dough were higher than the free extracts both by the straight-dough (1134.73 mg GAE/100g) and by sponge-dough method (1558.94 mg GAE/100g). The REGMF sample by straight dough showed greater antioxidant capacity by DPPH (3.69 mg/g), meanwhile the raw and extruded millet flour (REMF) sample was higher by FRAP (2133.0 µmole/g). The anti-hyperglycemic activity of millet bread was demonstrated by the concentration in which it was possible to inhibit at least 50% of the α-amylase enzyme (IC_{50}). The REMF sponge-dough sample presented the lowest concentration necessary to inhibit the enzyme (1,36 mg/mL), below solely to the free extract of wheat bread (control, 1,27 mg/mL). It was concluded that although gluten-free breads do not demonstrate similarity to wheat breads in terms of specific volume, this product were nutritionally more adequate than commercial breads, demonstrating that the combination of the processes was beneficial for the development of gluten-free breads.

Keywords: gluten-free breads, antioxidant capacity, antihyperglycemic capacity

Listra de Figuras

Lista de Tabelas

Lista de abreviaturas e siglas

AACC	American Associates of Cereal Chemist
AAE	Ascorbic Acid Equivalent
ABTS	2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
ADHD	Attention deficit hyperactivity disorder
CAGR	Compound annual growth rate
CD	Celiac disease
CF	Carbohydrate-to-fiber ratio
CP	Centipoise
CQI	Carbohydrate quality index
DNA	Deoxyribonucleic acid
DNS	3,5-Dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAO	Food and Agriculture Organization
FRAP	*Ferric Reducing Antioxidant Power*
GAE	Gallic acid equivalents
GF	Gluten-free
GI	Glycemic index
HCA	Hierarchical Clustering Analysis
HPMC	Hydroxy Propyl Methyl Cellulose
IgE	Imunoglobulina E
IDF	International Diabetes Federation
K-AMYL	Amylose Amylopectin Assay Kit Test
K-RSTAR	Resistant Starch Assay Kit
K-TSTA	Total Starch Assay Kit
LAB	*Lactobacillus*
NCDs	Chronic non-communicable diseases
OPAS	Organização Pan-Americana da Saúde
PCA	Principal Components Analysis
PSD	Particle size distribution of flours
RC	Radar Charts

ROS	Reactive oxygen species
TPA	Texture profile analysis
TPC	Total phenolic content
TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
WF	Wheat flour
WHO	World Health Organization

SUMÁRIO

PREFÁCIO

O presente trabalho segue as normas da dissertação no formato de artigo definido pelo Programa de Pós-Graduação em Alimentos e Nutrição (PPGAN) em 14 de maio de 2019. Portanto, essa dissertação está dividida em dois capítulos:

O primeiro capítulo consiste de um artigo de revisão bibliográfica que visa enfatizar o potencial tecnológico e nutricional do milheto, assim como técnicas de processamento favoráveis a biodisponibilidade e melhoramento das características físicas em pães sem glúten. O artigo é intitulado "Gluten-free millet-based bread: nutritional, health benefits and processing" submetido para publicação à revista "*Journal of Food Science and Technology"*.

O segundo capítulo apresenta o artigo original submetido para publicação à revista "*Food Research International*". O artigo é intitulado "Role of baking process on the physical properties and nutritional characteristics of pearl millet-based breads". Neste artigo, os pães elaborados em combinações binárias e ternárias de farinha de milheto (*Pennisetum glaucum* (L.) R. BR.) foram analisados quanto ao efeito combinado da extrusão, da germinação e de dois diferentes tipos de panificação sobre as propriedades físicas, químicas e nutricionais como atividade antioxidante e atividade anti-hiperglicemiante in vitro de pães à base de milheto e livres de glúten.

1 INTRODUÇÃO

O milheto é considerado um dos alimentos básicos da Ásia Central e Oriental, Europa, China, Índia e certas partes da África. Devido à sua capacidade de resistir a condições de estresse com abastecimento limitado de água e temperaturas elevadas pode ser uma cultura promissora para alcançar segurança alimentar e nutricional nessas regiões (Selladurai et al., 2023). O *Pennisetum glaucum* (L.) R. Br. é a espécie mais produzida mundialmente (Taylor, 2016).

Grãos de milheto apresentam alto potencial nutricional com teores de fibras, minerais, proteínas e compostos bioativos semelhantes ou até superiores aos encontrados em grãos tradicionais como arroz e milho (Dias-Martins et al., 2018). Além disso, o milheto vem ganhando destaque pelos seus aspectos benéficos à saúde devido as suas propriedades antioxidantes, capacidade hipoglicêmica (a partir da sua atividade inibição da enzima α-amilase) e por ser um alimento alternativo para celíacos e indivíduos sensíveis ao glúten (Hassan et al., 2021).

Nas últimas duas décadas o mercado global para consumo de alimentos isentos de glúten tem crescido significativamente (Woomer & Adedeji, 2020). Notavelmente, a indústria de panificação é o setor mais afetado com a exclusão do glúten, uma vez que a rede viscoelástica formada pelo glúten influencia no desenvolvimento de pães de maior volume, devido a retenção dos gases produzidos no processo de fermentação e no controle de umidade, após o processo de cozimento. No entanto, o amido também pode ser fator contribuinte para qualidade tecnológica de pães (Cappelli et al., 2020).

A maioria das estratégias relatadas pela literatura para melhoria das características físicas e sensoriais de pães sem glúten inclui aditivos, enzimas e outras substâncias como adição de proteínas de outras cadeias produtivas (laticínios, ovos, soja). O uso de processamentos (ou a combinação deles) como a extrusão, a germinação e a combinação de diferentes métodos de panificação vêm demonstrando efeitos positivos e qualidades desejáveis a produtos sem glúten. Além disso, o impacto dessas técnicas sobre a melhoria das características nutricionais tem sido alvo de estudos (Woomer & Adedeji, 2020; Rani et al., 2018).

O sistema alimentar global enfrenta muitos desafios complexos, como a fome, a subnutrição, os recursos naturais limitados e as alterações climáticas Uma possibilidade de solução para enfrentar esses desafios interligados seria melhorar a produção agrícola sustentável, cadeias de valor resilientes e acesso dos consumidores a dietas variadas e acessíveis. A Organização das Nações Unidas para a Alimentação e Agricultura (FAO) declarou 2023 como o ano Internacional do milheto, com o objetivo de avivar o milheto como alimento básico, assim como estimular o consumo humano em regiões onde ele ainda é subutilizado (FAO, 2023).

No Brasil ainda é usado apenas em sistemas de rotação para *commodities* importantes, como a soja, o milho e o algodão (Dias-Martins et al., 2018). No entanto, estimular o consumo desse cereal seria vantajoso para população devido a sua enorme qualidade nutricional. Dentro desse contexto, aplicar o uso desse cereal rico, na indústria de panificação sem glúten apresenta-se como uma boa alternativa.

REFERÊNCIAS

CAPELLI, A; OLIVA, N; CINI, ENRICO. A Systematic Review of Gluten-Free Dough and Bread: Dough Rheology, Bread Characteristics, and Improvement Strategies. **Applied Science**, v. 10, n. 18, 2020. Disponível em: https://www.mdpi.com/2076-3417/10/18/6559

DIAS-MARTINS, A. M., PESSANHA, K. L. F., PACHECO, S., RODRIGUES, J. A. S., CARVALHO, C. W. P. Potential use of pearl millet (*Pennisetum glaucum* (L.) R. Br.) in Brazil: Food security, processing, health benefits and nutritional products. **Food Research International**, v. 109, p. 175–186, 2018.

Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0963996918302941.

HASSAN, Z. M; SEBOLA, N. A; MABELEBELE, M. The nutritional use of millet grain for food and feed: a review. **Agriculture & Food Security**, v.10, 2021. Disponível em: https://agricultureandfoodsecurity.biomedcentral.com/articles/10.1186/s40066-020-00282-6

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). **International year of millets 2023**. Disponível em: https://www.fao.org/millets-2023/about/en.

RANI, S; SINGH, R; SEHRAWAT, R; KAUR, B. P; UPADHYAY, A. Pearl millet processing: a review. **Nutrition & Food Science**, v. 48, n. 1, p. 30-44, 2018. Disponível em: https://www.researchgate.net/publication/322278079_Pearl_millet_processing_a_review

SELLADURAI, M; PULIVARTHI, M.K; RAJ, A. S; IFTIKHAR, M; PRASAD, P. V. V; SILIVERU, K. Considerations for gluten free foods - pearl and finger millet processing and market demand. **Grain & Oil Science and Technology**, v. 6, p. 59–70, 2023. Disponível em: https://www.sciencedirect.com/science/article/pii/S2590259822000395

TAYLOR, J. R. N. **Millet pearl: Overview**. Encyclopedia of food grains. 2 ed. **Oxford: Academic Press**, p.190–198, 2016.WOOMER, J. S; ADEDEJI, A. A. **Critical Reviews in Food Science and Nutrition**, v. 61, n. 1, p. 14-24, 2021.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o efeito combinado da germinação, extrusão termoplástica e da panificação sobre as propriedades tecnológicas, químicas e atividade anti-hiperglicemiante *in vitro* de pães sem glúten à base de milheto (*Pennisetum glaucum* (L.) R. Br.).

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar as características físico-químicas de farinhas de milheto crua, extruda e germinada, assim como suas misturas binárias e terciárias;
- Avaliar o efeito das variáveis de processo da extrusão termoplástica para obtenção de farinhas extrudadas panificáveis;
- Avaliar efeito das variáveis de processo de panificação (razão farinha crua: farinha extrudada de milheto, tempo e temperatura de fermentação, uso de farinha germinada, de massa esponja e de massa direta) sobre as propriedades **tecnológicas** (análises reológicas das farinhas utilizadas, distribuição do tamanho de partícula das farinhas, volume específico dos pães, estrutura dos alvéolos, cor e textura instrumentais), **químicas** (composição centesimal dos pães, conteúdo de fibra dietética, de amido total, de amilose e de amido resistente) e **nutricionais** (atividade antioxidante e atividade anti-hiperglicemiante *in vitro*) de pães isentos de glúten elaborados com milheto (*Pennisetum glaucum* (L.) R. BR.).

CAPÍTULO 1 - GLUTEN-FREE MILLET-BASED BREAD: NUTRITIONAL, HEALTH BENEFITS AND PROCESSING

GLUTEN-FREE MILLET-BASED BREAD: NUTRITIONAL, HEALTH BENEFITS AND PROCESSING

Review article

Lorrany Miranda Marinho[a], Monique Barreto Santos[b], Carlos Wanderlei Piler de Carvalho[b], Cristina Yoshie Takeiti[a,b]

[a]*Post graduation Program of Food and Nutrition, Federal University of the State of Rio de Janeiro, UNIRIO, Rio de Janeiro, RJ, Brazil.*
[b]*Brazilian Agricultural Research Corporation, Embrapa Food Agroindustry, Rio de Janeiro, RJ, Brazil.*

*Corresponding author: e-mail: cristina.takeiti@embrapa.br

Abstract
Millet grains promote several health benefits due to good nutritional profile (high fiber content and bioactive compounds), low glycemic index and absence of gluten. As a result, millet flour has been used in the production of gluten-free products associated with various improvement strategies such as extrusion processing, fermentation and germination. In addition, different breadmaking methods are used in order to produce gluten-free bread with technological and nutritional quality. Replacing gluten is still a major challenge in the development of dough and bakery products. Gluten-free breads available on the market mostly have a low content of vitamins and minerals, fiber and proteins and are associated with high concentration of fat and carbohydrates including starches, gums and maltodextrins making it impossible to classify them as clean label products. In this context, the objective of this review is to summarize current knowledge regarding millet and its use as a raw material in the preparation of gluten-free breads.
Keywords: breadmaking process, gluten-free, bakery

1. Introduction

The development of chronic non-communicable diseases (NCDs) is closely related to poor diet, especially due to excessive intake of critical nutrients such as free sugars, sodium, total, saturated and trans fats to the detriment of reduced consumption of fruits, vegetables, oilseeds, seeds and foods rich in omega 3 (OPAS). Therefore, the importance of regular consumption of foods such as whole grains is due to the fact that contain an important source of carbohydrates, proteins, fiber, bioactive compounds, vitamins, and minerals (Comettant-Rabanal et al., 2021).

Millet offers enormous potential for use as an ingredient in the development of functional food products and food safety. Since this cereal has excellent nutritional quality, as a source of proteins (with an *in vitro* digestibility of almost 70%), fiber, calcium and iron, and its flavonoid composition exhibits phytochemical properties and if compared to other cereals, it is rich in starch resistant and slow to digest (Selladurai et al., 2023). In addition, millet is an alternative for gluten-related disorder diets and contributing with high content of phenolic compounds, generating characteristics of hypoglycemic properties, reducing the risk of incidence and management of diseases such as type 2 diabetes, obesity and coronary heart disease (Muthamilarasan et al., 2016).

At the same time, there is an increase in the prevalence of gluten-related disorders, especially celiac disease, which has become a public health problem globally (Alencar et al., 2021). Gluten-related disorders are triggered by the body's inability to digest gluten proteins that are present in cereals such as wheat, barley, rye, and in smaller grains such as triticale, spelled (Woomer & Adedeji, 2020) and oats that demonstrate controversial data due to limitations on its degree of purity. Contamination, which may be from wheat and barley, is unacceptable in the production of gluten-free foods (Smulders et al., 2018).

The absence of gluten in millet grains favors their use in the preparation of "gluten free" products, mainly in bakery products, that remaining a technological, sensorial and nutritional challenge, once gluten-free products have low levels of proteins and micronutrients, and high contents of lipids and sodium in addition to inadequate texture, low volume, dry mouth sensation and off-flavors (Centeno et al., 2021). The main ingredients frequently used in the manufacture of gluten-free breads are refined rice flour and corn, potato and cassava starches (Aguiar et al., 2023). These are ingredients with a lower content of micronutrients, proteins, dietary fiber and a high glycemic index. These are ingredients with a lower content of micronutrients, proteins, dietary fiber and a high glycemic index. Its consumption may increase the risk of developing NCDs (Romão et al., 2020).

The information available in the literature on millet processing conditions aiming to develop healthy food products with adequate physicochemical characteristics is still poorly understood and limited. In addition to the technological aspect, the application of this grain in food products that are easily accessible and accepted, proves to be an interesting strategy for its inclusion in the population's diet, spreading its use and adoption of the eating habits of specific populations that require blood glucose control, for instance, the diabetic population, as well gluten restriction diets.

2. Millets

Millets refer to a generic term that consider several botanical species of small grains belonging to the *Poaceae* family (Dias-Martins et al., 2018) and recognized worldwide as the sixth most important grain production (Di Stefano et al., 2017). The kernel structure of different millets consists of the pericarp, germ, and endosperm. The kernel of pearl millet is caryopsis, in which the pericarp is entirely attached to the endosperm (Figure 1) (Hassan et al., 2021).

Global production was estimated at 28.33 million tons in 2019 and increased to 30.08 million tons in 2021, according to Food and Agriculture Organization (FAO, 2021). India is the largest global producer with a market share of 33.3% in 2020. Aiming of revival the importance of millets as a staple food that had been declining over the last two decades, FAO approved India's proposal as the Year Millet International in 2023.

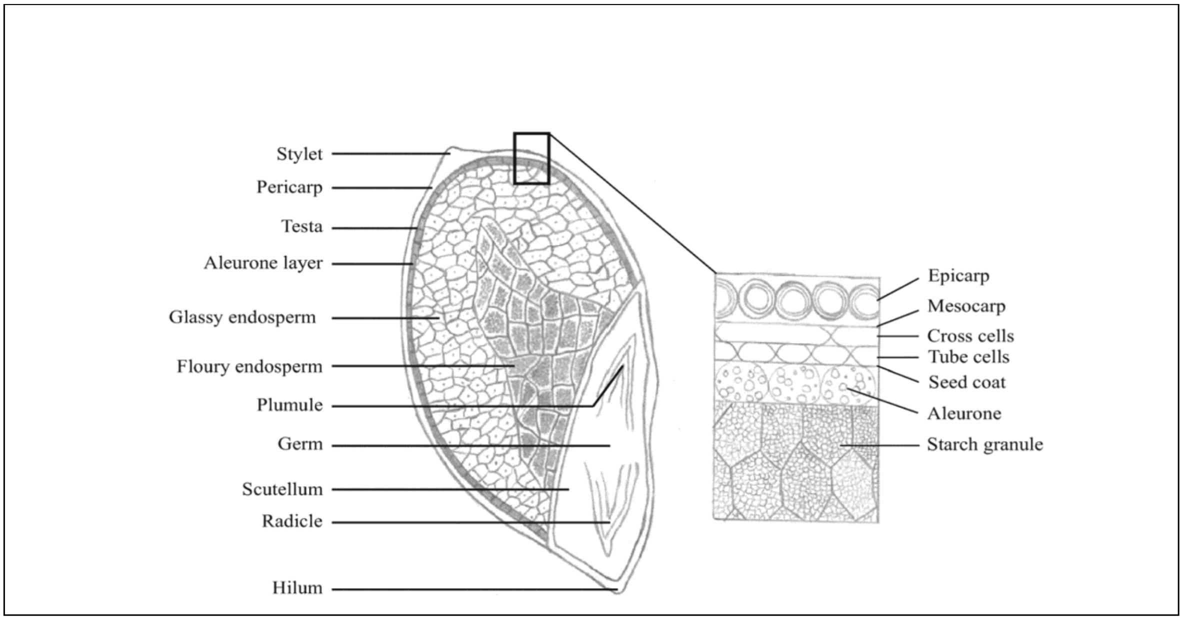


Figure 1. Grain structure of pearl millet (Hassan et al., 2021).

From a perspective of environmental and agricultural point of view, millets probably offer the best option once this specie favor profitability, adaptability and sustainability by (i) presenting the advantages of high tolerance against to increased temperatures, droughts and floods; (ii) have much lower water needs if compared to other crops; (iii) have a short rotation (65 days); (iv) the storage life is comparatively long (2 years or more); (v) cultivation requires small investment and (vi) the inputs added are mainly organic (Behera, 2017).

Millet grains have the ability to grow in drought conditions, and are capable to resist higher temperatures (Ambat & Sucharita, 2019), low soil fertility and high salinity (Dias-Martins et al., 2018) due to their extensive root system that allows the extraction of water and nutritional compounds from the deeper layers of the soil (Devisetti et al., 2014). Millets also have a low incidence of mycotoxin contamination compared to other crops such as wheat and corn (Dias-Martins et al., 2018). Mycotoxin concentrations result in host-specific differences in susceptibility to and pre-harvest infection by mycotoxin-producing of fungi. Maize crops subjected to drought stress tend to be contaminated by aflotoxin and fumonisin, leading to the millet cultivation preferable for these regions (Wilson et al., 2006).

The most known species are the pearl millet (*Pennisetum glaucum),* the foxtail millet (*Setaria italica*), the proso millet (*Panicum miliaceum*) and the finger fillet (*Eleusine coracana*) (Taylor, 2016) (Figure 2).

Pennisetum glaucum is the main millet harvested, accounting for 46% of the planted area worldwide (Gull et al., 2015) and in Brazil it was first introduced in 1929, adapted to the South, Southeast and Central-West regions (Dias-Martins et al., 2018). Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a cereal belonging to the genus *Setaria* of the family *Poaceae* and subfamily *Panicoideae* (Sharma & Niranjan, 2018). It is cultivated extensively in developing countries with semi-arid regions, as it presents health benefits and good yield using minimal agricultural inputs. It also presents good adaptation to different biotic and abiotic stresses, such as drought, salinity and fungal diseases, ranking fourth in terms of yield among all millets (Sun et al., 2019).

Proso millet (*Panicum miliaceum*) is one of the most suitable crops for the rainfed agricultural system due to its characteristics of shallow root system (90–120 cm) and short harvest duration (60–90 days). This allows farmers to harvest millet before sowing winter crops. Therefore, an important feature as winter wheat serves as the staple crop for most of the rainfed farming system. However, it is an underutilized crop in the human food market even though it is a source of a third of the protein and energy in developing countries (Das et al., 2019).

Figure 2. Anatomy photos of millet (A: *Pennisetum glaucum;* B*: Setaria italica*; C: *Panicum miliaceum;* D*: Eleusine coracana*). Credits: Karthics organics

3. Nutritional quality

Millets are a rich source of nutrients if compared to main cereals such as wheat, corn and rice, offering nutritional and subsistence security for populations in various regions of the world. Studies have shown that millet has a high percentage of nutritional compounds (proteins, fatty acids, vitamins) and bioactive compounds (Olamiti et al., 2020), consisting a rich source of phenolic compounds (phenolic acids and flavonoids) and natural antioxidants (ferulic, hydroxynamic, p-cumaric and sinapic acids) (Chandrasekara et al,, 2012; Taylor & Duodu, 2015).

The nutritional composition of millets is shown in Table 1. The *Pennisetum glaucum* specie is a good source of B vitamins, such as niacin, riboflavin, folate and thiamine, as well as essential amino acids, except lysine and threonine (Muthamilarasan et al., 2016; Singh et al., 2012), as expected for cereals.

Pennisetum glaucum has a high concentration of amylose (32.5%), which reflects on its adhesiveness properties, as it has a higher gelatinization temperature, that is a direct indication of greater resistance to swelling of starch granules (Annor et al., 2014; Punia et al., 2021). Furthermore, a large amount of amylose is associated with a strong tendency to retrogradation, which is the molecular interaction produced after gelatinization and cooling of the paste. After this process, the starch exhibits lower gelatinization and enthalpy compared to native starch because its crystalline structure has been weakened (Alcázar-alay & Meireles, 2015).

Concerning nutritional features, retrograde starch is classified as a form of resistant starch, demonstrating a strong relationship in promoting hypoglycemic activity. However, the content of resistant starch varies between genotypes and is influenced by the presence of other constituents, treatment or modification given to the starch (Mahajan et al., 2021). The molecular mass and degree of crystallinity are other factors that can influence the kinetics of enzymatic hydrolysis of millet starch, which is considered more resistant to hydrolysis if compared to rice starch due to the very rigid packaging of the millet starch granules (Mohan et al., 2005).

The nutritional composition of *foxtail millet* has a good content of essential amino acids, such as methionine, and thus it can be used to add value to products in the food industry. Dehulled grain flours have a higher protein content in comparison to polished flour and wholemeal flour due to the retention of proteins in the bran. The dietary fiber content corresponds to 20.8 g/100g in wholemeal flour, with a higher concentration of insoluble fiber (lignin, cellulose and hemicellulose), and 4.3 g/100g in polished flour (Devisetti et al., 2014). According to Kim et al. (2009), the amylose content in foxtail millet starch varied between 3.3 and 11.4%, and can be categorized into three types: (i) waxy starch (0-4%), (ii) low-amylose starch (8-16 %) and (iii) non-waxy starch (17-32%) (Mahajan et al., 2021).

The amylose concentration in finger millet corresponds to 15 to 20%, and the protein content ranges from 5.6 to 12.70% (Singh & Raghuvanshi, 2012). Regarding the amylose content in proso millet, a variation of 14.92 to 17.37% were identified in four different genotypes (Wen et al., 2014).

Table 1. Nutritional composition of millets (g/100g, except minerals mg/100g).

Cereais	Protein	Carbohydrates	Lipids	Dietary fiber	Raw fiber	Ash	Minerals	References
Pennisetum glaucum (pearl millet)	11.8	67.0	4.8	nd	2.3	2.2	nd	Muthamilarasan et al., 2016
	11.4	69.0	4.8	nd	2.0	2.1	nd	Kumar et al., 2018
	11.6	67.0	4.8	11.3	nd	nd	2.2	Hassan et al., 2021
Setaria itálica (foxtail millet)	13.5	60.2	4.1	14.0	7.8	4.2	60.2	Arora et al., 2023
Panicum miliaceum (proso millet)	11.0	-	3.5	8.5	9.0	3.6	-	Das et al., 2019
	12.5	70.4	3.1	nd	7.2	1.9	nd	Muthamilarasan et al., 2016
Eleusine coracana (finger millet)	7.3	68.0	2.7	nd	3.0	2.2	-	Gull et al., 2015
	7.7	75.0-83.3	1.8	15-22	nd	nd	2.7	Hassan et al., 2021
Wheat	13.78	69.8	2.81	nd	1.77	1.63	nd	Kumar et al., 2018
	14.4	64.0	2.3	12.1	2.9	1.9	nd	Das et al., 2019

4. Health benefits

Millet grains have several potential health benefits such as improving the digestive system, strengthening the immune system and reducing the risk of incidence of chronic non-communicable diseases (NCDs) (Gupta et al., 2012). The etiology of NCDs involves an interaction between biological systems and dietary variables, such as excessive consumption of quickly absorbed energy-rich foods to the detriment of nutrient-rich functional foods (Arora et al., 2023).

A large part of the world's population currently has a diet rich in refined products and fats that can trigger an increase in oxidative stress and an inflammatory state, causing damage to a variety of biomolecules, such as DNA, and favoring the development of diseases such as type 2 diabetes, obesity, cardiovascular diseases and cancer (Oliveira & Schoffen, 2010). According to the World Health Organization (WHO), high blood pressure, diabetes, overweight and obesity are classified as chronic non-communicable diseases (NCDs) and representing the main risk factors for death in the world population.

Diabetes mellitus is a chronic non-communicable disease characterized by high blood glucose concentrations; the main cause is the dysfunction of the beta cells of the pancreas, resulting in defects in the secretion and/or action of the hormone insulin (Mahan et al., 2012). Many factors influence the beta cell function, including hyperglycemia, glucotoxicity, lipotoxicity, autoimmunity, inflammation, insulin resistance, and oxidative stress (Cernea & Dobreanu, 2013). Evidence in the scientific literature is growing and oxidative stress plays an important role in diabetes complications (Núñez-Musa et al., 2020).

According to estimated data from the International Diabetes Federation (IDF, 2021), the global prevalence of diabetes in people between 20 and 79 years old is 10.5% (536.6 million) in 2021, with a projection of 12.2% (783.2 million) for 2045. Type 2 diabetes is the most prevalent, comprising around 90% of all diabetes cases worldwide. Brazil is the country that ranks fifth in the world incidence of diabetes, with 16.8 million adult patients, surpassed only by China, India, the United States of America and Pakistan.

Diet is considered the solid basis of the prevention and treatment strategy for glucose dysmetabolism and insulin resistance, and foods have significant effects on postprandial glycemia and the individual's overall physical health. The postprandial blood glucose level may contribute equally or even more than fasting blood glucose to the deleterious decrease in insulin sensitivity and insulin secretion observed in the development of type 2 diabetes. Therefore, establishing strategies to reduce postprandial glycemic excursions can be of fundamental relevance in the effort to mitigate the global burden of diseases (Bergia et al., 2022).

A variety of carbohydrate quality indicators have been proposed to classify foods and diets, such as the dietary glycemic index (GI), carbohydrate-to-fiber ratio (CF), and carbohydrate quality index (CQI) with the aim of relating their quality to the risk of chronic diseases and guide consumers in their food preference choices. The conventional indicator of carbohydrate quality, named the GI was proposed 40 years ago. The GI plays a substantial role in postprandial glucose excursions (Bergia et al., 2022; Cui et al., 2023), as it provides information about the relative glycemic response expected when consuming an amount of food that contains a fixed amount of carbohydrates. However, it is important to highlight that control of the glycemic response can be governed by the amount of food ingested (glycemic load) (Vega-López et al., 2018).

The source and quality of carbohydrates from the diet can enhance insulin action and thus influence the degree of insulin resistance, a fundamental metabolic characteristic in the pathogenesis of diabetes (McKeown, 2004). Food sources of fiber, magnesium and calcium reduce insulin resistance; in contrast, the increase occurs with foods with a high glycemic index and load, saturated fat, salt and alcohol (Papakonstantinou et al., 2022).

In this context, whole grains, such as millet, are recommended for diabetic patients based on glycemic index values and hypoglycemic effects, aiming to control blood glucose. Although the main constituent of whole foods is starch (present in the endosperm of grains), the presence of other bioactive compounds such as proteins, phenolic compounds, fibers and lipids help to adjust glycemic potential (Mondal et al., 2022).

Studies have demonstrated the effectiveness of millet in glycemic control with a decrease in fasting blood glucose concentration and postprandial increase, reduction of insulin resistance and the level of glycated hemoglobin. Palanisamy & Sree (2020) demonstrated that the postprandial glycemic response of replacing rice *dosa* with millet-based *dosa* significantly reduced postprandial plasma glucose levels, which was justified by the high levels of soluble dietary fiber, once its high viscosity slows down the digestion and absorption of carbohydrates.

The formation of helical complexes between lipids, including free fatty acids and monoglycerides, and amylose has been associated with significant decreases in starch hydrolysis rates and, consequently, lower millet digestibility (Kawai et al., 2012; Ai et al., 2013). Thus, the observed increased digestion resistance of starch when complexed with lipids is mainly due to reduced accessibility to enzymes, limiting enzyme absorption, penetration, binding and hydrolysis. However, it remains unclear whether starch-lipid complexes only affect the digestibility of the complexed amylose chains or the overall rate of starch digestion (Krishnan et al., 2021).

Another factor of fundamental relevance is that millet is among the gluten-free whole grains, a fact demonstrated in the food market by an increasing demand and consumption of gluten-free products, which is a trending niche in the global food sector (Alencar et al., 2021).

Gluten-related clinical disorders have affected an increasing number of individuals. The estimated prevalence of celiac disease (CD) is 1% of the population, more prevalent among women. Lately CD was a disease of Europeans, however, there is a gradual change in global distribution. In Brazil, according to a study carried out with 2,086 blood donors, a prevalence of 1.4% was reported, with a higher incidence in the Southeast region (Al-Toma et al., 2019; Peña & Rodrigo, 2015; Pereira et al., 2006). As for non-celiac sensitivity to gluten/wheat, it is believed to be the most common condition of the aforementioned disorders, however, there is still no reasonably reliable data on prevalence, even assuming a growing increase. The estimated prevalence may be up to six times higher than that of CD (Boarim, 2018).

Celiac disease is defined as a systemic process of autoimmune origin that manifests itself in genetically susceptible individuals. It is characterized by a chronic enteropathy of the small intestine precipitated by exposure to gluten (Peña & Rodrigo, 2015; Brouns et al., 2019). Approximately 40% of the population expresses the HLA-DQ2 or DQ8 haplotypes on the surface of antigen-presenting cells, which are related to susceptibility to CD. However, it is estimated that only 4% of these individuals actually develop the disease. Therefore, it is suggested that to trigger the onset of the disease, it is necessary to associate susceptibility with other environmental factors, such as high dosage of gluten at exposure (initial), changes related to diseases or drugs/alcohol in intestinal permeability, as well as exposure to antibiotics and viral infections (Lebwohl et al., 2018).

Dermatitis herpetiformis is the extra-intestinal manifestation of celiac disease. It is a benign, chronic and inflammatory clinical condition of the skin (Holanda et al., 2021). The etiology of the disease is known to be multifactorial, demonstrating a relationship with genetic, immunological and environmental components, with exposure to gluten being the environmental factor observed to aggravate the dermatosis. The resolution of lesions in both pathologies occurs after gluten withdrawal (Costin et al., 2019).

Non-celiac gluten/wheat sensitivity is a syndromic entity defined by presenting a variety of intestinal and/or extraintestinal clinical manifestations related to wheat ingestion in patients in whom celiac disease and wheat allergy have been excluded (Lionetti, 2017). The causes and underlying mechanisms for the development of non-celiac gluten/wheat sensitivity may not be the same for everyone, as reactions may be caused by different components of wheat or grains (products) and involve different individual factors. In fact, there are no sensitive biomarkers available for diagnostic testing for non-celiac sensitivity to gluten/wheat, therefore, rigorous and standardized monitoring of the patient during the elimination and reintroduction of gluten/wheat is necessary (Brouns et al., 2019).

Wheat allergy, depending on the route of allergen exposure and underlying immunological mechanisms, is classified into occupational asthma or respiratory allergy and rhinitis, immediate food allergy, contact urticaria, and wheat-dependent exercise-induced anaphylaxis. Although its sensitization assessed by serum IgE is more prevalent in adults, wheat allergy is more prevalent in children, especially in terms of immediate food allergy, which is commonly overcome at school age, as well as allergy to milk or eggs. In adult individuals, wheat-dependent exercise-induced anaphylaxis is the most common variant, in which symptoms are the result of the combination of ingestion of triggering foods and physical exercise (as well as non-steroidal anti-inflammatory drugs or alcohol) (Elli et al., 2015).

The only effective treatment suggested for people suffering with celiac disease, non-celiac gluten/wheat sensitivity and wheat allergy is to eliminate the gluten and/or wheat from their diet, as the absence of gluten can be beneficial through management of related symptoms. However, symptom recovery rates differ according to age and sex and longer duration of the diet appears to improve villus rehabilitation (Khoury, 2018).

5. Processing of millets

The searching for solutions to improve the physical aspects, functionality, nutritional profile and organoleptic properties of gluten-free products includes the use of ingredients (hydrocolloids, enzymes, starch, proteins) as well as technological advances by using of extrusion, germination and fermentation processing. These techniques aim to increase the physicochemical accessibility of nutrients, reduce the content of antinutrients, such as phytates, and/or increase the content of compounds that improve bioavailability (Saleh et al., 2013). Therefore, unveil mechanisms about the influence of processing on nutritional properties is of fundamental importance for the use of millet as an ingredient in the human diet.

5.1 Extrusion cooking

Extrusion cooking is a versatile and economical method that using high temperature and short time, very advantageous for thermosensitive foods due to low residence time, since heat treatment cause extensive changes in proteins, amino acids, vitamins, starches and enzymes (Kharat et al., 2018; Mościcki & Van Zuilichem, 2011). Thermoplastic extrusion is considered an integrated process that combines several operations (transport, mixing, twisting and cooking) in a single system that allows the native biopolymers present in cereals to be transformed into new functional biopolymers with new textures and shapes (Comettant-Rabanal et al., 2021).

The basic principle of the process is the conversion of a solid material into fluid, through the application of thermomechanical work and subsequent compression through a matrix (Marques et al., 2015). The diversity and proportion of ingredients, the amount of water, temperature and rotation speed of the extruder determine the characteristics of the final products (Mościcki & Van Zuilichem, 2011).

Furthermore, extrusion processing under the straight point of view in terms of energy efficiency is a viable tool on a large scale for the innovative products development, if compared to traditional processes since it has a low water and energy footprint, reduced effluent generation, although presents high initial cost of line processing implementation (Pessanha et al., 2021).

Regarding breadmaking process, the extrusion technology allows modification of the flour functionality. The molecular structure of starch is modified in terms of water absorption and the consequent development of the dough in order to offer wide application in the food industry as thickeners, gelling agents and fat substitutes, constituting an alternative to additives such as modified starches and hydrocolloids. Therefore, the use of extrusion as a strategy for modifying biopolymers such as starch and protein represents a new market trend aimed at developing clean label products (Espinosa-Ramírez, 2021).

Thermal processing can increase or decrease the total phenolic content (TPC) and antioxidant activity of whole grain flours. The likely mechanisms to explain these different effects include the release of phenolic compounds bound to the food matrix, thermal degradation, polymerization and oxidation of phenolics, depolymerization of high molecular weight phenolics such as condensed tannins and the products resulting from the Maillard reaction (Taylor & Duodu, 2015).

The literature reports that the extrusion process has two effects on the phenolic compounds in cereals: (i) degradation and polymerization of thermolabile phenolic compounds due to high process temperature; (ii) heat treatment triggers the disintegration of the cell wall matrix and consequent breakdown of covalent bonds in high molecular mass polyphenol complexes, favoring accessibility to phenolic compounds. Furthermore, if the feed moisture is low, the shear force is greater, and phenolic compounds are expected to be more susceptible to thermal degradation. However, with the high moisture content, even greater losses in the content of phenolic compounds were observed. Thus, the ideal extrusion feed moisture content allows the protection of phenolic compounds against thermal degradation, keeping them stable. Furthermore, extrusion conditions must be optimized depending on the food matrix (Rudra et al., 2015; Lopez et al., 2016; Ortiz-Cruz et al., 2020).

Ortiz-Cruz et al. (2020) observed a higher value of free phenolic compounds in sorghum grains (*Sorghum bicolor* (L.) Moench) using higher levels of feed moisture (35%) and temperature of the extruder end zone (180°C). Bangar et al. (2022) reported a high value of total phenolic compounds (327.70 mg GAE/100 g) in barley extruded at 30% of feed moisture and 150°C of end zone temperature. However, reducing moisture conditioning to 13% at 180°C of end zone temperature resulted in 275.60 mg GAE/100 g.

The literature reports few studies involving extrusion of non-traditional grains such as millets in order to obtain gluten-free bread flours. Gulati et al. (2016) evaluated the effects of flour moisture conditioning, screw speed and temperature on the physical characteristics and antioxidant activity of extruded proso millet flour. Results showed that an increase in moisture content resulted in a decrease in antioxidant capacity, according to a test of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), meanwhile increasing screw speed rotation showed an increasing on antioxidant activity. Shear resulted in greater breakdown of cell wall components and better interaction between the ABTS radical and insoluble antioxidant compounds, increasing its antioxidant capacity from 16.5 to 31.4 mmol g^{-1}. High shear also contributes to the production of Maillard reaction compounds, as well antioxidant activity.

Some authors reported that millet has great potential to be used in the development of extruded products with better digestibility, desirable texture quality and sensory acceptance (Balasubramanian et al., 2014; Kumar et al., 2020). However, it is essential to understand how the variables of the boundary conditions (heating zone temperatures, screw speed and effect of material moisture) affect the nutritional composition of millet flours in terms of total carbohydrates, proteins, lipids, dietary fiber and minerals, in addition to their influence on the glycemic index/load and antihyperglycemic activity (Kharat et al., 2018).

5.2 Germination

Germination was mainly used to produce fermentable extracts for beverage fermentation and distillation purposes. Currently, it has become a tool for producing ingredients with an improved nutritional profile. However, parameters such as germination time and temperature are crucial factors during this process, influencing the final chemical composition of the raw material (Horstmann et al., 2019).

Germination is a process in which grains are immersed in water until saturation and subsequent germination under controlled conditions. It presents good cost-benefit, as it is a simple and cheap technique that results in an increase in the activities of hydrolytic enzymes inherent to the grains, promoting biochemical changes, structural modification and synthesis of new compounds that can increase the nutritional value and stability of the grains. This process involves the hydrolysis of the main compounds, such as starch, fiber and protein. If compared to native grains, germinated millet grains had a higher content of proteins, vitamins and minerals (Annor et al, 2017; Theodoro et al., 2021; Li et al., 2017).

The protein content of the optimized foxtail millet after 46.5 h of germination showed a significant increase compared to non-germinated seeds, as well as the total dietary fiber which increased from 22.20 to 27.42% after germination. The protein content increases significantly with increasing time, due to the restructuring of seed cell wall polysaccharides, affecting the integrity of tissue histology and interrupting the protein-carbohydrate interaction during cell wall biosynthesis and, therefore, the production of new food fibers (Sharma et al., 2015).

Alpha-amylase activity is rapidly induced and increased as the germination process occurs. The increase in enzymatic activity promotes starch degradation and reduction in the mass of biomolecules with consequent changes in the viscoelastic properties of starch. As observed by Sharma et al. (2021), a significant decrease in peak viscosity (437 cP to 287.00 cP), final viscosity (728.0 cP to 368.0 cP) and breakdown was observed (35,0 cP para 29,0 cP). In addition to the reduction in setback, the use of germinated wheat flour to improve breadmaking performance should be evaluated. This trend may be of great interest, as a low rate of starch retrogradation and syneresis contributes to product quality, such as maintaining a soft crumb during bread storage by delaying aging, especially in gluten-free breads (Marti et al., 2017). It was observed that adding 10 or 20% sprouted rice flour to wheat flour suppressed staling of bread during storage (Watanabe et al., 2004).

The germination and fermentation process also improves the digestibility of carbohydrates by breaking down complex starch into simple soluble sugars. This tool is important in the development of food products with high energy density and easy digestion (Gowda et al., 2022).

Regarding the effects of processing on bioactives, it is observed that after the germination process there is an increase in these compounds, especially phenolic acids. This probably occurs due to the synthesis or activation of the most diverse enzyme systems present in the grain after they are soaked in water (Pradeep & Sreerama, 2015), positively influencing the antioxidant capacity. Sharma et al. (2021) suggested that the total antioxidant potential of the phenolic extract of kodo millet flour was significantly influenced by germination, in which the total antioxidant activity of the phenolic extracts increased from 45.34 to 67.23 mg AAE/g.

5.3 Fermentation

In developing countries, especially in most parts of Africa, the absence of sophisticated processing machines triggers the processing of millet using traditional methods as a way of ensuring food and nutritional security for these populations. Fermentation is practiced to diversify the use, improving the flavor, texture and palatability of food products (Adebiyi et al., 2016). Fermentation also plays a crucial role as in the absence of proper storage, extending the shelf life of foods (Onweluzo & Nwabugwu, 2009).

Fermentation promotes an increase in protein content, an improvement of amino acid balance, an increase in carbohydrate accessibility and a decrease in antinutritional factors such as tannin and phytic acid. Chinenye et al. (2017) when evaluating the effect of fermentation (natural and starter) on millet flour, observed that there was a significant reduction in tannin content from 2.8 to 1.85 (natural fermented millet) and 1,40 (starter fermented millet), and phytate from 1.78 to 0.12 (natural fermented millet) and 0.09 (starter fermented millet).

The reduction of antinutritional factors may be due to the leaching of antinutrients during soaking from the cleavage of tannin-protein, tannic acid-starch and tannin-iron complexes, thus releasing free nutrients that will invariably improve their availability. As a consequence of the reduction in tannin content, there is a reduced risk of intestinal, kidney, stomach irritation, liver damage and gastrointestinal pain associated with foods containing high tannin content (Onweluzo & Nwabugwu, 2009).

Modifications in the starch content and digestibility of the fermented product can be attributed to the effects of the amylolytic action of microorganisms in the fermentation mixture (Saleh et al., 2013). The other possibility would be associated with the decrease in phytic acid content during fermentation, since phytic acid had a significant negative correlation ($p<0.05$) with starch digestibility *in vitro*. The acidification of the dough during the fermentation process triggers the activation of endogenous phytases, promoting the dephosphorylation of phytic acid and the consequent release of divalent cations for absorption (Karkle, 2019).

Fermentation increases the bioconversion of phenolic compounds from their bound or conjugated forms to their free forms (Wang et al., 2014). In this way, endogenous and bacterial enzymes modify the constituents of the grain, affecting its structure, bioactivity and bioavailability (Muñoz et al., 2016). However, the temperature and types of microorganisms present in the material play a crucial role in this process, increasing its antioxidant capacity (Balli et al., 2020). The literature reports that fermentation of lactic acid bacteria increases nutrient levels (folate, soluble fiber) and total content of phenolic compounds in cereals, as well as improving protein digestibility (Hole et al., 2012).

Regarding the effects of fermentation on fiber content, Adebiyi et al. (2016) reported an increase in crude fiber (1.26–1.33%). Another study revealed that fermenting dietary fiber of foxtail millet bran with *Bacillus natto* increased the soluble dietary fiber content from 2.3% to 13.2%, and the soluble dietary fiber (SDF)/insoluble dietary fiber ratio (IDF) increased from 3.1% to 19.9%. The change in the type of dietary fiber after the fermentation process is attributed to the breakdown of cellulose and hemicellulose resulted in polysaccharides with a more porous structure (Chu et al., 2019).

6. Millet-based bread

The global gluten-free (GF) baking mixes market is estimated at US$477.1 million in 2022 (Markets and Markets, 2022) with a projection of US$681.7 million in 2027, with a compound annual growth rate (CAGR) of 7.4% between 2022 and 2027. Individuals diagnosed with celiac disease are not the only one driver in the "gluten-free" market. This market also includes individuals who are trying a gluten-free diet to alleviate symptoms related to asthma, attention deficit hyperactivity disorder (ADHD), irritable bowel syndrome, weight management, as well as people who have a perception that long-term gluten-free diet reduces health risks (Woomer & Adedeji, 2020).

Traditional bread, based on wheat flour, is one of the most important food products, having ancient significance in human nutrition, constituting a daily eating habit in different cultures. Therefore, developing non-gluten alternatives is a major technological challenge due to no raw material, ingredient or additive can completely replace gluten (Santos et al., 2019; Capriles et al., 2015). Alencar et al. (2021) report that some type of gluten-free bread was consumed daily by 93% of celiac patients, 61% by individuals who had other gluten-related disorders and 52% by people with a "gluten free lifestyle". However, percentages of low satisfaction or dissatisfaction among the three groups, varied between 82% to 91% for price, 74% to 82% for availability and 63% to 69% for the gluten-free bread variety. Regarding texture, almost 60% of consumers in each of the three groups were slightly satisfied or dissatisfied, and 50% is satisfied in relation to the taste.

Another factor to be considered is that gluten-free breads commonly have nutritional characteristics with a low content of vitamins and minerals, bioactive compounds, proteins and fiber, associated with a high content of different starches (Aguiar et al., 2023). In fact, gluten-free products available on the market are mostly made from polished rice flour combined with corn, potato and cassava starches, in the form of an isolated starch or as a mixture of combined starches. Furthermore, GF breads are known for their unsatisfactory presentation, texture and flavor, as well as low volume and short shelf life (Drub et al., 2021).

Due to the growing consumer demand for healthy and high-quality GF bakery products, the bakery industry is faced with the challenge of producing breads with better nutritional, physicochemical and sensory characteristics (Mariotti et al., 2014). As a result, there has been an increase in research into different types of GF cereals, including their nutritional factors, digestibility and applications (Woomer & Adedeji, 2020). Thus, alternative ingredients such as millet have induced interest in the scientific community in the GF bread sector, as they demonstrate that it is possible to obtain products based on millet flour, with high nutritional value and acceptable physical characteristics, with a soft texture and well-developed pores (Torbica et al., 2019). Furthermore, studies have shown that sprouted grains can be used as an alternative to reduce the hardness of the crumb of gluten-free breads. Natural enzymes expressed during this process can reduce or completely replace the amount of enzymes added in the bread formulation by modifying the rheology of the dough, promoting gas retention and contributing to the softness of the crumb (Marti et al., 2017).

Bread, which is traditionally based on wheat flour, has been made worldwide using other cereals, such as millet, either alone or in combination with wheat flour. The effects of adding millet flour (MF) to wheat flour (WF) on rheological properties were demonstrated by Maktouf et al. (2016). From the Mixolab test it was observed that the addition of MF (5%) to WF decreased both the development time and dough stability, reducing it from 4.31 to 1.18 min and from 11.12 to 9.46 min, respectively, leading to less work required during dough development. The addition level of 5% millet flour had the most significant positive effect ($p<0.05$) on the alveographic properties of the dough, in which strength (W) and elasticity-extensibility ratio (P/L) increased by 31% and 65% , respectively, compared to the control based on wheat flour.

Supplementation of whole wheat flour with raw millet flour adversely affects the handling and textural attributes of *chapatti* bread in terms of increased volume, plasticity and softness, as a consequence of the lack of viscoelastic properties. As a result, research has been developed with the implementation of various technological interventions, such as extrusion, with the aim of overcoming this limitation regarding the absence of gluten (Kumar et al., 2020). The extrusion process promotes the modification of the molecular structure of protein and starch, improving the viscoelastic properties of the dough, resembling the gluten network (Meng et al., 2019).

A study carried out with breads made from 100% wholemeal flour demonstrated that the formulation made with 50% raw millet flour and 50% extruded millet flour showed greater specific volume, height and uniform alveolus formation. Furthermore, it showed a high rate of volume increase during the fermentation process and maintenance of these characteristics after cooking. These results are directly related to greater softness identified from the texture profile analysis (Pessanha et al., 2021).

The addition of sprouted grains as a natural ingredient conduct to technological benefits to improve the quality of gluten-free breads, contributing to the gasification of CO_2 during fermentation and delaying the stale bread. Germinated millet (*Pennisetum glaucum* (L.) R. Br.) is an excellent option due to its high degree of germination ratio and considerable enzymatic activity compared to other grains (Horstmann et al., 2019). As demonstrated by Comettant-Rabanal et al. (2021), in which the incorporation of 5% germinated millet improved the appearance of the crumb of all gluten-free breads with a significant reduction ($p < 0.05$) in the hardness of the breads. These authors attributed these findings to the hydrolysis of the starch present in the samples by the enzyme system present in the germinated flour.

The type of grinding of flours also interfered in texture properties of breads made exclusively with millet proso flour. Bread based on flour ground in a hammer mill produced softer breads (17.28 N) compared to breads produced with flours from pin mills (26.44 N) and roller mills (25.85 N). The flour obtained by pin mill presented finer particles and breads with a low specific volume (2.40 cm^3/g) compared to roller mill (2.52 cm^3/g) and hammer mill (2.47 cm^3/g) (Prakash et al. 2022). In fact, the effect of particle size on the quality of gluten-free bread is already observed by other studies (De la Hera et al., 2013; Novotni et al., 2023).

In the baking industry, different baking methods have been proposed to produce bread with different qualities and palatability. Straight-dough and sourdough are the main breadmaking methods that have been traditionally practiced. The straight-dough breadmaking method involves fermentation by baker's yeast, whereas the sourdough method is primarily based on lactic acid bacteria fermentation. A third method that can leads to benefits of technological and nutritional characteristics is the sponge dough method (Awulachew, 2020).

The use of sponge dough method in bakery production showed a great importance. It has the advantages of producing a more extensible dough, promoting yeast activation, facilitating dough formation, transmitting superior aroma and flavor, generating soft and regular crumb texture (Cavanagh et al., 2010). Furthermore, the longer fermentation process of sponge dough results in a more acidic dough, which has been shown to be very useful for improving the texture and palatability of fiber-rich products, and also for stabilizing or improving the levels of bioactive compounds (Luiz & Vanin, 2022).

Sourdough fermentation is one of the most traditional food biotechnologies, it has favorable effects due to the retention of carbon dioxide produced by yeast, which promotes improvement in the structure of the bread crumb, volume, flavor, nutritional values, prolonging the shelf life with delayed staling and greater durability (Wang et al., 2020). Nami et al. (2019) proved the benefits of using various sourdoughs, based on combinations of four species of *Lactobacillus* (LAB), for the quality and shelf life of gluten-free millet bread. However, the type of starter culture used positively and significantly influences bread height, specific volume, porosity and baking loss. Single species of LAB starters had a greater effect than multi-species starters on loaf height and specific volume, demonstrated a greater value when *Lactobacillus brevis* was used. The inclusion of sourdough induces changes in the rheology of the dough, specifically greater elasticity and less rigidity, which results in an increase in the bread expansion capacity. It is possible that these changes occur from the proteolytic activity of LAB altering the nature of the protein network in the dough. The softness of the dough has the effect of improving its ability to retain the carbon dioxide generated during fermentation.

Controlling the parameters of the gluten-free bread manufacturing process is essential to obtain breads with good quality characteristics, such as high specific volume, cellular structure of the crumb and desirable instrumental textural properties (Villarino et al., 2014). However, there are currently few studies in the literature evaluating the effect of different breadmaking methods on the technological quality of gluten-free breads, as well as on phytochemicals and their contribution to the antioxidant capacity of these products.

7. Conclusion and future prospects

Millet grains have enormous potential, due to either sustainable characteristics and as a source of macro/micronutrients and bioactive compounds constituting an alternative to ensure nutrition in a climate change scenario, as well specific dietary necessities for gluten-related disorders patients relating to physical characteristics or nutritional imbalance of products. The development of more studies relating the effects of different types of processing and digestion represents fundamental importance for the inclusion of this cereal in human nutrition.

Funding

This work was funded by the Carlos Chagas Filho Research Support Foundation of the State of Rio de Janeiro, E-26/210.022/2020; and National Council for Scientific and Technological Development, 308416/2019-8.

Declaration of competing interest

The authors attest that there are no interests that competed with the objective, interpretation, and presentation of the results.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Credit authorshipp contribution statement

Lorrany Miranda Marinho: Conceptualization, Methodology, Data curation, Writing- original draft, preparation, Visualization, Investigation, Writing - review & editing. **Monique Barreto dos**

Santos: Visualization, Investigation, Writing - review & editing. **Carlos Wanderlei Piler Carvalho**: Supervision, Writing -review & editing. **Cristina Yoshie Takeiti**: Supervision, Validation, Writing - review & editing.

References

Adebiyi, J. A; Obadina, A. O; Mulaba-Bafubiandi, A. F; Adebo, O. A; Kayitesi, E. (2016). Effect of fermentation and malting on the microstructure and selected physicochemical properties of pearl millet (*Pennisetum glaucum*) flour and biscuit. J. Cereal Sci., 70:132-139. doi.org/10.1016/j.jcs.2016.05.026

Adebiyi, J. A; Obadina, A. O; Mulaba-Bafubiandi, A. F; Adebo, O. A; Kayitesi, E. (2017). Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (*Pennisetum glaucum*) flour. *Food Chem.*, *232: 210–217*. doi:10.1016/j.foodchem.2017.04.020

Aguiar, E. V; Santos, F. G; Krupa-Kozak, U; Capriles, V. D. (2023). Nutritional facts regarding commercially available gluten-free bread worldwide: Recent advances and future challenges. Crit. rRev. Food. Sci., 63 (5): 693-705. doi.org/10.1080/10408398.2021.1952403

Ai, K., Hasjim, J., Jane, J-L. (2013). Effects of lipids on enzymatic hydrolysis and physical properties of starch. Carbohydrate Polymers, 92: 120– 127. doi: doi.org/10.1016/j.carbpol.2012.08.092

Alcázar-Alay, S. C; Meireles, M. A. A. (2015). Physicochemical properties, modifications and applications of starches from different botanical sources. Food Sci. Techn., 35(2): 215-236. doi: dx.doi.org/10.1590/1678-457X.6749

Alencar, N. M. M; Araújo, V. A; Faggian, L; Araújo, M. B. S; Capriles, V. D. (2021) What about gluten-free products? An insight on celiac consumers opinions and expectations. J. Sens. Stud., 36. doi.org/10.1111/joss.12664.

Al-Toma, A; Volta, U; Auricchio, R; Castillejo, G; Sanders, D. S. S; Cellier,C; Mulder, C. J; Lundin, K. E. A. (2019). European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. United European Gastroenterology Journal 7(5).

Ambat, K., Sucharita, K. V. (2019). Millets-review on nutritional profiles and health benefits. Intern. Jour. Recent Scient Res., 10 (7): 33943-33948. doi: http://dx.doi.org/10.24327/ijrsr.2019.1007.3786

Annor, G. A; Marcone, M; Bertoft, E; Seetharaman, K. (2014). Physical and Molecular Characterization of Millet Starches. Cereal Chem., 91(3): 286–292. dx.doi.org/10.1094/CCHEM-08-13-0155-R

Annor, G.A., Tyl, C., Marcone, M., Ragaee, S., Marti, A. (2017) Why do millets have slower starch and protein digestibility than other cereals? Trends in Food Science & Technology, 66: 73-83. doi: doi.org/10.1016/j.tifs.2017.05.012

Arora, L; Aggarwal, R; Dhaliwal, I; Gupta, O. P; Kaushik, P. (2023). Assessment of sensory and nutritional attributes of foxtail millet-based food products. Front Nutr, 10:1146545.doi: 10.3389/fnut.2023.1146545.

Awulachew, M. T. (2020). Properties of Wholegrain Bread and Nutritional Quality Baked with Different Methods: A Review. Adv. Food Nutr. Res., 3 (2): 5-15. orcid.org/0000-0002-9215-9582

Balasubramanian, S., Kaur, J., & Singh, D. (2014). Optimization of weaning mix based on malted and extruded pearl millet and barley. J.Food Sci. Technol., 51 (4): 682–690. doi: 10.1007/s13197-011-0579-6

Balli, D.; Bellumori, M.; Pucci, L.; Gabriele, M.; Longo, V.; Paoli, P.; Mellani, F.; Mulinacci, N; Innocenti, M. (2020). Does fermentation really increase the phenolic content in cereals? A study on millet. Foods, 9: 303. doi: 10.3390/foods9030303

Bangar, S.P.; Sandhu, K.S.; Trif, M.; Rusu, A.; Pop, I.D.; Kumar, M. (2022). Enrichment in different health components of barley flour using twin-screw extrusion technology to support nutritionally balanced diets. Front. Nutr., 8, 823148. doi: 10.3389/fnut.2021.823148

Behera, M. K. (2017). Assessment of the state of millets farming in India. MOJ Ecol. & Envir. Sci., 2. doi: 10.15406/mojes.2017.02.00013

Bergia, R. B; Giacco, R; Hjorth, T; Biskup, I; Zhu, W; Costabile, G; Vitale, M; Campbell, W. W; Landberg, R; Riccardi, G. (2022). Differential Glycemic Effects of Low - versus High - Glycemic Index Mediterranean - Style Eating Patterns in Adults at Risk for Type 2 Diabetes: The MEDGI-Carb Randomized Controlled Trial. Nutrients.14(3):706. doi: 10.3390/nu14030706

Boarim, D. S. F. (2018). Non-Celiac Gluten Sensitivity. International Journal of Nutrology, 11(3): 71-792018. doi: 10.1055/S-0039-1681015

Brouns, F; Rooy, G. V; Shewry, P; Rustgi, S; Jonkers, D. (2019). Adverse Reactions to Wheat or Wheat Components. Comprehensive Reviews in Food Science and Food Safety, 18 (5): 1437-1452. doi.org/10.1111/1541-4337.12475

Capriles, V.D., Santos, F.G., Arêas, J.A.G. (2015) Gluten-free breadmaking: Improving nutritional and bioactive compounds. Journal of Cereal Science, 67:83–91. doi.org/10.1016/j.jcs.2015.08.005.

Cavanagh, C. R; Taylor, J; Larroque, O; Coombes, N; Verbyla, A. P; Nath, Z; Kutty, I;..., Newberry, M. (2010). Sponge and dough bread making: genetic and phenotypic relationships with wheat quality traits. Theor Appl Genet, 121: 815–828. doi: 10.1007/s00122-010-1352-3

Centeno, A. C. L; Aguiar, E; Santos, F; Queiroz, V; Conti-Silva, A; Krupa-Kozak, U; Capriles, V. D. (2021). Defining Whole Grain Sorghum Flour and Water Levels to Improve Sensory and Nutritional Quality of Gluten-Free Bread — A Factorial Design Approach. Appl. Sci., 1: 8186. doi.org/10.3390/app11178186.

Cernea, S., Dobreanu, M. (2013). Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. Biochem. Medica, 23 (3): 266–80. doi: 10.11613/bm.2013.033

Chandrasekara, A., Naczk, M., Shahidi, F. (2012). Effect of processing on the antioxidant activity of millet grains. Food Chem., 133(1): 1–9.

Chinenye, O. E; Ayodeji, O. A; Baba, A. J. (2017). Effect of Fermentation (Natural and Starter) on the Physicochemical, Anti-nutritional and Proximate Composition of Pearl Millet Used for Flour Production. Am. J. Biosci. Bioieng., 5(1): 12-16. doi: 10.11648/j.bio.20170501.13

Chu, J; Zhao, H; Lu, Z; Lu, F; Bie, X; Zhang, C (2019). Improved physicochemical and functional properties of dietary fiber from millet bran fermented by *Bacillus natto*. Food Chem., 294: 79-86. doi:10.1016/j.foodchem.2019.05.035

Comettant-Rabanal, R; Carvalho, C. W. P; Ascheri, J. L. R; Chávez, D. W. H; Germani, R. (2021). Extruded whole grain flours and sprout millet as functional ingredients for gluten-free bread. LWT - Food Sci Techn, 150: 112042. doi.org/10.1016/j.lwt.2021.112042

Costin, A., António, A. M., Furtado, C., Bártolo, E. (2019). Dermatitis herpetiformis in an adolescent patient. Anais brasileiros de dermatologia, 94(4): 495– 496. doi: doi.org/10.1590/abd1806-4841.20198227

Cui, Z., Wu, M., Liu, K., Wang, Y., Kang, T., Meng, S., Meng, H. (2023). Associations between Conventional and Emerging Indicators of Dietary Carbohydrate Quality and New - Onset Type 2 Diabetes Mellitus in Chinese Adults. Nutrients, 15(3) 6: 7. doi: 10.3390/nu15030647.

Dagostin, C. T; Rigo, F. K; Damázio, L. S. (2019). Associação entre Alimentação Vegetariana e a Prevenção do Câncer Colorretal: Uma Revisão de Literatura. Revista Contexto & Saúde, 19 (37): 44-51. doi.org/10.21527/2176-7114.2019.37.44-51

Das, B; Khound, R; Santra, M; Santra, D. K. (2019). Beyond Bird Feed: Proso Millet for Human Health Envir. Agric., 9 (3): 64. doi:10.3390/agriculture9030064

De la Hera, E, Martinez, M, Gomez, M. (2013). Influence of flour particle size on quality of gluten-free rice bread. LWT — Food Sci. Technol, 54 (1): 199 – 206. dx.doi.org/10.1016/j.lwt.2013.04.019.

Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G., & Priyadarisini, V. B. (2014). Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: A review. J. Food Sci. Technol., 51 (6): 1021–1040. doi: 10.1007/s13197-011-0584-9.

Devisetti, R; Yadahally, S. N; Bhattacharya, S. (2014). Nutrients and antinutrients in foxtail and proso millet milled fractions: Evaluation of their flour functionality. LWT - Food Sci. Techn, 59: 889-895. doi.org/10.1016/j.lwt.2014.07.003

Dias-Martins, A. M., Pessanha, K. L. F., Pacheco, S., Rodrigues, J. A. S., Carvalho, C. W. P. (2018). Potential use of pearl millet (*Pennisetum glaucum* (L.) R. Br.) in Brazil: Food security, processing, health benefits and nutritional products. Food Res Intern., 109: 175–186. doi: 10.1016/j.foodres.2018.04.023.

Di Stefano, E., White, J., Seney, S., Hekmat, S., Mcdowell, T., Sumarah, M., REID, G. (2017). A novel millet-based probiotic fermented food for the developing world. Nutrients, 9: 529. doi: 10.3390/nu9050529

Dlamini, N. R; Taylor, J. R. N; Rooney, L. W. T. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. Food Chem., 105 (4), 1412-1419. doi.org/10.1016/j.foodchem.2007.05.017

Drub, T. F; dos Santos, F.G; Centeno, A. C. L. S; Capriles, V. D. (2021). Sorghum, millet and pseudocereals as ingredients for gluten-free whole-grain yeast rolls. Int. J. Gastron. Food Sci., 23: 100293. doi.org/10.1016/j.ijgfs.2020.100293

Elli, L; Branchi, F; Tomba, C; Villalta, D; Norsa, L; Ferretti, F; Roncoroni, L; Bardella, M. T. (2015). Diagnosis of gluten related disorders: Celiac disease, wheat. World J Gastroenterol, 21 (23): 7110-7119. doi: 10.3748/wjg.v21.i23.7110.

Espinosa-Ramírez, J; Rodríguez, A; Rosa-Millan, J; Heredia-Olea, E; Pérez-Carrillo, E; Serna-Saldívar; S. O. (2021). Shear-induced enhancement of technofunctional properties of whole grain flours through extrusion. Food Hydrocoll., 111: 106400. doi.org/10.1016/j.foodhyd.2020.106400

FAOSTAT. Agriculture Organization of the United Nations Statistics. (2021). Division. Data–Crops-Yield. Available in: http://www.fao.org/faostat/en/#data/QC. Access at: 15.03.22.

Gowda, N. A. N; Siliveru, K; Prasad, P. V. V; Bhatt, Y; Netravati, B. P; Gurikar, C. (2022) Modern Processing of Indian Millets: A Perspective on Changes in Nutritional Properties. Foods, 11: 499. doi.org/10.3390/foods11040499

Gulati, P; Weier, S. A; Santra, D; Subbiah, J; Rose, D. L. (2016) Effects of feed moisture and extruder screw speed and temperature on physical characteristics and antioxidant activity of extruded proso millet (*Panicum miliaceum*) flour. International Journal of Food Sci.Technol, 51: 114–122. doi:10.1111/ijfs.12974

Gull, A., Prasad, K., Kumar, P. (2015). Evaluation of functional, antinutritional, pasting and microstructural properties of Millet flours. Food Meas., 10: 96–102. doi.org/10.1007/s11694-015-9281-0

Gupta, N; Srivastava, A. K; Pandey, V. N. (2012). Biodiversity and Nutraceutical Quality of Some Indian Millets. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. 82(2): 265–273. doi: 10.1007/s40011-012-0035-z

Habiyaremye, C; Matanguihan, J. B; Guedes, J. D. A; Ganjyal, G. M; Whiteman, M. R; Kidwell, K. K; Murphy, K. M. (2017). Proso Millet (*Panicum miliaceum* L.) and Its Potential for Cultivation in the Pacific Northwest, U.S.: A Review. Front. Plant Sci., 7. doi: 10.3389/fpls.2016.0196

Hassan, Z. M; Sebola, N. A; Mabelebele, M. (2021). The nutritional use of millet grain for food and feed: a review. Agric & Food Secur, 10. doi.org/10.1186/s40066-020-00282-6

Holanda, S. M. M. S. C; Nunes, R. M. O; Silva, L. A. S; de Barros, M. M; Silva, P. H. F; Assunção, A. J. T; Moura, A. C; Galvão, E. M. L. N. F. (2021). Theoretical-practical approach to dermatitis herpetiformis as the main cutaneous manifestation of celiac disease: Integrative review. . Res., Soc. Dev., 10 (6). doi: dx.doi.org/10.33448/rsd-v10i6.15276

Hole, A. S.; Rud, I.; Grimmer, S.; Sigl, S.; Narvhus, J.; Sahlstrøm, S. (2012). Improved Bioavailability of Dietary Phenolic Acids in Whole Grain Barley and Oat Groat following Fermentation with Probiotic Lactobacillus acidophilus, *Lactobacillus johnsonii*, and *Lactobacillus reuter*. J. Agric. Food Chem., 60: 6369-6375. doi: 10.1021/jf300410h

Horstmann S. W; Atzler , J. J; Heitmann , M; Zannini , E; Lynch, K. M; Arendt, E. K. (2019). A comparative study of gluten-free sprouts in the gluten-free bread-making process. Eur. Food Res. Technol., 245: 617–629. doi.org/10.1007/s00217-018-3185-2

International Diabetes Federation. IDF Diabetes Atlas, 10th end. Brussels, Belgium: 2021.Avaible at: https://www.diabetesatlas.org. Acess at: November 22, 2022.

Karkle, E., N., L. (2019). Process and ingredient options to improve the nutritional value of bread. Brazilian Food and Nutrition Society (SBAN).

Kawai, K., Takato., S., Sasaki, T., Kajiwara, K. (2012). Complex formation, thermal properties, and *in-vitro* digestibility of gelatinized potato starch–fatty acid mixtures. Food Hydrocoll., 27(1):228-234. doi: doi.org/10.1016/j.foodhyd.2011.07.003

Kharat, S., Medina-Meza, I.G., Kowalski, R.J., Hosamani, A; C.T., R., Hiregoudar, S., Ganjyal, G.M. (2018). Extrusion processing characteristics of whole grain flours of select major millets (Foxtail, Finger, and Pearl). Food Bioprod. Process., 114 (3): 60–71. doi.org/10.1016/j.fbp.2018.07.002.

Khoury, D. E; Balfour-Ducharme, S; Joye, I. J. (2018). A Review on the Gluten-Free Diet: Technological and Nutritional Challenges. Nutrients, 10 (10):1410. doi:10.3390/nu10101410

Kim, S-K; Sohn, E-Y; Lee, I-J. (2009). Starch Properties of Native Foxtail Millet, *Setaria italica* Beauv. J. Crop Sci. Biotech., 12 (1): 59-62. doi: 10.1007/s12892-009-0073-0

Krishnan, V; Mondal, D; Thomas, B; Singh, A; Praveen, S. (2021). Starch-lipid interaction alters the molecular structure and ultimate starch bioavailability: A comprehensive review. Int J Biol Macromol, 182: 626–638. doi.org/10.1016/j.ijbiomac.2021.04.030

Kumar, A; Tomer, V; Kaur, A; Kumar, V; Gupta, K. (2018). Millets: a solution to agrarian and nutritional challenges. Agric & Food Secur, 7. doi.org/10.1186/s40066-018-0183-3

Kumar, P; Kaur, C; Jambh, H. K. (2020). Use of extruded finger millet flour to enhance quality characteristics of barley chapatti. Int. J. Chem. Stud., 8(3):507-513. doi: 10.22271/chemi.2020.v8.i3f.9262

Kumar, P; Kaur, C; Jambh, H. K. (2021). Rheological, textural, and technological modifications in wheat unleavened flatbread substituted with extruded finger millet. Journal Texture Studies, 52, 400–409. doi: 10.1111/jtxs.12595

Lebwohl, B., Sanders, D. S., Green, P. H. R. (2018). Coeliac disease. Lancet, *391 (10115)*: 70–81. doi: doi.org/10.1016/S0140-6736(17)31796-8

Li, C., Oh, S.-G., Lee, D.-H., Baik, H.-W., Chung, H.-J. (2017). Effect of germination on the structures and physicochemical properties of starches from brown rice, oat, sorghum, and millet. Int. J. Biol. Macromol., 105: 931–939. doi:10.1016/j.ijbiomac.2017.07.123

Lionetti, E; Pulvirenti, A; Vallorani, M; Catassi, G; Verma1, A. K; Gatti, S; Catassi, C. (2017). Re-challenge Studies in Non-celiac Gluten Sensitivity: A Systematic Review and Meta-Analysis. Frontiers in Physiology, 8. doi: 10.3389/fphys.2017.00621

Lopez, N. J. S; Loarca-Piña, G; Campos-Vega, R; Martínez, M. G; Sánchez, E. M; Esquerra-Brauer, J. M; Gonzalez-Aguilar, G. A; Sánchez, M. R. (2016). The Extrusion Process as an Alternative for Improving the Biological Potential of Sorghum Bran: Phenolic Compounds and Antiradical and Anti-Inflammatory Capacity. J. Evid.Based Complementary Altern. Med. doi.org/10.1155/2016/8387975

Luiz, R. O; Vanin, F. M. (2022). Effect of straight dough X pre-fermented dough method on composite wheat breads characteristics. Food Sci. Technol., 42, e64420. doi: doi.org/10.1590/fst.64420

Mahan, L. K.; Escott-Stump, S.; Raymond, J.L. (2012). Krause: Alimentos, Nutrição e Dietoterapia. 13ª ed. Rio de Janeiro: Elsevier. 1227 p.

Mahajan, P., Bera, M. B., Panesar, P. S., Chauhan, A. (2021). Millet starch: A review. Intern. Journ. Biolog. Macrom., 180: 61–79. doi: doi.org/10.1016/j.ijbiomac.2021.03.063

Markets and Markets. Gluten-free products market by type (Baked products, Snacks & RTE, Condiments and dressings, Pizzas and pasta), Distribution channel (Conventional stores, Specialty stores and Drugstores & Pharmacies), Form and Region – (2020-2025). Published: September 2020. Available at: https://www.marketsandmarkets.com/Market-Reports/gluten-free-products-market-738.html

Maktouf, S; Jeddou , K. B; Moulis, C; Haji, H; Remaud-simeon, M; Ellouz-Ghorbel, R. (2016). Evaluation of dough rheological properties and bread texture of pearl millet-wheat flour mix. J. Food. Sci. Technol., 53(4):2061–2066. doi: 10.1007/s13197-015-2065-z

Mariotti, M; Garofalo, C; Aquianti, L; Osimani, A; Fongaro, L; Tavoletti, S; Hager, A. S; Clementi, F. (2014). Use of barley flour in sourdough baking: A technological, nutritional and

sensorial evaluation. LWT - Food Sci. Technol., 59: 973-980. doi.org/10.1016/j.lwt.2014.06.052
Marques, E. C; Marques, R. C; Costa, S. R. R. (2015). Aspects of thermoplastic extrusion technology in food on consumer health. Revista Eletrônica Gestão & Saúde, 6 (2): 1935-51.
Marti, A., Cardone, G., Nicolodi, A., Quaglia, L., Pagani, M. A. (2017). Sprouted wheat as an alternative to conventional flour improvers in bread-making. LTW - Food Sci. Technol., 80, 230-236. doi: 10.1016/j.lwt.2017.02.028
McKeown, N. M; Meigs, J. D; Liu, S; Saltzman, E; Wilson, P. E. S; Jacques, P.F. (2004). Carbohydrate Nutrition, Insulin Resistance, and the Prevalence of the Metabolic Syndrome in the Framingham Offspring Cohort. Diabetes Care, 27 (2):538-546. doi: 10.2337/diacare.27.2.538.
Meng, Y; Guan, X; Liu, X; Zhang, H. (2019). The rheology and microstructure of composite wheat dough enriched with extruded mung bean flour. LWT - Food Sci. Technol., 109: 378–386. doi.org/10.1016/j.lwt.2019.03.095
Mohan, B. H., Gopal, A., Malleshi, N. G., Tharanathan, R. N. (2005). Characteristics of native and enzymatically hydrolyzedragi (*Eleusine coracana*) and rice (*Oryza sativa*) starches. Carbohyd. Polym., 59: 43–50. doi: doi.org/10.1016/j.carbpol.2004.08.022
Mondal, D; Awana, M; Aggarwal, S; Das, D; Thomas, B; Singh, S. P; Satyavathi C, T; Sundaram, R. M; Anand, A; Singh, A; Sachdev, A; Praveen, A; Krishnan, V. (2022). Microstructure, matrix interactions, and molecular structure are the key determinants of inherent glycemic potential in pearl millet (*Pennisetum glaucum*). Food Hydrocoll., 127, 107481
Mościcki, L; Van Zuilichem D. J. (2011). Extrusion-Cooking and Related Technique. In Moscicki, L (Org.), Extrusion-Cooking Techniques. 1 ed. Lublin: Wiley-VCH.
Muñoz, R., De Las Rivas, B., De Felipe, F. L., Reverón, I., Santamaría, L., Esteban-Torres, M., ... Landete, J.M. (2016). Biotransformation of Phenolics by *Lactobacillus plantarum* in Fermented Foods. *In:* Frias, J; Martinez-Villaluenga, C; Peñas, E (org). Fermented Foods as a Source of Healthy Constituents. Elsevier: Amsterdam, 417–432.
Muthamilarasan, M., Dhaka, A., Yadav, R., Prasad, M. (2016). Exploration of millet models for developing nutrient rich graminaceous crops. Plant Sci., 242: 89-97. doi: 10.1016/j.plantsci.2015.08.023.
Nami, Y; Gharekhani, M; Aalami, M; Hejazi, M. A. (2019). Lactobacillus-fermented sourdoughs improve the quality of gluten-free bread made from pearl millet flour. J. Food Sci. Technol., 56 (9): 4057–4067. doi: 10.1007/s13197-019-03874-8
Novotni, D; Najara, L; Strkalj, L; Drakula, S; Mustac, N. C; Voucko, B; Curic, D. (2023). Influence of Particle Size and Xylanase Pretreatment of Proso Millet Bran on Physical, Sensory and Nutritive Features of Gluten-Free Bread. Food Technol. Biotechnol., 61 (1): 73-84. doi.org/10.17113/ftb.61.01.23.7776
Núñez-Musa, R., Núñez-Sellés, A. J., Rossi, W. M., Marmolejos, R. G., Martínez-Sánchez, G., Selman-Almonte, A. (2020). Posibilidades de la terapia antioxidante en diabetes mellitus tipo 2. Estudio del estrés oxidativo en una muestra poblacional de pacientes diabéticos. Cienc. Salud, 4 (3). doi: doi.org/10.22206/cysa.2020.v4i3.pp71-73
Olamiti G., Takalani, T. K., Beswa, D., Jideani, A. I. O. (2020). Effect of malting and fermentation on colour, thermal properties, functional groups and crystallinity level of flours from pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*). Heliyon, 6.

Oliveira, M. C; Schoffen, J. P. F. (2010). Oxidative Stress Action in Cellular Aging. Braz. Arch. Biol. Technol., 53 (6): 1333-1342. doi.org/10.1590/S1516-89132010000600009

Onweluzo, J. C; Nwabugwu, C. C. Fermentation of Millet (*Pennisetum americanum*) and Pigeon Pea (*Cajanus cajan*) Seeds for Flour Production: Effects on Composition and Selected Functional Properties. Pak. J. Nutr., 8 (6): 737-744. doi: 10.3923/pjn.2009.737.744

Ortiz-Cruz, R. A; Ramírez-Wong, B; Ledesma-Osuna, A. N; Torres-Chávez, P. I; Sánchez-Machado, D. I; Montaño-Leyva, B; López-Cervantes, J; Gutiérrez-Dorado, R. (2020). Effect of Extrusion Processing Conditions on the Phenolic Compound Content and Antioxidant Capacity of Sorghum (*Sorghum bicolor* (L.) Moench) Bran. Plant Foods for Hum. Nutr., 75:252–257. doi.org/10.1007/s11130-020-00810-6

Pan American Health Organization (2016). Risk factors for chronic non-communicable diseases in the Americas: Considerations on strengthening regulatory capacity. REGULA Technical Reference Document. Washington, DC.

Pessanha, K. L.F; Menezes, J. P; Silva, A. A; Ferreira, M. V. S; Takeiti, C. Y; Carvalho, C. W. P. (2021). Impact of whole millet extruded flour on the physicochemical properties and antihyperglycemic activity of gluten free bread. LWT - Food Sci. Technol., 147: 111495. doi.org/10.1016/j.lwt.2021.111495

Palanisamy, T; Sree, R. (2020). Efficacy of millets (foxtail, kodo, small, barnyard and pearl millet) varieties on post prandial glycaemic response in patients with type 2 diabetes. Eur J Biomedp. Sci., 7 (7): 443–449.

Papakonstantinou, E; Oikonomou, C; Nychas, G; Dimitriadis, G. D. (2022). Effects of Diet, Lifestyle, Chrononutrition and Alternative Dietary Interventions on Postprandial Glycemia and Insulin Resistance. Nutrients, 14(4): 823. doi: 10.3390/nu14040823

Peña, A. S; Rodrigo, L. Epidemiology of Celiac Disease and Non-Celiac Gluten-Related Disorders. *In:* Arranz, E; Fernández-Bañares, F; Rosell, C. M; Rodrigo, L; Peña, A. S (org). Advances in the Understanding of Gluten related Pathology and the Evolution of Gluten-Free Foods. 1st edition. Omnia Science. Available in: https://www.omniascience.com/books/index.php/monographs/catalog/book/83. Access at: 22 de novembro de 2022.

Pereira, M. A; Ortiz-Agostinho C. L; Nishitokukado I; Sato, M. N; Damião, A. O. M. C; Alencar, M. L; Abrantes-Lemos, C. P; Cançado, E. L. R; de Brito, T; Ioshii, S. O; Valarini, S. B. M; Sipahi, A. M. (2006). Prevalence of celiac disease in an urban area of Brazil with predominantly European ancestry. World J Gastroenterol, 12: 6546-50. doi: 10.3748/wjg.v12.i40.6546
Pessanha, K. L.F; Menezes, J. P; Silva, A. A; Ferreira, M. V. S; Takeiti, C. Y; Carvalho, C. W. P. (2021). Impact of whole millet extruded flour on the physicochemical properties and antihyperglycemic activity of gluten free bread. LWT - Food Sci. Technol., 147: 111495. doi.org/10.1016/j.lwt.2021.111495
Pradeep P.M., Sreerama Y. N. Impact of processing on the phenolic profiles of small millets: Evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia. Food Chem., 169: 455-463, 2015. doi: 10.1016/j.foodchem.2014.08.010

Prakash, D. S; Nkurikiye, E; Rajpurohit, B; Li, Y; Siliveru, K. (2023). Significance of different milling methods on white proso millet flour physicochemical, rheological, and baking properties. J. Texture. Stud., 54:92-104. doi: 10.1111/jtxs.12717

Punia, S; Kumar, M; Siroha, A. K; Kennedy, J. F; Dhull, S. B; Whiteside, W. S. (2021). Pearl millet grain as an emerging source of starch: A review on its structure, physicochemical properties, functionalization, and industrial applications. Carbohyd. Polym., 260: 17776.

Ragaee, S., Abdel-Aal, E. M., Noaman, M. Antioxidant activity and nutriente composition of selected cereals for food use. Food Chem., 98: 32–38. doi:10.1016/j.foodchem.2005.04.039

Romão, B., Botelho, R. B. A., Alencar, E. R., da Silva, V. S. N., Pacheco, M. T. B. and Zandonadi, R. P. (2020). Chemical composition and glycemic index of gluten-free bread commercialized in Brazil. Nutrients, 12(8), 1-12. https://doi.org/10.3390/nu12082234

Rudra, S. G; Jakhar, N; Nishad, J; Saini, N; Sen, S; Bhardhwaj , R; Jaiswal, R; Suneja , P; Singh, S; Kaur, C. (2015). Extrusion Conditions and Antioxidant Properties of Sorghum, Barley and Horse Gram Based Snack. Int. J. Plant Res., 28 (2): 171-182. 10.5958/2229-4473.2015.00053.1

Saleh, A. S. M., Zhang, Q., Chen, J., Shen, Q. (2013). Millet Grains: Nutritional Quality, Processing, and Potential Health Benefits. Compr. Rev. Food Sci. Food Saf., 12:281-295. doi: doi.org/10.1111/1541-4337.12012.

Santos, F.G; Aguiar, E. V; Capriles, V. D. (2019). Analysis of ingredient and nutritional labeling of commercially available gluten-free bread in Brazil. Int J Food Sci. Nutr, 70 (5): 562-569. https://doi.org/10.1080/09637486.2018.1551336

Selladurai, M; Pulivarthi, M. K; Raj, A. S; Iftikhar, M; Prasad, P. V. V; Siliveru, K. (2023). Considerations for gluten free foods - pearl and finger millet processing and market demand. Grain & Oil Sci Tech, 6: 59–70. doi.org/10.1016/j.gaost.2022.11.003

Sharma, S; Saxena, D. C; Riar, C. S. (2015). Antioxidant activity, total phenolics, flavonoids and antinutritional characteristics of germinated foxtail millet (*Setaria italica*). Cogent Food Agric., 1:1, 1081728. doi: 10.1080/23311932.2015.1081728

Sharma, N; & Niranjan, K. (2018). Foxtail millet: Properties, processing, health benefits, and uses. Food Rev. Intern., 34(4): 329-363. doi:10.1080/87559129.2017.1290103

Sharma, S; Jan, R; Riar, C. S; Bansal, V. (2021). Analyzing the effect of germination on the pasting, rheological, morphological and in- vitro antioxidant characteristics of kodo millet flour and extracts. Food Chem., 361: 130073. doi.org/10.1016/j.foodchem.2021.130073

Singh, K.P., Mishra, A., Mishra, H.N. (2012). Fuzzy analysis of sensory attributes of bread prepared from millet-based composite flours. LWT - Food Sci. Techn., 48 (2): 276-282. doi.org/10.1016/j.lwt.2012.03.026

Singh, P; Raghuvanshi, R. S. (2012). Finger millet for food and nutritional security. African Journ. Food Sci., 6(4): 77-84. doi: 10.5897/AJFSX11.010

Smulders, M. J. M; van de Wiel, C. C. M; van den Broeck, H. C; van der Meer, I. M; Israel-Hoevelaken, T.P. M; Timmer, R. D; van Dinter, B.J; Braun, S; Gilissen, L. J. W. J. (2018). Oats in healthy gluten-free and regular diets: A perspective. Food Res Intern, 110: 3–10. doi.org/10.1016/j.foodres.2017.11.031

Sun, J; Luu, N. S; Chen, Z; Chen, B; Cui, X; Wu, J; Zhang, Z; Lu, T. (2019). Generation and Characterization of a Foxtail Millet (*Setaria italica*) Mutant Library. Front. Plant Sci., 10. doi.org/10.3389/fpls.2019.00369

Taylor, J. R. N; Duodu, T. K. (2015). Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. J Sci Food Agric, 95: 225–237. doi: 10.1002/jsfa.6713

Taylor, J. R. N. (2016). Millet pearl: Overview encyclopedia of food grains (second edition). Oxford: Academic Press: 190–198.
Theodoro, J. M. V; Martinez, O. D. M; Grancieri, M; Toledo, R. C. L; Dias Martins, A. M; Dias, D. M; Carvalho, C. W. P; Martino, H. S. D. Germinated millet flour (*Pennisetum glaucum* (L.) R. Br.) reduces inflammation, oxidative stress, and liver steatosis in rats fed with high-fat high-fructose diet. J. Cereal Sci., 99: 103207. doi.org/10.1016/j.jcs.2021.103207
Torbica, A; Belović, M; Tomić, J. Novel breads of non-wheat flours (2019). Food Chem., 282: 134–140. doi.org/10.1016/j.foodchem.2018.12.113
Vega-López, S., Venn, B. J., , J. L. (2018). Relevance of the Glycemic Index and Glycemic Load for Body Weight, Diabetes, and Cardiovascular Disease. Nutrients, Oct; 10(10): 1361. doi: 10.3390/nu10101361.
Villarino, C. B; Jayasena, V; Coorey, R; Chakrabarti-Bell, S; Johnson, S. (2014). The effects of bread-making process factors on Australian sweet lupin-wheat bread quality characteristics. Int. J. Food Sci. Technol., 49: 2373–2381. doi:10.1111/ijfs.12595
Wang, T.; He, F.; Cheng, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. J. Funct. Foods, 7:101–111. doi.org/10.1016/j.jff.2014.01.033
Wang, X; Zhao, R; Yuan, W. (2020). Type I sourdough steamed bread made by retarded sponge-dough method. Food Chem., 311:126029. doi.org/10.1016/j.foodchem.2019.126029
Wankhede, D. B; Shehnaj, S; Raghavendra Rao, M. R. (1979). Carbohydrate composition of finger millet (*Eleusine coracana*) and foxtail millet (*Setaria italica*). Qualitas plantarum, 28: 293–303.
Watanabe, M; Maeda, T; Tsukahara, K; Kayahara, H; Morita, N. (2004). Application of Pregerminated Brown Rice for Breadmaking. Cereal Chem, 81(4): 450–455. doi: doi.org/10.1094/CCHEM.2004.81.4.450
Wen, Y; Liu, J; Meng, X; Zhang, D; Zhao, G. (2014). Characterization of Proso Millet Starches from Different Geographical Origins of China. Food Sci. Biotechno, 23(5): 1371-1377. doi 10.1007/s10068-014-0188-z
Wilson, J. P., Jurjevic, Z., Hanna, W. W., Wilson, D. M., Potter, T. L., Coy, A. E. (2006). Host-specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and corn. Mycopath., 161: 101–107. doi: 10.1007/s11046-005-0170-7.Woomer, J. S; Adedeji, A. A. (2020). Current applications of gluten-free grains – a review. Crit Rev Food Sci Nutr, 61(1):14-24. doi.org/10.1080/10408398.2020.1713724

CAPÍTULO 2 - ROLE OF BAKING PROCESS ON THE PHYSICAL PROPERTIES AND NUTRITIONAL CHARACTERISTICS OF PEARL MILLET-BASED BREADS

ROLE OF BAKING PROCESS ON THE PHYSICAL PROPERTIES AND NUTRITIONAL CHARACTERISTICS OF PEARL MILLET-BASED BREADS

Original Article

Lorrany Miranda Marinho[a], Monique Barreto Santos[b], Maria Eugênia Araújo Silva Oliveira[a], Renata Marenda Ferreira[a], Mariana Larraz Simões Ferreira[a], Carlos Wanderlei Piler de Carvalho[b], Cristina Yoshie Takeiti[a,b]

[a] *Post graduation Program of Food and Nutrition, Federal University of the State of Rio de Janeiro, UNIRIO, Rio de Janeiro, RJ, Brazil.*

[b] *Brazilian Agricultural Research Corporation, Embrapa Food Agroindustry, Rio de Janeiro, RJ, Brazil*

*Corresponding author: E-mail: cristina.takeiti@embrapa.br

ABSTRACT

The absence of gluten-forming proteins leads to a major technological challenge in bakery production. This study investigated the effect of the role of different baking processes on the phenolic profile and physicochemical properties, such as antioxidant capacity and antihyperglycemic capacity, of breads made exclusively with millet flour. In addition, it was evaluate the inhibition of the α-amylase enzyme of millet-based breads. The breads were processing by straight and sponge-dough methods using different proportions of raw (RMF), extruded (EMF) and germinated millet flour (GMF). The formulation made with a mix of raw, extruded and germinated millet flour (REGMF) using sponge-dough demonstrated higher values of dietary fiber (10.83g/100g), and phenolic compounds (4131.8 mg GAE/100g) whith antioxidant capacity of DPPH = 3.06 mg/g. However, this sample present the highest hardness value (19.85 N), that could be correlated with its higher amylose value (41.18%). The formulation made with a mix of raw and extruded millet flour (REMF) sponge-dough sample showed greater antioxidant capacity (2133.0 µmole/g) by the FRAP method. Interestingly, only the refined-wheat sample (control) showed α-amylase inhibition activity in the free and bound extracts. Among the millet bread samples, the REMF sponge-dough sample demonstrated the lowest IC_{50} (1.36 mg/mL), that is, lower concentration needed to inhibit 50% of the enzyme α-amylase. Results suggest that millet-based breads consist in a good food resource for health promotion.

Keywords: gluten-free, breadbaking process, phenolic compounds, antihyperglycemic activity.

1. Introduction

Bread is one of the most representative food in the world, consisting an important part of the modern human diet (Yano, 2019). Bread is commonly obtained by cereals, notably by use of wheat, in which mixed with water and salt forms a viscoelastic matrix that provides the necessary development of the dough for the desirable development of the bread. However, the market driver for gluten-free products, especially bread is due to the diagnosis of diseases associated with the presence of gluten or the consumption trend based on the popular belief that these products are healthier. Therefore, there is a necessity to produce these analogue products using other raw materials (Selladurai et al., 2023) that present mimetic characteristics in terms of gluten matrix development.

Breadmaking involve physicochemical changes that occur during the steps of mixing, fermentation, baking, and cooling. Massive efforts are given to gluten-free breads, gluten-free dough lacks a viscoelastic network, compromising product quality, especially texture, which makes it necessary to design matrices that mimic breadmaking requirements (Santos & Capriles, 2021). In addition, there is a necessity to improve the nutritional quality once these products commonly have a high content of sugar and fat in order to compensating the absence of some technological properties, increasing the risk factor for the development of chronic non-communicable diseases (Gobbetti et al., 2018).

In this context, grains of millet can be considered a good source of alternative material due to high nutritional value and hypoglycemic properties. Millets are rich in proteins (6% to 13%), calcium (10–348 mg/100 g) and iron (2.2–17.7 mg/100 g). Dietary fiber content (18%) has a significantly higher value compared to rice and wheat providing a positive effect on the glycemic profile of millet-based breads (Kumar et al., 2020; Kaur et al., 2019). Furthermore, it contains significant amounts of phytochemicals that are associated with antioxidant and hypoglycemic properties by inhibiting the activity of pancreatic α-glucosidases and α-amylase enzymes (Li et al., 2020). However, the type of processing affect the concentration and bioavailability of nutrients in terms of phytochemicals such as polyphenols (Abiyoe et al., 2022). Pearl millet grains have low concentrations of benzoic acid derivatives (hydroxybenzoic acid, gallic acid, p-hydroxybenzoic, vanillic, syringic and protocatechuic), but high levels of cinnamic acid derivatives (hydroxycinnamic, coumaric, ferulic, sinapic) (Chandrasekara & Shahidi, 2011). According to

Nani et al. (2015), pearl millet contained *p*-coumaric (1350.0 µg/g), ferulic acid (199.0 µg/g), hydroxycinnamic acid (41.3 µg/g), gallic acid (15.3 µg/g), syringic acid (7.4 µg/g), and.

Although the effect of different millet processing on nutritional quality improvement has been established, the comparison of baking process on the physical properties and nutritional characteristics of pearl millet-based breads is not reported in the literature. Hence, the present study was conducted in order to evaluate the impact of different bread-making process (straight and sponge dough) on the physical properties, nutritional quality, bioactive compounds profile, antioxidant capacity and antihyperglycemic activity.

2. Materials and methods

2.1 Materials

Pearl millet (*Pennisetum glaucum (L.)* R. Br.), ADR 9070 cultivar, was provided by Atto Sementes (Rondonópolis, Brazil). Whole wheat flour (WWF), refined wheat flour (RWF) and other ingredients (eggs, sugar, palm fat, salt and dry yeast) were acquired at a market in Rio de Janeiro, Brasil. Acarbose® was purchased from Sigma-Aldrich (Pharmaceutical Secondary Standard, ref. 56180-94-0, Darmstadt, Germany). Enzyme α-amylase from Porcine Pancreas (Sigma-Aldrich ref. A3176, Saint Louis, USA),

2.2 Technological flour characterization

2.2.1 Preparation of flour

Grains were cleaned and ground using a disc mill for breaking grains followed by the use of a hammer mill LM3100 (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm opening screen for obtaining fine raw millet flour.

2.2.2 Germination of millet

Grains were soaked in water (1:3) (grain:water) during 1 h and then drained. The grains were allowed to germinate in a fermentation cabinet (Eight doors fermentation cabinet, National Mfg. Co., Lincoln, USA) at a controlled temperature of 30 °C and relative humidity of 90%. After 24 h, the grains were dried in a fan oven (model DMS-G.E, Macanuda, Joinville, Brasil) at 45 °C/24 h, and ground using the same procedure as mentioned above for raw millet flour in order to obtain germinated millet flour (GMF).

2.2.3 Extrusion of millet

Prior to extrusion, the pearl millet flour was preconditioned to reach final moisture content to 15% (wb). The flour was extruded in a single-screw laboratory extruder (Plasti-Corder® Lab-Station 19/25, Brabender, Duisburg, Germany). The extruder was equipped with 3:1 compression screw, 3 mm circular die, rotation speed of 200 rpm, temperature profile heating zones (40°C, 80°C, 90°C). The extrudates were dried in a tray dryer at 55°C for 10 h, followed by grinding the dried extrudates in order to obtain a extruded millet flour (EMF) using the same conditions as raw flour preparation.

2.2.4 Particle size distribution (PSD)

Flour particle size was measured by the laser diffraction using an S3500 series particle size analyzer (Microtrac Inc., Montgomeryville, USA) according to method 55–40.01 (AACC, 1999) using deionized water. Particle sizes were expressed in terms of mean particle size of D [4,3] (volume or mass moment mean) calculated by the Flex Software, version 11.0.0.3 (Microtrac, USA).

2.2.5 Paste viscosity

The viscoelastic properties of samples were analyzed by Rapid Viscosity Analyser (RVA-4, Newport Scientific Pty Ltd., Warriewood, Austrália). Three grams of the flour adjusted to 14% of moisture content (wb) were placed with 25 mL of distilled water in the sample holder (aluminum cup). The equipment starts the analysis at 25 °C for 2 minutes; heats at a rate of 14 °C/min until reaching 95 °C and remains at this temperature during 3 minutes; cool to 25 °C at the same rate and maintain this temperature (25 °C) for another 5 minutes, totalizing 20 minutes of analysis. The following apparent viscosity properties were evaluated as a function of temperature: paste temperature (T_p) (°C), cold viscosity at the beginning 25 ˚C (CV, cP), peak viscosity (PV, cP), breakdown viscosity (BDV= PV-TV, cP), final viscosity (FV, cP), and setback viscosity (SBV= FV-TV, cP).

2.3 Bread elaboration

A straight and sponge dough method were used in preparation of breads according methods 10-10.03 and 10-11.01 (AACC, 1999), with modifications. Breads were made following the formulations presented in Table 2. The maximum water absorption was determined using a 50g FD0234H Farinograph® (Brabender, Duisburg, Alemanha) according to method 54-21.02 (AACC International, 2000). The breads were made exclusively with millet flour and free from additives. the use of additives tends to increase the cost of production, which makes the product inaccessible to many consumers, in addition to not covering the market of consumers looking for clean label products, a current trend.

Dough preparation was performed with a 35 g Micromixer (National MFG. CO., Lincoln, U.S.A.) (Figure 3). Instant yeast (Fleischmann, Pederneiras, Brazil) was previous activated with deionized water (1/3 of the total formulation water) at 35 ˚C and placed in a fermentation chamber at 85% relative humidity during 10 min for activation. In case of straight-dough (st) method, the dry ingredients were added and mixed for 2 minutes before pouring the liquid ingredients and palm fat. After mixing all the ingredients, the dough was portioned in 20 g, shaped, and placed into previously greased and floured steel molds of 45 mL capacity, then were placed in a fermentation cabinet at 30 °C and 85% relative humidity for 45 min (Crescepão ACT20, Venâncio Metalúrgica, Venâncio Aires, Brasil). Finally, breads were baked using a convection oven Maxiconv Skymsen (Metalúrgica Siemsen, Brazil), at 180 °C for 12 min and then cooling during 1h at room temperature for further analysis in comparison to breads control made with commercial flour (whole wheat flour, Control 1; refined wheat flour, Control 2). The sponge dough (sp) process involves two stages: first the creation of a light, airy ‘sponge’ by mixing a portion of the flour, water, instant yeast and fermenting for 4 h. The rest of the ingredients are incorporated into the 'sponge' and the resulting 'dough' is divided, shaped and submitted to final fermentation (45 min) before baking. Tests were carried out according to the following in both straight (st) and sponge (sp) dough methods: (i) raw millet flour (100%) (RMF); (ii) extruded millet flour (100%) (EMF); (iii) raw and extruded millet flour (REMF) (1:1); (iv) raw, extruded and germinated millet flour (REGMF) (1:1:0.05); (v) extruded and germinated millet flour (EGMF) (1:0.05) and (vi) raw and germinated millet flour (RGMF) (1:0.05). In addition, control samples were prepared with whole (WWF) and refined (RWF) wheat flour. These formulations (millet breads and control) were evaluated in terms of color analysis, specific volume, proximate composition and dietary fiber content in comparison to commercial samples (whole wheat bread (WWB) and gluten-free bread (GFB) in order to screening samples submitted to nutritional characterization analyses due to these analyzes presented a great cost and long operating time.

Figure 3. Bread dough made with millet flour.

Table 2. Bread formulations and particle size distribution millet flours.

	Ingredients (%)											
Samples	RMF	EMF	GMF	WWF	RWF	Fat	Yeast	Sugar	Eggs	Salt	Water absorption*	D [4,3]
1	100	-	-	-	-	6	3	6	35	2	45.01	$164.85^{c} \pm 2.6$
2	0	100	-	-	-	6	3	6	35	2	51	$148.08^{c} \pm 26.7$
3	50	50	-	-	-	6	3	6	35	2	70.8	$173.60^{c} \pm 12.7$
4	47.5	47.5	5.0	-	-	6	3	6	35	2	47.74	$263.65^{b} \pm 28.6$
5	95	-	5	-	-	6	3	6	35	2	45.29	$150.01^{c} \pm 6.4$
6	-	95	5	-	-	6	3	6	35	2	54.3	$272.90^{ab} \pm 11.2$
Control 1	-	-	-	100	-	6	3	6	35	2	55	$314.63^{a} \pm 46.9$
Control 2	-	-	-	-	100	6	3	6	35	2	55	$75.92^{d} \pm 3.6$

RMF: raw millet flour; EMF: extruded millet flour; GMF: germinated wheat flour; WWF: whole wheat flour; RMF: refined wheat flour; *Water absorption was based on farinograph parameters showed in Appendix; D [4,3]: Volume or Mass Moment Mean.

2.4 Bread technological characterization

2.4.1 Color measurements

The color of crust (ct) and crumb (cb) of breads were measured by reflectance with a portable colorimeter CR-400 (Konica Minolta, Tokyo, Japan) used illuminant D65/2°. The results were expressed according to the CIEL*a*b* colour space parameters. The colour values were expressed as lightness (L) and measure black to white (0 to 100); a* indicate hue angle (H°) on green (-80 to 0) to red (zero to+100) axis and b* indicate H° on blue (-100 to zero) to yellow (zero to+70) axis (Martinez-Giron et al., 2017). The changes in the bread crust and crumb colour were estimated according to the browning index (BI) as follows:

$$BI = \frac{100\ (x-0.31)}{0.17} \tag{1}$$

Where:

$$x = \frac{a+1.75L}{5.645L+a-3.01b} \tag{2}$$

2.4.2 Specific volume analysis

Loaf volume was determined using a standard seed displacement method n. 10–05.01 (AACC, 2000), using millet seeds. Each bread was weighed and the volume was measured 1h after baking. The specific volume was calculated as bread volume divided by bread weight (cm^3/g).

2.4.3 Texture analysis

The texture profile analysis (TPA) evaluated parameters of bread namely hardness (Hd, N), Adhesiveness (Ad, g·s), cohesiveness (Co), springiness (Sp), chewiness (Ch, N), and resilience (R) were measured by Texture Analyser TA-XT Plus (Stable Micro Systems, Surrey, U K) equipped with a 5 kg load cell and a 15 mm aluminum cylindrical probe. Each dough sample was studied in 12 replications. Run was carried out using the center of the bread crumb slices with a thickness of 20 mm. The analysis was controlled by the Exponent software version 6.1.11.0 (Stable Micro Systems, Surrey, UK) at a compression of 50% and 30s cycle according to Comettant-Rabanal et al., 2021.

2.5 Bread nutritional characterization

2.5.1 Chemical composition

The chemical composition of bread and commercial samples were performed according to the AOAC (2000) methods. The protein content was calculated using method 2001.11 (factor of 5.75), fat (method 945.38), ash (method 923.03), total dietary fiber (method 991.43) and moisture (method 925.09). Carbohydrate content was calculated by diference.

2.5.2 Total starch, amylose and resistant starch contents

Total starch, amylose and resistant starch (RS) contents of breads were determined using a Megazyme Assay kits K-TSTA-100A by method 76-13.01 (AACC, 2000), K-AMYL by method 79-13 (AACC, 2000) and K-RSTAR by methods 32-40.01 (AACC, 2002) respectively. The results were expressed in percentage.

2.6 Determination of phenolic content and antioxidant capacity

2.6.1 Sequential extraction of free and bound phenolic compounds

Phenolic compounds of liofilized breads were extracted in triplicate according to the method reported by Santos et al. (2019) with modifications. Free phenolics were extracted by manual maceration of 210 mg of each sample and 50 mg of Celite in the presence of 80% ethanol. Samples were stirred (200 rpm, 10 min, 25 °C) and centrifuged (5000-× *g*, 10 min, 25 °C). The supernatants were retained and the extraction was repeated. The supernatants of each replicate were dried in an evaporator centrifuge (Savant, SpeedVac Concentrator, ThermoFisher Scientific, USA). The pellets resulting from extraction of free phenolics were re-suspended with 4M NaOH and then placed in an ultrasonic bath (42 kHz) for 90 min at 40 °C. After the alkaline hydrolysis, the pH was adjusted to below 2.0 with concentrated HCl and the samples were centrifuged at 2.000 x g for 5 min. The supernatant was washed three times with ethyl acetate and centrifuged between each step (10000 ×*g*, 5 min, 10 °C). All supernatants were collected and dried. All dried extracts were re-suspended in 1.5 mL of Solution A containing 2% methanol, 5% acetonitrile and 93% ultrapure water and then filtered (13 mm, 0.22 µm), transferred to vials and stored at - 20 °C until analysis.

2.6.2 Determination of total phenolic content (TPC)

Total phenolic content were estimated by measuring their capacity to reduce Folin-Ciocalteu reagent in triplicate according to Singleton et al. (1999), adapted to microplates. Absorbance was determined at 750 nm on a microplate reader (FlexStation III, Molecular Devices, LLC., San Jose, USA). The phenolic content was calculated using a gallic acid standard (5–130 mg/L) calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample (dry basis).

2.6.3 DPPH Free Radical Scavenging Capacity

DPPH radical-scavenging ability of bread extracts were evaluated according to Pires et al. (2017). The stock solution was prepared by dissolving DPPH powder in methanol solvent, and the absorbance range was maintained between 0.9 – 1.1. Briefly, extract was mixed with DPPH solution, and incubated for 30 min in the dark at room temperature after shaking vigorously. The DPPH solution containing distilled water was considered the blank sample. Samples were analyzed at 517 nm using a microplate reader (FlexStation III, Molecular Devices, LLC., San Jose, USA). Antioxidant capacity was calculated using a standard curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid – Trolox) and the results were expressed in mg/g of dried sample.

2.6.4 Determination of ferric reducing antioxidant power (FRAP)

The reducing capacity of the bread extract was evaluated according to Urrea-Victoria et al. (2016). The FRAP assay is based on the reduction of the Fe (III) –TPTZ complex to the ferrous form at low pH. FRAP reagent was prepared daily and consisted of acetate buffer, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution and ferric chloride. Absorption was measured at 595 nm using a microplate reader, after 30 min incubation at 37 °C. Antioxidant capacity was calculated using a standard curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid – Trolox) and the results were expressed in µmol Trolox/100 g of dried sample.

2.7 Antihyperglycemic activity of breads

The inhibition assay for the α-amylase activity was based on Aleixandre et al. (2022), with some modifications. Briefly, the enzyme α-amylase from Porcine Pancreas (Sigma-Aldrich ref. A3176, Saint Louis, USA) was dissolved in deionized water. The substrate (6.25 mg/ml) was prepared from a solution of wheat starch in sodium phosphate buffer (0.02 M, pH 6.9 containing NaCl 6 mM) followed by gelatinization in a water bath at 100 °C for 20 min and keep at 60 ºC until used in the reaction. 50 µL of sample extract was added to a test tube and placed in a water bath at 37 ºC, followed by the addition of 50 µL of enzyme and homogenization. The tubes were kept in incubation for 10 minutes and then added 400 µL of substrate for another 10 minutes. After this time, 0.5 ml of DNS (3,5-Dinitrosalicylic acid) was added, followed by a new incubation at 100 ºC for 10 minutes, followed by the addition of deionized water (9 ml) and subsequent readings. Samples were analyzed at 540 nm in a spectrophotometer (Weblabor model wuv-m51, Milano, Itália).

Solutions without extract and without enzymes were analyzed as control and blank, respectively. The inhibition rate of the extracts was calculated according to the Equation (1). A positive control sample was done using the commercial hypoglycemic drug (Acarbose®, Pharmaceutical Secondary Standart, ref 56180-94-0, Sigma-Aldrich, Darmstadt, Alemanha). The IC_{50} value is defined as the concentration required for 50% inhibition of the α-amylase activity. From a linear regression curve obtained by different test dilutions.

$$\%\ \text{Enzyme inhibition} = \left[1 - \frac{(Abs_{\text{sample}} - Abs_{\text{negative control}})}{Abs_{\text{positive control}} - Abs_{\text{blank}}}\right] \times 100 \quad (1)$$

where: *Abs*sample is the absorbance of sample (extract + substrate + enzyme), *Abs*negative control is the absorbance obtained without enzyme, *Abs*positive control the reaction is the absorbance without extracts, and *Abs*blank is the absorbance of substrate.

2.8 Statistical analysis

Statistical analysis was performed with one-way ANOVA followed by a Tukey's HSD test ($p < 0.05$) using XLSTAT software (version 2023.2.0, Lumivero, Denver, CO, USA). Principal Components Analysis (PCA), Hierarchical Clustering Analysis (HCA) and Radar Charts (RC) was carried out by XLSTAT and a correlogram for correlation analysis and the significance test were generated in the R package "corrplot" by RStudio software (version 1.2.5042, RStudio, Boston, USA).

3 Results and Discussion

3.1 Flour characterization

3.1.1 Particle size distribution of flours (PSD)

The particle size of EGMF and REGMF were significantly ($p < 0.05$) larger D [4.3] = 272.9 µm and D [4.3] = 263.6 µm, respectively, among samples millet flour (Table 2). Particle size is an important factor related to bakery products, as it affects the rheological characteristics of the dough and the final product texture. It has already been shown that the particle size of bran used in breadmaking generally affects the technological, sensory and nutritional quality of bread. As demonstrated by Novotni et al. (2023), breads elaborated with coarse millet bran presented greater specific volume. Flours with larger particle sizes increase the amount of damaged starch, i. e. more starch is accessible to enzymatic reaction. Greater starch accessibility promotes a high degree of gelatinization and starch retrogradation that favors the formation of a three-dimensional network, and thus mimicking the viscoelastic properties of gluten contributing to the final structure of the gluten-free dough (Ferreira et al., 2016).

3. 1. 2 Pasting profile of flours

Extruded millet flour, isolated or combined with germinated flour, presented values of paste properties (PV, BDV, SBV and FV) significantly lower than RMF ($p<0.05$) (Supplementary material, Table S.1). These finding can be explained due to the starch granules swelling and breaking during the extrusion process. The crystalline arrangement and cold-water solubility changed, promoting a decrease in viscosity (Meng et al., 2019). This observation was in accordance with the results obtained by Patil et al. (2016) and Kumar et al. (2020) in which there was a significant decreasing trend in the same pasting properties of millet. The addition of GMF to EFM also contributing to the decreases of viscosities observed.

The REGMF mixture (Figure 4) demonstrated lower BDV, which may characterize advantageous effects of using this flour, as lower BDV values indicate that the starch is more suitable to resist heating and shear stress during cooking, besides showed a lower tendency to retrograde. Maximum values of pasting properties were observed for refined wheat flour. This can be justified by the high gluten content in wheat, resulting in greater adhesion of starch granules and a consequent increase in apparent viscosity.

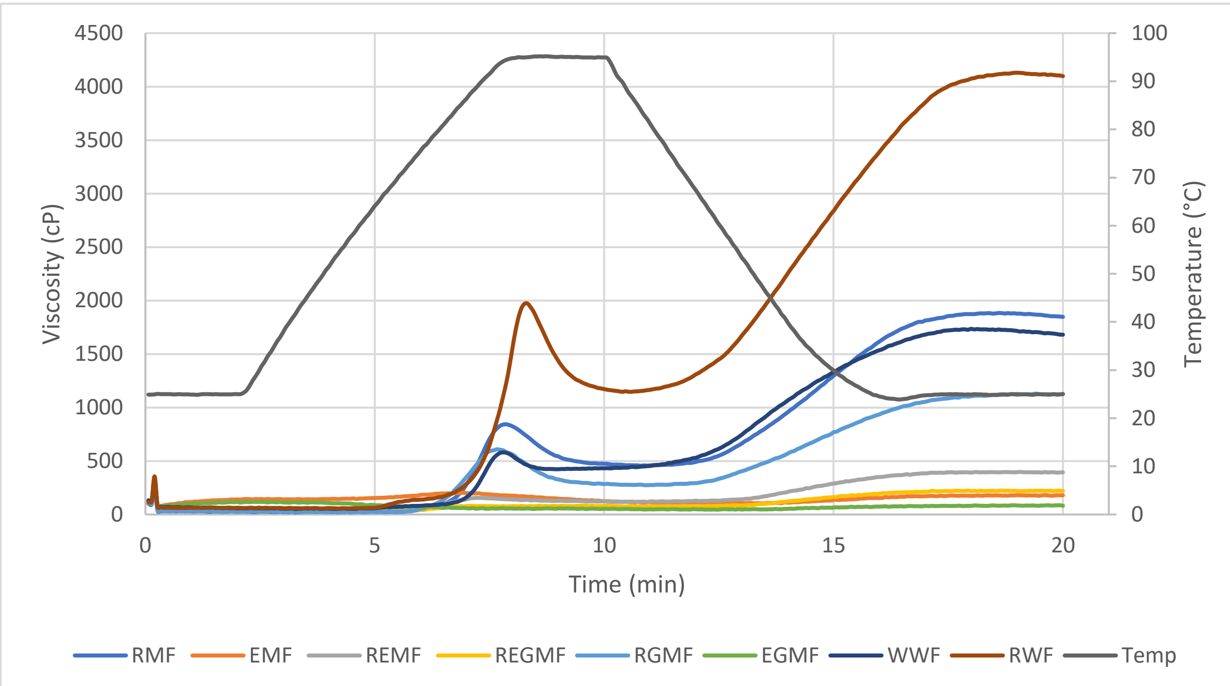


Figure 4. Paste viscosity properties of (i) RMF; (ii) EMF; (iii) REMF; (iv) REGMF; (v) RGMF; (vi) EGMF; (vii) WWF (commercial whole wheat flour), and (viii) RWF (commercial refined wheat flour).

3. 2 Quality evaluation of bread

3. 2. 1 Instrumental color analysis

The crumb and crust color parameters of breads are presented in Table 3. As expected, the crumb of refined-wheat flour bread presented the highest value of L* and the lowest BI ($p<0.05$) in both breadmaking processes. In general, all millet breads showed lower values of L* and BI than the controls (whole and refined-wheat breads) as much as crumb or crust and that can be attributed to the polyphenol content present in the pericarp, in the cells of the aleurone layer and in the endosperm regions of the millet grain (McDonough et al., 1989). A similar decrease of L* values of bread with finger millet flour substitution was also reported Mudau et al. (2021). However, the L* of sponge-dough crust breads is higher ($p<0.05$) than straight-dough samples. Higher values in the crust compared to the crumb are derived from the *Maillard* and caramelization reactions, i.e. a non-enzymatic browning that is influenced by the distribution of water and the reaction between reducing sugars and amino acids during baking step. Color is an important parameter that drive consumer´s preferences mainly related to purchase intentions, since gluten-free breads commonly have a pale color, with an artificial appearance, differing from wheat bread expectances (Pessanha et al., 2023; Herculano et al, 2021).

3. 2. 2 Specific volume

Loaf volume is considered as the one main desirable bread characteristics since it provides a quantitative measurement of baking performance and acceptance by consumers. Bread images and specific volume are shown in Figure 5 and Table 3. The specific volume ranged from 1,14 to 3,83 cm^3/g in which all formulations that used millet flour had a lower significant values ($p<0.05$) than control samples, as expected. Chandrasekar et al. (2022) also reported that although the specific volume of fermented finger millet breads was lower than the commercial samples, it was higher than the specific volume of unfermented millet flour breads. Gluten free breads formulated with proso millet adding xantam gum (XG), guar gum (GG), and hydroxypropyl methylcellulose (HPMC) at various concentrations ranging from 0.5 to 2.0 g/100 g, showed higher specific volume values. The highest specific volume was observed in HPMC 2.0 (2,88 cm^3/g). Hernández Aguirre et al. (2019) attributing to the use of improvers, such as HPMC that promoting the association of the gum's hydrophilic groups with the OH- groups of starch and water by hydrogen bonds. The hydrophobic component acts as a surfactant between the starch components and the air cell interphase in the food matrix, reinforcing this structure. However, our work delivery a clean label option since celiac disease (CD) consumers are in favor of foods with minimum chemical additives following the worldwide trend (Gobbetti et al., 2018).

RMF and REGMF samples demonstrated an increase in specific volume values in both baking methods. However, this difference was not statistically significant ($p<0.05$). The positive correlation between specific volume and parameters breadmaking processings (mixing dough, fermentation and baking) was observed during elaboration of Australian sweet lupin-wheat bread reported by Villarino et al. (2014). These authors presented specific volume ranging between 2.6 – 4.3 cm^3/g.

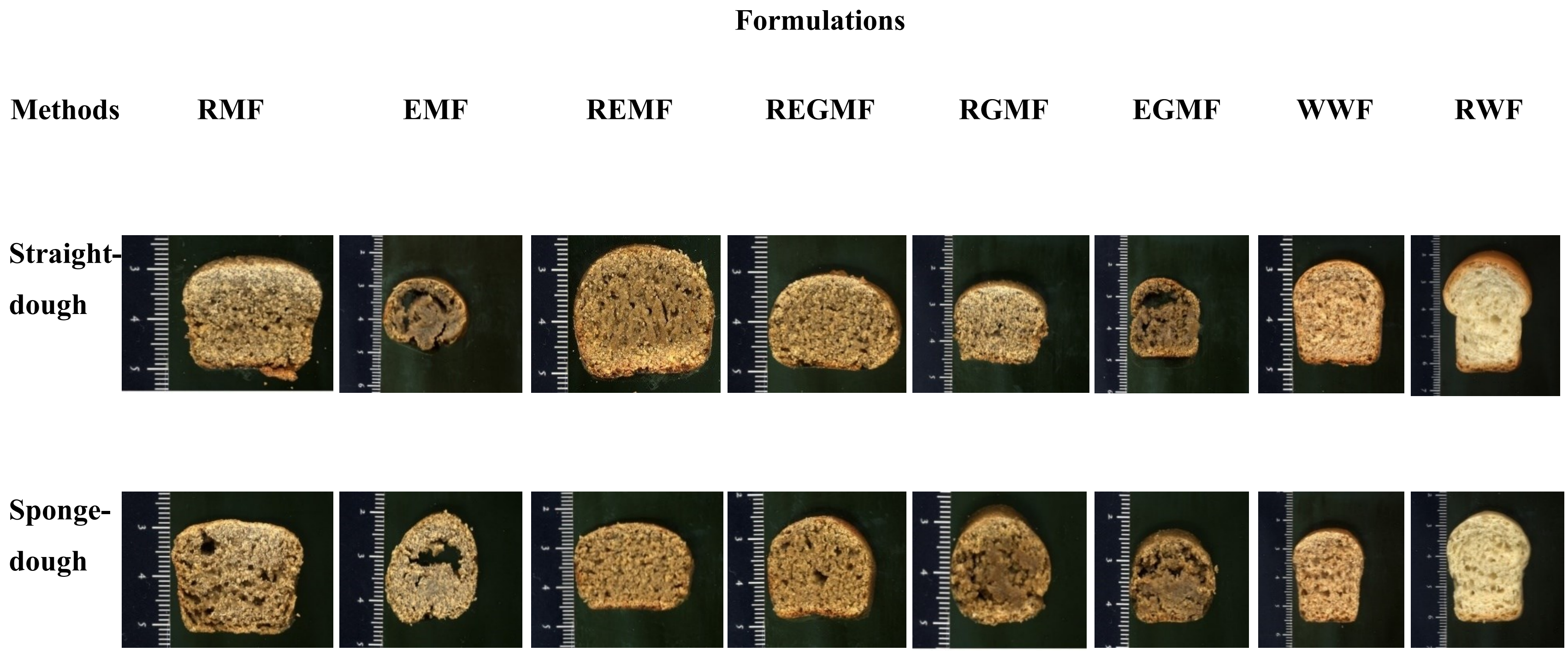


Figure 5. Photographs of millet breads and controls (wheat whole flour and refined wheat flour) obtained by different breadmaking processes.

3. 2. 3 Texture profile analysis (TPA)

As expected, RWF exhibited the lowest hardness value ($p<0.05$) in both methods (Table 4). Millet breads showed increase in hardness and chewiness in accordance with Wang et al. (2020). In general, sponge-dough millet breads showed the high values of hardness (15.33-19.85 N) in relation to straight-dough (12-41-12.97 N) that can be related to the increase in dietary fiber content (Table 5). The greater amount of fibers thickens the walls surrounding the gas cells, promoting greater hardness of the dough (Patil et al., 2016). Hardness is mainly related to the density and chewiness represents the strength of the internal resistance of the food structure (Li et al., 2020).

Concerning this aspect, amylose presented high correlation with hardness and chewiness by correlation analysis (Figure 6) that can be explained due to the re-association and reordering of amorphous amylose, which was largely responsible for the short-term retrogradation of starch (Zhu, 2016).

The addition of germinated flour in bread formulations did not significantly affect the elasticity and resilience parameters. However, a strong correlation was observed between these parameters (R=0.98), indicating that the bread has softness and quick ability to recover after removing the applied stress. Except the control bread, the REGMF bread by straight-dough method showed the highest cohesiveness in accordance to reported by Chandrasekar et al. (2022). High cohesiveness is desirable because during chewing it forms a cake instead of disintegrated shape (Onyango, 2011).

Table 3. Instrumental color parameters and specific volume of millet breads.

Samples		Crumb color				Crust color				SV (cm^3/g)
		L*	a*	b*	BI	L*	a*	b*	BI	
Straight-dough	**RMF**	$50.67^{fgh} \pm 0.82$	$3.34^{cde} \pm 0.17$	$11.53^{e} \pm 0.19$	$30.22^{de} \pm 1.28$	$40.07^{g} \pm 2.83$	$9.35^{b} \pm 0.34$	$12.06^{efg} \pm 2.08$	$52.27^{d} \pm 2.56$	$1.39^{ef} \pm 0.05$
	EMF	$54.87^{d} \pm 0.85$	$3.26^{de} \pm 0.03$	$12.52^{de} \pm 0.09$	$29.80^{def} \pm 0.28$	$41.86^{defg} \pm 0.17$	$6.14^{bc} \pm 0.09$	$11.92^{efg} \pm 0.37$	$43.77^{de} \pm 1.18$	$1.63^{cdef} \pm 0.07$
	REMF	$62.59^{c} \pm 1.17$	$2.78^{e} \pm 0.15$	$15.69^{bc} \pm 0.11$	$31.56^{bcde} \pm 0.63$	$42.24^{defg} \pm 0.94$	$8.44^{bc} \pm 0.41$	$12.93^{def} \pm 0.35$	$50.62^{d} \pm 0.81$	$1.45^{def} \pm 0.12$
	REGMF	$53.34^{defg} \pm 0.17$	$3.67^{bcd} \pm 0.06$	$14.14^{cd} \pm 0.27$	$35.32^{abcd} \pm 0.64$	$40.49^{fg} \pm 1.65$	$9.30^{b} \pm 1.29$	$9.46^{fg} \pm 1.10$	$42.86^{de} \pm 0.57$	$1.43^{def} \pm 0.10$
	EGMF	$50.64^{fgh} \pm 0.04$	$3.51^{bcde} \pm 0.03$	$11.25^{e} \pm 0.17$	$29.76^{def} \pm 0.45$	$41.83^{defg} \pm 0.72$	$7.38^{bc} \pm 0.27$	$9.03^{g} \pm 0.49$	$36.80^{d} \pm 0.33$	$1.73^{ef} \pm 0.15$
	RGMF	$51.55^{defgh} \pm 1.81$	$3.14^{de} \pm 0.19$	$11.91^{de} \pm 0.45$	$30.27^{de} \pm 0.36$	$41.65^{efg} \pm 2.51$	$8.89^{bc} \pm 2.25$	$12.29^{efg} \pm 1.24$	$50.10^{e} \pm 3.10$	$1.57^{def} \pm 0.06$
	WWF	$65.24^{c} \pm 0.78$	$5.81^{a} \pm 0.26$	$17.61^{ab} \pm 0.13$	$37.49^{a} \pm 1.09$	$47.05^{cde} \pm 2.38$	$15.18^{a} \pm 0.15$	$16.63^{cd} \pm 2.33$	$66.25^{bc} \pm 3.95$	$1.96^{cd} \pm 0.23$
	RWF	$77.80^{a} \pm 0.91$	$-0.46^{f} \pm 0.02$	$17.33^{ab} \pm 0.04$	$24.15^{f} \pm 0.37$	$54.01^{b} \pm 0.0$	$15.43^{a} \pm 0.45$	$25.93^{ab} \pm 0.62$	$84.65^{a} \pm 2.64$	$3.83^{a} \pm 0.52$
Sponge-dough	**RMF**	$54.42^{de} \pm 0.42$	$3.20^{de} \pm 0.14$	$12.30^{de} \pm 0.45$	$29.49^{def} \pm 1.50$	$46.53^{cdef} \pm 0.21$	$9.12^{b} \pm 0.49$	$18.09^{c} \pm 0.26$	$62.77^{c} \pm 1.32$	$1.69^{cde} \pm 0.13$
	EMF	$49.72^{gh} \pm 1.29$	$3.62^{bcd} \pm 0.18$	$11.49^{e} \pm 0.22$	$31.16^{cde} \pm 0.62$	$42.88^{defg} \pm 0.16$	$7.56^{bc} \pm 0.01$	$12.32^{efg} \pm 0.23$	$46.30^{de} \pm 0.56$	$1.54^{def} \pm 0.16$
	REMF	$53.50^{def} \pm 1.50$	$3.48^{bcde} \pm 0.07$	$13.44^{cde} \pm 0.40$	$33.19^{abcde} \pm 0.20$	$42.82^{defg} \pm 0.55$	$6.76^{bc} \pm 0.07$	$12.73^{efg} \pm 0.50$	$46.32^{de} \pm 0.82$	$1.14^{f} \pm 0.06$
	REGMF	$50.83^{efgh} \pm 0.37$	$4.06^{bc} \pm 0.02$	$13.48^{cde} \pm 0.16$	$36.16^{abc} \pm 0.07$	$42.96^{defg} \pm 0.45$	$8.13^{bc} \pm 0.17$	$12.87^{defg} \pm 0.60$	$48.97^{d} \pm 2.85$	$1.61^{def} \pm 0.20$
	EGMF	$49.62^{h} \pm 0.24$	$4.12^{b} \pm 0.27$	$13.09^{de} \pm 0.83$	$36.20^{abc} \pm 2.45$	$43.69^{defg} \pm 0.26$	$6.56^{bc} \pm 0.31$	$12.69^{efg} \pm 0.42$	$44.82^{de} \pm 1.57$	$1.36^{ef} \pm 0.06$
	RGMF	$53.79^{def} \pm 0.27$	$3.38^{bcde} \pm 0.01$	$13.07^{de} \pm 0.06$	$31.95^{abcde} \pm 0.35$	$47.79^{cd} \pm 1.38$	$5.45^{c} \pm 0.67$	$15.47^{cde} \pm 0.12$	$46.89^{d} \pm 3.00$	$1.41^{ef} \pm 0.09$
	WWF	$63.68^{c} \pm 0.25$	$5.65^{a} \pm 0.25$	$17.00^{ab} \pm 0.24$	$37.07^{ab} \pm 0.62$	$51.28^{bc} \pm 1.11$	$13.29^{a} \pm 0.53$	$22.53^{b} \pm 0.79$	$75.39^{ab} \pm 0.26$	$2.16^{c} \pm 0.09$
	RWF	$71.03^{b} \pm 1.14$	$-1.04^{f} \pm 0.50$	$18.09^{a} \pm 1.92$	$27.62^{ef} \pm 4.57$	$67.46^{a} \pm 2.99$	$5.95^{bc} \pm 1.94$	$29.63^{a} \pm 0.06$	$62.97^{c} \pm 5.97$	$2.75^{b} \pm 0.07$

RMF: raw millet flour; EMF: extruded millet flour; GMF: germinated wheat flour; REMF: raw and extruded millet flour; REGMF: raw, extruded and germinated millet flour; EGMF: extruded and germinated millet flour; RGMF: raw and germinated millet flour; WWF: commercial whole-wheat flour; RWF: commercial refined-wheat flour; BI: browning index; SV: specific volume. Values are mean ± standard deviation. Values followed by the same letters in the same columns are not significantly different ($p < 0.05$).

Table 4. Texture profile analysis (TPA) of crumb millet breads by straight and sponge-dough methods.

	Samples	**Hardness (N)**	**Adhesiveness (g.s)**	**Cohesiveness (-)**	**Springiness (-)**	**Chewiness (N)**	**Resilience (-)**
Straight-dough	**REMF**	$12.97^{c} \pm 1.62$	$43.05^{ab} \pm 39.56$	$0.22^{d} \pm 0.03$	$0.80^{a} \pm 0.40$	$2.35^{c} \pm 1.86$	$0.08^{d} \pm 0.01$
	REGMF	$12.41^{c} \pm 0.61$	$66.92^{ab} \pm 27.18$	$0.30^{c} \pm 0.04$	$0.71^{a} \pm 0.25$	$2.87^{abc} \pm 1.35$	$0.09^{d} \pm 0.0$
	WWF	$9.25^{d} \pm 0.82$	$11.55^{a} \pm 4.11$	$0.47^{b} \pm 0.03$	$0.94^{a} \pm 0.02$	$4.03^{ab} \pm 0.29$	$0.15^{c} \pm 0.01$
	RWF	$7.55^{de} \pm 1.11$	$4.94^{b} \pm 3.92$	$0.58^{a} \pm 0.04$	$0.97^{a} \pm 0.02$	$4.28^{a} \pm 0.61$	$0.22^{b} \pm 0.01$
Sponge-dough	**REMF**	$15.33^{b} \pm 1.50$	$40.25^{ab} \pm 34.74$	$0.27^{cd} \pm 0.09$	$0.70^{a} \pm 0.19$	$3.02^{abc} \pm 1.72$	$0.09^{d} \pm 0.0$
	REGMF	$19.85^{a} \pm 2.78$	$20.95^{ab} \pm 28.41$	$0.20^{d} \pm 0.04$	$0.79^{a} \pm 0.18$	$3.31^{abc} \pm 1.14$	$0.06^{d} \pm 0.01$
	WWF	$5.87^{e} \pm 1.31$	$4.27^{ab} \pm 3.22$	$0.44^{b} \pm 0.02$	$0.96^{a} \pm 0.03$	$2.60^{bc} \pm 0.64$	$0.15^{c} \pm 0.01$
	RWF	$3.24^{f} \pm 0.83$	$3.10^{a} \pm 0.85$	$0.58^{a} \pm 0.05$	$1.05^{a} \pm 0.24$	$1.90^{c} \pm 0.77$	$0.26^{a} \pm 0.03$

REMF: raw and extruded millet flour; REGMF: raw, extruded and germinated millet flour; WWF: commercial whole-wheat flour; RWF: commercial refined-wheat flour; Values are mean ± standard deviation, n = 12 (TPA). Values followed by the same letters in the same columns are not significantly different ($p < 0.05$)

3.3 Characterization of nutritional composition

3.3.1 Proximate composition

The WWF sample by sponge-dough method presented the highest protein content (11.23 g/100g) ($p < 0.05$) among the samples, followed by REGMF which showed a significant increase in comparison with the methods, ranging between 9.82g/100g to 10.86g/100g (Table 5). The increase in crude protein values was due to protein accumulation and production of some additional amino acids in the samples as a result of fermentation (Adebiyi at al., 2017). RMF sponge-dough showed lipid content significantly greater (9.69 g/100g) and straight-dough EMF was significantly lower (4.78 g/100g). The fat apparent reduction can be attributed to the formation of amylose-lipid complexes after the extrusion process that cannot be extracted by the petroleum ether solvent (Espinosa-Ramírez et al., 2021). Literature report lower values of protein and lipids in millet breads produced without additives (Torbica et al., 2019).

The carbohydrate content ranged from 49.45 to 61.1 g/100 g, and RWF straight-dough showed the highest value ($p < 0.05$), which was attributed to the partial removal of the pericarp after the milling process, causing a reduction of dietary fiber content. Dietary fiber contents are highlighted for RGMF and REGMF, ranging between 12.6 and 10.8 g/100 g, respectively. Similar values were found by Horstmann et al. (2018) for breads obtained using germinated flour. The germination process promotes less interaction between protein and carbohydrate, favoring the biosynthesis of the cell wall and consequent increase in dietary fiber (Gowda et al., 2022).

The ash content was higher in REGMF by sponge-dough. Germination process has considerable influence on the final chemical composition of the raw material. The highest moisture content of EMF sample by straight method was observed. However, it is statistically smaller than the commercial gluten-free sample. The moisture of a product can affect its processing, as it interferes with stability, quality and composition. It can also impact its useful life as it is one of the main factors in accelerating chemical and enzymatic reactions (Gutkoski & Jacobsen Neto, 2002). Furthermore, complete gelatinization of starch is observed in gluten-free products, which may partially explain why these formulations aging quickly (Gobbetti et al., 2018).

3.3.2 Total starch, resistant starch and amylose contents

Concerning total starch, RWF presented the highest values in both methods. However, the REGMF sample by sponge-dough method demonstrated a significant ($p<0.05$) reduction (12%) in starch content if compared to the straight-dough sample (Table 6). A reduction on starch content after the fermentation process may be associated with the amylolytic action of microorganisms in the fermented dough (Rani et al., 2018). In addition, the total starch values of millet-based breads are comparable to the WWF.

In terms of resistant starch content, significant difference ($p<0.05$) was not observed in relation to millet-based breads, except the RWF by sponge-dough processing (1.18 g/100g).

The amylose content was significant ($p<0.05$) higher in the REGMF by sponge-dough sample (41.18%). These finding was also reported by Pessanha et al. (2021). REGMF obtained by sponge-dough method showed the highest hardness and chewiness values (Table 3), although did not show the higher setback and FV values among samples (Table 4), demonstrating the impact of processing rather than solely the rheological characteristics of the flour. The increase in amylose content is probably related to the decrease in the lipid content as a result of the fermentation process, an effect had already reported by Chinenye et al. (2017). Consequently, less interaction and formation of starch-lipid complexes. Although the tendency to form binary complexes is due to the helical structure of amylose, it still depends on the availability of lipids in the matrix (Krishnan et al., 2021)

Table 5. Chemical composition (g/100g) and total calories (kcal/100 g) of millet flour breads.

	Samples	Moisture	Ash	Protein	Lipids	Carbohydrates	Dietary fiber	Total calories
Straight-dough	**RMF**	$26.94^{i} \pm 0.12$	$2.37^{ab} \pm 0.08$	$10.14^{def} \pm 0.03$	$7.55^{def} \pm 0.41$	$53.0^{cd} \pm 0.16$	6.96	292.62
	EMF	$34.10^{c} \pm 0.04$	$2.11^{cde} \pm 0.09$	$9.48^{g} \pm 0.16$	$4.78^{k} \pm 0.12$	$49.52^{i} \pm 0.34$	6.37	253.58
	REMF	$28.94^{fg} \pm 0.31$	$2.34^{abc} \pm 0.03$	$10.06^{defg} \pm 0.08$	$7.02^{efgh} \pm 0.18$	$51.64^{def} \pm 0.61$	6.42	284.34
	REGMF	$30.55^{e} \pm 0.48$	$2.24^{bcd} \pm 0.04$	$9.82^{efg} \pm 0.16$	$7.94^{cde} \pm 0.04$	$49.45^{i} \pm 0.55$	6.34	283.17
	EGMF	$28.23^{gh} \pm 0.28$	$2.25^{bcd} \pm 0.04$	$10.06^{defg} \pm 0.08$	$5.98^{ijk} \pm 0.25$	$53.49^{c} \pm 1.0$	7.72	277.18
	RGMF	$26.24^{ij} \pm 0.18$	$2.33^{abc} \pm 0.13$	$10.37^{cde} \pm 0.12$	$8.26^{bcd} \pm 0.02$	$52.8^{cd} \pm 0.16$	7.55	296.66
	WWF	$22.03^{m} \pm 0.00$	$2.03^{de} \pm 0.04$	$11.23^{ab} \pm 0.20$	$8.60^{bc} \pm 0.27$	$56.11^{b} \pm 0.03$	5.44	324.99
	RWF	$20.78^{l} \pm 0.09$	$1.65^{g} \pm 0.05$	$9.83^{efg} \pm 0.08$	$6.61^{fghi} \pm 0.20$	$61.14^{a} \pm 0.43$	4.5	325.37
Sponge-dough	**RMF**	$25.94^{j} \pm 0.09$	$2.48^{ab} \pm 0.04$	$10.83^{bc} \pm 0.12$	$9.69^{a} \pm 0.02$	$51.06^{efgh} \pm 0.09$	7.07	306.53
	EMF	$32.36^{d} \pm 0.03$	$2.25^{bcd} \pm 0.06$	$9.68^{fg} \pm 0.20$	$5.58^{jk} \pm 0.20$	$50.14^{ghi} \pm 0.43$	4.71	270.7
	REMF	$28.92^{fg} \pm 0.22$	$2.40^{ab} \pm 0.01$	$10.60^{cd} \pm 0.03$	$7.29^{efg} \pm 0.09$	$50.79^{fgh} \pm 0.26$	9.75	272.12
	REGMF	$27.86^{h} \pm 0.17$	$2.50^{a} \pm 0.04$	$10.86^{bc} \pm 0.07$	$7.81^{cde} \pm 0.18$	$50.97^{fgh} \pm 0.48$	10.83	274.33
	EGMF	$29.15^{f} \pm 0.09$	$2.40^{ab} \pm 0.07$	$10.29^{cdef} \pm 0.0$	$5.72^{jk} \pm 0.11$	$52.45^{cde} \pm 0.28$	8.05	270.28
	RGMF	$26.33^{ij} \pm 0.12$	$2.44^{ab} \pm 0.04$	$10.86^{bc} \pm 0.07$	$9.12^{ab} \pm 0.25$	$51.25^{efg} \pm 0.0$	12.61	280.12
	WWF	$23.20^{k} \pm 0.07$	$2.26^{abcd} \pm 0.06$	$11.61^{a} \pm 0.32$	$6.13^{hij} \pm 0.14$	$56.81^{b} \pm 0.45$	9.94	289.13
	RWF	$21.95^{l} \pm 0.07$	$1.72^{fg} \pm 0.02$	$9.88^{efg} \pm 0.16$	$6.32^{ghij} \pm 0.66$	$60.13^{a} \pm 0.45$	3.84	321.60
Commercial	**WWB**	$37.05^{b} \pm 0.07$	$2.01^{de} \pm 0.03$	$10.78^{bc} \pm 0.28$	$0.47^{m} \pm 0.09$	$49.70^{hi} \pm 0.14$	7.18	217.42
	GFB	$39.70^{a} \pm 0.24$	$1.94^{ef} \pm 0.05$	$3.44^{h} \pm 0.07$	$2.92^{l} \pm 0.06$	$52.0^{def} \pm 0.43$	6.43	222.32

RMF: raw millet flour; EMF: extruded millet flour; GMF: germinated millet flour; REMF: raw and extruded millet flour; REGMF: raw, extruded and germinated millet flour; EGMF: extruded and germinated millet flour; RGMF: raw and germinated millet flour; WWF: commercial whole-wheat flour; RWF: commercial refined-wheat flour; WWB: commercial whole wheat bread; GFB: commercial gluten-free bread. Values are mean ± standard deviation. Different letters in the same columns indicate statistic differences ($p < 0.05$) among samples

Table 6. Total starch, resistant starch, amylose and amylopectin contents of millet-based breads.

	Amostras	Total starch (g/100g)	Resistant starch (g/100g)	Amylose (%)	Amylopectin (%)
	REMF	$57.51^{bc} \pm 0.21$	$0.64^{b} \pm 0.01$	$22.44^{d} \pm 1.57$	$77.55^{a} \pm 1.57$
	REGMF	$59.36^{b} \pm 0.63$	$0.50^{b} \pm 0.03$	$28.39^{cd} \pm 1.11$	$71.60^{ab} \pm 1.11$
Straight-dough	**WWF**	$53.50^{bc} \pm 0.21$	$0.77^{ab} \pm 0.01$	$35.79^{ab} \pm 2.40$	$64.20^{cd} \pm 2.40$
	RWF	$71.02^{a} \pm 1.38$	$0.97^{ab} \pm 0.08$	$32.75^{bc} \pm 1.58$	$67.24^{bc} \pm 1.58$
	REMF	$53.38^{bc} \pm 2.54$	$0.61^{b} \pm 0.08$	$36.87^{ab} \pm 0.03$	$63.12^{cd} \pm 0.03$
Sponge-dough	**REGMF**	$52.08^{c} \pm 1.45$	$0.45^{b} \pm 0.05$	$41.18^{a} \pm 1.07$	$58.81^{d} \pm 1.07$
	WWF	$55.30^{bc} \pm 0.64$	$0.71^{b} \pm 0.07$	$29.02^{c} \pm 1.22$	$70.97^{b} \pm 1.22$
	RWF	$74.68^{a} \pm 2.17$	$1.18^{a} \pm 0.07$	$31.97^{bc} \pm 2.60$	$68.02^{bc} \pm 2.60$

REMF: raw and extruded millet flour; REGMF: raw, extruded and germinated millet flour; WWF: commercial whole-wheat flour; RWF: commercial refined-wheat flour; Values are mean ± standard deviation, n = 2. Different letters in the same columns indicate statistic differences ($p < 0.05$) among samples.

3.3.3 Total phenolic content (TPC) and antioxidant properties of breads

Table 7 shows the TPC of the millet breads by process straingh dough and sponge dough. An analysis of each extract showed that the bound extract had more phenolic content than the free extract. As already documented in other studies on both millet and wheat samples (Chandrasekara & Shahidi, 2010; Liyana-Pathirana & Shahidi, 2006). The Folin-Ciocalteu method is a non-specific method and although it may present interference due to the reaction of other reducing molecules present in the extracts, it is commonly applied by scientists to determine total phenolic compounds. In this work, the total phenolic content was estimated by measuring the reducing capacity of the obtained extracts, enriched in phenolic compounds, considering free and bound phenolics (Górnas et al., 2016).

When each sample is evaluated using different baking processes, it is noted that there is a decrease in the TPC content in the free extracts (except REGMF samples) and an increase in the bound extracts, which consequently influenced the total TPC values. Data that conflict with those obtained in the study by Santetti et al. (2022) in which there was an increase in free phenolics and a decrease in bound phenolics. Justification for the apparent increase in free extracts would be due to the effect of the fermentation process and cooking temperature, which can lead to the hydrolysis of some complex phenols. The action of microorganisms that use soluble and fermentable fibers for their growth promotes the release of phenolic compounds “mechanically trapped” from the polymeric fiber structure (Wang et al., 2022). Furthermore, the Maillard reaction in the bread manufacturing process can promote the incorporation of certain compounds into melanoidins, promoting an increase in bound compounds (Abdel-Aal & Rabalski, 2013). The TPC value of the REGMF sample was significantly higher than all samples, including the control breads. A significant increase was observed when the sponge method (4131.8 mg GAE/100g) was compared to the direct method (3160.2 mg GAE/100g). It was reported the cell wall-degrading enzymes of the grain become active during germination, which may contribute to the cell wall structure modification leading to the release of bound phenolic compounds. In addition, that the key enzymes involved in biosynthesis of phenols get activated during germination of seeds (Kumar et al., 2022). Other studies with gluten-free breads also demonstrated positive effects on the content of phenolic compounds when using sprouted flour (Yaver, 2022; Carnejo et al., 2015).

Data of the antioxidant properties of the processed millet determined by DPPH and FRAP confirmed that all of the processed millet had higher peroxide scavenging capacity than the control (Table 7). Data consistent with the study by Sharma (2017) where the incorporation of millet flour in chapati breads demonstrated a significant increase in antioxidant activity.

Antioxidant activity calculated by DPPH was higher in the REGMF sample, however showing a decrease when compared to the direct sponge method. This can be justified by interference due to the reaction of other reducing molecules present in the extracts, probably a result of the germination process. Using the FRAP method, the sample with the highest antioxidant activity was the REMF sample, demonstrating a significant increase when prepared using the sponge dough method. Corroborating the hypothesis that gluten-free breads made with millet flour have high antioxidant activity, especially when compared to wheat breads.

Free radicals are continuously produced in the body as a part of the normal aerobic respiration and substrate oxidation and when uncontrolled, damage to the vital biomolecules. Oxidative stress caused by excessive production of reactive oxygen species (ROS) plays a role in the pathogenesis of a number of age related diseases. Thus, regulation of oxidative stress is of much importance in ameliorating and prevention such diseases, by supplying exogeneous antioxidants that can minimize the effects of stimulators that produce ROS in the body (Chandrasekara et al., 2011).

3.3.4 Anti-hyperglicemic activity

Only one free extract sample (RWF – sponge dough) showed an inhibition effect on the α-amylase enzyme. These findings can be justified by previous studies that observed a high amount of ferulic acid and its isoforms in refined wheat flour (Santos et al., 2022). *In vivo* studies have shown that ferulic acid has the ability to neutralize free radicals present in the pancreas induced by streptozotocin facilitates the proliferation of β-cells that secrete insulin, which in turn enhance the use of glucose by extra hepatic tissues, thus reducing blood glucose levels (Balasubashini et al., 2004).

It was observed that the extract-bound millet bread samples exhibited inhibitory activity (IC 50%) at a significant ($p<0.05$) lower concentration than the control sample extract. The lowest

IC_{50} value for REMF sample (2.06 mg/mL-straight dough and 1.36 mg/mL-sponge dough) means that the inhibition capacity of the extracts is greater if compared to wheat-based breads. All samples presented an IC_{50} significant higher than Acarbose as expected (Table 6). These results are in agreement with the antioxidant capacity values, in which the values of the bound extracts increased significantly if the breadmaking methods are compared. REMF sample revealed an increase of 1.29 mg/g (straight-dough) to 2.02 mg/g (sponge-dough) by DPPH and 460.8 µmole/g (straight-dough) to 1145.5 µmole/g (sponge-dough) by FRAP method. The REGMF sample also showed an increase of 1.84 mg/g (straight-dough) to 1.93 mg/g (sponge-dough) by DPPH and 630.2 µmole/g (straight-dough) to 925.2 µmole/g (sponge-dough) by FRAP method, however the intensity of this increase is lower than the formulation that does not contain GMF addition.

The interaction between phenolic compounds and the starch digestion process is widely studied. Two main mechanisms have been proposed, that was classified (i) as the modulation of glycolytic enzymes and (ii) the formation of supramolecular complexes between starch and phenolic compounds. The first mechanism modulates the enzyme directly through competitive or non-competitive inhibition; and the latter may act indirectly, limiting the accessibility of starch to the enzymes α-amylase and α-glucosidase and/or affecting rheological and solubility properties. These mechanisms probably play a complementary role, not excluding the other (Giuberti et al., 2020).

Hyperglycemia is a factor associated with an increased risk of developing metabolic diseases, such as Type 2 Diabetes. The 10th Edition of the International Diabetes Federation Atlas estimates that in 2045, 784 million people in the world will present diabetes (IDF, 2021). Health loss has become a growing burden from chronic non-communicable diseases (NCDs). Risk factors are responsible for half of the years of healthy life lost worldwide. Given this scenario, it becomes increasingly necessary to adopt strategies to reduce the occurrence of these pathologies. Reducing exposure to inappropriate behavioral and metabolic risks would bring enormous health benefits, hence the enormous relevance of researching food products with nutritional characteristics such as the one presented in the current study.

Table 7. TCP, antioxidant capacity and IC_{50} of the straight and sponge-dough breadmaking methods.

		Total phenols as gallic acid equivalent (mg GAE/100g)	**DPPH (mg/g)**	**FRAP (µmole/g)**	**IC_{50} (mg/m)**
Straight-dough	REMF	$3085.04^{b}\pm130.2$	$2.41^{c}\pm0.10$	$869.3^{b}\pm20.9$	$2.06^{cd} \pm 0.03$
	REGMF	$3160.2^{b}\pm115.7$	$3.69^{a}\pm0.12$	$1041.0^{b}\pm72.9$	$1.46^{d} \pm 0.04$
	WWF	$1619.8^{c}\pm93.2$	$1.70^{d}\pm0.05$	$979.7^{b}\pm60.2$	$7.29^{a} \pm 0.18$
	RWF	$492.4^{d}\pm22.3$	$0.75^{f}\pm0.03$	$516.9^{c}\pm51.4$	$2.50^{c} \pm 0.05$
Sponge-dough	REMF	$3210.6^{b}\pm217.5$	$3.04^{b}\pm0.06$	$2133.0^{a}\pm236.9$	$1.36^{d} \pm 0.20$
	REGMF	$4131.8^{a}\pm284.2$	$3.06^{b}\pm0.17$	$1923.3^{a}\pm85.8$	$1.78^{cd} \pm 0.05$
	WWF	$1289.5^{c}\pm54.4$	$1.42^{e}\pm0.03$	$861.5^{b}\pm23.5$	$5.4^{b} \pm 0.06$
	RWF	$287.3^{d}\pm5.5$	$0.92^{f}\pm0.04$	$344.2^{c}\pm10.2$	$7.69^{a} \pm 0.02$
	RWF free	-	-	-	1.27 ± 0.02
	Acarbose®	-	-	-	0.024 ± 0.001

REMF: raw and extruded millet flour; REGMF: raw, extruded and germinated millet flour; WWF: commercial whole-wheat flour; RWF: commercial refined-wheat flour; Values are mean ± standard deviation. Values followed by the same letters in the same columns are not significantly different ($p < 0.05$)

4 Pairwise correlation coefficient (*r*) and Principal component analysis (PCA)

In order to explore relations between texture profile and starch, amylose and amylopectin content in breads samples, a correlation matrix was calculated (Figure 6). The correlogram indicates a positive relationship between total starch and resistant starch with cohesiveness (0.78 and 0.82, respectively), springiness (0.62 and 0.69, respectively) and resilience (0,85 and 0,89, respectively), but a negative correlation with hardness (-0.68 and -0.66, respectively). A positive correlation was also found between amylose content with hardness (0.40) and chewiness (0.50).

The PCA was applied to evaluate the relationship among the 17 variables related to breads characteristics (Figure 7). PC1 and PC2 explained 65.26% of the total variance among three quality characteristics of breads (specific volume, colour, and proximate composition). PC analysis evidenced the differences between the millet and control breads. Dim 1 is described by total calories, lipids, a*(ct), b* (ct), L* (ct), C* (ct), BI crust, b*(cb), L* (cb), C* (cb) and specific volume. In contrast, the other variables are described by Dim 2 are carbohydrates, protein, dietary fiber, ash. The parameters of the close vectors are positive and correlated.

A hierarchical tree by the principal components of PCA was used to better explain the relationship between the variables (physicochemical composition) and factors (millet samples). HCPC formed two sample groups according to their similarities (Figure 8). Cluster 1 (EMFsp, RMFst, REMFsp, EMFst, REGMFst) was composed of samples with similar carbohydrate values. Cluster 2 was composed of the samples (RGMFst, RMFsp, REGMFsp, RGMFsp) that presented values close to ash, cluster 3 grouped the RWF samples showing greater correlation with the variables a*(ct), b* (cb), C* (cb), BI crust, L* and total calories. Cluster 4 was formed by values from RWF samples that demonstrated similar values of specific volume, C* (ct), b* (ct), L* (ct).

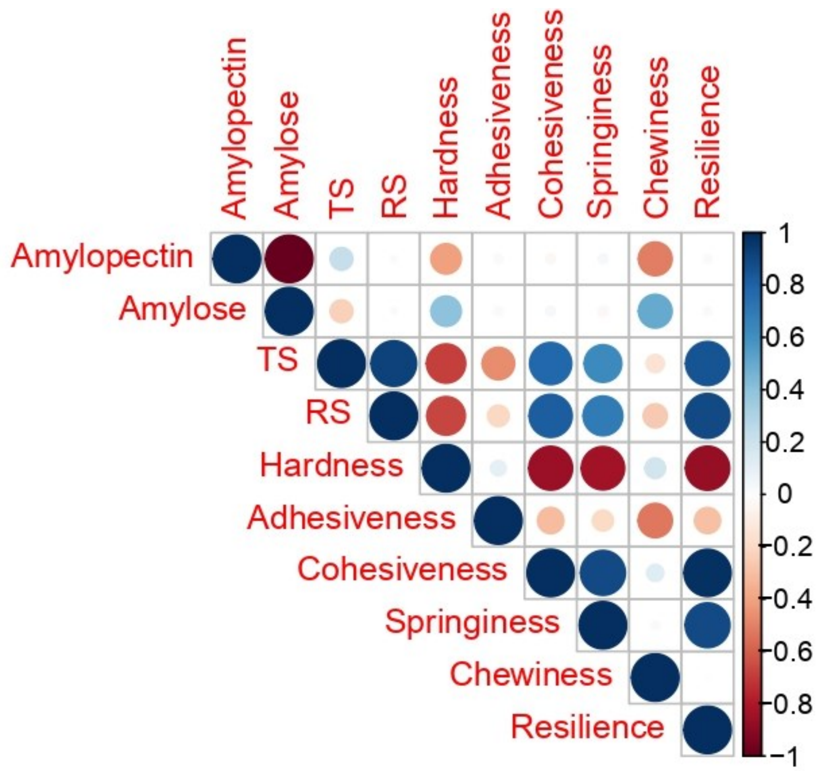


Figure 6. Correlogram of texture parameters and starch properties of millet breads.

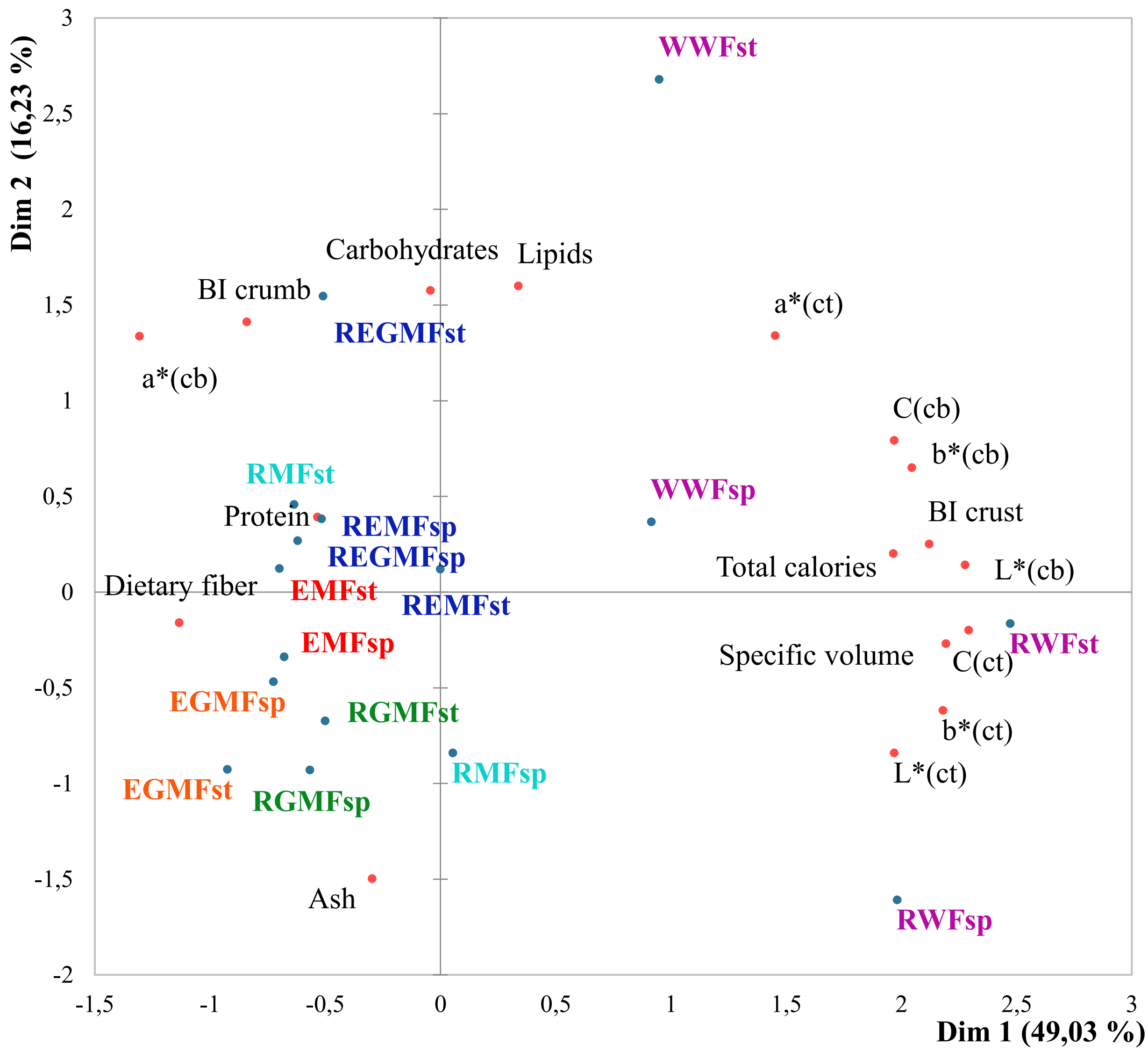


Figure 7. Two-dimensional loading plot from principal component analysis (PCA) using PCA Dimension 1 (Dim 1) and PCA Dimension 2 (Dim 2). Loading plot based on different variables of millet bread properties.

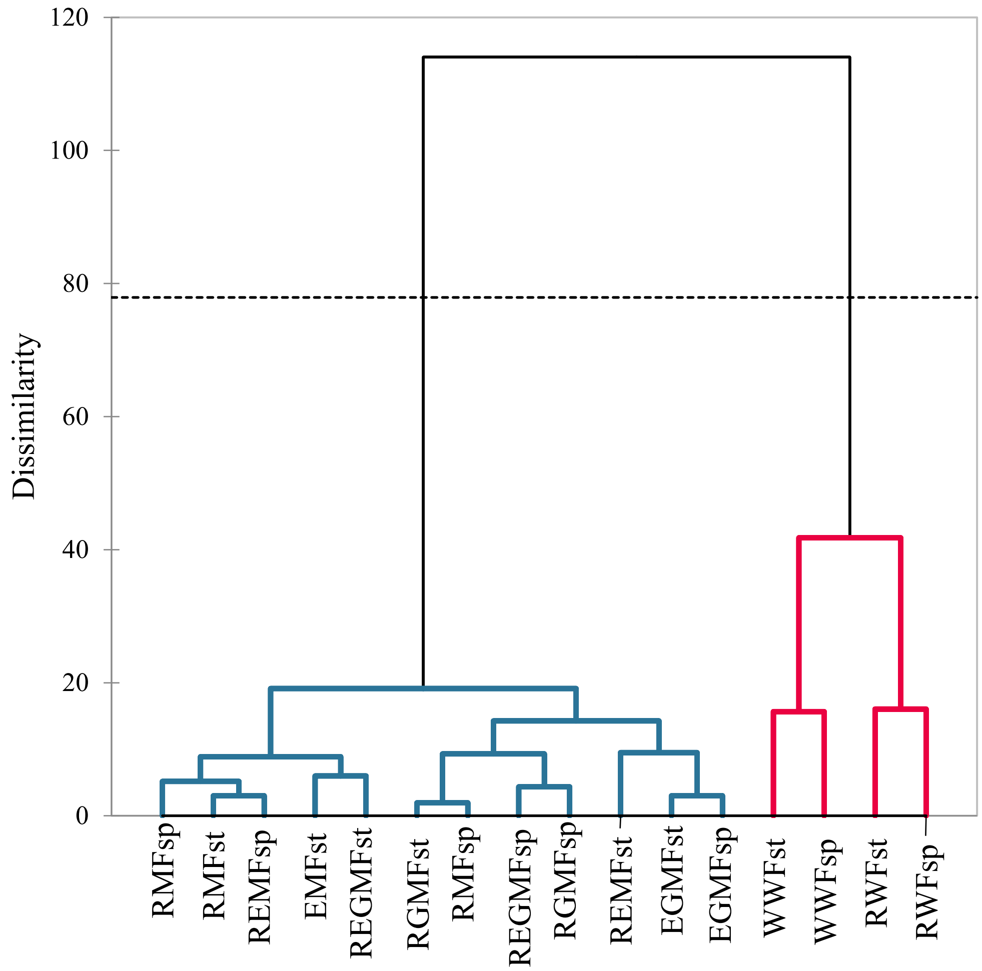


Figure 8. Hierarchical tree and individual factor map by the principal components of PCA.

5. Conclusion

The results of this study indicated that the different types of breadmaking processing had a positive impact on the nutritional characteristics of gluten-free millet-based breads nutritionally enriched with high dietary fiber (10.83g/100g) and amylose content (41.18%). Although the millet-based samples presented a significantly lower specific volume than the controls, they demonstrated a higher value in terms of ash, protein, fiber and less content of moisture than commercial gluten-free breads. Furthermore, the content of phenolic compounds and antioxidant capacity were significant higher by least 50% compared to wheat-based formulations ($p<0.05$). Millet-based formulations demonstrated positive effects in inhibiting the activity of the pancreatic enzyme α-amylase, especially in case of sponge-dough breadmaking process. The ternary formulation of millet (raw, extruded and germinated millet flour) demonstrated better effects mainly in terms of the content of phenolic compounds and the nutritional characteristics of antioxidant capacity. Therefore, it can be concluded that millet has the potential to be used in gluten-free baking with adequate nutritional and technological characteristics in order to be useful for the diet management. As a perspective, it will be interesting to evaluate the sensorial acceptance of bakery products obtained by the use of millet under different processing and baking processes.

Funding

This work was funded by the Carlos Chagas Filho Research Support Foundation of the State of Rio de Janeiro, E-26/210.022/2020; and National Council for Scientific and Technological Development, 308416/2019-8.

Declaration of competing interest

The authors attest that there are no interests that competed with the objective, interpretation, and presentation of the results.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Credit authorshipp contribution statement

Lorrany Miranda Marinho: Conceptualization, Methodology, Data curation, Writing- original draft, preparation, Visualization, Investigation, Writing - review & editing. **Monique Barreto dos Santos**: Visualization, Investigation, Writing - review & editing. **Carlos Wanderlei Piler Carvalho**: Supervision, Writing -review & editing. **Cristina Yoshie Takeiti**: Supervision, Validation, Writing - review & editing.

References

Abioye, VF., Babarinde, GO., Ogunlakin, GO., Adejuyitan, JA., Olatunde, SJ., & Abioye, AO. (2022). Varietal and processing influence on nutritional and phytochemical properties of finger millet: A review. Heliyon, 8, Article e12310. doi.org/10.1016/j.heliyon.2022.e12310

Aleixandre, A., Gisbert, M., Sineiro, J., Moreira, R., & Rosell, CM. (2022). In vitro inhibition of starch digestive enzymes by ultrasound-assisted extracted polyphenols from *Ascophyllum nodosum* seaweeds. Journal of Food Science, 87, 2405–2416.

AMERICAN ASSOCIATES OF CEREAL CHEMIST INTERNATIONAL. **AACC**. Measurement of bread firmness —Compression Test, 1999.

AMERICAN ASSOCIATES OF CEREAL CHEMIST INTERNATIONAL. **AACC**. Approved methods of analysis of the AACC. 10 ed. St. Paul, 2000.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS INTERNATIONAL. **AACC**. Approved Methods of Analysis, 11 ed. Saint Paul, MN, USA, 2010.

BRASIL, MINISTERIO DA SAUDE. (2003). Agência nacional de vigilância sanitária resolução - RDC nº 360, de 23 de dezembro de 2003. Diário Oficial da União.

Chandrasekara, A., & Shahidi, F. (2010). Content of Insoluble Bound Phenolics in Millets and Their Contribution to Antioxidant Capacity. Journal of Agricultural and Food Chemistry, 58, 6706–6714. doi:10.1021/jf100868b

Chandrasekara, A., & Shahidi, F. (2011). Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. Journal of Functional Foods**,** 3 (3), 159-170. doi.org/10.1016/j.jff.2011.03.008

Chandrasekar, V., Nambi, EV., Sultan, S., & John, SG. (2022). Effect of operating conditions of a solid-state fermenter on structural and pasting properties of finger millet flour as applied to bread quality. Journal of Food Process Engineering, 45. doi.org/10.1111/jfpe.14017

Chinenye, OE., Ayodeji, OA; & Baba, AJ. (2017). Effect of Fermentation (Natural and Starter) on the Physicochemical, Anti-nutritional and Proximate Composition of Pearl Millet Used for Flour Production. American Journal of Bioscience and Bioengineering, 5(1), 12-16.

Ferreira, SMR., de Mello, AP., dos Anjos, MCR., Krüger, CCH., Azoubel, PM., & Alves, MAO. (2016). Utilization of sorghum, rice, corn flours with potato starch for the preparation of gluten-free pasta. Food Chemistry, 191, 174-151. doi.org/10.1016/j.foodchem.2015.04.085

Giuberti, G., Rocchetti, G., & Lucini, L. (2020). Interactions between phenolic compounds, amylolytic enzymes and starch: an updated overview. Current Opinion in Food Science, 31, 102-113. doi.org/10.1016/j.cofs.2020.04.003

Gobbetti, M., Pontonio, E., Filannino, P., Rizzello, CG., De Angelis, M., & Di Cagno, R. (2018). How to improve the gluten-free diet: The state of the art from a food science perspective. Food Research International, 110, 22-32. doi.org/10.1016/j.foodres.2017.04.010

Górnas, P., Dwiecki, K., Siger, A., Tomaszewska-Gras, J., Michalak, M., & Polewski, K. Contribution of phenolic acids isolated from green and roasted boiled-type coffee brews to total coffee antioxidant capacity. European Food. Research and Technology, 242, 641–653. doi: 10.1007/s00217-015-2572-1

Hernández-Aguirre, MA., Islas-Hernández, JJ., Sánchez-Pard, ME., Rodríguez-Ambriz, SL., & Osorio-Díaz, P. (2019). Response surface methodology for optimization of gluten-free bread made with unripe banana flour. Journal of Food Measurement and Characterization, 13, 1652–1660. doi.org/10.1007/s11694-019-00082-y

Herculano, LFL., Pontes, ACB., Rodrigues, CLJ., Barroso, LP., de Oliveira, MN., Medeiros, SRA., Raymundo, ACSNM. (2021). Development of gluten-free breads from under-exploited flours. Brazilian Journal of Development, 7 (6), 62905-62924. doi:10.34117/bjdv7n6-595

International Diabetes Federation. IDF Diabetes Atlas, 10th end. Brussels, Belgium: 2021.Avaible at: https://www.diabetesatlas.org. Access at: November 22, 2022.

Kaur, P., Purewal, SS., Sandhu, KS., Kaur, M., & Salar, RK. (2019). Millets: a cereal grain with potent antioxidants and health benefits. Journal of Food Measurement and Characterization, 13, 793–806. doi.org/10.1007/s11694-018-9992-0

Kumar1, P., Kaur, C., Sethi, S., & Jambh, HK. (2020). Effect of extruded finger millet on dough rheology and functional quality of pearl millet based unleavened flatbread. Cereal chemistry, 97, 991-998. doi.org/10.1002/cche.10321

Krishnan, V., Mondal, D., Thomas, B., Singh, A., & Praveen, S. (2021). Starch-lipid interaction alters the molecular structure and ultimate starch bioavailability: A comprehensive review. International Journal of Biological Macromolecules, 182, 626–638.

Li, Y., Lv, J., Wang, L., Zhu, Y., & Shen, R. (2020). Effects of Millet Bran Dietary Fiber and Millet Flour on Dough Development, Steamed Bread Quality, and Digestion In Vitro. Applied. Sciences, 10, 912. doi:10.3390/app10030912

Liyana-Pathirana, C., & Shahidi, F. (2006). Importance of insoluble-bound phenolics to antioxidant properties of wheat. Journal Agricultura Food Chemistry, 54 (4), 1256–1264. doi: 10.1021/jf052556h

Martinez-Giron J., Figueroa-Molano, AM., & Ordonez-Santos, LE. (2017). Effect of the addition of peach palm (*Bactris gasipaes*) peel flour on the color and sensory properties of cakes. Food Science Technology, 37(3), 418–424. doi. org/ 10. 1590/ 1678- 457X. 14916

McDonough, CM., & Rooney LW. (1989). Structural characteristics of *Pennisetum americanum* (Pearl Millet) using scanning electron and florescence microscopy. Food Structure, 8 (1), 16.

Meng, Y., Guan, X., Liu, X., & Zhang, H. (2019). The rheology and microstructure of composite wheat dough enriched with extruded mung bean flour. LWT - Food Science and Technology, 109, 378–386. doi.org/10.1016/j.lwt.2019.03.095

Mudau, M., Ramashia, S. E., Mashau, M. E., & Silungwe, H. (2021). Physicochemical characteristics of bread partially substituted with finger millet (*Eleusine corocana*) flour. Brazilian Journal of Food Technology, 24, Article e2020123. doi.org/10.1590/1981-6723.12320

Nani, A., Belarbi, M., Ksouri-Megdiche, W., Abdoul-Azize, S., Benammar, C., Ghiringhelli, F., Hichami, F., & Khan, N. A. (2015). Effects of polyphenols and lipids from *Pennisetum glaucum* grains on T-cell activation: modulation of Ca2+ and ERK1/ ERK2 signaling. BMC Complementary and Alternative Medicine, 15: 426. doi 10.1186/s12906-015-0946-3

Novotni1, D., Nanjara, L., Strkalj, L., Drakula, S., Mustac, NC., Voucko, B., & Curic, D. (2023). Influence of Particle Size and Xylanase Pretreatment of Proso Millet Bran on Physical, Sensory and Nutritive Features of Gluten-Free Bread. Food Technology & Biotechnology, 61 (1), 73-84. doi.org/10.17113/ftb.61.01.23.7776

Onyango, C; Mutungi, C; Unbehend, G; Lindhauer, M. G. (2011). Rheological and textural properties of sorghum-based formulations modified with variable amounts of native or pregelatinised cassava starch. LWT - Food Science and Technology, 44 (3), 687–693. doi.org/10.1016/j.lwt.2010.08.019

Patil, S. S., Rudra, SG., Varghese, E., & Kaur, C. (2016). Effect ofextruded fingermillet (*Eleusine coracan* L.) on textural properties and sensory acceptability of composite bread. Food Bioscience, 14, 62–69. doi.org/10.1016/j.fbio.2016.04.001 2212-4292/& 2016ElsevierLtd.Allrightsreserv

Pessanha, KLF., de Menezes, JP., Silva, AA., Ferreira, MVC., Takeiti, CY., & Carvalho, CWP. (2021). Impact of whole millet extruded flour on the physicochemical properties and antihyperglycemic activity of gluten free bread. LWT - Food Science and Technology, 147,111495. doi.org/10.1016/j.lwt.2021.111495

Pessanha, KLF,, Santos, MB ., Freitas-Sá, DGC ., Takeiti, CY., & Carvalho, CWP. (2023). Impact of whole millet extruded four on the physicochemical properties and antihyperglycemic activity of gluten free pasta. Jounal of Food Science and Technology, 60(6), 1738-1748.

Pires, J., Torres, PB., dos Santos, DYAC., & Chow, F. (2017). Microplate assay of antioxidant potential using the DPPH free radical scavenging method for algae extracts. Institute of Biosciences, University of São Paulo.

Rani, S., Singh, R., Sehrawat, R., Kaur, BP., & Upadhyay, A. (2018). Pearl millet processing: a review. Nutrition & Food Science, 48 (1), 30-44. doi.org/10.1108/NFS-04-2017-0070

Santetti, GS., Lima, LRS., Biduski, B., Santos, MCB., D'Almeida, CTS., Cameron, LC., Gutkoski, LC., Ferreira, MSL., & Amboni, RDM. (2022). Untargeted metabolomics analysis reveals improved phenolic profile in whole wheat bread with yerba mate and the effects of the bread-making process. Food Research International, 159, Article 111635. doi.org/10.1016/j.foodres.2022.111635

Santos, MCB., da Silva Lima, LR., Nascimento, FR., do Nascimento, TP., Cameron, LC., & Ferreira, MSL. (2019). Metabolomic approach for characterization of phenolic compounds in different wheat genotypes during grain development. Food Research International, 124, 118-128. doi: 10.1016/j.foodres.2018.08.034

Santos, F G., & Capriles, VD. (2021). Relationships between dough thermomechanical parameters and physical and sensory properties of gluten-free bread texture during storage. LWT - Food Science and Technology, 139 Article 110577. doi.org/10.1016/j.lwt.2020.110577

Selladurai, M., Pulivarthi, MK., Raj, AS., Iftikhar, M., Prasad, PVV.,& Siliveru, K. (2023). Considerations for gluten free foods - pearl and finger millet processing and market demand. Grain & Oil Science and Technology, 6, 59–70. doi.org/10.1016/j.gaost.2022.11.003

Urrea-Victoria, V., Pires, J., Torres, PB., dos Santos, DYAC., & Chow, F. (2016). Microplate Antioxidant Iron Reducing Power (FRAP) Assay for Algae Extracts. Institute of Biosciences, University of São Paulo.

Villarino, CB., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., & Johnson, S. (2014). The effects of bread-making process factors on Australian sweet lupin-wheat bread quality characteristics, International Journal of Food Science & Tecnology, 49, 2373–2381.

Wang, X., Zhao, R., & Yuan, W. (2020). Type I sourdough steamed bread made by retarded sponge-dough method. Food Chemistry, 311, Article 126029. doi.org/10.1016/j.foodchem.2019.126029

Wang, H., Fu, Y., Zhao, Q., Hou, D., Yang, X., Bai, S., Diao, X., Xue, Y., & Shen, Q. (2022). Effect of Different Processing Methods on the Millet Polyphenols and Their Anti-diabetic Potential. Frontiers in Nutrition., 9, Article 780499. doi: 10.3389/fnut.2022.780499

Yano, H. (2019). Recent practical researches in the development of gluten-free breads. Npj Science of Food, 7.

Zhu, F. Staling of Chinese steamed bread: Quantification and control. (2016). Trends in Food Science & Technology*, 55*, 118–127. doi.org/10.1016/j.tifs.2016.07.009

CONCLUSÃO GERAL

O alto potencial nutricional assim como sua cultura sustentável tem sido base para estimular o milheto para o consumo humano. Seja para mitigar a insegurança alimentar que ainda é presente no nosso país ou como alternativa para patologias associadas direta ou indiretamente à alimentação e/ou como estratégia no manejo de hábitos saudáveis. O estilo da vida moderna é caracterizado por estar frequentemente associada ao consumo de alimentos de alta densidade energética, ao perfil elevado e excessivo de açúcar no sangue pós-prandial, à diminuição do gasto energético e ao estilo de vida sedentário, o que tem grande relação com a epidemia das doenças crônicas não transmissíveis que vivemos atualmente.

Neste trabalho, a combinação dos processamentos de extrusão termoplástica, germinação e métodos de panificação apresenta-se como uma proposta de valorização do milheto como rica matriz alimentar para o consumo humano seja no contexto de tratamento de distúrbios relacionados ao glúten ou ao diabetes, como na adequação de dietas saudáveis com o objetivo de diminuição do risco de desenvolvimento de doenças crônicas não transmissíveis.

A partir dos resultados do presente estudo foi demonstrado que a fermentação, a germinação e a extrusão favoreceram o aumento do perfil de fibra alimentar, de compostos fenólicos assim como da capacidade antioxidante desses compostos bioativos, ocorrido tanto por biotransformação como por liberação dos compostos da matriz alimentícia. Além disso, demonstrou-se que o milheto na forma de produto final, como é o caso do pão, mantém as características de atividade anti-hiperglicemiante que tem sido alvo de muitos estudos, porém em sua maioria apenas em farinhas cruas de milheto. Finalmente, este estudo contribui para o entendimento do impacto do processamento de alimentos frente os benefícios oferecidos ao consumidor a nível de suas características físicas, químicas, sensoriais e nutricionais.

Supplementary material

Table S.1 Pasting properties characteristics by RVA.

Samples	T_p (°C)	CV (cP)	PV (cP)	Breakdown (cP)	Setback (cP)	FV (cP)
RMF	84.7^{d}±0.4	33.5^{c}±4.9	844.5^{b}±21.9	389.0^{a}±15.5	1384.5^{b}±6.3	1840.0^{b}±0.0
EMF	84.7^{d}±0.4	143.0^{a}±12.7	203.0^{d}±2.8	91.0^{c} ±2.8	67.5^{d}±4.9	179.5de±4.9
REMF	154.7^{a}±0.3	57.5^{bc}±3.5	156.5^{de}±6.3	41.0^{cd}±2.8	279.0^{d}±15.5	394.5^{d}±19.09
REGMF	90.8^{b}±0.3	47.5^{bc}±9.1	80.5^{e}±3.5	3.5^{d}±3.5	142.0^{d}±0.0	219.0^{de}±0.0
EGMF	28.2^{f}±0.8	117.5^{a}±2.1	120.0^{de}±1.4	43.0^{cd}±1.4	38.5^{d}±3.5	81.5^{e}±4.9
RGMF	81.6^{e}±0.5	35.5^{bc}±6.3	609.5^{c}±30.4	337.5^{a}±7.7	855.0^{c}±72.1	1127.0^{c}±94.7
WWF	87.2^{c}±0.4	73.0^{b}±5.6	592.0^{c}±7.07	171.5^{b}±13.4	1260.5^{b}±86.9	1681.0^{b}±107.4
RWF	85.8^{cd}±05	68.5^{bc}±20.5	1232.0^{a}±69.2	85.0^{c}±33.9	4097.5a±156.2	4097.5^{a}±156.2

RMF: raw millet flour; EMF: extruded millet flour; REMF: raw and extruded millet flour; REGMF: raw, extruded and germinated millet flour; EGMF: extruded and germinated millet flour; RGMF: raw and germinated millet flour; WWF: commercial whole-wheat flour; RWF: commercial refined-wheat flour. $T_{p:}$ pasting temperature, CV: cold viscosity at the beginning 25 ◦C, PV: peak viscosity, FV: final viscosity. Values are mean ± standard deviation in replicates. Values followed by the same letters in the same columns are not significantly different ($p < 0.05$).

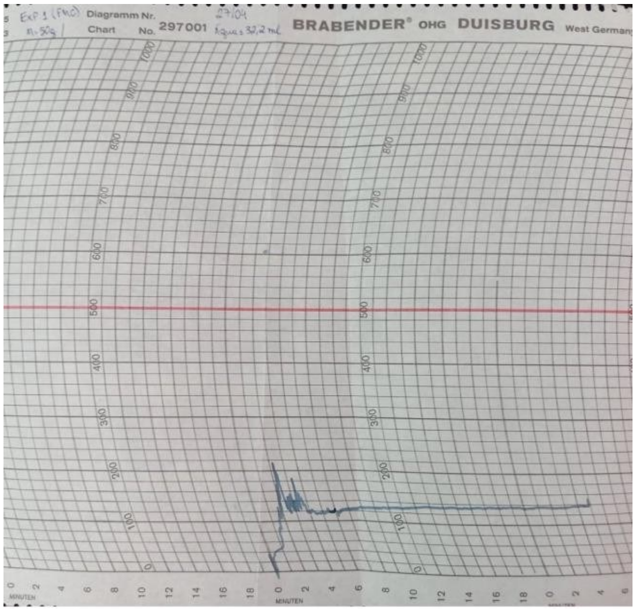
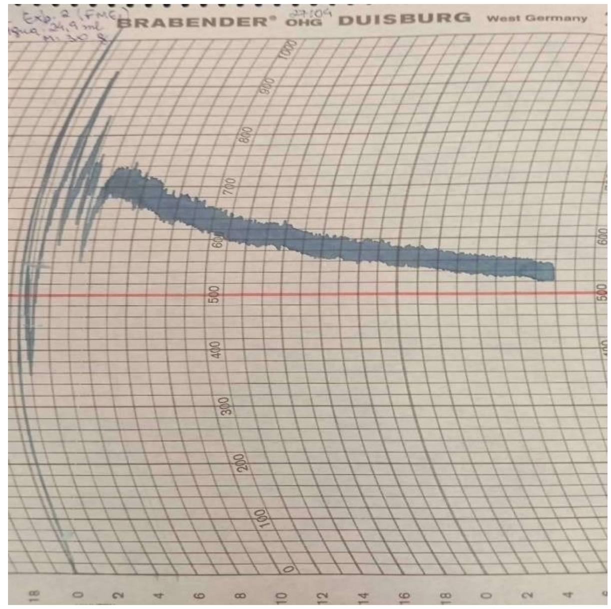
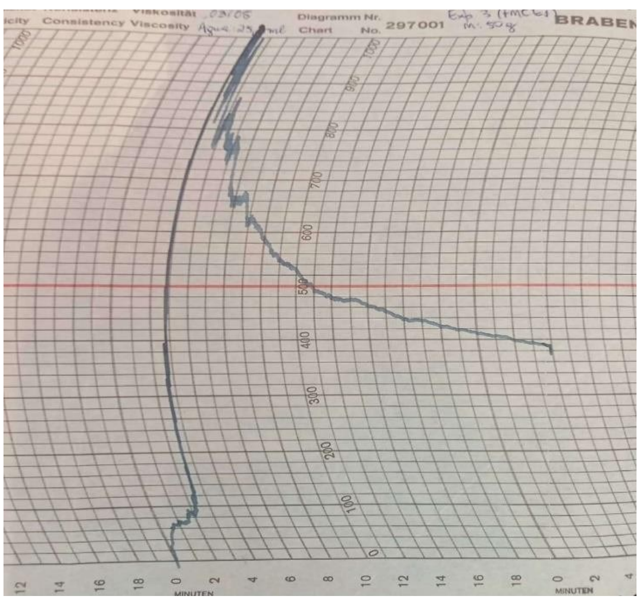


Diagramm Nr.
Chart No. 297001
BRABENDER® OHG DUISBURG West Germany
MINUTEN

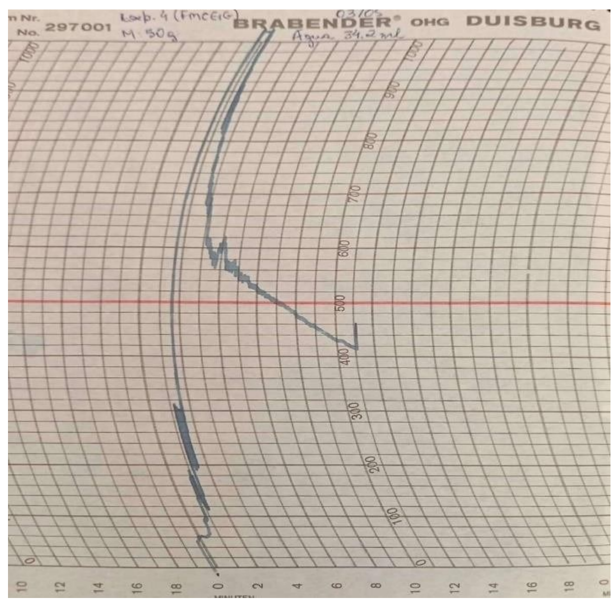
(a)


BRABENDER® OHG DUISBURG West Germany

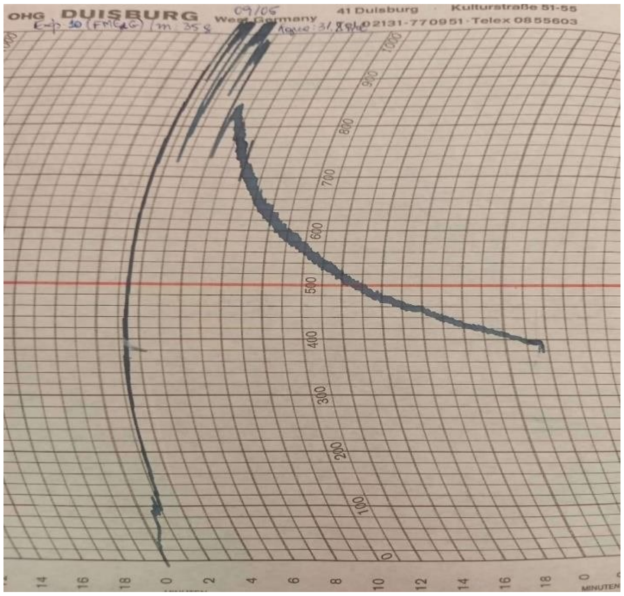
(b)


Consistency Viscosity
Diagramm Nr.
Chart No. 297001
MINUTEN

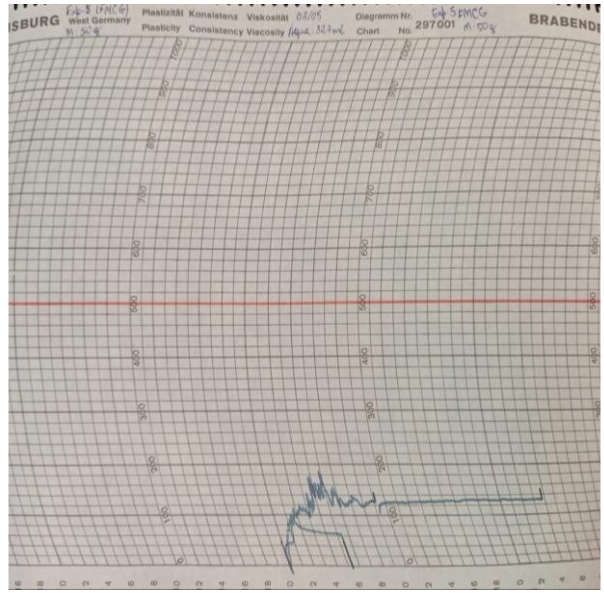
(c)


No. 297001
BRABENDER® OHG DUISBURG

(d)



(e)



(f)

Figure S.1: Farinograph profile of millet flours: (a) RMF; (b) EMF; (c) REMF; (d) REGMF; (e) EGMF; (f) RGMF.