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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

TESE

PÃES INTEGRAIS ISENTOS DE GLÚTEN PRODUZIDOS COM FARINHAS INTEGRAIS MULTICEREAIS OBTIDAS POR EXTRUSÃO TERMOPLÁSTICA

Raúl Comettant Rabanal

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO

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RAÚL COMETTANT RABANAL

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Tese submetida como requisito para obtenção do grau de **Doutor em Ciência e tecnologia de Alimentos** no Curso de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de concentração em Tecnologia de Alimentos.

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DEDICATORIA

A meus pais

Josefa Rabanal Cabanillas e meu pai **Franklin Comettant Campos**

Pelo ensino na vida inteira, por me dar o suporte e respaldo emocional incondicionalmente para me tornar um homem de fé com valores morais para a sociedade e por sua entrega total aos seus filhos.

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RESUMO

COMETTANT-RABANAL, Raúl. **Pães integrais isentos de glúten produzidos com farinhas integrais multicereais processadas por extrusão termoplástica. 115 p**. Tese (Doutorado em Ciência de Alimentos). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro - Seropédica, outubro, 2022.

O pão é um produto ancestral feito originalmente de grãos de cereais como centeio, cevada, einkorn, *emmer*, espelta e trigo sarraceno. Mas estes foram substituídos pelo trigo hexaplóide (AABBDD) que apresenta melhores e notáveis características tecnológicas de sua massa quando hidratada (viscoelasticidade) que, anos mais tarde com a tecnologia de moagem e peneiração, para separação do farelo, tornou-se amplamente consumido na forma de farinha refinada, principalmente para a produção de pão “branco”, assim reduzindo o teor de fibra alimentar, vitaminas e minerais. Com o objetivo de simular essas características tecnológicas e tentar excluir completamente o trigo das formulações de pão, a fim de atender às demandas atuais, que estão associadas a doenças como as autoimunes (doença celíaca), alergias não celíacas e sensibilidades de glúten, que são desencadeadas pelo consumo de frações proteicas (chamadas de prolaminas de glúten) intrinsecamente presentes no trigo. Neste sentido, o uso de grãos inteiros sem glúten são uma alternativa atraente, pois eles oferecem vantagens nutricionais adicionais, mantendo todos os seus constituintes, tais como fibras (insolúveis e solúveis), minerais, vitaminas e fitoquímicos, em comparação com grãos refinados e seus derivados. Neste contexto, o presente trabalho visa desenvolver protótipos laboratoriais de pães sem glúten à base de farinhas pré-cozidas por extrusão termoplástica de grãos inteiros de arroz, de milho e de sorgo, que foi dividido em quatro capítulos. No capítulo 1, foi elaborada uma revisão de literatura sobre processos físicos aplicados a grãos e farinhas sem glúten para alcançar certas propriedades viscoelásticas simulando o glúten de trigo, livre de aditivos. Os resultados neste campo são escassos, mostrando que ainda é um desafio desenvolver pães isentos de farinha de trigo sem aditivos (hidrocoloides e amidos), portanto, a tecnologia de extrusão termoplástica pode ser promissora, já que modifica os biopolímeros nativos nos cereais com características técnico-funcionais para serem aplicados na indústria de panificação e massas. O objetivo do capítulo 2 foi utilizar a tecnologia de extrusão termoplástica como um pré-tratamento para a farinha de grãos integrais (milho, arroz parboilizado e sorgo) e a incorporação de 5% de farinha de grãos de milheto germinados para a produção de pão sem glúten como fonte também de enzimas. As farinhas foram caracterizadas quanto a composição química e distribuição granulométrica, a massa foi determinada quanto as propriedades reológicas, tanto empíricas quanto fundamentais e os pães analisados quanto volume, textura e macroestrutura por análise de imagens. A extrusão termoplástica permitiu o desenvolvimento de maior absorção de água (105-153%) e melhoria das propriedades viscoelásticas da massa. Este processo resultou também no aumento do volume específico (66, 33 e 82%, respectivamente para o arroz, milho e sorgo), bem como na formação de uma melhor distribuição interna das células (alvéolos) de ar nos três diferentes pães produzidos, especialmente no pão de sorgo. Além disso, a farinha de arroz integral parboilizado apresentou massa atípica com elevadas absorções de água e desenvolvimento de propriedades reológicas relacionadas com a viscoelasticidade da massa como consistência farinográfica e tan δ, que também afetou as características de textura, macroestrutura e volume específico do pão. A incorporação de 5% de milheto germinado aumentou a maciez da massa do pão em todas as amostras, particularmente para a farinha de arroz parboilizada integral extrudada adicionada da farinha germinada de milheto, que apresentou valores de dureza (7,3 N) e elasticidade (0,97) semelhantes aos da farinha de trigo integral. No capítulo 3, foi estudada a funcionalidade das farinhas pré-tratadas por extrusão branda e as interações entre arroz, milho e sorgo por meio da aplicação do delineamento experimental de mistura simplex-centroide, onde foram estabelecidos os tratamentos comparando-se com as farinhas únicas, misturas de farinhas binárias (dois cereais) e misturas de farinhas ternárias (três cereais), esta última denominada multigrãos. Os resultados permitiram-se encontrar que o uso da farinha única de sorgo a que apresentou melhores características em termos de volume e macroestrutura do pão, em comparação com as outras farinhas únicas de milho e de arroz. As misturas binárias e multigrãos não apresentaram melhorias consideráveis, possivelmente devido ao teor de fibras do milho e à excessiva modificação da fração amilácea presente no arroz parbolizado, que ainda foi extrudado. Entretanto, quando as massas obtidas a partir dos grãos foram misturadas, observou-se melhorias na estrutura dos miolos de pão multigrão. Finalmente, no capítulo 4 estudou-se a digestibilidade *in vitro* do amido por determinação dos açúcares redutores (RS) e a bioacessibilidade dos bioativos nos pães tais como, compostos fenólicos totais (TPC), taninos condensados totais (TCT), capacidade antioxidante (ABTS^{+}), bem como os ácidos fenólicos e flavonóides.

Palavras-chave: Bioacessibilidade, compostos bioativos, cozimento por extrusão, multigrão, reologia.

ABSTRACT

Bread is an ancestral product made from cereals such as rye, barley, eirkon, *emmer*, spelt and buckwheat; these grains were displaced by hexaploid wheat (AABBDD) which has outstanding technological characteristics (tenacity, extensibility and viscoelasticity) which, years later with milling and sieving technology, became widely consumed as refined flour, mainly for the production of light white bread. Aiming to simulate these technological characteristics and trying to completely exclude wheat from bread formulations, in order to meet current demands, which are associated with diseases such as autoimmune diseases (celiac disease), non-celiac allergies and gluten sensitivities, which are triggered by the consumption of protein fractions (called gluten prolamins) intrinsically present in wheat, barley, rye and oats. In this sense, the use of gluten-free grains in their whole state offers an attractive alternative because they offer additional nutritional advantages by maintaining all their constituents such as fibre (soluble and insoluble), minerals, vitamins and phytochemicals compared to refined grains and their derivatives. In this context, the present work aimed to develop laboratory prototype gluten-free breads based on extruded whole grain flours from corn, rice, and sorghum, which was divided into four chapters. In chapter 1, a literature review was carried out on physical and thermal processes applied to gluten-free grains and flours to achieve certain viscoelastic properties that mimic wheat gluten free of chemical additives. The results in this field were scarce, showing that it is still a challenge to develop gluten-free breads free of additives (hydrocolloids and starches), therefore, thermoplastic extrusion technology is a promising technology that can transform native biopolymers into modified ones with techno-functional characteristics to be applied in the bakery and pastry industry. The objective of chapter 2 was to use thermoplastic extrusion technology as a pre-treatment for whole grain flour (millet, parboiled brown rice and sorghum) and the incorporation of 5% germinated millet to produce gluten-free bread. The flours were characterized (chemical composition and granulometric distribution), the doughs were evaluated (paste, rheological, empirical, and fundamental properties) and the quality characteristics of the bread were analyzed (physical, structural, and textural measurements). The thermoplastic extrusion allowed the development of consistency, better water absorption (105-153%) and viscoelastic properties of the dough. This process resulted in an increase of the specific volume (66, 33 and 82%, respectively for millet, rice and sorghum bread), and the formation of a better internal distribution of the air cell in the three different breads produced, especially in sorghum bread. In addition, the parboiled brown rice presented atypical paste and dough rheological properties, which also affected the quality characteristics of the bread. The incorporation of 5% sprouted millet improved the dough softness of the bread in all the samples, particularly for the extruded rice flour added with sprouted flour, which presented hardness (7.3 N) and elasticity (0.97) values similar to those of whole wheat flour. In chapter 3, we studied the functionality of flours pre-treated by bland extrusion and the interactions between whole grain corn, parboiled brown rice, and sorghum cereals using simplex-centroid mixture design, where we established treatments or formulations comparing pure flours, binary mixtures (two cereals) and ternary mixtures (three cereals) or also called multigrains. The results allowed us to establish that sorghum as pure flour was the cereal with the best characteristics in terms of volume and macrostructure compared to the other pure wholemeal corn and rice flours. The binary and ternary mixtures did not show considerable improvements, possibly due to the fibre content of the corn and the excessive consistency of the extruded parboiled rice. However, when the doughs obtained from the pure grains were mixed, improvements in the structure of the multigrain gluten-free bread millets were observed. Finally, chapter 4 studied the in vitro starch digestibility by determination of reducing sugars (RS) and the bioaccessibility of bioactives in gluten-free breads by determination of total phenolic compounds (TPC), total condensed tannins (TCT), antioxidant capacity ($ABTS^{+}$), as well as the quantification of phenolic acids and flavonoids of the gluten-free breads.

Keywords: in vitro digestibility, Bioaccessibility, Bioactive compounds, Extrusion cooking, Multigrain, Rheology.

SUMMARY

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LIST OF ABBREVIATIONS AND SYMBOLS

AACC: American Association of Cereal Chemist
ABTS2,2’-azinobis, 3-etilbenzotiazolina-6-ácido sulfônico
Ad: Adhesiveness (g·s)
AOAC Association of Official Agricultural Chemists
AT: arrival time (min)
BDV: Breakdown viscosity (PV-TV, cP)
BB: Binary bread
BU: Brabender units
CD: Celiac disease
CE: Catechin equivalent
Ch: Chewiness (N)
Co: Cohesiveness (adimensional)
CONAB: Companhia Nacional de Abastecimento
CV: Cold viscosity at the beginning 25 °C (cP)
DDT: dough development time (min)
DST: dough stability time (min)
DT: departure time (min)
DPPH: 2,2-difenil-1-picril-hidrazil
D-BB: Digested binary bread
D-SB: Digested sorghum bread
D-MB: Digested multigrain bread
D-WB: Digested whole bread
D-WWB: Digested whole wheat bread
EWCF: Extruded whole corn flour
EPBR: Extruded parboiled brown rice
EWSF: Extruded whole sorghum flour
FV: Final viscosity (cP)
GAE: Gallic acid equivalents
GF: Gluten free
GFBs: Gluten-free breads
G’: Elastic or storage modulus (Pa)
G”: Viscous or loss modulus (Pa)
H: Height (mm)
Hardness (Hd, N)
HCPC: Hierarchical Clustering on Principle Components
LM: Hammer mill
LVR: Linear viscoelastic regime
MB: Multigrain bread
MTI: mixing tolerance index
MV: Minimum viscosity after heating
NCGS: Non-autoimmune and non-allergic disorders also called nonceliac gluten sensitivity
P: Porosity (TCA/TBA, %)
PAs: Phenolic acids
PCA: Principal component analysis
PCs: Principal components
PBR: Parboiled brown rice
PM: peak maximum of consistency (BU)

pTem: pasting temperature
PV: Peak viscosity at 95 °C (cP)
PZD: Particle size distribution
r: Pearson´s coefficient
R^2: Coefficient of determination
R: Resilience (adimensional)
RVA: Rapid Visco Analyser
SA: Solid area (TBA – TCA, mm^2)
SBV: Setback viscosity (FV-TV, cP)
SB: Sorghum bread
Sp: Springiness (adimensional)
tan δ (G''/G'): Angle of displacement
TBA: Total area of bread slice (mm^2)
TCA: Total cell area (mm^2)
TCT: Total condensed tannins
TE: Trolox equivalent
TEAC: Trolox equivalent antioxidant capacity
TPC: Total phenolic compounds
TV trough viscosity or holding strength (cP)
WB: White bread
WCF: Whole corn flour
WI: Water solubility index
WSF: Whole sorghum flour
WWF: Whole wheat flour
WSF: Whole sorghum flour
µm: Micrometer
λ: Box-Cox transformation factor

INTRODUÇÃO GERAL

O consumo de pão à base de cereais integrais é ancestral e data do início das primeiras civilizações, onde o trigo deslocou outros cereais utilizados na panificação, pois tinha características tecnológicas únicas necessárias para desenvolver massas viscoelásticas que permitissem reter o CO_2 durante a fermentação para obter pães leves com miolo com alvéolos ocos bem distribuídas e macias. O uso do trigo se intensificou com os avanços tecnológicos (moagem e peneiramento) para obter farinhas brancas refinadas sem pericarpo e hoje constitui 95% da produção total de trigo que é utilizada principalmente para a panificação. Além disso, nas últimas décadas, foram identificados grupos de indivíduos que têm sensibilidade para o trigo, especificamente para suas frações proteicas conhecidas como "prolaminas de glúten", e, portanto, requerem produtos sem glúten que sejam nutritivos, baixos em calorias e com características semelhantes àquelas feitas com trigo.

Neste contexto, o uso de grãos integrais sem glúten é uma alternativa nutricional que, ao diferente de sua contraparte (farinha de trigo refinado), fornece maiores quantidades de fibra alimentar, que, sendo moléculas não digeríveis, pode reduzir a digestibilidade do amido e o índice glicêmico no sangue e assim ajudar a prevenir doenças associadas ao metabolismo da glicose ou também conhecidas como desordens metabólicas. A maioria dos fitoquímicos, incluindo pigmentos (antocianinas, carotenoides, betalainas etc.), taninos e compostos fenólicos, que conferem capacidade antioxidante comprovada e estão relacionados com a prevenção de doenças cardiovasculares e alguns tipos de câncer através de mecanismos de sequestro de espécies reativas de oxigênio (ROS) no organismo humano, estão localizados junto com as fibras (na testa dos grãos).

Neste sentido, milho integral, arroz e sorgo foram utilizados devido à sua fácil disponibilidade no mercado e baixo custo, já que são os cereais isentos de glúten mais amplamente produzidos no Brasil. Entre seus benefícios nutricionais e nutracêuticos como grãos integrais, o milho amarelo brasileiro (*Zea mays* L) possui carotenóides, é rico em fibras e lipídios com a presença de ácidos graxos polinsaturados e tocoferóis; o arroz (*Oryza sativa* L.) parbolizado entre todos os cereais sem glúten tem os mais altos níveis de lisina e suas prolaminas são de menor alergenicidade; o sorgo pigmentado (*Sorghum bicolor* (L) Moench ssp.) é por excelência o cereal com maior variedade de fitoquímicos com alto poder antioxidante devido a suas altas concentrações de ácidos fenólicos, flavonóides e taninos. Estes grãos individualmente ou em conjunto oferecem um alto potencial como matéria-prima ou ingredientes para o desenvolvimento de pães funcionais sem glúten.

O processo de extrusão termoplástica é uma tecnologia múltipla que combina principalmente efeitos térmicos e de cisalhamento (termomecânico) pela dupla ação de aquecimento dentro de um barril e a rotação dentro do barril de um ou dois parafusos que transportam, misturam, comprimem, pressurizam e derretem o material ao entrar no barril até ser forçado a sair por um pequeno orifício. Dependendo das condições de operação, vários fenômenos simultâneos ocorrem, como cozimento, pré-gelatinização, dextrinização, plastificação e conversão dos componentes da matéria-prima para gerar produtos expandidos, granulados e texturizados, que podem ser utilizados como ingredientes técnico-funcionais com propriedades viscoelásticas na indústria do pão sem glúten. Portanto, o objetivo desta tese é desenvolver pães nutritivos sem glúten a partir de farinhas integrais de milho, arroz parboilizado e sorgo, processados por extrusão termoplástica. Os objetivos específicos eram:

1. Desenvolver farinhas extrudadas de grãos integrais e adição de milheto germinado como ingredientes funcionais para pães isentos de glúten.
2. Avaliar a funcionalidade das farinhas integrais pré-tratadas por extrusão e sua interação para produzir pães veganos isentos de glúten
3. Estudar a digestibilidade *in vitro* do amido e a bioacessibilidade de compostos bioativos em pães isentos glúten de farinhas extrudadas de grãos integrais e suas misturas.

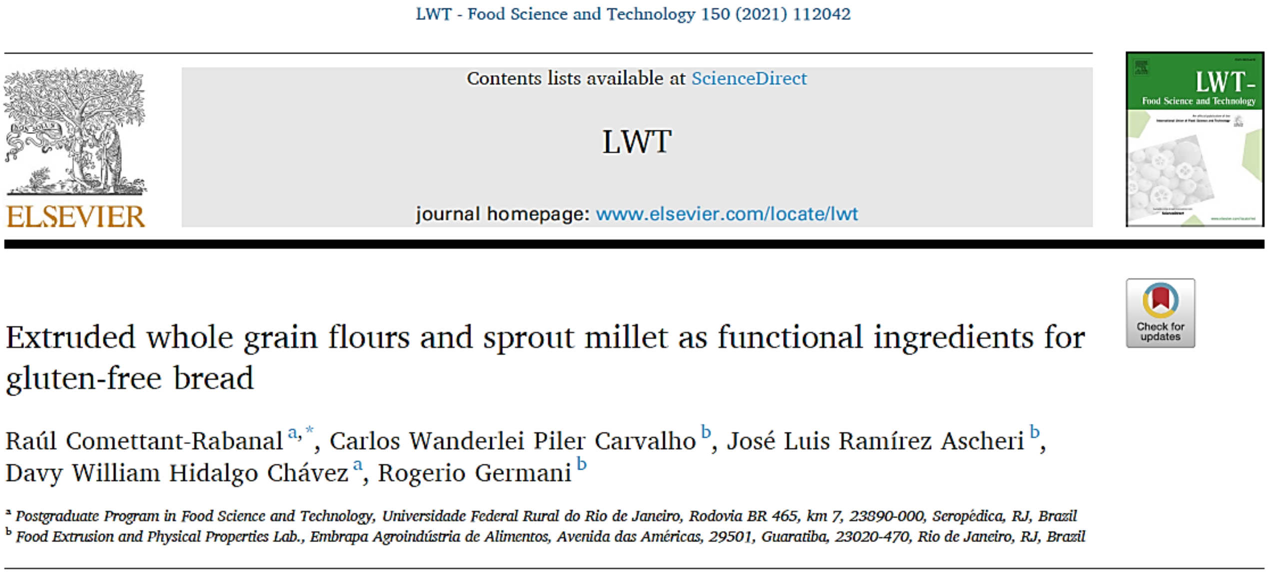
ESTRUTURA DA TESE

A tese foi desenvolvida em três capítulos, como se segue:

Chapter	Title	Production until defense date
1	Extruded whole grain flours and sprout millet as functional ingredients for gluten-free bread	Original article published in LWT- Food Science and Technology on June 2021
2		
3	Avaliação da funcionalidade das farinhas integrais pré-tratadas por extrusão e sua interação para produzir pães veganos isentos de glúten	(Em submissão)
4	Estudo da digestibilidade *in vitro* do amido e a bioacessibilidade de compostos bioativos em pães sem glúten de farinhas extrudadas de grãos integrais e suas misturas	(Em submissão)

CHAPTER I AND II

EXTRUDED WHOLE GRAIN FLOURS AND SPROUT MILLET AS FUNCTIONAL INGREDIENTS FOR GLUTEN-FREE BREAD



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Extruded whole grain flours and sprout millet as functional ingredients for gluten-free bread

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Resumo

Este trabalho teve como objetivo utilizar a tecnologia de extrusão termoplástica como um pré-tratamento para farinhas de grãos integrais (milho, arroz integral parboilizado e sorgo) e a incorporação de milheto germinado a 5% para produzir pão sem glúten. O estudo caracterizou-se a farinhas (composição química e distribuição granulométrica), avaliou-se a massa (pasta, propriedades reológicas empíricas e fundamentais) e analisaram-se as características de qualidade do pão (medidas físicas, estruturais e texturais). A extrusão de termoplástico permitiu o desenvolvimento da consistência, melhor absorção de água (105 a 153%) e propriedades viscoelásticas das massas. Este processo provocou um aumento do volume específico (66, 33 e 82%, respectivamente para pão de milho, arroz e sorgo), e a formação de uma melhor distribuição interna da célula de ar nos três diferentes pães produzidos, especialmente no pão de sorgo. Além disso, o arroz integral parboilizado mostrou uma colagem atípica e propriedades reológicas da massa, o que também afetou as características de qualidade do pão. A incorporação de 5% de milheto germinado melhorou a maciez do pão em todas as amostras, particularmente para a farinha de arroz extrudado adicionada de farinha de milheto germinado, que apresentou valores de dureza (7,3 N) e de elasticidade (0,97) semelhantes aos da farinha de trigo integral.

Palavras-chave: farinha sem glúten, reologia, processo termomecânico, propriedades viscoelásticas

Abstract

This work aimed to use thermoplastic extrusion technology as a pretreatment for whole grain flours (corn, parboiled brown rice, and sorghum) and the incorporation of germinated millet at 5% to produce gluten-free bread. The study characterized the flour (chemical composition and particle size distribution), evaluated the dough (pasting, empirical and fundamental rheological properties) and analyzed the bread quality characteristics (physical, structural, and textural measurements). Thermoplastic extrusion enabled the development of consistency, improved water absorption (105 to 153%) and viscoelastic properties of the doughs. This process caused an increase of the specific volume (66, 33 and 82%, respectively for corn, rice and sorghum made bread), and formation of better internal air cell distribution in the three different breads produced, especially in the sorghum bread. In addition, parboiled brown rice showed atypical pasting and rheological properties of the dough, which also affected the quality characteristics of the bread. The incorporation of 5% germinated millet enhanced breadcrumb softness in all samples, particularly for extruded rice flour added of germinated millet flour sample, which presented similar hardness values (7.3 N) and springiness (0.97) to whole wheat flour.

Keywords: non-gluten flour, rheology, thermomechanical process, viscoelastic properties

INTRODUCTION

Gluten-free (GF) products are indispensable for individuals affected by gluten ingestion (STAMNAES; SOLLID, 2015). Prolamins are gluten fractions directly related to gluten-related disorders such as celiac disease, wheat allergy, and non-celiac gluten sensitivity (SCHERF; KOEHLER; WIESER, 2016). Furthermore, the high consumption of refined wheat-based products is attributed to low cost, wide availability, and singular properties that favor the production of bulky loaves. These modern habits result in the loss of many micronutrients and phytochemicals in the human diet, since often are separated during milling and are directly related to health benefits (KIKUCHI; NOZAKI; MAKITA *et al.*, 2018).

That is why today the intake of whole grains (WG) and/or their derivatives that contain all their original components in the same proportion is being valued, since they constitute an important source of carbohydrates, proteins, fiber, bioactive phytonutrients, vitamins of the group. B and minerals (OLDWAYS WHOLE GRAINS COUNCIL, Undated). Among the gluten-free whole grains that are grown mainly in Brazil are corn, rice and sorghum (CONAB, 2019). Whole corn has various bioactive constituents, such as carotenoids, anthocyanins (pigment depending) and phenolic compounds that have many health-promoting and disease-preventing properties (SINGH; SINGH; SHEVKANI, 2019). Parboiled brown rice is a hypoallergenic grain with bioactive components including dietary fibers, ɣ-oryzanols, and phytosterols (CHO; LIM, 2016). Sorghum is a gluten-free ancient grain that stands out for having high levels of dietary fiber and phytochemicals including phenolic acids (especially 3-deoxyanthocyanidins), condensed tannins, polyflavanols (procyanidins), anthocyanins, phytosterols and policosanols, presented in the pericarp and are of considerable interest due to their possible health benefits (AWIKA; ROONEY; WU *et al.*, 2003; DYKES, 2019).

In order to produce gluten-free breads some pretreatments have been tried. Among them, extrusion cooking is considered an integrated process that combines various operations (conveying, mixing, shearing, and cooking) into one only system which enables transform native biopolymers present in cereals into new functional biopolymers (GANJYAL, 2020). This process manages to modify the molecular structure of starch, which leads to an increase in its functional properties such as water absorption and the development of dough consistency (ESPINOSA-RAMÍREZ; RODRÍGUEZ; DE LA ROSA-MILLÁN *et al.*, 2021). Likewise, recent studies have shown that extrusion promotes structural changes in corn proteins (zein) and also favors synergic interactions with starch that enhance dough viscoelasticity (FEDERICI; JONES; SELLING *et al.*, 2020). In addition, there is ample evidence of other favorable effects of extrusion on the conversion of insoluble to soluble fibers (AKTAS-AKYILDIZ; MASATCIOGLU; KÖKSEL, 2020), reduction of antinutrients, increase of minerals bioavailability, flour stabilization by inactivation of lipolytic enzymes and increased antioxidant activity through the release of phenolic compounds bound in insoluble fibers (PESSANHA; DE MENEZES; DOS ANJOS SILVA *et al.*, 2021). Few previous works have used extrusion under conventional temperature conditions to develop viscoelasticity and try to mimic the effect of wheat gluten to produce GF products (CLERICI; AIROLDI; EL-DASH, 2009; MARTÍNEZ; MARCOS; GÓMEZ, 2013; TORBICA; BELOVIC; TOMIC, 2019).

On the other hand, one problem of GF bread is crumb hardness, but this can be reduced with the addition of germinated grains. This natural ingredient additionally contributes to CO_2 gasification during the fermentation and the retardation of bread staling (MARTI; CARDONE; NICOLODI *et al.*, 2017). Germinated millet (*Pennisetum glaucum* (L.) R. Br.) would allow technological benefits due its high degree of germination ratio and considerable enzymatic activity compared to other grains (HORSTMANN; ATZLER; HEITMANN *et al.*, 2019).

In the aforementioned works related to effect of extrusion on changing starch functionality, they used high process temperatures that ranged from 140 to 220 °C to produce extruded flours that were employed at range from 10 to 70% in the GF bread formulations, whereas the present work only whole grain extruded flours were used in GF bread. Therefore, unconventional extrusion temperatures (<110 °C) can induce slight modifications in the biopolymers contained in cereal-based flours to generate potential nutritious functional ingredients that can be used 100% in GF bread formulations. The objective of this work was to evaluate the effect of thermoplastic extrusion on the pasting properties modification and development rheological properties of WG flours to improve the quality characteristics of GF bread with and without the incorporation of 5% germinated pearl millet.

MATERIAL AND METHODS

Whole grain flour characterization

Flour preparation

Corn grains were donated by Indústrias de Alimentos Granfino (Nova Iguaçú, Brazil). Sorghum grains (red pericarp, low tannin) were donated by Embrapa Milho e Sorgo (Sete Lagoas, Brazil). Parboiled brown rice and whole wheat flour (WWF) were acquired at a market in Rio de Janeiro. WGs were cleaned and ground using a hammer mill LM3100 (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm opening screen for obtaining fine whole corn flour (WCF), parboiled brown rice flour (PBRF), and whole sorghum flour (WSF).

Germination of pearl millet

Grains of pearl millet hibrid ADR9070 were kindly donated by Atto Sementes (Rondonópolis, Brazil). Grains with a 99% germination index were soaked in water (1:3 grain to water) for 4 h, the water was replaced hourly and then drained following the methodology of Dias-Martins et al (2019). The grains were allowed to germinate in a fermentation cabinet (National Mfg. Co., Lincoln, USA) at a controlled temperature of 30 ºC and relative humidity of 90%. After 24 h, the grains were dried in a fan oven at 30 °C/24 h, until reaching a final moisture content lower than 12%, then they were ground using the same procedure as mentioned above for WG flour.

Chemical composition analysis

The chemical composition of raw and extruded flours was performed according to the AOAC (2000) official analytical methods: moisture (method 925.09), fat (method 945.38), total protein (method 2001.11, factor of 5.75), ash (method 923.03), total dietary fiber (soluble and insoluble) (method 991.43), and the carbohydrate was determined by the difference. The quantification of macro and micro elements was determined following the method 990.08, item 9.2.39 of AOAC (2000).

Particle size distribution (PZD)

The PZD of the raw flours was determined in duplicates, using a S3500 series particle size analyzer (Microtrac Inc., Montgomeryville, USA) according to the modified method 55-40.01 (AACC, 1999) with deionized water, using three size ranges: <0.1 mm, from 0.1 to 0.5 mm, and from > 0.5 to 1.7 mm.

Extrusion conditions

In this work, an Evolum HT25 co-rotating, intermeshing twin-screw extruder (Clextral Inc., Firminy, France) was used. The screw diameter was 25 mm, with a diameter ratio of 40:1, ten heating zones (25, 40, 60, 80, 100, 110, 110, 90, 80 and 70 °C), and the screw speed was set at 200 rpm. WG flours were fed through a twin-screw gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany) at a constant rate of 10 kg/h, and the process was monitored by Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). Deionized water was injected between the first and second modular zones through a port with a 5.25 mm internal diameter using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China) set to Adjust in-barrel moisture content to 25% in the samples and provide a final 25% moisture content. The collected extrudates were dried in a forced air oven at 55 °C/10 h. Then, they were ground under the same conditions as of the flour preparation to obtain fine extruded whole grain flours of corn (EWCF), parboiled brown rice (EPBRF), and sorghum (EWSF).

Rheological evaluation

Pasting properties

A Rapid Visco Analyzer series 4 RVA (Newport Scientific Pty Ltd., Warriewood, Australia) was used to measure the paste viscosity of the raw and extruded flours according to the methodology reported by RAGAEE e ABDEL-AAL (2006). Three grams of the gluten-free whole grain flour adjusted to 14% of moisture (wet basis) were placed along with 25 mL of distilled water in the sample holder (aluminum cup) of the equipment. The test conditions were: mixing at 160 rpm at 25 °C for 2 min, heating up to 95 °C at a constant rate of 14 °C/min and kept it for 3 min and then cooled to 25 °C in 5 min at the same rate, with a total time of 20 min. The pasting property readings measured were pasting temperature (PTem, cP), cold viscosity at the beginning 25 °C (CV, cP), peak viscosity (PV, cP), trough viscosity or holding strength (TV, cP), breakdown viscosity (BDV= PV-TV, cP), final viscosity (FV, cP), and setback viscosity (SBV= FV-TV, cP). Measurements were performed in duplicates.

Farinograph measurement

Dough resistance to mixing was performed using the Farinograph© model FD0234H (Brabender, Duisburg, Germany). The readings were obtained according to the method 54-21.01 AACC (2000b) with the following modifications: For 30 grams of flour, four levels of water addition were tested (between 88 to 106.4, 100.8 to 122.2 and 93.8 to 102.9% for flours of corn, rice and sorghum, respectively) until obtaining the highest dough consistency with optimal hydration. The consistencies obtained from each cereal superpased the 500 Brabender Units (BU) wheat standard. From the farinograms the following readings were considered: water absorption (WA, %), farinographic consistence (BU), dough development time (DDT, min), dough stability time (DST, min) and mixing tolerance index (MTI, min), determined at 5 min after peak. Measurements were performed in triplicates.

Dynamic mechanical properties

The dynamic mechanical properties of the raw and extruded flours were performed using a rotational rheometer HAAKE Mars II (Thermo Fisher Scientific, Karlsruhe, Germany). Prior to the rheological readings, the dough of each cereal was mixed with water in the farinograph using the water absorption (WA) and dough development time (DDT) obtained from farinograms. All analyzes were performed according to KORUS; WITCZAK; ZIOBRO e JUSZCZAK (2009) at 25 °C using a 35 mm diameter parallel plate geometry. Three grams of dough were loaded onto the bottom plate and the top plate approached onto the dough at speed of 0.6 mm/min to a gap of 2 mm. The excess dough was trimmed by removing from the outer edge and then coated with mineral oil to prevent drying during measurement. For each type of dough, a dynamic oscillatory frequency sweep was conducted at constant strain amplitude (γ) within the linear viscoelastic regime (LVR) and frequencies ranged from 0.1 to100 Hz. Values of elastic or storage modulus (G'), viscous or loss modulus (G'') and tan δ (G''/G') were obtained at 1 Hz. Readings were performed in duplicates.

Bread making and quality evaluation

Formulation and bread making procedure

Breads were made following the proportions shown in Table 1. Dough preparation was performed with a 35 g micromixer (National MFG. CO., Lincoln, U.S.A.). 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 for 15 min for activation. All dry ingredients were homogenized for 2 min, prior to adding the palm fat and liquid ingredients. Mixing times that were obtained from the farinograms for each WG flour varied: sorghum: 1.5 min, rice: 3.0 min, and corn: 2 min. Portions of 20 g were cut, formed, and placed into previously greased and floured steel molds of 45 mL capacity; after which they were placed in a fermentation cabinet at 30 °C and 85% RH for 60 min. Finally, they were put into a convection oven FVT5D (Venâncio, Venâncio Aires, Brazil) at 200 °C/14 min, then allowed to cool at room temperature. Bread analyzes were performed after 24 h, using two controls for comparison: commercial whole wheat flour (Control 1) and the mixture of non-extruded and extruded rice flour in the proportion of 50:50% (Control 2).

Table 1. Gluten-free bread formulations.

Samples	Raw flour (%)	Extruded flour (%)	Fat (%)	Yeast (%)	Sugar (%)	Salt (%)	Water (%)	Germinated millet (%)
Control 1	100	-	6	3	3	1.5	70.0	-
Control 2	50	50	-	-	-	-	127.5	-
EWCF	-	100	6	3	3	1.5	100.5	-
EPBRF	-	100	6	3	3	1.5	100.8	-
EWSF	-	100	6	3	3	1.5	96.9	-
EWCF+5% GM	-	95	6	3	3	1.5	100.5	5
EPBRF+5% GM	-	95	6	3	3	1.5	100.8	5
EWSF+5% GM	-	95	6	3	3	1.5	96.9	5

Control 1-commercial whole wheat flour, control 2-mixture of raw and extruded parboiled brown rice flour in the proportion of 50:50%, EWSF-extruded whole sorghum flour, EPBRF-extruded parboiled brown rice flour, and EWCF-extruded whole corn flour, and 5% GM-incorporation of 5% of germinated millet, weight flour basis.

Specific volume analysis

Bread volume was determined using a modified standard seed displacement method 10-05.01 (AACC, 2000a), using millet seeds. The recipient used to do the calculation was a parallelepiped with dimensions of 8.5 cm x 8.4 cm x 9.2 cm (width×length×height). Bread specific volume (cm^3/g) was calculated as bread volume divided by bread weight measured at 24 h after baking.

Bread crumb structure

Images of bread slices with dimensions of approximately 33 x 35 mm were captured using an Epson Perfection 1240U scanner (Seiko, Nagano-Ken, Japan), recording images at 300 dpi resolution (170 mm wide x 60 mm high). Subsequently, the images were analyzed using the ImageJ software (v.1.51j8, Wayne Rasband, National Institute of Health, USA) following the method of CROWLEY; GRAU e ARENDT (2000). Firstly, the region of interest was cropped, then, 8-bit images with dimensions (30.0 x 33.4 mm) were generated and adjusted to threshold. Finally, total area of bread slice (TBA, mm^2), total cell area (TCA, mm^2), solid area (SA = TBA – TCA, mm^2), porosity (P = TCA/TBA, %), and height (H, mm) were determined.

Texture measurement

The texture profile analysis (TPA) was carried out using the center of the bread crumb slices with a thickness of 20 mm using a Texture Analyser TA-XT Plus (Stable Micro Systems, Surrey, U K) equipped with a 5 kg load cell and a 15 mm cylindrical aluminum probe. The analysis was controlled by the Exponent software version 6.1.11.0 (Stable Micro Systems, Surrey, UK) at a compression of 50% and 30 s cycle according to MARTÍNEZ e GÓMEZ (2017). TPA was performed in order to measured hardness (Hd, N), Adhesiveness (Ad, g·s), cohesiveness (Co), springiness (Sp), chewiness (Ch, N), and resilience (R) were measured.

Statistical analysis

Analysis of variance (one-way ANOVA) and LSD Fisher multiple range tests were used to determine the differences among samples. A paired T-test was used to evaluate the extrusion process effect of each cereal and for the incorporation of the germinated millet in the GF bread. The Shapiro-test and Bartlett-test were used to confer normality distribution and homoscedasticity, respectively. The Box-Cox transformation was performed only for those variables that lacked a normal distribution or homoscedasticity by the use of the lambda (λ) to achieve the normal distribution (BOX; COX, 1964). A significant level of 5% was used for all statistical tests.

Multivariate analysis was applied on the paste, farinographic and dynamic mechanical properties of the doughs (1) and on the physical, structural, and textural properties of the breads (2). Principal Component Analysis (PCA) was used to evaluate the relationship between analyzed samples. Pearson´s coefficient (r) was calculated to evaluate the correlations among variables, the strongest correlation was evaluated according to the scale reported by TELES; CHÁVEZ; OLIVEIRA *et al.* (2019). Finally, the Hierarchical Clustering on Principal Components (HCPC) was performed by applying the Euclidean distance and Ward's grouping methods. All analyses were performed using the software R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Whole grain flour characterization

Chemical composition analysis

The WSF protein content was the highest ($p < 0.05$) among the three cereals followed by WCF (7.53 g/100g) and PBRF (6.73 g/100g), respectively (Table 2). WCF showed the highest lipid content ($p < 0.05$) of 4.19 g/100g against WSF (3.46 g/100g) and WCF (2.42 g/100g). The carbohydrate content ranged from 61.7 to 71.6 g/100g, and PBRF showed the highest value ($p < 0.05$), which was attributed to the partial removal of the pericarp after the parboiling process, causing a reduction of dietary fiber content. Dietary fiber stood out for WCF and WSF, with values of 13.2 and 10.8 g/100g, respectively. The soluble fiber fraction in both the WCF and WSF samples were the same (1.80 g/100g), while PBRF showed a little less at 1.14 g/100g, whereas for insoluble fiber, WCF and WSF showed higher values 11.4 and 9.0 g/100g than PBRF, 4.52 g/100g. Similar values of protein, lipids, and dietary fiber content were found by TOLEDO; CARVALHO; VARGAS-SOLÓRZANO *et al.* (2020). The high proportion of insoluble fiber present in WCF and WSF gave rise to high levels of total phenolic compounds, ferric acid content, and in the case of WCF it additionally showed high antioxidant potential, thus consolidated whole grains as a functional ingredient (GUO; BETA, 2013).

All cereals presented high amounts of macro elements such as potassium, magnesium and phosphorus and micro elements such as zinc (Table 2). WSF presented the highest ($p < 0.05$) amounts of iron, manganese, copper, and zinc. PBRF showed high ($p < 0.05$) amounts of manganese and copper, and corn had high ($p < 0.05$) amounts of zinc. According to MARRIOTT; BIRT; STALLINGS e YATES (2020) these important concentrations of macro and micro elements mentioned above, contribute to between 20 to 50% of the iron, 36 to 100% of the magnesium, 15 to 61% of the zinc, 24 to 73% of the copper and 100% of the manganese needs for the daily intake requirements of both children and adults.

Table 2. Chemical composition of gluten-free whole grain flours of corn (WCF) parboiled brown rice (PBRF), and sorghum (WSF).

Components	Whole grain flours		
	Corn	Parboiled brown rice	Sorghum
Moisture (g/100 g)	12.29^{b} ± 0.04	12.36^{b} ± 0.08	11.73^{a} ± 0.02
Ash (g/100 g)	1.07^{a} ± 0.04	1.26^{b} ± 0.02	1.40^{c} ± 0.02
Protein (g/100 g)	7.53^{b} ± 0.13	6.73^{a} ± 0.04	10.47^{c} ± 0.04
Lipids (g/100 g)	4.19^{c} ± 0.00	2.42^{a} ± 0.01	3.46^{b} ± 0.04
Carbohydrates (g/100 g)	61.74^{a} ± 0.13	71.58^{b} ± 0.07	62.14^{a} ± 0.08
Dietary fiber (g/100 g)	13.19	5.66	10.81
Soluble fiber (g/100 g)	1.81	1.14	1.80
Insoluble Fiber (g/100 g)	11.38	4.52	9.01
Total calories (kcal/100 g)	314.67^{a} ± 0.03	335.22^{c} ± 0.39	321.64^{b} ± 0.21
Minerals			
Na (mg/100 g)	6.37^{a} ± 0.06	6.56^{a} ± 2.85	6.39^{a} ± 0.08
K (mg/100 g)	349.25^{b} ± 2.64	244.77^{a} ± 2.66	346.07^{b} ± 5.30
Mg (mg/100 g)	84.56^{a} ± 1.25	127.40^{b} ± 2.85	148.82^{c} ± 1.15
Ca (mg/100 g)	2.43^{a} ± 0.18	7.58^{c} ± 0.14	5.51^{b} ± 0.19
P (mg/100 g)	243.04^{a} ± 4.58	316.66^{b} ± 0.88	351.36^{c} ± 0.33
Mn (mg/100 g)	0.39^{a} ± 0.00	3.21^{c} ± 0.00	1.01^{b} ± 0.02
Fe (mg/100 g)	1.61^{b} ± 0.01	0.83^{a} ± 0.01	3.59^{c} ± 0.24
Zn (mg/100 g)	1.99^{b} ± 0.03	1.68^{a} ± 0.03	1.97^{b} ± 0.00
Cu (mg/100 g)	0.18^{a} ± 0.00	0.22^{b} ± 0.01	0.22^{b} ± 0.01
Particle size (%)			
<0.1 (mm)	7.82^{a}	6.83^{a}	12.20^{b}
0.1-0.5 (mm)	71.63^{b}	40.40^{a}	79.02^{c}
0.5-1.7 (mm)	20.60^{b}	52.81^{c}	8.86^{a}

Values represent the mean ± SD (n=2). Different letters in the same row indicate statistic differences ($p < 0.05$) among samples.

Particle size distribution

WCF and WSF presented the highest percentage of particle size within the range of 0.1 to 0.5 mm. On the other hand, PBRF showed a bimodal behavior between the ranges of 0.1 to 0.5 mm and 0.5 to 1.7 mm, the latter range being the predominant one due to compaction of the endosperm and the sealing of the caryopsis (NAMBI;

MANICKAVASAGAN; SHAHIR, 2017). Only 7 to 12% of the particles in all whole grain flours were found in the range of <0.1.

Rheological evaluation of the doughs

Pasting properties

The raw flours, particularly WSF and WCF presented similar pasting properties such as Ptemp, CV, TV, BDV, but differed in SBV, and FV (Figure 1). On the other hand, WCF and WSF showed a PV of 626 and 696 cP, respectively. Whereas, the PBRF sample presented a peculiar significant increase ($p < 0.05$) of PV, FV, SBV values after the extrusion process. This atypical behavior was also found by CHENG; GAO; WU *et al.* (2020), in buckwheat flour processed by thermoplastic extrusion at 100 °C and high moisture (58 and 70%). The authors attributed this behavior to an increase in granular rigidity resulting from an increase in the crystalline order and the interactions of the starch chain within the amorphous regions, which together caused the increase in peak viscosity due to heat resistance and shear of the modified starch granules (WANG; WANG; WANG; WANG, 2017).

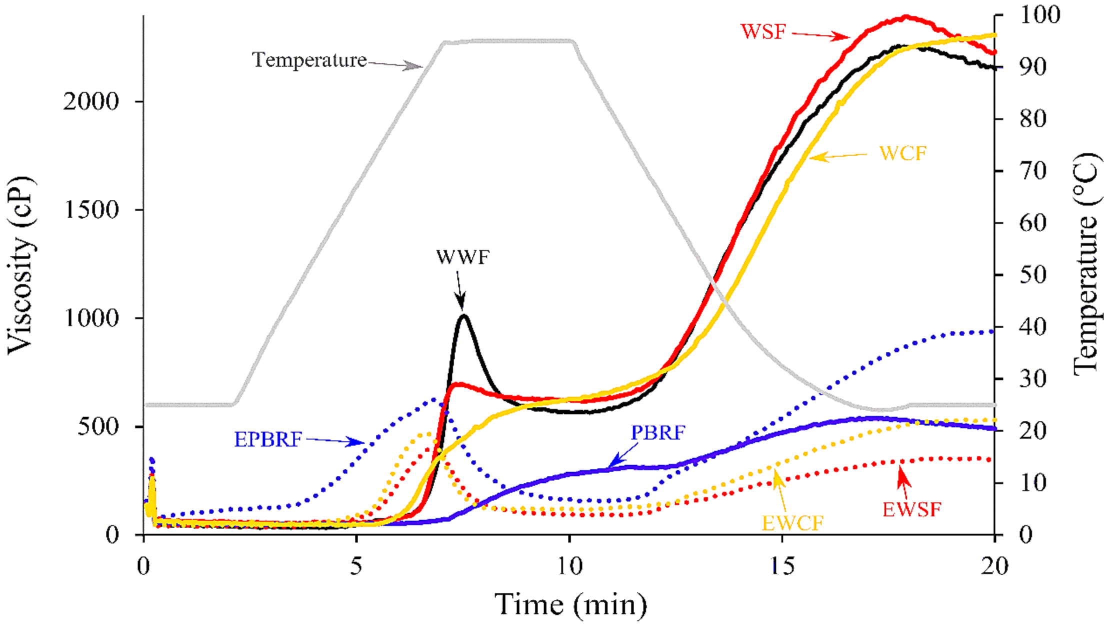

Fig. 1. Paste viscosity properties of WWF (commercial whole wheat flour), raw flours (WCF-whole corn flour, PBRF-parboiled brown rice flour, and WSF-whole sorghum flour) and extruded flours (EWCF-extruded whole corn flour, EPBRF-extruded parboiled brown rice flour, and EWSF-extruded whole sorghum flour).

The thermal stability of all samples, during the holding at 95 °C (TV), decreased ($p < 0.05$) after the extrusion process, while the BDV increased ($p < 0.05$). The EPBRF sample showed the highest values in all pasting properties in comparison with EWCF and

EWSF. On the other hand, FV and SBV showed a significant typical decrease ($p < 0.05$) for the WSF and WCF samples. This behavior is associated with the disruption of the molecular order of the starch granules during the extrusion process causing a loss of their integrity and crystallinity (LINKO; LINKO; OLKKU, 1983).

Farinographic properties

The water absorption levels ranged from 88.0 to 122.0% (Fig. 2a), on a flour weight basis, and within this range it was possible to detect a good dough formation and handling. The extruded flours showed shorter DDT (Table 3), due to the partial modification of their components as was evidenced in the pasting properties (Fig. 1). Namely, rapid water absorption was observed by the extruded flour, which can reduce bread processing time and energy costs. Samples EWCF and EWSF presented a significant increase ($p < 0.05$) in both DST and MTI in comparison with EPBRF (Table 3), and they showed an increased resistance to mechanical work but without affecting the structure of the dough throughout the bread-making process. The increment in both properties may be associated to the reduction of the particle size by the insoluble fiber, which consequently causes an increase in the dough viscosity (LIU; MA; LI; WANG, 2019). The EPBRF sample presented opposite results, possibly due to its degree of starch conversion prior to the extrusion process caused by the parboiling process, which allowed high farinographic consistency, but reduced stability when compared to PBRF.

As previously mentioned, the extrusion process had a positive impact on the increase of the WA and farinographic consistency (Fig. 2a and 2b); a similar behavior was observed by BOUREKOUA; BENATALLAH; ZIDOUNE e ROSELL (2016) for rice and corn flours when they underwent hydrothermal treatment. Furthermore, the water absorption capacity of all WG flours after the extrusion process was very similar, but their corresponding levels of consistency were very variable.

All flour samples demonstrated that water levels below the optimum caused a loss of consistency of the dough due to a lack of hydration, while doughs with water levels above the optimum caused rapid absorption leading to low consistency development and water exudation. In both cases, the absence of consistency may be associated with the higher affinity of the fiber for water, which hinders both the interaction between water and the biopolymers (mainly starch) and the consequent formation of the dough consistency. Furthermore, since EPBRF had a parboiling pretreatment, it exhibited higher levels of consistency compared to EWCF, EWSF, and WWF (Fig. 2b).

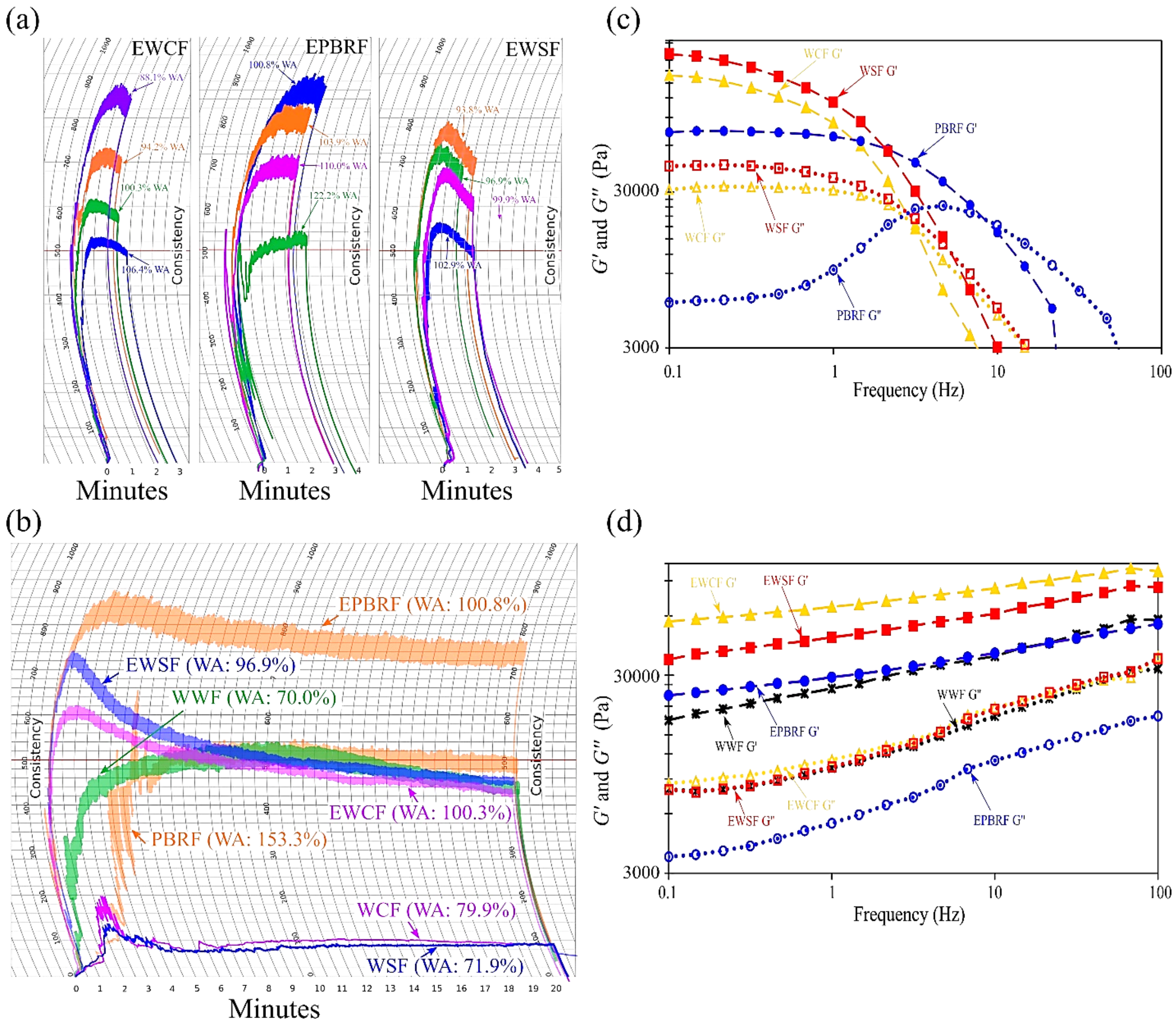

Fig. 2. Empirical and fundamental properties of raw and extruded flours. (a) optimal water absorption of extruded whole grain flours, (b) farinograms of whole grain flour of corn (WCF) parboiled brown rice (PBRF) and sorghum (WSF); extruded whole grain flours (EWCF, EPBRF, and EWSF); and whole wheat flour (WWF), (c) behavior of gluten-free whole grain flours and (d) development of elastic (G′) and viscous modulus (G″) in extruded flours.

Dynamic mechanical properties

Raw flours showed a rapid collapse of the biopolymeric structures when subjected to oscillatory shears between the ranges of 0.1 to 1 Hz (Fig. 2c). Whereas PBRF exhibited type III behavior, which is a characteristic of some molten polymers (HYUN; WILHELM; KLEIN *et al.*, 2011; WAN; CLIFFORD; GAO *et al.*, 2005) where *G'* decreases and *G''* increases followed by a decrease.

Table 3. Empirical and fundamental dynamic mechanical properties of raw and extruded whole grain flours.

Samples	Farinographic properties				Dynamic mechanical properties		
	WA (%)	DDT (min)	DST (min)	MTI (BU)	Critical stress (τ, Pa)	Critical Strain (γ)	tan δ (G''/G', at 1 Hz)
WWF	70.0	9.8^{d} ± 0.4	9.0^{d} ± 1.4	45.0^{c} ± 7.1	60.8^{a} ± 0.9	0.002^{a} ± 0.0000	0.402^{f} ± 0.003
WCF	80.0	$3.0^{c,\alpha}$ ± 0.7	$0.6^{a,\alpha}$ ± 0.0	$121.5^{f,\alpha}$ ± 4.9	$971.6^{b,\alpha}$ ± 45.8	$0.010^{b,\alpha}$ ± 0.0001	$0.370^{f,\alpha}$ ± 0.007
EWCF	100.5	$1.9^{ab,\beta}$ ± 0.1	$1.3^{b,\beta}$ ± 0.0	$101.5^{e,\beta}$ ± 3.5	$888.7^{b,\beta}$ ± 1.1	$0.017^{c,\beta}$ ± 0.0002	$0.170^{b,\beta}$ ± 0.001
PBRF	153.3	$11.5^{e,\alpha}$ ± 0.7	$10.0^{d,\alpha}$ ± 2.1	$21.0^{b,\alpha}$ ± 1.4	$1456.0^{d,\alpha}$± 55.2	$0.021^{d,\alpha}$ ± 0.0014	$0.129^{a,\alpha}$ ± 0.001
EPBRF	100.8	$2.6^{bc,\beta}$ ± 0.1	$3.8^{c,\beta}$ ± 0.2	$82.5^{d,\beta}$ ± 3.5	$3501.5^{e,\beta}$ ± 265.2	$0.142^{e,\beta}$ ± 0.0143	$0.183^{c,\beta}$ ± 0.005
WSF	71.9	$1.8^{ab,\alpha}$ ± 0.4	$0.7^{ab,\alpha}$ ± 0.2	$6.5^{a,\alpha}$ ± 0.7	$1287.0^{cd,\alpha}$ ± 69.3	$0.010^{b,\alpha}$ ± 0.0000	$0.330^{e,\alpha}$ ± 0.008
EWSF	96.9	$1.5^{a,\beta}$ ± 0.1	$1.0^{ab,\beta}$ ± 0.1	$20.5^{b,\beta}$ ± 2.1	$1033.2^{bc,\beta}$ ± 57.8	$0.020^{c,\beta}$ ± 0.0003	$0.222^{d,\beta}$ ± 0.007
Parametric assumptions							
Shapiro test	-	0.61	0.04	0.99	0.06	0.00	0.51
Bartlett test	-	0.43	0.00	0.69	0.01	0.00	0.56
Box-Cox							
λ	-	-	0.06	-	-0.42	-0.75	-

WA-water absorption, unique value without variation. DDT-Dough development time (min), DST- Dough stability time (min), BU-Brabender units, and MTI-Mixing tolerance index - 5 min after peak (BU). WWF- commercial whole wheat flour, WCF-whole corn flour, PBRF-parboiled brown rice flour, WSF-whole sorghum flour, EWCF-extruded whole corn flour, EPBRF-extruded parboiled brown rice flour, and EWSF-extruded whole sorghum flour. Values represent the mean ± SD (n=3). Different letters in the same column indicate statistical differences ($p < 0.05$) among samples. Greek letters indicate paired t-tests for each type of cereal before and after the extrusion process. Box-Cox transformation factor (λ) for non-parametric data.

The extrusion process promoted a significant reduction ($p < 0.05$) of τ for EWCF and EWSF (Table 3), indicating an early disintegration of the new biopolymeric structures in comparison with the native structures. Also, it was found that the extruded flours tolerated a higher γ. Regarding the raw flours, the γ values were 0.010, 0.021, and 0.010 for WCF, PBRF, and WSF respectively, while the extruded samples presented 0.017, 0.142 and 0.020 for EWCF, EPBRF, and EWSF, respectively (Table 3). This increase in γ caused a positive effect of the extrusion process on the transformation of raw materials forming a novel biopolymeric structures with differentiated functional properties.

At frequencies (or angular velocities) greater than 1 Hz, a fall and crossover of the modulus G' and G'' was observed in the WCF and WSF, such behavior is typical in non-crosslinked polymers (Fig. 2c). The opposite effect was shown by those flours subjected to the extrusion process (Fig. 2d). The crosslinking phenomenon is typical in some extruded biopolymers (i.e. cereal melts) which display an ascending linear behavior of the elastic (G') and viscous (G'') modules (BRENT; MULVANEY; COHEN; BARTSCH, 1997).

All extruded flours (Fig. 2d) presented a predominant elastic component ($G' > G''$). The tan δ of EWSF and EWCF decreased (Table 3), indicating an increase in the elasticity, which can be attributed to the plasticizing effect promoted by extrusion on the formation of binding zones among carbohydrate polymers (BRENT; MULVANEY; COHEN; BARTSCH, 1997). Another possible effect could be due to the starch complexation with proteins and lipids (WU; LI; WANG *et al.*, 2010), because those samples presented higher values (Table 2). On the contrary, tan δ of PBRF increased after the extrusion process, probably due to the previous parboiling process that caused the increase and resistance of granular stiffness of rice starch.

Principal components analysis for flours

The two first principal components explained 70.3% of the total variance among a total of 13 variables that represent three groups of results (pasting viscosity, empirical, and fundamental rheology). PC analysis evidenced the differences between the GF cereals and wheat (Fig. 3a). The main variables that characterized these three raw flours were the pasting properties of FV, SBV, and tan δ (Fig. 3b), while WWF presented high BDV (Fig. 1).

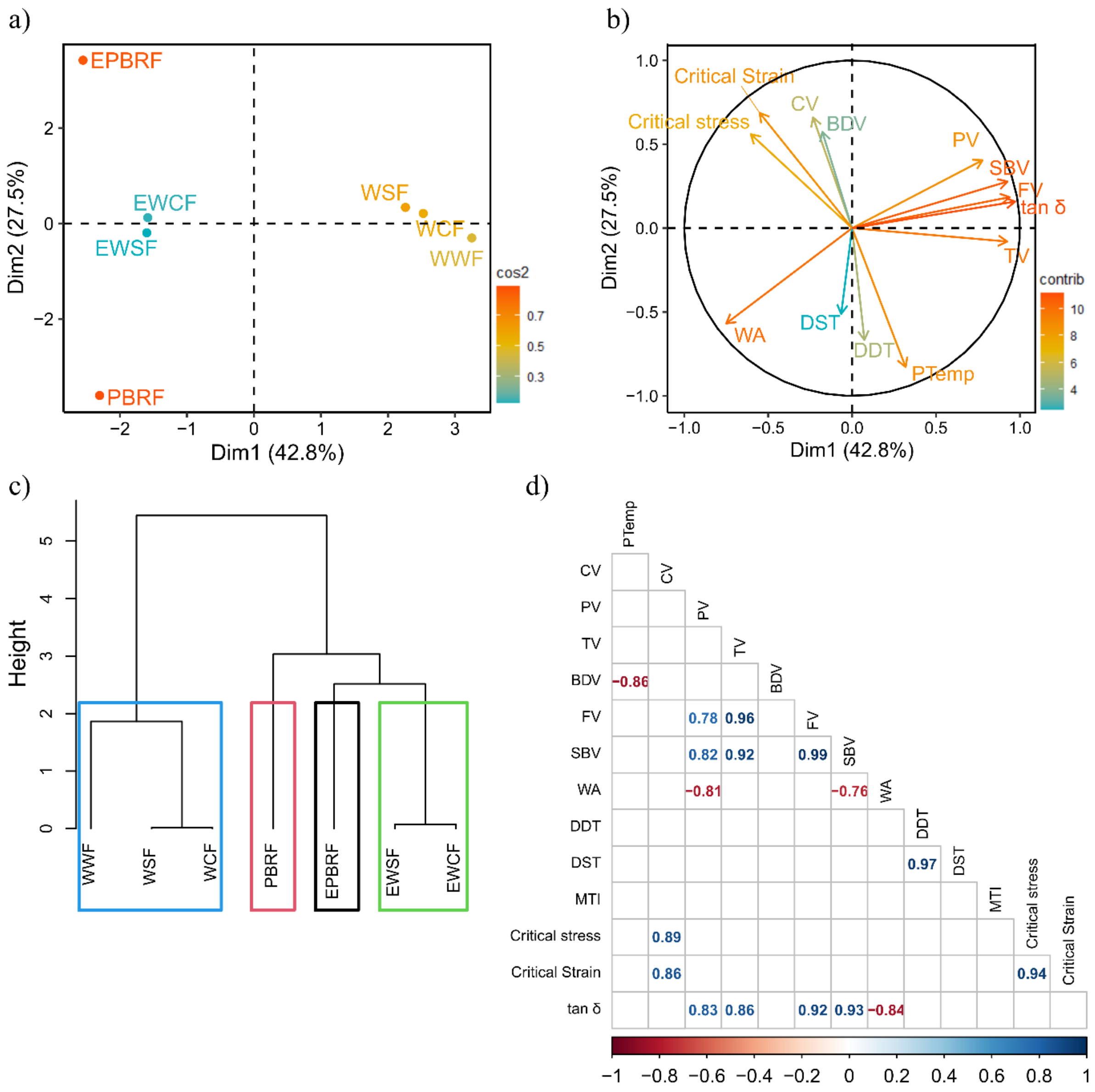

Fig. 3. Principal components analysis (PCA) of whole grain flours. a) score plot for flour samples, b) PCA loading plot for response variables, c) hierarchical clustering on principal components of raw and extruded flours and d) Pearson's correlation coefficients among pasting viscosity, empirical and fundamental rheology properties.

Furthermore, considering the high values of the rheological properties of WA, τ, γ, and CV and the low values of PTemp and PV, it was possible to identify and differentiate a cereal with previous heat treatment such as EPBRF and raw cereals. On the other hand, the variables with the least contribution in the PC1 and PC2 were BDV, DST, and DDT. Finally, the PCs on the raw and extruded flours were used to apply HCPC (Fig. 3c), resulting in 4 groups depending on the type of heat treatment to which the flours were subjected, as follows: raw flour samples (WWF, WSF, and WCF), parboiled brown rice flour (PBRF), the extruded brown parboiled rice flour (EPBRF) and the extruded flours (EWSF and EWCF).

Very high positive correlations ($0.90 > r \leq 0.99$) were detected between FV-TV, SBV-tan δ, SBV-TV, SBV-tan δ, DST-DDT and τ-γ (Fig. 3d). Also, there were high positive correlations ($0.70 > r \leq 0.90$) between the variables PV-FV, PV-SBV, PV-tan δ, CV- τ, CV- γ, and TV-tan δ. High negative correlations between BDV-PTemp and WA-PV, SBV, and tan δ were seen as well as negative correlations between BDV-PTemp and WA-PV, SBV, and tan δ. These correlations could be explained by the effect of the extrusion process on the pasting properties, the WA and on the dynamic mechanical properties. These effects caused a disruption of the biopolymeric structures, as evidenced by an increasing τ and γ (Table 3) produced by the formation of hydrophilic bonds and protein crosslinking (BRENT; MULVANEY; COHEN; BARTSCH, 1997).

Quality evaluation of gluten-free (GF) bread

Specific volume

The specific volume of the bread from the extruded flours were 1.04, 1.30 and 1.42 cm^3/g for EPBRF, EWCF and EWSF, respectively. In the case of sorghum, the results coincide with TORBICA; BELOVIC e TOMIC (2019) where the sorghum bread was obtained by a combined thermal process. GF breads made from the extruded whole flours had a significant increase ($p < 0.05$) in their specific volume, which were 66% for EWCF, 33% for EPBRF and 82% for EWSF compared to Control 2 (Table 4). This could indicate the favorable effect of extrusion, corroborating the studies of MARTÍNEZ; MARCOS e GÓMEZ (2013) and CLERICI; AIROLDI e EL-DASH (2009). In addition, this increase in specific volume is related to the improvement in the dough consistency (Fig. 2b), which in turn may be attributed to the conversion of biopolymeric structures leading to an increase of water absorption, favoring the crosslinking phenomenon through the formation of molecular bonds (BARBIROLI; BONOMI; CASIRAGHI *et al.*, 2013). However, GF breads made from extruded flours presented lower specific volume values ($p < 0.05$) than Control 1.

Table 4. Specific volume and structure properties of gluten-free bread.

Samples	Specific volume, SV (cm^3/g)	Total area of bread slice, TBA (mm^2)	Total cell area, TCA (mm^2)	Solid area, SA (mm^2)	Porosity, P (%)	Height, H (mm)
Control 1	$2.23^{f} \pm 0.10$	$825.48^{e} \pm 78.63$	$118.745^{e} \pm 23.04$	$735.44^{e} \pm 38.03$	$13.49^{b} \pm 2.69$	$32.7^{d} \pm 2.0$
Control 2	$0.78^{a} \pm 0.05$	$339.22^{a} \pm 1.90$	$7.47^{a} \pm 3.82$	$331.70^{a} \pm 5.63$	$1.96^{a} \pm 0.82$	$19.4^{a} \pm 0.4$
EWCF	$1.30^{d,\alpha} \pm 0.04$	$504.32^{d,\alpha} \pm 2.32$	$88.62^{bc,\alpha} \pm 21.32$	$422.93^{d,\alpha} \pm 33.88$	$16.29^{bc,\alpha} \pm 6.50$	$24.1^{c,\alpha} \pm 0.1$
EWCF+5% GM	$1.15^{c,\beta} \pm 0.01$	$373.31^{ab,\beta} \pm 36.45$	$26.44^{a,\beta} \pm 2.47$	$362.87^{abc,\beta} \pm 0.66$	$6.87^{a,\beta} \pm 0.28$	$19.5^{a,\alpha} \pm 0.9$
EPBRF	$1.04^{b,\alpha} \pm 0.06$	$410.61^{bc,\alpha} \pm 14.37$	$77.87^{bc,\alpha} \pm 4.02$	$331.41^{a,\alpha} \pm 1.99$	$18.93^{bc,\alpha} \pm 0.05$	$21.7^{b,\alpha} \pm 0.2$
EPBRF+5% GM	$1.11^{bc,\beta} \pm 0.04$	$434.73^{bcd,\beta} \pm 6.62$	$71.92^{b,\alpha} \pm 3.73$	$343.81^{ab,\beta} \pm 4.50$	$16.26^{bc,\alpha} \pm 1.01$	$22.7^{bc,\beta} \pm 0.1$
EWSF	$1.42^{e,\alpha} \pm 0.03$	$478.41^{cd,\alpha} \pm 22.85$	$101.96^{cd,\alpha} \pm 3.19$	$383.04^{bcd,\alpha} \pm 35.36$	$20.10^{c,\alpha} \pm 3.48$	$23.2^{bc,\alpha} \pm 0.4$
EWSF+5% GM	$1.12^{bc,\beta} \pm 0.06$	$497.31^{cd,\beta} \pm 0.97$	$92.06^{bcd,\beta} \pm 6.99$	$405.25^{cd,\beta} \pm 6.02$	$18.50^{bc,\alpha} \pm 1.36$	$23.6^{bc,\beta} \pm 0.5$
Parametric assumptions						
Shapiro test	0.84	0.03	0.12	0.06	0.81	0.28
Bartlett test	0.18	0.01	0.26	0.08	0.09	0.18
Box-Cox						
λ	-	-0.54	-	-	-	-

Control 1-bread of whole wheat flour, Control 2-mixture of non-extruded and extruded rice flour in the proportion of 50:50%, EWCF-extruded whole corn flour, EPBRF-extruded parboiled brown rice flour, EWSF-extruded whole sorghum flour, and 5% GM-incorporation of 5% of germinated millet. Values represent the mean ± SD (n=6). Different letters in the same column indicate statistical differences ($p < 0.05$) among samples. Greek letters indicate paired t tests for each type of cereal with incorporation of 5% germinated millet. Box-Cox transformation (λ) for non-parametric data.

The incorporation of 5% GM had a negative effect ($p < 0.05$) on the specific volume of EWCF and EWSF (Table 5), due to the natural hydrolysis of the germinated millet starch, which causes a dilution effect and weakening of the dough. On the other hand, the 5% GM showed a positive effect ($p < 0.05$) on EPBRF, which may be due to its weakening biopolymeric structures with high cohesiveness (Table 3), characteristics of parboiled rice samples (BARBIROLI; BONOMI; CASIRAGHI; IAMETTI *et al.*, 2013).

Bread crumb structure

The EPBRF exhibited a very significant collapse of the internal air cells (Fig 4). On the other hand, EWSF and EWCF presented a better distribution and less collapse. The extruded samples presented significant increases in TBA, TCA, P, and H than Control 2 (Table 4), and this shows that extruded flours allow the development of structural properties associated with the gas retention capacity. On the other hand, the SA in EWCF and EWSF showed significant increases ($p < 0.05$) compared to Control 2. The EPBRF presented a similar ($p > 0.05$) value, due to the significant collapse of its internal air cells, which hindered the formation of a porous structure. This may be associated with a low value of the G'' in the dough (Fig. 2c) and by the possible high air permeability generated by the larger particle size in the parboiled rice sample (Table 2).

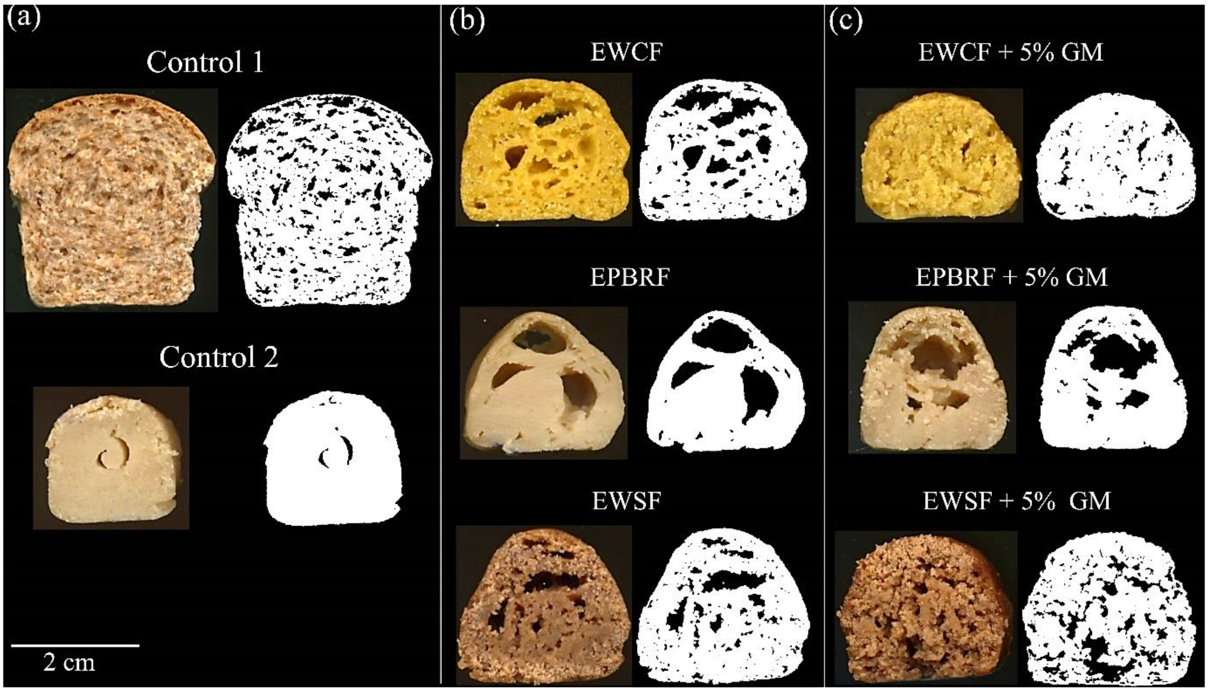

Fig. 4. Structure of the bread crumb. (a) bread controls-control 1 (commercial whole wheat flour, WWF) and control 2 (mixture of non-extruded and extruded rice flour in the proportion of 1:1), (b) GF-bread made from extruded whole flours of corn (EWCF), parboiled brown rice flour (EPBRF) and sorghum (EWSF) and (c) GF-bread with incorporation of 5% germinated millet, (5% GM).

EWSF and EWCF showed lower structural collapse and better air cell distribution, which indicates that the extrusion process greatly favored the formation of internal air cells and porous structure in GF whole grain breads. However, this was not enough to obtain GF bread with similar crumb structure to Control l. The incorporation of 5% GM improved the appearance of the bread crumbs for all GF breads (Fig. 4c) EWCF+5% GM was the most affected ($p < 0.05$) by the incorporation of 5% GM causing a reduction ($p < 0.05$) in TBA, TCA, and SA (Table 4), followed by EWSF+5% GM that had a significant decrease ($p < 0.05$) in TCA. However, EPBRF+5% GM was the bread sample that presented significant increases in TBA, SA, and H.

Texture profile analysis (TPA)

Hardness values were 15.9, 16.4 and 18.8 N for EPBRF, EWSF and EWCF, respectively (Table 5). These values could be highly related to the dietary fiber content (Table 2), indicating that dietary fiber contributes to increase the specific volume and crumb softness of the GF bread (PHIMOLSIRIPOL; MUKPRASIRT; SCHOENLECHNER, 2012).

The hardness values, found in EWSF breads, were lower than the values obtained by TORBICA; BELOVIC e TOMIC (2019), who used thermal pretreatments and additives to improve their gluten-free sorghum breads. The crumb hardness values of EPBRF breads were lower than those reported by SCIARINI; RIBOTTA; LEÓN e PÉREZ (2008) in gluten-free rice breads. Breads made with EWCF exhibited lower hardness values than those found by KOTANCıLAR; GÜDÜK e SEYYEDCHERAGHı (2018), who used corn flour with the addition of eggs and yogurt as natural additives.

The incorporation of 5% GM had a positive effect, mainly on the significant reduction ($p < 0.05$) of the hardness and cohesiveness of the bread crumbs (Table. 5), which could be attributed to the enzymatic system that hydrolyzed the starch present in the samples, weakening the microstructure and therefore reducing crumb rigidity. These findings differ from those found by HORSTMANN; ATZLER; HEITMANN; ZANNINI *et al.* (2019) when adding 5% of sprouted brown millet in gluten-free bread. On the other hand, in the same investigation, similar results to ours were reported, when sprouts of amaranth, quinoa, and corn were incorporated. The springiness was similar ($p > 0.05$) in breads made from EPBRF and EWSF, indicating that the addition of 5% GM did not significantly affect this parameter.

Table 5. Texture profile analysis (TPA) of gluten-free breads of corn, parboiled brown rice and sorghum.

Samples	Hardness (N)	Adhesiveness (g.s)	Cohesiveness (-)	Springiness (-)	Chewiness (N)	Resilience (-)
Control 1	7.77^{b} ± 0.38	-14.41^{bc} ± 1.47	0.41^{d} ± 0.01	0.95^{d} ± 0.03	2.74^{c} ± 0.45	0.12^{b} ± 0.00
Control 2	12.66^{c} ± 0.62	-1.25^{de} ± 0.50	0.22^{b} ± 0.04	0.73^{b} ± 0.02	2.02^{c} ± 0.44	0.12^{b} ± 0.03
EWCF	$18.83^{e,\alpha}$ ± 1.05	$-33.97^{ab,\alpha}$ ± 12.22	$0.30^{c,\alpha}$ ± 0.03	$0.94^{d,\alpha}$ ± 0.03	$5.21^{d,\alpha}$ ± 1.31	$0.10^{ab,\alpha}$ ± 0.00
EWCF+5% GM	$5.42^{a,\beta}$ ± 0.22	$-0.21^{e,\beta}$ ± 0.18	$0.08^{a,\beta}$ ± 0.01	$0.47^{a,\beta}$ ± 0.01	$0.21^{a,\beta}$ ± 0.02	$0.04^{a,\beta}$ ± 0.00
EPBRF	$15.99^{d,\alpha}$ ± 1.32	$-5.79^{cd,\alpha}$ ± 1.84	$1.00^{e,\alpha}$ ± 0.05	$1.00^{d,\alpha}$ ± 0.03	$8.97^{e,\alpha}$ ± 1.32	$0.27^{ab,\alpha}$ ± 0.08
EPBRF+5% GM	$7.31^{b,\beta}$ ± 1.31	$-4.60^{cd,\alpha}$ ± 1.62	$0.12^{a,\beta}$ ± 0.01	$0.97^{d,\alpha}$ ± 0.09	$0.77^{b,\beta}$ ± 0.03	$0.06^{ab,\beta}$ ± 0.01
EWSF	$16.45^{d,\alpha}$ ± 0.11	$-54.93^{a,\alpha}$ ± 5.10	$0.19^{b,\alpha}$ ± 0.02	$0.81^{bc,\alpha}$ ± 0.06	$2.44^{c,\alpha}$ ± 0.37	$0.08^{ab,\alpha}$ ± 0.01
EWSF+5% GM	$5.16^{a,\beta}$ ± 0.66	$-2.37^{d,\beta}$ ± 1.30	$0.07^{a,\beta}$ ± 0.02	$0.90^{cd,\alpha}$ ± 0.14	$0.35^{ab,\beta}$ ± 0.18	$0.03^{ab,\beta}$ ± 0.01
Parametric assumptions						
Shapiro test	0.59	0.03	0.56	0.80	0.80	0.00
Bartlett test	0.31	0.04	0.61	0.38	0.06	0.01
Box-Cox						
λ	-	0.26	-	-	-	0.22

Control 1-bread of whole wheat flour, Control 2-mixture of non-extruded and extruded rice flour in the proportion of 50:50%, EWCF: extruded whole corn flour, EPBRF: extruded parboiled brown rice flour, EWSF: extruded whole sorghum flour, and 5% GM-incorporation of 5% of germinated millet. Values represent the mean ± SD (n=8). Different letters in the same column indicate statistical differences ($p < 0.05$) among samples. Greek letters indicate paired t tests for each type of cereal with incorporation at 5% of germinated millet. Box-Cox transformation (λ) for non-parametric data.

In contrast, the springiness decreased ($p < 0.05$) for EWCF breads; this is probably associated with its low protein content, which reduces the degree of crosslinking, and the high content of insoluble fiber that causes network weakening.Principal component analysis for GF bread

PC1 and PC2 explained 74.1% of the total variance among a total of 12 variables that represent three kinds of bread quality characteristics (physical, structural and textural properties). The EWCF and EWSF breads (Fig. 5a) were characterized by high values of the textural variables (Hd and Ad) in Figure 5b.

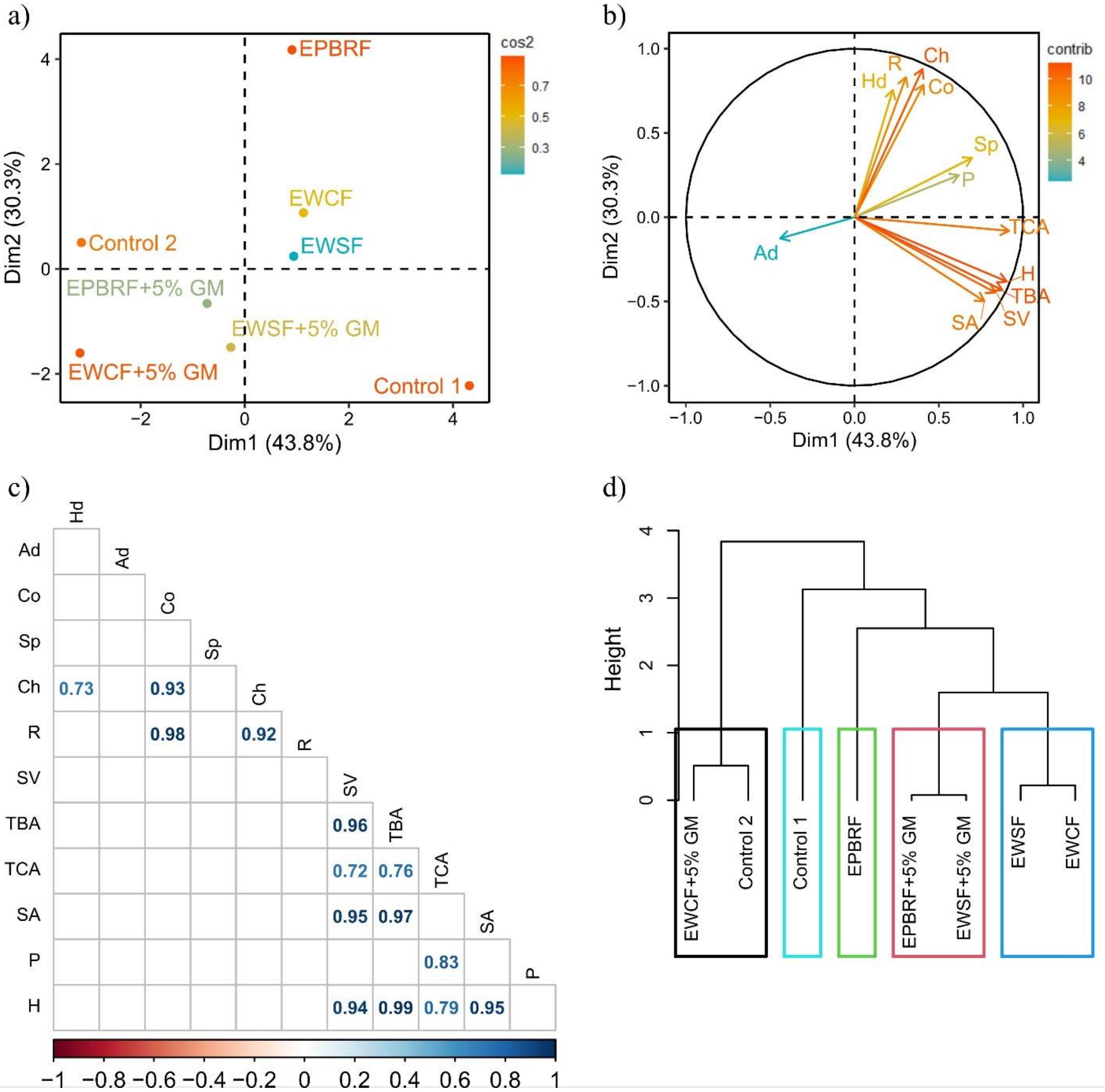

Fig. 5. Principal components analysis (PCA) of gluten-free bread. a) score plot for samples, b) PCA loading plot for response variables, c) Pearson’s correlation at $p < 0.05$ and d) hierarchical clustering on principal components.

In addition, the EWSF bread was also represented by variables in the structure (P). Likewise, the EWCF+5% GM and EWSF+5% GM bread samples had the lowest values of the textural variables (Hd and R) in comparison to EWCF and EWSF.

The EPBRF bread presented the maximum values for the textural properties of Co, Ch and R due to the parboiling followed by extrusion. This indicates that the combined effect of the processes caused functional changes at the molecular level of the rice starch. On the other hand, the lower textural variables (Co, Ch and R) indicate the high impact that the incorporation of 5% GM had on the quality properties of corn bread (EWCF+5% GM). There was a very high correlation ($0.90 > r \leq 0.99$) between Co-Ch and R, Ch-R, SV-TBA and SA, TBA-SA and H, SA-H. Also, high significant ($p < 0.05$) positive correlations ($0.70 > r \leq 0.90$) were observed between Hd-Ch and TCA-SV, TBA, P, and H (Fig. 5c). Finally, the controls were characterized by the physical (SV), structural (TBA, TCA, SA and H) and textural (Sp) variables, with the highest and lowest values for Control 1 and Control 2, respectively.

HCPC formed five sample groups (Fig. 5d). The first group was composed of EWCF+5% GM and Control 2 samples and was characterized by lower values of physical (SV) and structural (TBA, SA, and H) properties for Control 2 and only textural (Ch, Co, and R) for EWCF + GM 5%. The second group was formed only by Control 1, which showed the maximum values in the physical (SV), structural (TBA, TCA, SA, and H) and textural (Sp) variables among all the samples (Table 4). The third group represented by EPBRF showed the maximum values in the texture (Co, Ch, and R), indicating that the parboiled brown rice sample subjected to extrusion developed high cohesion and resistance forces in comparison to EWCF and EWSF. The fourth group was composed of breads with the inclusion of 5% GM (EWSF+5% GM and EPBRF+5% GM), with the lower values for the texture (Hd and R). Furthermore, such patterns were found for EWCF+5% GM, but the grouping technique did not consider it because it had a strong decline in the other structural and textural properties (Table 4 and 5), leaving this category without effect.

CONCLUSIONS

Whole sorghum was the cereal that presented the highest amounts of iron, manganese, and copper. Parboiled brown rice showed high amounts of manganese and copper, and corn had high amounts of zinc. The modification of the paste viscosity profile in whole grain flours caused by the extrusion process was evidenced by the decrease in all the pasting properties of raw flours without pretreatment. On the other hand, the parboiled brown rice sample presented an unexpected increase in the peak viscosity, final viscosity, and seatback viscosity. Extruded flours showed high increases of water absorption and consistency as well as reduced dough development times (DDT). GF breads presented good increase in volume: 66%, 33% and 82% for EWCF, EPBRF and EWSF samples compared to the control 2, being the EWSF sample the one that approximated the bread made from wheat flour but could not reach a desired analogous volume. Addition of 5% germinated millet enhanced bread-crumb softness in all samples, being the EPBRF + 5% GM sample the one that presented hardness and springiness similar to WWF.

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CHAPTER III

FUNCTIONALITY OF WHOLE GRAIN FLOURS PRE-COOKED BY EXTRUSION TO PRODUCE GLUTEN-FREE BREADS

Resumo

Extrusão branda foi utilizada para tratar farinhas de grãos inteiros sem glúten e avaliar a funcionalidade de cada uma delas e sua interação entre misturas binárias (1:1) e misturas multigrão (1:1:1:1) utilizando o desenho de mistura simplex-centroide para produzir pães sem glúten e modelar as variáveis. Para testar a funcionalidade das farinhas, foram utilizados os métodos reológicos de pasta, propriedades farinográficas e mecânico-dinâmicas (oscilatórias), e para pães sem glúten foram avaliados o perfil de textura, perda de cozimento e volume específico. Através da adaptação das técnicas farinográficas e mecânico-dinâmicas (oscilatórias) foi possível padronizar os níveis de absorção de água e a consistência ótima para a conformação da massa e seus correspondentes módulos elástico (G") e viscoso (G"). Os modelos de regressão foram significativos com comportamento linear e quadrático para variáveis reológicas (exceto tan δ) com coeficientes ótimos de determinação para propriedades de pasta (ajustado R^2 = 0,90-099), farinográficas (ajustado R^2 0,91-0). 98) e mecânicas dinâmicas (R^2 ajustado = 0,90-099), enquanto para pães foi possível modelar as variáveis de dureza (R^2 ajustado baixo = 0,60), coesão e mastigabilidade (R^2 ajustado bom = 0,83), e resiliência (R^2 ótimo = 0,90) dentro do perfil de textura. O arroz integral parboilizado (PBR ou T1) foi a amostra que influenciou fortemente em todas as variáveis avaliadas que foram significativas e que se alcançaram seus modelamentos. Por outro lado, o sorgo apresentou a menor dureza de miolo com menores perdas no cozimento, indicando maior retenção de água causada pela menor permeabilidade da massa durante o cozimento e menor retrogradacão de seu amido, como evidenciado no perfil de propriedades da pasta. Por outro lado, o milho mostrou boa funcionalidade da massa, mas seu alto teor de fibras afetou a resistência do miolo e levou a uma estrutura mais quebradiça.

Palavras-chave: Pão, Massa, Extrusão, Perfil Textura, Multigrão, Reologia, Superfície de resposta.

Abstract

Bland extrusion was used to treat gluten-free whole grain flours and evaluate the functionality of each of them and their interaction between binary (1:1) and multigrain (1:1:1:1) mixtures using single-centroid mix design to produce gluten-free breads and model the variables. To test the functionality of the flours, the rheological methods of paste, farinographic and mechanical-dynamic (oscillatory) properties were used, and for gluten-free breads the texture profile, baking loss and specific volume were evaluated. Through the adaptation of the farinographic and mechanical-dynamic (oscillatory) techniques, where it was possible to standardize the levels of water absorption and the optimal consistency for dough conformation and their corresponding elastic (G') and viscous (G'') moduli. The regression models were significant with linear and quadratic behaviour for rheological variables (except tan δ) with optimal coefficients of determination for paste (adjusted R2 = 0.90-099), farinographic (adjusted R2 0.91-0.98) and dynamic mechanical (adjusted R2 = 0.90-099) properties, while for breads it was only possible to model hardness (low adjusted R^2 = 0.60), cohesiveness and chewiness (good adjusted R^2 = 0.83), and resilience (optimal R^2= 0.90) within the texture profile. Parboiled brown rice (PBR or T1) was the sample that strongly influenced in most variables that were significant to be modelled. On the other hand, sorghum showed the lowest crumb hardness with lower cooking losses, indicating higher water retention caused by lower dough permeability during cooking and less retrogradation of its starch, as evidenced in the paste property profile. On the other hand, corn showed good dough functionality, but its high fibre content affected crumb strength and led to more crumbling.

Keywords: Bread, Dough, Extrusion, Texture profile**,** Multigrain, Rheology**,** Surface response

INTRODUCTION

Gluten-free (GF) products are a relatively new, attractive, and challenging market for the baking industry since the grains used do not possess the unique characteristics of wheat. This market is mainly represented by a segment of the world's population, which may have some sensitivity to storage proteins (called gluten prolamins) found in the endosperm of wheat, barley, rye and in some cases may be found in oats by cross contamination. Gluten sensitivities (GS) are classified into three groups, each mediated by various immunological mechanisms, which are categorized into autoimmunogenic (including coeliac disease), allergic and innate (non-coeliac gluten sensitivity - NCGS) responses to wheat (CABANILLAS, 2020; SCHERF; KOEHLER; WIESER, 2016). Together these disorders constitute the main target market for the development of GF products. In addition, there are groups of individuals who do not manifest GS, but who reject the consumption of wheat and its derivatives and, consequently, adhere to GF diets because of trend alignment, empathy with the unhealthy and misleading associations of wheat components of various indoles.

However, obtaining gluten-free breads (GFBs) is the most difficult challenge for the breadmaking industry, since it requires the formation of an impermeable network with viscoelastic properties like those of wheat gluten to capture CO_2 during fermentation to achieve light breads with soft crumb and well-distributed cavities. On the other hand, gluten-free bread formulations generally use refined raw materials with addition of economic ingredients in high proportions such as potato starch, corn, rice and cassava and/or their mixtures, which contribute technologically to increase the viscosity and consistency of the dough by means of high-water absorption mechanisms (MANCEBO; MERINO; MARTÍNEZ; GÓMEZ, 2015). This makes them highly caloric products with poor nutritional intake due to the removal of macronutrients such as proteins (albumins and globulins) and lipids that are mainly located in the pericarp and germ of the grains, as well as micronutrients (minerals and B vitamins), phytonutrients and phytochemicals that include fibres that reduce starch absorption and glycaemic index in blood during digestion (BHAR; BOSE; DUTTA; MANDE, 2022); as well as bioactive compounds that have antioxidant capacity to prevent degenerative diseases.

For these reasons, the use of whole grain corn, rice and sorghum allows integrating and taking advantage of the nutritional and nutraceutical benefits of each of these cereals from several continents. Yellow corn is a grain from the Americas, rich in carotenoids, dietary fiber with a slightly but significantly higher lipid content among cereals, including monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids, as well as antioxidant compounds such as tochols. Parboiled brown rice is native to Asia, rich in carbohydrates, minerals (Ca, Mg, P, Cu and Fe) (OLIVEIRA; COIMBRA; GALDEANO *et al.*, 2022) and storage protein (high lysine content) of lowest molecular weight prolamins and is considered the most hypoallergenic among cereals. Finally, sorghum is native to Africa and stands out for having the greatest variety and quantity of bioactive compounds beneficial to health among all cereals, but because of its low-digestible proteins and carbohydrates, it can currently be considered a hypocaloric grain to prevent overweight and obesity.

Such whole grains under physical processing can acquire technological properties useful for the gluten-free baking industry. Therefore, the use of clean and sustainable technologies with low energy and water consumption and no effluent generation such as thermoplastic extrusion can be used to transform the native and intact components of cereals into functional ingredients (BOUVIER; CAMPANELLA, 2014; GANJYAL, 2020). Thermal and mechanical treatments are the main phenomena governing the thermoplastic extrusion process and can be manipulated by control of temperature, water injection and rotation of the screw. Previous studies by CLERICI; AIROLDI e EL-DASH (2009); MARTÍNEZ; MARCOS e GÓMEZ (2013); SAITO; OKOUCHI; YAMAGUCHI *et al.* (2022); TORBICA; BELOVIC e TOMIC (2019) used thermal treatment or conventional extrusion in refined flours to obtain gluten-free breads at extruded flour proportions of 10-30%, while COMETTANT-RABANAL; CARVALHO; ASCHERI *et al.* (2021), using bland extrusion in whole grain flours achieved promising progress using 100% whole grain flours in the formulation of gluten-free breads. Therefore, the aim of this work was to study the interaction between extruded whole grains by designing mixtures for the optimization of gluten-free breads and to evaluate their physical, rheological, textural, and macrostructural characteristics.

MATERIALS AND METHODS

Plant materials

Whole grains (WGs) such as corn were donated by Indústrias de Alimentos Granfino (Nova Iguaçú, Brazil), sorghum (red pericarp, low tannin) was donated by Embrapa Milho e Sorgo (Sete Lagoas, Brazil), parboiled brown rice and ingredients (non-hydrogenated palm fat, yeast, sucrose, and salt) were acquired in a market on Rio de Janeiro. WGs were cleaned and ground using a hammer mill LM3100 (Perten

Instruments, Huddinge, Sweden) equipped with a 0.8 mm opening screen for obtaining fine corn, parboiled brown rice (PBRF), and sorghum flours.

Extrusion conditions

An Evolum HT25 (Clextral Inc., Firminy, France) twin-screw, co-rotating, geared extruder was used to process the raw whole grain flours (corn, parboiled brown rice and sorghum) under the parameters used by COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021). The screw diameter was 25 mm, with a length-to-diameter ratio of 40:1, ten heating zones (from feed to outlet: 25, 40, 60, 80, 100, 110, 110, 110, 90, 80 and 70 °C), and the screw speed was set at 200 rpm. WG flours were fed through a model GRMD15 twin-screw gravimetric feeder (Schenck Process, Darmstadt, Germany) at a constant rate of 10 kg/h, and the process was monitored with Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). Deionized water was injected between the first and second modular zones through a port with an internal diameter of 5.25 mm using a Clextral plunger metering pump (Clextral Inc., Firminy, France) adjusted to compensate for moisture differences in the samples and provide a final moisture content of 25%. Collected extrudates were dried in a forced-air oven at 55 °C for 10 h. The unexpanded extrudates were dried at 55 °C for 5 h and milled into fine flours to be used to produce gluten-free breads (GFB) in an LM3100 hammer mill (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm aperture sieve.

Pasting properties

The paste viscosity of the extruded wholemeal flours was analyzed in triplicate according to the methodology described by RAGAEE e ABDEL-AAL (2006) with some modifications, using a Rapid Visco Analyzer series 4 RVA (Newport Scientific Pty Ltd., Warriewood, Australia). Three grams of sample adjusted to 14% moisture (wet basis) along with 25 mL of distilled water were placed in the sample holder (aluminium beaker) of the equipment. The test conditions were mixing at 160 rpm at 25 °C for 2 minutes, heating to 95 °C at a constant rate of 14 °C/min and holding for 3 minutes and then cooling to 25 °C in 5 minutes at the same rate, with a total time of 20 minutes. Paste properties were measured: paste temperature (PTem, cP), cold viscosity at onset at 25 °C (CV, cP), peak viscosity (PV, cP), trough viscosity or holding strength (TV, cP), break viscosity (BDV= PV-TV, cP), final viscosity (FV, cP) and setback viscosity (SBV= FV-TV, cP).

Farinography measurement

The farinographic 54-21.01 AACC (2000b) method for 50 g mixture chamber was adapted for gluten-free samples thermally and mechanically pre-treated by extrusion, from the results reported by Comettant (2021) and taking as reference 33 g of extruded flour and the average of the water absorption percentages (99.33%) of the three grains studied by the author in order to integrate the water absorption (WA) for use in binary and multigrain mixtures based on corn, parboiled rice and sorghum, without taking as reference the 500 Brabender units (BU) traditionally used for wheat. Previously, the moisture content of each sample was determined to calculate the amount of flour standardized to 14% moisture on a wet basis (wb) and the amount of water to be added, using the following equations:

$$Flour\ weight\ (g) = \frac{33\ (g) x\ 86(\%)}{100 - moisture\ (\%)}$$

$$Water\ absoption\ (g) = \frac{99.33\ x\ flour\ weight\ (g)}{100}$$

From the farinograms the following readings were considered: WA, arrival time (AT, min), departure time (DT, min), dough development time (DDT, min), dough stability time (DST, min), peak maximum of consistence (PM, BU) and mixing tolerance index (MTI), determined at 5 min after peak.

Dynamic mechanical properties

Dynamic mechanical properties of the extruded flours were performed with a HAAKE Mars II rotational rheometer (Thermo Fisher Scientific, Karlsruhe, Germany) at 25 °C using a 35 mm diameter parallel plate geometry (PP35 Ti). Prior to rheological readings, the dough of each cereal was obtained by mixing the flour samples with water in the farinograph using the water absorption (WA) and dough development time (DDT) obtained from the farinograms, according to COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021). Three grams of dough was loaded on the bottom plate and the top plate was approached to the dough at a speed of 0.6 mm/min to a separation of 2 mm. Excess dough was carefully trimmed off and mineral oil was applied to the edges to prevent dehydration of the doughs during measurement. Then, an amplitude sweep was performed to determine the strain rate (γ) value within the linear viscoelastic region (LVR). Subsequently, we proceeded with the frequency sweep determination between 0.1-100 Hz, using the strain rate (γ) value of each sample. The values of elastic or storage modulus (G′, Pa), viscous or loss modulus (G″, Pa), shear strain (ɣ), shear stress (τ, Pa) and tan δ (G"/G') were obtained at 1 Hz. The readings were performed in triplicate.

Formulation and bread making procedure

The extruded whole grain flours were used to produce gluten-free breads (GFB) based on 100% of each cereal and their binary and multigrain mixes, following the proportions of the simple centroid mix design (Table 1). The formulations followed the method of COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021) with some alterations, consisting of non-hydrogenated palm fat (3%), sugar (3%), salt (1.5%), water (99.33%) and yeast (1%) on a flour weight basis. The dough was prepared in a 35 g micromixer (National MFG. CO., Lincoln, USA), in which the dry ingredients were mixed for 2 min, then the liquid ingredients were incorporated during a kneading time of 3 min. 20 g portions were fermented at 39 °C and 85% RH for 2 h and baked at 200 °C for 19 min in an electric oven, grill model (Fischer SA., Santa Catarina, Brazil).

Texture profile analysis (TPA)

The TPA was carried out using the centre of the bread crumb slices with a thickness of 20 mm using a Texture Analyzer TA-XT Plus (Stable Micro Systems, Surrey, U K) equipped with a 5 kg load cell and a 15 mm aluminium cylindrical probe controlled by the Exponent software version 6.1.11.0 (Stable Micro Systems, Surrey, UK) according to COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021). The was configure with the modifications of the distance set at 50% compression and waiting time between the first and second compression cycle of 30 s. TPA crumb hardness (N), Adhesiveness (g·s), cohesiveness (-), springiness (-), chewiness (N), and resilience (-) were measurement.

Physic quality characteristics of bread

Specific volume analysis of breads was determined using a modified standard seed displacement method 10–05.01 (AACC, 2000a), using millet seeds. The recipient used to do the calculation was a parallelepiped with dimensions of 8.5 cm × 8.4 cm x 9.2 cm (width × length × height). Bread specific volume (cm^3/g) was calculated as bread volume divided by bread weight measured at 24 h after baking.

Statistical analysis

A simple centroid mixture design was used to evaluate the interaction between the three cereals. The interactions were composed of 7 types of gluten-free breads (GFB) with 2 replicates in the centre point (CP) or equal proportions, based on pure extruded corn, parboiled brown rice (PBR) and sorghum flour, as well as their binary (1:1) and multigrain mixtures (equal proportions). To determine the differences of each response variable between treatments, analysis of variance (one-way ANOVA) and Tukey's test for comparisons were used, considering a significance level of 95%. R software version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical evaluations. The Bartlett-test and Shapiro-test were used to confer homoscedasticity and normality distribution, respectively. The Box-Cox transformation was performed only for those variables that lacked a normal distribution or homoscedasticity using lambda (λ) to achieve the normal distribution (BOX; COX, 1964). Determination coefficients (R^2) were categorized according to TOLEDO; CARVALHO; VARGAS-SOLÓRZANO; ASCHERI *et al.* (2020) as: low adjustment (R^2 = 0.5–0.69), well adjustment (R^2 = 0.70–0.89), optimum adjustment (R^2 = 0.90–0.99), and those adjusted models ≥0.70 were considered relevant.

Table 1. Simplex centroid mixture design for proportion of whole grain flours pre-treated by extrusion

Treatment	Corn	Sorghum	Parboiled brown rice
T1	1	0	0
T2	0	1	0
T3	0	0	1
T4	0.5	0.5	0
T5	0.5	0	0.5
T6	0	0.5	0.5
T7	0.3	0.3	0.3
T8	0.3	0.3	0.3
T9	0.3	0.3	0.3

RESULTS AND DISCUSSION

Functionality of gluten-free whole grain flours pre-treated by extrusion.

Pasting properties

The paste profile of the flours and their blends pre-treated by extrusion showed slight modifications (Fig. 1), mainly evidenced by the absence of CV and only in samples T3 and T5 (Table 2) composed of parboiled brown rice showed higher CV ($p<0.05$)

associated to the higher degree of cooking of the rice starch, given the parboiling process that this sample underwent. In addition, formation of PV were observed in all samples, indicating that much of the starch remained intact and/or with little fragmentation and exhibited slightly decreased gelatinization properties compared to raw materials as reported by COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021), due to the bland conditions of the process. Parboiled brown rice showed significant increases in PV, due to a special behavior of starch in this sample, associated with the increase in granular stiffness during the parboiling process, which is usually observed in annealing and extrusion-treated polymers (CHENG; GAO; WU; GAO *et al.*, 2020; WANG; WANG; WANG; WANG, 2017).

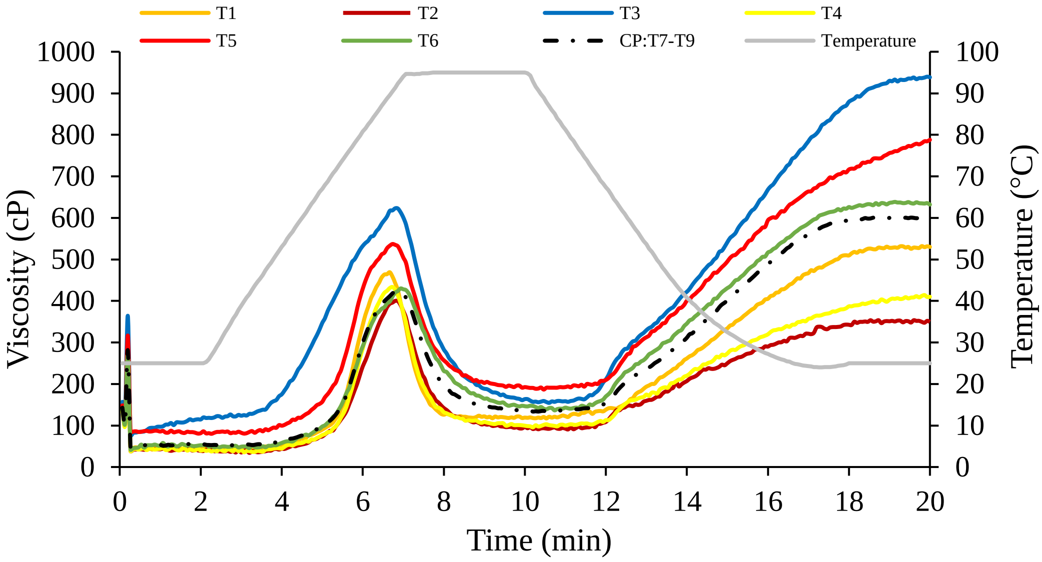

Fig. 1. Pasting profile of gluten-free whole grain flours pre-treated by extrusion. Pure flours (T1: corn, T2: sorghum and T3: parboiled brown rice), binary mixtures 1:1 (T4: corn-sorghum, T5: corn-parboiled brown rice and T6: parboiled brown rice-sorghum) and multigrain mixtures in equal proportions (T7-T9= corn-parboiled brown rice-sorghum).

However, TV and BDV parameters were found as evidence of starch modifications, since in the raw corn and whole grain sorghum samples these parameters were not evident, and FV values were found to be reduced in relation to the raw samples, with T2 and T4 (with higher proportion of sorghum) showing the lowest FV values (Table 2), due to the characteristics of sorghum starch and its retrogradation qualities compared to other cereal starches.

Table 2. Paste viscosity properties of gluten-free whole grain flours pre-treated by extrusion.

Treatment	PTemp (°C)	CV (cP)	PV (cP)	TV (cP)	BDV (cP)	FV (cP)	SBV (cP)
T1	57 ± 0.04 bc	49 ± 4.95 c	469 ± 2.83 c	116 ± 0.00 de	353 ± 2.83 b	530 ± 0.00 e	414 ± 0.00 d
T2	62 ± 0.57 a	49 ± 4.95 c	400 ± 5.66 e	90 ± 4.24 f	310 ± 1.41 d	355 ± 9.90 g	265 ± 5.66 f
T3	47 ± 0.07 e	106 ± 6.36 a	627 ± 9.90 a	160 ± 7.07 b	467 ± 2.83 a	939 ± 3.54 a	779 ± 3.54 a
T4	59 ± 0.07 b	48 ± 6.36 c	429 ± 9.19 d	98 ± 6.36 ef	331 ± 2.83 c	410 ± 4.24 f	313 ± 2.12 e
T5	51 ± 0.71 d	74 ± 1.41 b	526 ± 5.66 b	191 ± 10.61 a	336 ± 4.95 c	779 ± 2.83 b	589 ± 7.78 b
T6	55 ± 1.38 c	59 ± 2.12 bc	430 ± 4.24 d	146 ± 4.24 bc	284 ± 0.00 ef	632 ± 5.66 c	486 ± 9.90 c
T7	54 ± 0.64 c	45 ± 1.41 c	419 ± 2.12 de	122 ± 2.83 d	297 ± 4.95 de	581 ± 5.66 d	459 ± 8.49 c
T8	54 ± 0.67 c	44 ± 1.41 c	400 ± 2.12 e	119 ± 3.54 de	281 ± 5.66 f	582 ± 9.90 d	464 ± 13.44 c
T9	56 ± 0.07 c	54 ± 1.41 c	427 ± 7.07 d	135 ± 2.12 cd	293 ± 4.95 ef	592 ± 2.83 d	458 ± 4.95 c
p-anova	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F-cal	89.07	50.25	300.45	67.17	457.40	1830.98	834.77
F-tab (8,9)	3.23	3.23	3.23	3.23	3.23	3.23	3.23
CV	1.15	6.81	1.32	4.15	1.17	0.97	1.56
Shapiro (Norm.Res)	0.57	0.24	0.45	0.98	0.08	0.58	0.96
Durbin-Watson (Independence.Re)	0.94	0.37	0.73	0.94	0.36	0.84	0.91
LeveneTest (Var.Homoge)	---	---	---	---	---	---	---
Lambda (λ)	1.39	0.63	-0.30	0.06	1.88	1.88	0.51

pTemp: pasting temperature, CV: cold viscosity at beginning of the run at 25 °C, PV: maximum peak viscosity, TV: through viscosity, BDV: break down viscosity, FV: final viscosity and SBD: setback viscosity. Results represent the mean ± SD (n=3). Lower case letters indicate differences between treatments ($p<0.05$). Box-Cox transformation factor (λ) for non-parametric data.

Farinographic measurement

The amounts of water absorption (Table 3) were calculated with their corresponding consistency evolutions in the samples (Fig. 2a-g), thus demonstrating the functionality of extrusion-treated wholemeal flours, without the need to incorporate hydrocolloids into gluten-free flours, or to attach special compartments to facilitate farinographic readings, as was done by SAHIN; WIERTZ e ARENDT (2020). The AT of the samples ranged from 0.98-1.75 min, indicating rapid water absorption of the flours due to increased hydrogen bonding caused by the thermal and shearing effect on starch fragmentation and solubility during the extrusion process.

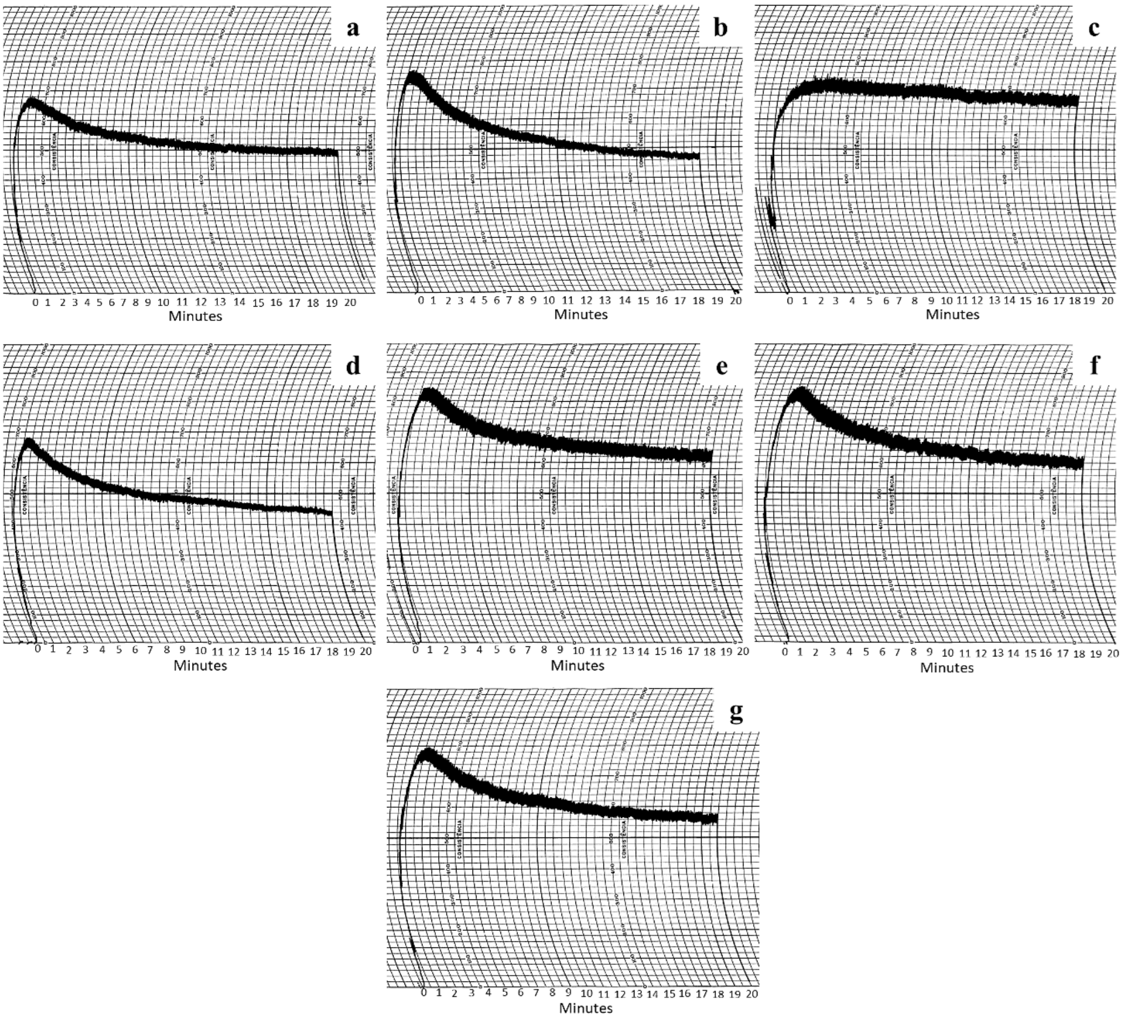

Fig. 2. Farinograms of gluten-free whole grain flours pre-treated by extrusion. Pure flours: a) corn (T1), b) sorghum (T2) and c): parboiled brown rice (T3); binary mixtures 1:1: d) corn-sorghum (T4), e) corn-parboiled brow rice (T5) and f) parboiled brown rice-sorghum (T6) and multigrain mixtures in equal proportions: g) CP=T7-T9= corn-parboiled brown rice-sorghum).

Among the pure samples, T1 (corn) and T2 (sorghum) showed the lowest AT ($p<0.05$) (Table 2), while T3 (parboiled brown rice) showed the highest values due to its lower hydration capacity. In the case of the binary mixtures (T4-T6) and multigrain (T7-T9) the AT and were intermediate and similar to the sorghum sample (T2) in the ease of water absorption.

Among the samples, T3 showed high stability during mixing, evidenced by its higher DT, DDT, DST and PM (Table 2) compared to corn (T1) and sorghum (T2) flours, but all of them showed farinograms with PM levels above 500 BU, which is very difficult to achieve in gluten-free flours and is only possible with the addition of proteins and hydrocolloids (ĆURIĆ; NOVOTNI; TUŠAK *et al.*, 2007; VAN RIEMSDIJK; VAN DER GOOT; HAMER, 2011). This is evidence that in the rice sample (T3) the parboiling and extrusion processes led to a special behavior and conferred resistance of its biopolymers to disintegration under mechanical work, possibly due to the formation of cross-linked complexes or aggregates between starch and proteins/lipids. While T1 and T2 extruded flours that were not pre-treated by parboiling showed lower DT, DDT, DST, PM and higher MTI ($p<0.05$), indicating that corn and sorghum flours exhibited functionality, but with lower tolerances to mechanical mixing work leading to rapid destructuring of their constituents. Progress with improved farinographic profiles was evidenced by BIAN; XING; YANG *et al.* (2022) in gluten-free flours with the addition of soy protein isolate where and its contribution of cysteine in interaction with water generates disulphide bonds, which improve dough stability and dough formation (VAN RIEMSDIJK; VAN DER GOOT; HAMER, 2011).

Among the binary mixtures, it was observed that the interaction between corn and sorghum in sample T4 significantly reduced DT, DDT, DST and PM ($p<0.05$), due to the impact of dietary fibre in both samples that caused interference in dough conformation and cohesiveness. In the case of T5 and T6, the presence of rice contributed to the farinographic properties of DDT, DST and PM, thus demonstrating the strong influence of parboiled and extruded rice on farinographic properties. In the multigrain samples, most of the properties were similar to those of the binary mixtures ($p>0.05$), with the exception of sample T4, which did not have rice as a component.

Table 3. Faronographic properties of gluten-free whole grain flours pre-treated by extrusion.

Treatment	FW* (g)	WA* (mL)	AT (min)	DT (min)	DDT (min)	DST (min)	PM (BU)	MTI (-)
T1	31.3	31.1	1.45 ± 0.07 ab	2.45 ± 0.07 bc	2.05 ± 0.07 bc	1.00 ± 0.14 bc	680 ± 0 g	116 ± 3.54 e
T2	31.3	31.1	1.05 ± 0.07 bc	2.05 ± 0.07 c	2.00 ± 0.14 bc	1.00 ± 0 bc	761 ± 1.41 f	169 ± 2.12 a
T3	31.0	30.8	1.75 ± 0.07 a	6.90 ± 0.14 a	3.10 ± 0.14 a	5.15 ± 0.21 a	883 ± 3.54 a	85 ± 0 f
T4	31.2	31.0	1.05 ± 0.07 bc	2.00 ± 0.28 c	1.60 ± 0.14 c	0.95 ± 0.21 c	683 ± 1.41 g	144 ± 0 c
T5	31.2	30.9	0.98 ± 0.04 c	2.60 ± 0.10 bc	2.10 ± 0.14 b	1.57 ± 0.11 b	848 ± 8.49 b	132 ± 6.36 d
T6	30.9	30.8	1.35 ± 0.21 abc	2.78 ± 0.04 b	2.45 ± 0.07 b	1.43 ± 0.25 bc	857 ± 2.12 b	156 ± 0.71 b
T7	31.1	30.9	1.25 ± 0.07 bc	2.50 ± 0 bc	2.10 ± 0.14 b	1.25 ± 0.07 bc	799 ± 1.41 d	140 ± 2.83 cd
T8	30.9	30.7	1.10 ± 0.14 bc	2.45 ± 0.07 bc	2.35 ± 0.07 b	1.40 ± 0.14 bc	815 ± 7.07 c	134 ± 2.12 cd
T9	30.9	30.7	1.15 ± 0.07 bc	2.25 ± 0.07 c	2.15 ± 0.07 b	1.10 ± 0 bc	780 ± 0 e	141 ± 1.41 cd
p-anova	---	---	7e-04	0	3e-05	0	0	0
F-cal	---	---	11.3701	306.48	25.10	153.60	644.51	142.11
F-tab (8,9)	---	---	3.23	3.438	3.23	3.23	3.23	3.23
CV	---	---	8.37	4.24	5.22	9.2	0.51	2.1
Shapiro (Norm.Res)	---	---	0.20905	0.46	0.003	0.40	0.39	0.98
Durbin-Watson (Independence.Re)	---	---	0.7552	0.40	0.95	0.75	0.80	0.60
LeveneTest (Var.Homoge)	---	---	---	---	---	---	---	---
Lambda (λ)	---	---	-0.02	1.15	1.19	0.70	-2	1.35

*Unique values without variability.

FW: Flour weight, WA: Water absorption, arrival time (AT, min), departure time (DT, min), dough development time (DDT, min), dough stability time (DST, min), peak maximum of consistence (PM, BU) and mixing tolerance index (MTI), determined at 5 min after peak. Pure flours (T1: corn, T2: sorghum and T3: parboiled brown rice), binary mixtures 1:1 (T4: corn-sorghum, T5: corn-parboiled brown rice and T6: parboiled brown rice-sorghum) and multigrain mixtures in equal proportions (T7-T9= corn-parboiled brown rice-sorghum). Results represent the mean ± SD (n=3). Lower case letters indicate differences between treatments ($p<0.05$). Box-Cox transformation factor (λ) for non-parametric data.

Dynamic mechanical properties

In all samples, continuous elastic (G') and viscous (G") modulus development was evidenced with increasing and predominantly elastic nature (G'>G") (Fig. 3a-c). These functional properties indicate that the use of heat treatment together with shearing can modify the components (starch, proteins, lipids and fibres) of the grains and/or establish cross-linked complexes between them leading to the formation of new polymers with techno-functional characteristics useful in the gluten-free industry that require viscoelastic properties for their production (noodles and baking) (GÓMEZ; MARTÍNEZ, 2016; MARTÍNEZ; DÍAZ; GÓMEZ, 2014; MARTÍNEZ; CALVIÑO; ROSELL; GÓMEZ, 2014).

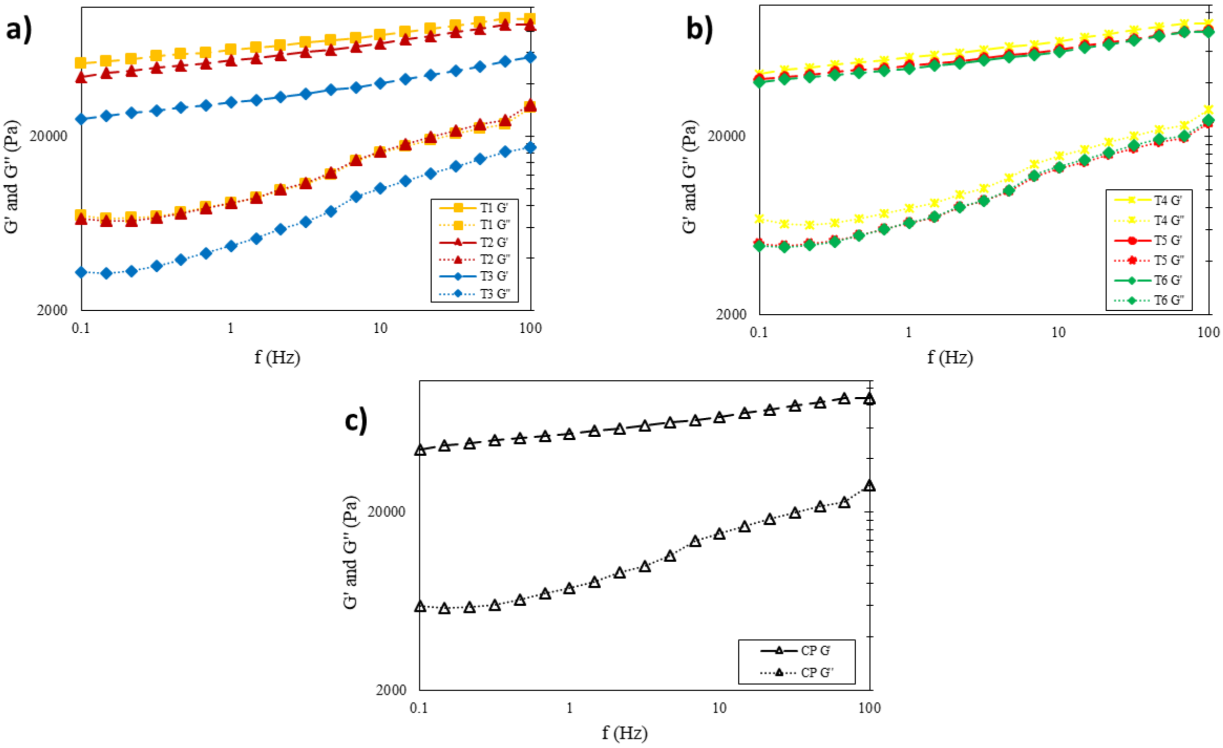

Fig. 3. Dynamic mechanical properties of gluten-free whole grain flours pre-treated by extrusion. a) Pure flours (T1: corn, T2: sorghum and T3: parboiled brown rice), b) binary mixtures 1:1 (T4: corn-sorghum, T5: corn-parboiled brow rice and T6: parboiled brown rice-sorghum) and c) multigrain mixtures in equal proportions (CP=T7-T9= corn-parboiled brown rice-sorghum). Results represent the mean ± SD (n=3).

In the pure samples, T1 (62543.33 Pa) based on extruded corn was the one with the highest G' values among all treatments ($p<0.05$), followed by T2 (47680 Pa) based on sorghum (Table 4), but at G" both T1 and T2 were similar to each other and to the results reported by COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021) for wheat. While T3 based on parboiled rice showed the lowest G' and G" values close to reported by BIAN; XING; YANG; FAN *et al.* (2022) for wheat flour (Fig.1a).In the binary mixtures T4 and T5 (50266.67 and 41280 Pa, respectively) it was observed that the extruded corn -sorghum and corn -parboiled brown rice interaction had the highest G' and G"(Table 4), due to the influence of extruded corn, while T6 showed the lowest G' among the binary samples due to the presence of extruded parboiled brown rice. The multigrain samples T7-T9 G' values ranged from 39780-44466.67 Pa, indicating that the elastic modulus values were intermediate and similar to the binary samples T5 and T6 ($p>0.05$).

The parameters γ (shear stress) and τ (shear strain) determined both at the end of the LVR (beginning of destructuring) and in the critical state (Table 4), show the degree of structuring of the constituents of each sample against mechanical-dynamic or oscillating stresses. Where T3 showed the highest $\tau = 4644.33$ Pa (equivalent to $\gamma = 0.21$) ($p<0.05$), indicating a higher degree of structuring or strength of its constituents than T1 and T2 (Table 4), while among the binary samples, the parboiled corn-parboiled brown rice interactions (T5) showed the highest $\tau = 2913.33$ Pa ($\gamma = 0.08$), followed by parboiled brown rice-sorghum mixtures with $\tau = 2601.33$ ($\gamma = 0.08$), indicating the strong influence of brown rice on rheological parameters due to its double cooking by parboiling and extrusion, which caused atypical modifications rarely reported in starch and its other components, associated with phenomena of starch granular stiffness (LIU; HAO; CHEN; GAO, 2019; YEUM; CHOI; KIM *et al.*, 2021) and starch/protein or starch/lipid cross-linking (WU; LI; WANG; ÖZKAN *et al.*, 2010). Multigrain blends (T7-T9) showed intermediate τ values between 1990.33-2092.33 Pa, which may be useful to regulate the prominent properties of T3.

The angle displacement $\tan \delta$ (G"/G'), the smaller it is, the higher the elasticity of a sample, where samples T1 (pure), T5 (binary) showed the lowest values of this parameter (0.13). Finally, the parameters G'=G" (crossover) and their equivalents in γ and τ measured in the non-linear region indicate the behavior of the samples when their components (mainly polymers) are affected by stresses that cause their destructuring but can give important information in those investigations that focus on this type of parameters in the non-linear region.

Table 4. Dynamic mechanical properties of Extruded flours and blends.

Treatment	amplitude sweep								Frequency sweep
	LVR plateau	End of LVR		Critical value		Crossover			
	G’ (Pa)	ɣ	τ (Pa)	ɣ	τ (Pa)	G’=G’’ (Pa)	ɣ	τ (Pa)	tan δ (G’’/G’) at 1 Hz
T1	62543.33 ± 317.23 a	0.02 ± 0 d	1056.33 ± 39.27 f	0.02 ± 0.00 d	1361.00 ± 19.97 f	7355.33 ± 46.61 a	1.34 ± 0.04 e	13756.67 ± 151.77 fg	0.13 ± 0 cd
T2	47680.00 ± 1558.97 c	0.02 ± 0 d	775.67 ± 4.04 g	0.03 ± 0.00 d	1139.00 ± 67.82 g	5569.67 ± 56.45 e	1.74 ± 0.07 d	14203.33 ± 30.55 f	0.15 ± 0 a
T3	25473.33 ± 989.97 h	0.17 ± 0.01 a	3968.67 ± 64.36 a	0.21 ± 0.01 a	4644.33 ± 88.49 a	3617.67 ± 52 g	4.71 ± 0.22 a	23816.67 ± 72.34 a	0.15 ± 0 a
T4	50266.67 ± 135.77 b	0.02 ± 0 d	682.00 ± 22.11 g	0.02 ± 0.00 d	1021.33 ± 21.03 g	6071.33 ± 36.75 d	1.54 ± 0.02 de	13126.67 ± 355.57 g	0.14 ± 0 b
T5	41280.00 ± 294.62 ef	0.06 ± 0 b	2105.67 ± 57.14 b	0.08 ± 0.00 b	2913.33 ± 35.23 b	6103.33 ± 72.28 cd	2.65 ± 0.14 b	22043.33 ± 510.03 b	0.13 ± 0 d
T6	38366.67 ± 1062.28 g	0.06 ± 0 b	1961.33 ± 35.22 c	0.08 ± 0.00 b	2601.33 ± 21.22 c	5387.00 ± 79 f	2.92 ± 0.07 b	21753.33 ± 581.58 b	0.14 ± 0 c
T7	44466.67 ± 489.93 d	0.04 ± 0 c	1577.33 ± 15.7 d	0.06 ± 0.00 c	1990.33 ± 80.13 de	6266.00 ± 60.32 b	2.32 ± 0.11 c	19790 ± 20 c	0.15 ± 0 a
T8	42733.33 ± 118.46 de	0.04 ± 0 c	1583.00 ± 18.52 d	0.06 ± 0.00 c	2092.33 ± 4.16 d	6245.00 ± 11.53 bc	2.86 ± 0 b	18873.33 ± 50.33 d	0.14 ± 0 b
T9	39780.00 ± 439.66 fg	0.04 ± 0 c	1441.33 ± 20.53 e	0.06 ± 0.00 c	1949.33 ± 6.66 e	5692.67 ± 23.07 e	2.23 ± 0 c	17070 ± 86.6 e	0.14 ± 0 b
p-anova	0.00	0	0	0	0	0	0	0	0
F-cal	518.31	1541.73	2242.41	388.76	1570.79	1071.65	301.51	550.17	92.41
F-tab (8,9)	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	3.23
CV	1.74	4.13	2.14	7.35	2.23	0.91	4.07	1.6	0.88
Shapiro (Norm.Res)	0.17	6e-04	0.86	0.00	0.15	0.50	0.06	0.01	0.45
Durbin-Watson (Independence.Res)	0.30	0.14	0.69	0.97	0.51	0.22	0.99	0.96	0.61
LeveneTest (Var.Homoge)	0.76	0.11	0.69	0.09	0.47	0.93	0.18	0.30	---
Lambda (λ)	---	---	---	-0.26	---	---	---	0.02	2

Results represent the mean ± SD (n=3). Lower case letters indicate differences between treatments ($p<0.05$). Box-Cox transformation factor (λ) for non-parametric data. ɣ: shear strain or strain rate, τ: shear stress, tan δ: angle of displacement, G’: elastic or storage modulus, G’’: viscous or loss modulus.

All mathematical models corresponding to paste properties were significant ($p<0.05$) and showed no lack of fit (Table 5), making these models useful for predicting the behavior of the variables by means of linear and quadratic regressions (Fig. 4a-f). Among the variables corresponding to the paste properties pTemp and FV, as well as PV, BDV and SBV showed linear and quadratic models, respectively and with optimal coefficients of determination (adjusted R^2) between 0.90-0.99. Whereas CV (adjusted $R^2 = 0.66$) and TV (adjusted $R^2 = 0.59$) showed low R^2, so the models corresponding to these variables can be taken only to explain trends. The higher the proportion of parboiled and extruded brown rice (PBRF), the lower the pTemp, but the higher the PV, TV, BDV, FV and SBV, while the higher the proportion of extruded sorghum flour, the opposite was true for the same variables.

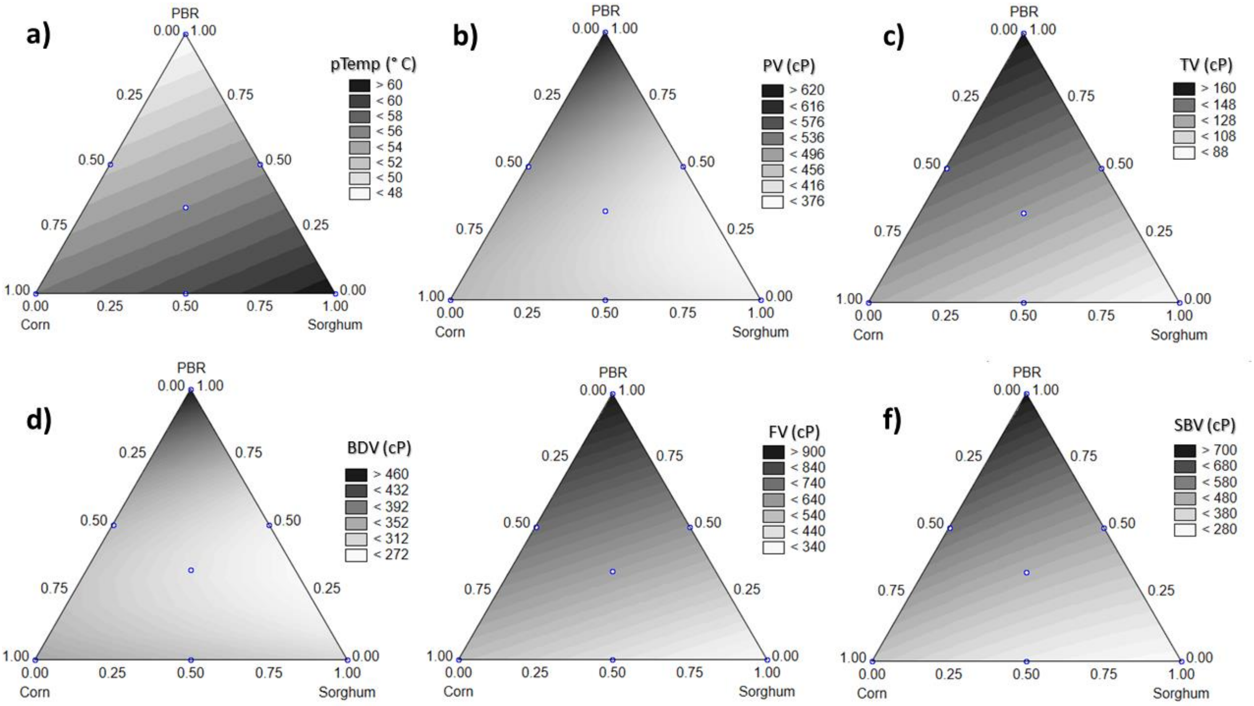

Fig. 4. Contour plot for pasting properties of flours.

Likewise, all mathematical models of farinography properties were significant ($p<0.05$) and showed no lack of fit (Table 5) and most of the variables (AT, DT, DST and MTI) showed quadratic models (Fig. 5a-f) with an optimal adjusted R^2 (0.91-0.98) (only AT showed 0.87) and DDT and PM showed linear models with a good adjusted R^2 (0.73-0.80). As in the case of paste properties, the influence of parboiled and extruded brown rice was predominant in the behaviour of farinographic properties and their modelling, as the higher the proportion of rice, the higher the TA, DT, DDT, DST and PM, but the lower the MTI. High corn proportions generated the lowest DDT, DST (together with sorghum) and PM. High sorghum proportions resulted in the lowest DT and DST, as well as intermediate values of DDT and PM. In addition, high sorghum proportions and binary mixtures of corn and parboiled brown rice generated the lowest AT values.

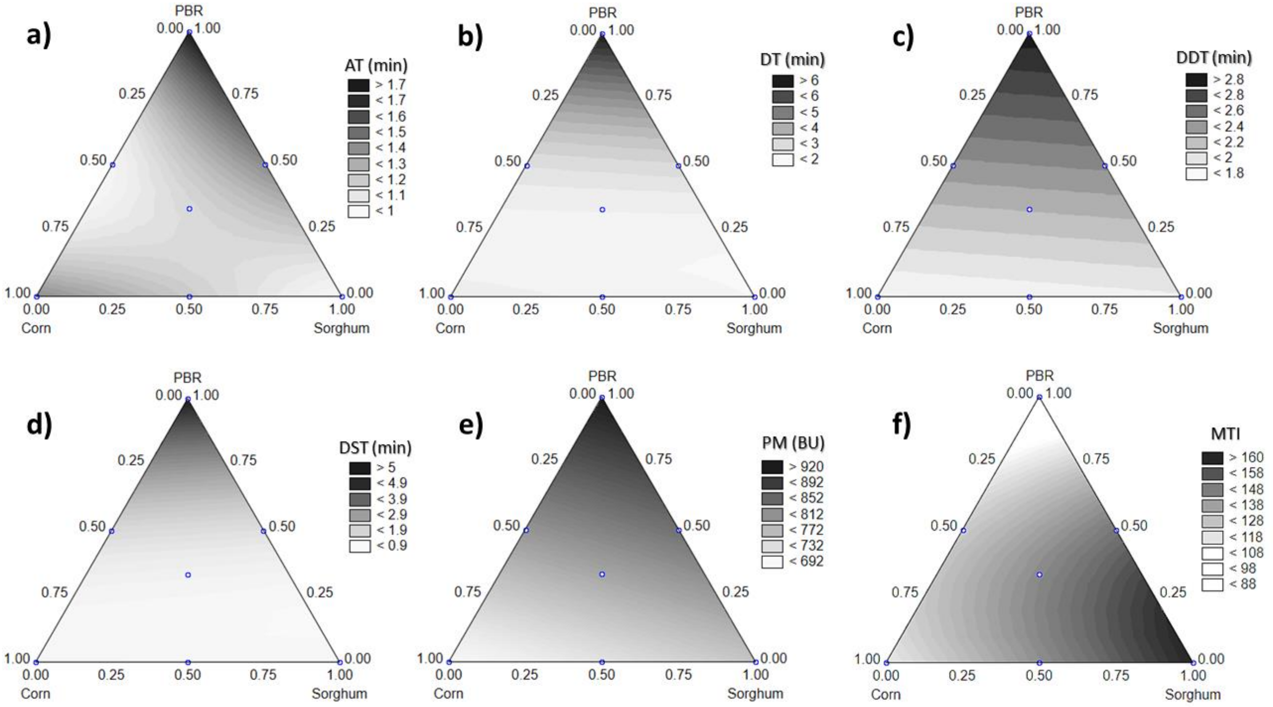

Fig. 5. Contour plot for farinographic properties of flours.

The models for the mechanical-dynamic properties were significant (G', ɣ, τ, and G'=G") and had no lack of fit, except for tan δ (G"/G') where the model was non-significant. The variables G' together with ɣ and τ, both at the LVR and critical end, showed optimal adjusted R^2 adjusted (0.90-0.99) with linear and quadratic models, while G'=G" and τ (crossover) had good adjusted R^2 of 0.78 and 0.82, respectively. Higher corn ratios led to higher G' (LVR) and G'=G", and lower ɣ and τ (both at the LVR, critical and crossover ends) (Fig. 6a-i), indicating that the extruded corn sample loses its structure more easily than the sorghum and parboiled rice samples, due to its higher fibre content, which exerts a shearing effect between the continuous network of the dough, thus decreasing the cohesive forces of the dough conformation (COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.*, 2021). While the rice sample showed the highest ɣ and τ values, indicating its predominant and interesting contribution to the dough resistance to oscillatory stresses.

Table 5. Regression models for rheology characteristic of gluten-free whole grain flours pre-treated by extrusion.

Variable	Mathematical equation	Model	Model significance (p-value)	Lack off fit (p-value)	R^2	R^2ajust
Paste properties						
pTemp	$56.04X_1+61.85X_2+47.05X_3$	Linear	0.000012	0.66	0.98	0.97
CV	$43.79X_1+37.58X_2+93.78X_3$	Linear	0.016	0.14	0.75	0.66
PV	$463.44X_1+394.45X_2+632.33X_3-418.86X_2X_3$	Quadratic	0.007	0.18	0.95	0.90
TV	$125.22X_1+86.62X_2+179.82X_3$	Linear	0.03	0.11	0.69	0.59
BDV	$352.73X_1+309.72X_2+467.16X_3-300.29X_1X_3-420.29X_2X_1$	Quadratic	0.000003	0.93	0.99	0.99
FV	$527.67X_1+328.88X_2+943.27X_3$	Linear	0.00001	0.05	0.98	0.98
SBV	$411.67X_1+264.61X_2+776.17X_3-96.40X_1X_3-131.40X_2X_3$	Quadratic	0.0000	0.28	0.99	0.99
Farinography						
AT	$1.39X_1+0.98X_2+1.74X_3-2.21X_1X_3+0.09X_2X_3$	Quadratic	0.02	0.26	0.93	0.87
DT	$2.39X_1+1.99X_2+6.85X_2-7.41X_1X_3-5.9086956521739X_2X_3$	Quadratic	0.0003	0.18	0.99	0.98
DDT	$1.78X_1+1.88X_2+2.96X_3$	Linear	0.008	0.24	0.80	0.73
DST	$1.00X_1+1.01X_2+5.11X_3-5.30X_1X_3-5.90X_2X_3$	Quadratic	0.0003	0.31	0.99	0.98
PM	$689.58X_1+757.77X_2+920.97X_3$	Linear	0.003	0.18	0.85	0.80

MTI	$115.03X_1+168.02X_2+86.71X_3+95.18X_1X_3+85.17X_2X_3$	Quadratic	0.006	0.15	0.95	0.91
Reometry (dynamic mechanical properties) amplitude sweep						
G' (LVR)	$58860.29X_1+45804.29X_2+24444.30X_3$	Linear	0.0004	0.23	0.93	0.90
ɣ (end of LVR)	$0.02X_1+0.02X_2+0.16X_3-0.14X_1X_3-0.14X_2X_3$	Quadratic	0.000057	0.06	0.99	0.99
τ (end of LVR)	$976.48X_1+695.81X_2+3971.44X_3-1517.62X_1X_3-1533.61X_2X_3$	Quadratic	0.0001	0.26	0.99	0.98
ɣ (Critical value)	$0.02X_1+0.03X_2+0.21X_3-0.13X_1X_3-0.14X_2X_3$	Quadratic	0.000005	0.15	0.99	0.99
τ (Critical value)	$1231.95X_1+929.55X_2+4490.61X_3$	Linear	0.000005	0.23	0.98	0.97
G'=G"	$7471.64X_1+5756.58X_2+4207.77X_3$	Linear	0.005	0.29	0.83	0.78
ɣ (Crossover)	$1.25X_1+1.68X_2+4.50X_3$	Linear	0.0001	0.86	0.95	0.94
τ (Crossover)	$14357.04X_1+14598.37X_2+25855.70X_3$	Linear	0.002	0.41	0.87	0.82
Frequency sweep						
tan δ (G"/G')*	$0.13X_1+0.15X_2+0.15X_3+0.02X_1X_2-0.03X_1X_3-0.04X_2X_3$	Quadratic	0.574859	0.338232	0.64	0.05

* Variables that showed lack of fit or that the models were not significant.

pTemp: pasting temperature, CV: cold viscosity, PV: maximum peak viscosity, TV: thought viscosity, BDV: breakdown viscosity, FV: final viscosity and SBD: setback viscosity. AT: arrival time, DT: departure time, DDT: dough development time, DST: dough stability time, PM: peak maximum of consistency and MTI: mixing tolerance index. G′: elastic or storage modulus, G″: viscous or loss modulus, ɣ: shear strain, τ: shear stress, LVR: linear viscoelastic region and tan δ: angle of displacement.

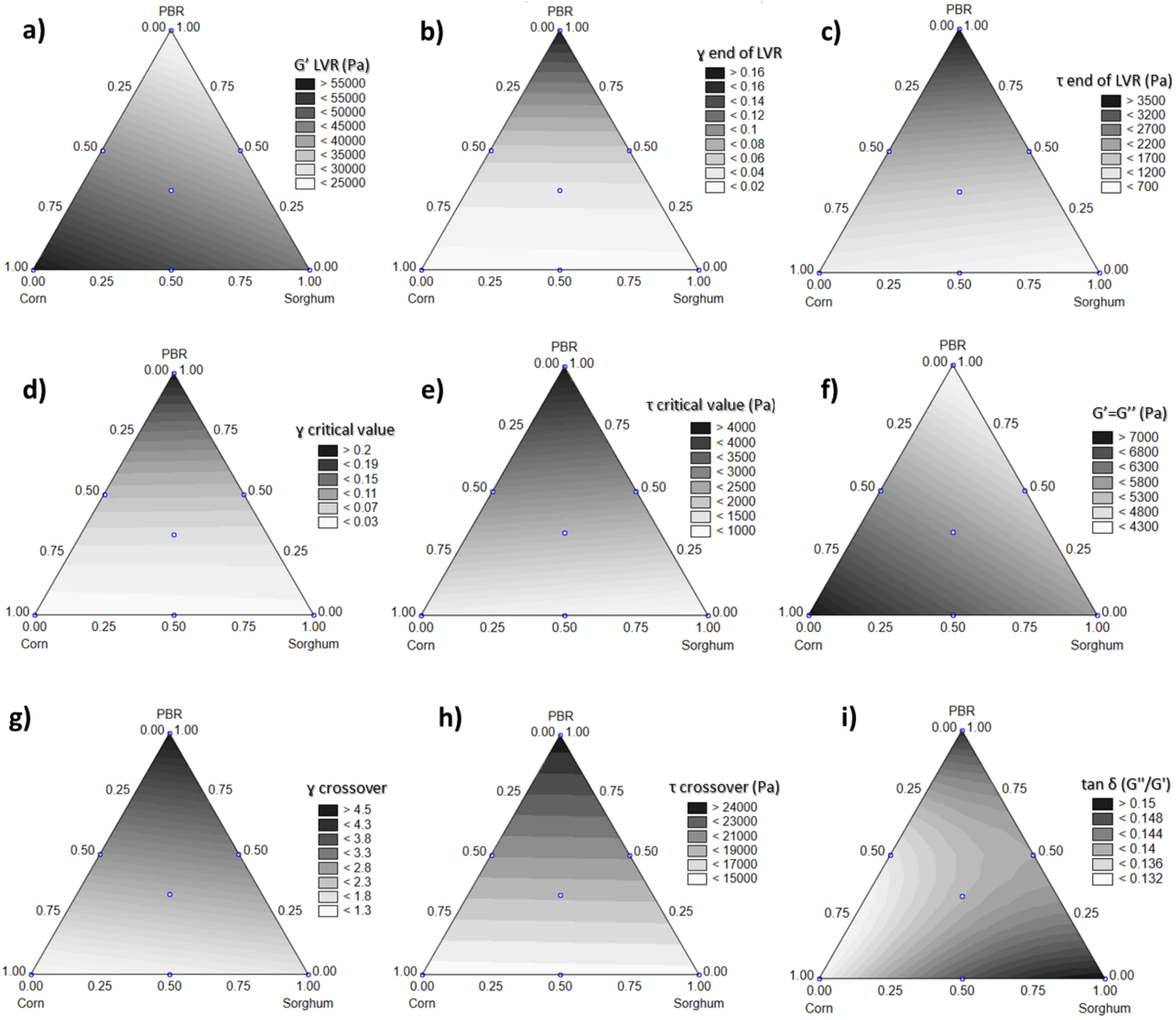

Fig. 6. Contour plot for mechanical dynamic properties of flours.

Bread characterization

Of the texture profile parameters, hardness, cohesiveness, chewiness, and resilience were significant and had no lack of adjustment, so they can be used as reliable and accurate models in predicting and explaining the behavior of these variables. Among these variables, hardness showed a linear behaviour (Fig. 6), but with a low adjusted $R^2 = 0.60$ (Table 6), so it can be considered to explain trends, while other authors as GENEVOIS e DE ESCALADA PLA (2021); SANTOS; FRATELLI; MUNIZ e CAPRILES (2018) did not achieve significance in this parameter which is the most relevant instrumental texture for gluten-free breadcrumb. In this sense, we could estimate that the higher the proportion of sorghum, the lower the hardness of the breads evaluated ($p<0.05$) (Table 7). While high proportions of parboiled brown rice or corn led to increases in hardness as reported by MANCEBO; MERINO; MARTÍNEZ e GÓMEZ (2015) for gluten-free breads with predominant rice starch incorporation.

Likewise, cohesiveness and chewiness showed a linear behavior and a good adjusted R^2 of 0.83 for both, where the highest cohesiveness was reached with the highest proportion of parboiled brown rice, intermediate values with high proportions of sorghum and the lowest with high proportions of corn, which due to its high fibre content affected the strength of the breadcrumb and caused a higher crumbling of the breadcrumb. The highest chewiness was achieved with higher proportions of parboiled rice, while with high proportions of sorghum or corn, this parameter reached its lowest values. Resilience had a quadratic behavior with an optimal adjusted R^2 equal to 0.91 and reached its highest values at high proportions of parboiled rice, indicating a better crumb recovery capacity; the opposite effect was caused by high proportions of corn and intermediate values by majority proportions of sorghum.

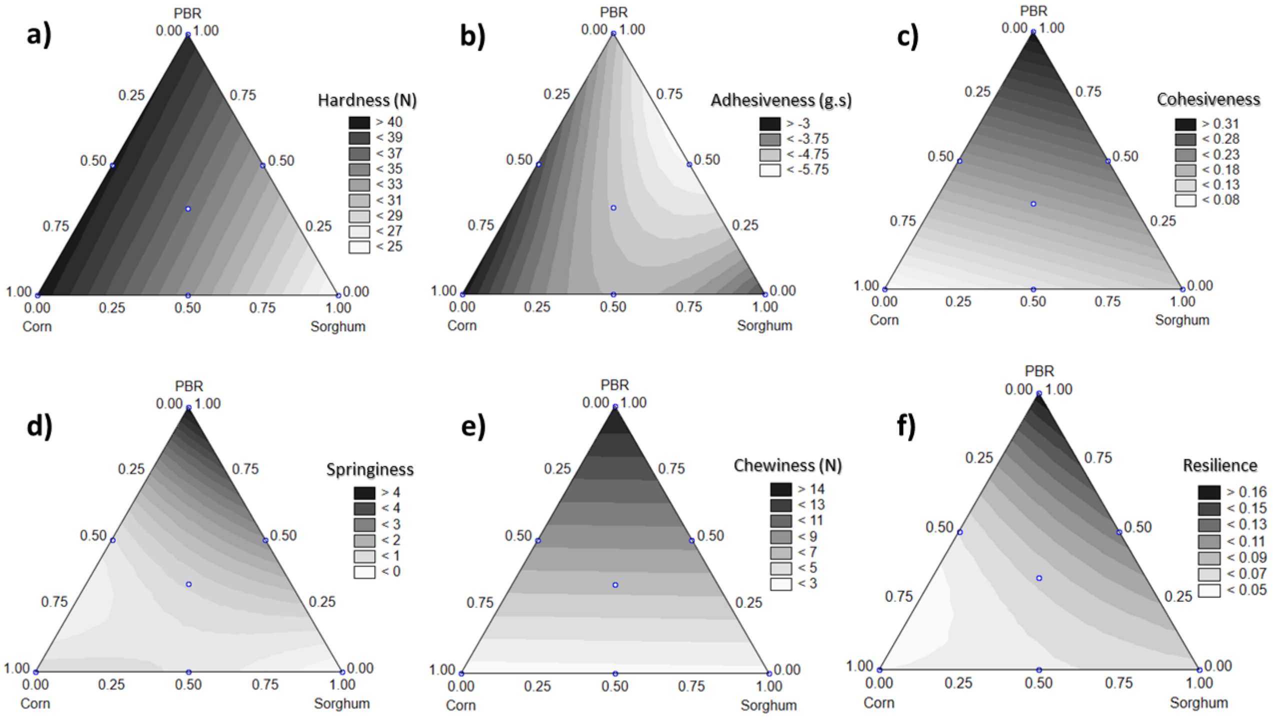

Fig. 7. Contour plot for texture of gluten-free bread.

Adhesiveness and springiness were texture parameters that had a lack of fit (Table 7), so they were not considered in the interpretation of the regression models (Fig. 8), but for both texture parameters, it was T3 that expressed outstanding values. Likewise, the physical quality parameters of the gluten-free breads such as baking loss and specific volume could not be modelled (Table 7), but the results can be interpreted, where the sample T2 (sorghum-based) showed lower baking loss, indicating lower evaporation loss and better water retention (Table 8). The specific volume between samples ranged from 1-1.5 cm3/g and was statistically equal (p>0.05), and was close to those found by SANDRI; SANTOS; FRATELLI e CAPRILES (2017) when incorporating a fibre-rich ingredient such as chia, resulting in dense gluten-free breads.

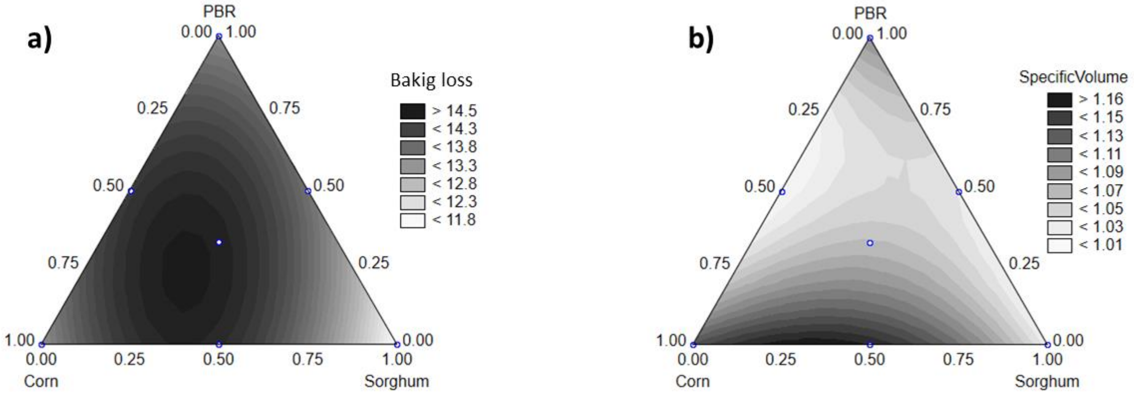

Fig. 8. Contour plot for physical quality of gluten-free bread.

Table 7. Regression models for characteristic of gluten-free bread.

Variable	Mathematical equation	Model	Model significance (p-value)	Lack off fit (p-value)	R^2	R^2ajust
Texture						
Hardness	$41.82X_1+24.84X_2+40.87X_3$	Linear	0.03	0.46	0.70	0.60
Adhesiveness*	$-2.37X_1-3.14X_2-4.62X_3-7.11X_1X_2+1.77X_1X_3-7.60X_2X_3$	Quadratic	0.25	0.01	0.25	0.00
Cohesiveness	$0.07X_1+0.15X_2+0.35X_3$	Linear	0.002030	0.30	0.87	0.83
Springiness*	$0.93X_1-0.02X_2+4.92X_3+0.90X_1X_2-8.89X_1X_3$	Quadratic	0.001870	0.000114	0.98	0.97
Chewiness	$2.49X_1+2.70X_2+14.21X_3$	Linear	0.002	0.24	0.87	0.83
Resilience	$0.050X_1+0.06X_2+0.17X_3-0.19X_1X_3$	Quadratic	0.013	0.76	0.94	0.91
Physic quality characteristics						
Baking loss*	$13.32X_1+11.74X_2+13.39X_3+8.01X_1X_2+4.04X_1X_3+4.54X_2X_3$	Quadratic	0.22	0.08	0.77	0.39
Volume specific*	$1.13X_1+1.02X_2+1.09X_3-0.41X_1X_3$	Quadratic	0.11	0.63	0.89	0.72

* Variables that showed lack of fit or that the models were not significant.

Table 8. Texture profile and physical analysis of gluten-free breads.

Treatment	Hardness (N)	Adhesiveness (g.s)	Cohesiveness (-)	Springiness (-)	Chewiness (N)	Resilience (-)	Baking loss (g)	Specific volume (cm^3/g)
T1	38.78 ± 3.64 bc	-2.98 ± 0.43 bc	0.08 ± 0.01 g	0.88 ± 0.1 b	2.63 ± 0.29 e	0.04 ± 0.01 e	13.17 ± 0.38 b	1.13 ± 0.03 ab
T2	26.85 ± 3.33 d	-3.75 ± 0.53 c	0.12 ± 0.02 f	0.78 ± 0.09 b	2.73 ± 0.39 e	0.06 ± 0.01 de	11.58 ± 0.18 c	1.03 ± 0.06 ab
T3	40.62 ± 4.7 ab	-5.23 ± 0.79 d	0.37 ± 0.04 a	5.72 ± 0.64 a	16.11 ± 2.06 a	0.17 ± 0.02 a	13.23 ± 0.38 b	1.09 ± 0.06 ab
T4	32.7 ± 3.32 cd	-2.1 ± 0.37 b	0.14 ± 0.01 ef	0.90 ± 0.08 b	4.17 ± 0.83 de	0.07 ± 0.01 d	15.17 ± 1.15 a	1.15 ± 0.05 a
T5	45.25 ± 3.7 a	-0.62 ± 0.08 a	0.15 ± 0.02 ef	0.92 ± 0.05 b	6.19 ± 0.88 bc	0.06 ± 0.01 de	15 ± 0.5 a	1.00 ± 0.01 b
T6	26.66 ± 2.88 d	-3.35 ± 0.51 bc	0.25 ± 0.02 b	0.96 ± 0.04 b	6.51 ± 0.67 bc	0.12 ± 0.01 b	14.33 ± 0.29 ab	1.02 ± 0.05 ab
T7	33.53 ± 3.02 c	-7.3 ± 1.03 e	0.23 ± 0.03 bc	0.90 ± 0.07 b	6.94 ± 0.89 bc	0.06 ± 0.01 de	14.67 ± 0.29 a	1.05 ± 0.06 ab
T8	40.81 ± 2.49 ab	-6.2 ± 0.96 de	0.2 ± 0.03 cd	0.89 ± 0.09 b	7.48 ± 1.44 b	0.09 ± 0.01 c	13.97 ± 0.16 ab	1.05 ± 0.06 ab
T9	37.42 ± 2.57 bc	-6.43 ± 1.32 de	0.18 ± 0.02 de	0.90 ± 0.08 b	5.46 ± 0.81 cd	0.07 ± 0.01 cd	13.93 ± 0.08 ab	1.10 ± 0.04 ab
p-anova	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
F-cal	21.79	50.79	84.71	300.56	88.01	64.86	15.9505	3.6851
F-tab (8,9)	2.15	2.15	2.15	2.15	2.15	2.15	2.51	2.51
CV	9.36	-18.06	11.98	15.96	16.23	14.19	3.48	4.44
Shapiro (Norm.Res)	0.10	0.51	0.45	0	0.11	0.30	0.03231	0.19256
Durbin-Watson (Independence.Res)	0.09	0.92	0.76	0.99	0.99	0.43	0.6686	0.1932
LeveneTest (Var.Homoge)	0.98	0.005	0.03	0.001	0.06	0.11	0.82009	0.95722
Lambda (λ)	---	x_negativo	-0.101	-0.101	---	---	-2	---

Results represent the mean ± SD (n=8 for texture and n=6 for physical properties). Lower case letters indicate differences between treatments ($p<0.05$). Box-Cox transformation factor (λ) for non-parametric data.

CONCLUSIONS

It was possible to demonstrate the functionality of gluten-free whole grain flours pre-treated by extrusion through the adaptation of the farinographic technique used only for wheat flour and through mechanical-dynamic (oscillatory) techniques, where it was possible to standardize the levels of water absorption and the optimal consistency for dough conformation and their corresponding elastic (G') and viscous (G") modulus. Parboiled rice (PBR or T1) was the sample that strongly influenced the dynamic properties of the paste, farinographic and mechanical properties, as well as the textural parameters of hardness, cohesiveness, chewiness, and resilience that were significant to be modelled. On the other hand, sorghum showed the lowest crumb hardness with lower cooking losses, indicating higher water retention caused by lower dough permeability during cooking and less retrogradation of its starch, as evidenced in the paste property profile. However, maize showed good dough functionality, but its high fibre content affected the crumb strength (lower cohesion) and led to more crumbling of the crumb. The regression models were significant with linear and quadratic behaviour for rheological variables (except tan δ) with optimal coefficients of determination for paste (adjusted R^2 = 0.90-099), farinographic (adjusted R^2 0.91-0.98) and dynamic mechanical (adjusted R^2 = 0.90-099) properties, while for breads it was only possible to model hardness (low adjusted R^2 = 0.60), cohesiveness and chewiness (good adjusted R^2 = 0.83), and resilience (optimal R^2= 0.90) within the texture profile.

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CHAPTER IV

STARCH *In Vitro* DIGESTIBILITY AND BIOACTIVE COMPOUNDS BIOACCESSIBILITY OF GLUTEN-FREE BREADS FROM EXTRUDED WHOLE GRAIN FLOURS AND THEIR BLENDS

Resumo

O objetivo desta pesquisa foi avaliar a digestão *in vitro* do amido pela liberação de açúcares redutores e a bioacessibilidade dos compostos fenólicos e flavonóides em pães sem glúten (GFBs) de farinhas extrudadas em comparação com pães de trigo (integral e branco). Foram elaborados três GFBs: sorgo (SB), binário (BB) feito a partir da mistura milho/sorgo (1/:1) e pães multigrão (MB) usando arroz integral parboilizado/milho /sorgo (1:1:1). Os pães e seus extratos digeridos foram avaliados para compostos fenólicos totais (TPC), capacidade antioxidante in vitro ($ABTS^+$), taninos condensados totais (TCT), flavonóides e perfil de ácido fenólico (PAs) por cromatografia líquida de alto desempenho (HPLC). Os pães sem glúten mostraram retenções interessantes de fenólicos totais e taninos com boa capacidade antioxidante, onde se destacaram SB e BB junto com a WWB ($p<0,05$). Após a digestão, os extratos de GFBs mostraram aumentos significativos ($p<0,05$) na redução de açúcares liberados do amido (127-173 vezes). A digestão aumentou significativamente os bioativos (TPC, $ABTS^+$ e TCT) e os ácidos fenólicos liberados (protocatecúrico e 4-hidroxibenzóico) juntamente com grandes liberações da catequina flavonóide, principalmente no pão de sorgo digerido (D-SB), seguido pelo binário digerido (D-BB) e pão integral digerido (D-WWB). Além disso, se evidenciou a degradação total dos ácidos vanílico, clorogênico e sináptico, assim como degradação parcial dos ácidos ferúlico (ácido fenólico predominante em cereais), 4-hidroxibenzóico, cafeico e p-cumárico (somente GFBs) após a digestão.

Palavras-chave: digestão gastrointestinal *in vitro*, Fitoquímicos, Multigrão, Açúcares redutores, Pão de trigo

Abstract

The aim of this research was to evaluate the in vitro digestion of starch by the release of reducing sugars and the bioaccessibility of phenolic compounds and flavonoids in gluten-free breads (GFBs)of extruded flours compared to wheat breads (wholemeal and white). It was elaborated three GFBs: sorghum (SB), binary (BB) made from mixture corn/sorghum (1/:1) and multigrain (MB) breads using corn/parboiled brown rice/sorghum (1:1:1). The breads and their digested extracts were evaluated for total phenolic compounds (TPC), *in vitro* antioxidant capacity (ABTS$^+$), total condensed tannins (TCT), flavonoids and phenolic acid profile (PAs) by high performance liquid chromatography (HPLC). Gluten-free breads showed interesting retentions of total phenolic and tannins with good antioxidant capacity, where SB and BB together with the WWB stood out ($p<0.05$). After digestion, the extracts from GFBs showed significant increases ($p<0.05$) in reducing sugars released from the starch (127-173 times). Digestion significantly increased bioactives (TPC, ABTS$^+$ and TCT) and the release phenolic acids (protocatechuic and 4-hydroxybenzoic) along with large releases of the flavonoid catechin, mainly in the digested sorghum bread (D-SB), followed by the digested binary (D-BB) and digested whole wheat bread (D-WWB). In addition, total degradation of vanillic, chlorogenic and synaptic acids, as well as partial degradation of ferulic (predominant phenolic acid in cereals), 4-hydroxybenzoic, caffeic and *p*-coumaric (only GFBs) acids after digestion.

Keywords: *in vitro* gastrointestinal digestion, Phytochemicals, Multigrain, Reducing sugars, Wheat bread

INTRODUCTION

Gluten-free (GF) products are a food gamma within the cereal processing industry that carefully and strictly exclude from their formulations grains such as wheat, barley, rye and sometimes oats, because contains proteins called gluten prolamins, which produce or trigger health problems associated to gluten in some genetically predisposed or sensitive individuals classified according to their immune responses involved in gluten ingestion into autoimmune (coeliac disease, IgA/IgG-mediated), gluten allergies (IgE-mediated) and non-coeliac gluten sensitivity (IgG) disorders (SCHERF; KOEHLER; WIESER, 2016). Among the large variety of foods, gluten-free breads (GFBs) are part of the biggest technological challenges, as they are formulated from GF cereals such as rice, corn, sorghum, and millet (generally stripped of their pericarp to improve their technological performance), pulses and pseudocereals, which content low-allergenic proteins, but unfavorably exhibit poor viscoelastic properties as those containing gluten to form light, soft and porous breads (ROSELL; BARRO; SOUSA; MENA, 2014).

Therefore, to simulate these properties in GF grains, high proportions of starches, hydrocolloids, proteins, enzymes, soluble fibres and enzymes are added to GFBs formulation, but it does not result in fully similar to those made from wheat. Few studies have focused on the use of physical and thermal treatments such as thermoplastic extrusion to modify the native polymers of cereals into novel raw materials with improved techno-functionalities (COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.*, 2021).

On the other hand, GFBs made from refine grains (corn, millet, rice, and sorghum) are often calorie-dense and deficient in healthy nutrients such as vitamins, minerals, and phytochemicals. In this context, whole grains (WG) or their derivatives (including WG-GFBs), must keep the natural nutrients and phytochemicals of the whole grain in their almost original proportions (MIR; BOSCO, 2019). Despite their enormous potential, WGs by themselves are not capable of providing health benefits to consumers unless they are processed to be edible. In addition, phenolics are the main phytochemicals found in WGs, including phenolic acids, flavonoids, anthocyanidins and phytosterols (BETA; CAMIRE, 2018). About 60-85% of the phenolic compounds in WGs are bound to the cell wall polysaccharides of the insoluble fibre matrix that makes up the bran layers (ZHANG; ZHANG; DONG *et al.*, 2019).

Phenolics are considered bioactive compounds because they have recognized health-promoting properties associated with antioxidant, anti-inflammatory and chemopreventive mechanisms, nowadays they are highly valued for reducing the risk of various diseases (TOMÉ-SÁNCHEZ; PEÑAS; HERNÁNDEZ-LEDESMA; MARTÍNEZ-VILLALUENGA, 2022). Therefore, different by-products from plants, fruits, pulses and others are incorporated into the GFBs (DJEGHIM; BOUREKOUA; RÓŻYŁO *et al.*, 2021), but very few studies have demonstrated the genuine nutraceutical potential of WGs on products like GFBs that are thermally processed.

In this regard, research efforts have mainly focused on technological development, but few efforts have focused on the production of fibre-rich GFBs with proven retention of bioactive compounds (phytochemicals) that manage to remain stable after undergoing thermal processes including extrusion and baking. Therefore, the aim of this research was to evaluate starch digestibility through the release of reducing sugars and bioaccessibility through the retention of phenolic compounds and flavonoids in GFB obtained by extrusion and baking compared to traditional whole and refined wheat breads.

MATERIAL AND METHODS

Plant material and flour preparation

Whole corn and sorghum grains were donated by Indústrias de Alimentos Granfino (Nova Iguaçú, Brazil) and EmbrapaMilho e Sorgo (SeteLagoas, Brazil), respectively; parboiled brown rice, wholemeal and refined wheat flour, and the ingredients (fat, sucrose, salt, yeast, egg, HPMC and DATEM) were purchased in the local market of Rio de Janeiro (Brazil).Grains were cleaned and milled on a hammer mill LM3100 (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm aperture sieve to obtain whole grain flours (WG).

Extrusion process

A co-rotating, intermeshing twin-screw extruder Evolum HT25 (Clextral Inc., Firminy, France) was used to process the whole grain flours of corn, parboiled rice, and sorghum. The screw diameter was 25 mm, with a length:diameter of 40:1, ten heating zones (from feeding to outlet: 25, 40, 60, 80, 100, 110, 110, 90, 80 and 70 °C), and the screw speed was set at 200 rpm. WG flours were fed through a twin-screw gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany) at a constant rate of 10 kg/h, and the process was monitored by Schenck Process Easy Serve software (Schenck

Process, Darmstadt, Germany). Deionized water was injected between the first and second modular zones through a port with a 5.25 mm internal diameter using a plunger metering pump Clextral (Clextral Inc., Firminy, France) set to compensate moisture differences in the samples and provide a final moisture content of 25%. The unexpanded extrudates were dried in a forced air oven at 55 °C for 5 h and milled into fine flours to be used to produce gluten-free breads (GFBs) in a hammer mill LM3100 (Perten Instruments, Huddinge, Sweden) equipped with a 0.8 mm aperture sieve.

Formulation and bread making procedure

The extruded whole grain flours were used to produce three types of gluten-free breads (GFBs) based on 100% sorghum flour (SB), binary mixture between corn and sorghum (BB, 1:1) and the multigrain mixture composed by corn, parboiled rice, and sorghum (MB, 1:1:1:1). The formulation was based on the one proposed by COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021) with some variations that consisted in the incorporation of additives and natural ingredients such as sugar (3%), salt (1.5%), non-hydrogenated palm fat (3%), whole egg (35%), water (86%), yeast (1%), DATEM (0.5%) and HPMC (4%) based on flour weight. The dough was prepared in a 35 g micro-mixer (National MFG. CO., Lincoln, USA), where the dry ingredients were mixed for 2 min, then the liquid ingredients were incorporated for a kneading time of 3 min. 20 g portions were fermented at 39 °C and 85% RH for 2 h and baked at 200 °C for 19 min in an electric oven, model grill (Fischer SA., Santa Catarina, Brazil).

Chemical composition

The chemical composition of breads was performed according to the AOAC (2000) official analytical methods: moisture (method 925.09), ash (method 923.03), total protein (method 2001.11, factor of 5.75), fat (method 945.38), total dietary fibre (soluble and insoluble) (method 991.43), and the carbohydrate was determined by the difference.

Simulation of in vitro gastrointestinal digestion

The fresh bread was crushed with a mortar until fragments between 0.15 and 2 mm were obtained to simulate the chewing process. Then, 1 g of each sample was taken for *in vitro* gastrointestinal simulation, comprising three phases: oral (2 min at pH 7), gastric (2 h at pH 3) and intestinal (2 h at pH 7), following the INFOGEST 2.0 protocol (BRODKORB; EGGER; ALMINGER *et al.*, 2019). In parallel, a blank (without sample) was prepared under the same procedure and containing the same fluids. The oral phase was performed at pH 7.0 for 2 min and for this purpose 4 mL of saliva simulation fluid, 475 µL of ultrapure water with 25 µL of $CaCl_2$ solution (0.3 mol/L) and 500 µL of salivary amylose solution (1000 mg/4.072 mL ultrapure water) were added sequentially. It was quickly homogenized and incubated at 37 C° in a water bath for 2 min (100 rpm), then with the help of ice water or by reducing the pH to the gastric phase we ceased this step.

The gastric phase was performed by adding 8.5 mL of gastric fluid and 5 µL of $CaCl_2$ solution on top of the oral phase mixture. It was further adjusted to pH 3 by adding 1M HCl and ultrapure water (995 µL-volume of HCl used to adjust pH). Then, 500 µL of pepsin solution (1.120 g/4.168 mL ultrapure water) was added, homogenized, and incubated rapidly in a 37 °C water bath under stirring at 100 rpm for 2 h. After time, enzymatic activity was stopped with the help of ice water.

The intestinal phase was performed by adding to the gastric phase mixture 8.5 mL of intestinal fluid, 40 µL of $CaCl_2$ and incorporating 1M NaOH until pH 7 was reached. A volume of water of 3.960 mL was then added to which the volume of base used to adjust the pH was subtracted. Next, 2.5 mL of bile solution (1.880 g/20.328 mL of intestinal fluid) together with 5 mL of pancreatin solution (5.200 g/40.304 mL of intestinal fluid) were added, homogenized, and incubated in a water bath at 37 °C under agitation at 100 rpm for 2 h. At the end, the intestinal phase was interrupted with ice-cold water and the tubes were centrifuged at 3000 rpm for 10 min at 4 °C. Supernatants were recovered and kept frozen (-18 °C) for further analysis. The experiment was done in triplicate.

Preparation of the breads and their digested extracts for the analysis

The breads and extracts obtained after the *in vitro* digestion were analyzed to assess starch digestibility by (1) reducing sugars released from the starch and (2) quantification of the bioactive compounds and antioxidant capacity. For the bread samples prior digestion, 1 g of crushed bread was used, and 36 mL of ultrapure water was added, stirred in a vortex for 1 min and left in extraction for 1 h. Then, it was centrifuged at 3000 rpm (1008 G-force) for 10 min at 4 °C and the supernatants were recovered for analysis. For the digested bread extracts, the aliquots were taken directly from the supernatant after centrifugation as previously described.

Determination of total reducing sugars (RS)

1 g of crushed bread was used, and 36 mL of ultrapure water was added, stirred in a vortex for 1 min and left in extraction for 1 h. Then, it was centrifuged at 3000 rpm (1008 G-force) for 10 min at 4 °C and the supernatants were recovered for analysis. For the digested bread extracts, the aliquots were taken directly.

The determination of total reducing sugars were carried out according to the modified methodology of MILLER (1959). 50 µL of sample was taken and mixed with 1.45 mL of distilled water to obtain a 1:30 dilution. For the reducing sugar reaction, 1 mL of the diluted sample (1.30) was taken, and 1 mL of DNS solution was added, shaken, and placed in boiling water bath at 100 °C for 5 min. After time, the reaction was stopped with ice water and readings were taken in a spectrophotometer at 540 nm wavelength. A glucose standard curve between 0-10 µmol glucose/mL was developed. Preparing a solution of 0.180 g glucose standard solution in 100 mL of distilled water to obtain a concentration of 10 µmol glucose/mL in the proportions (0.1, 0.2, 0. 3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1) with addition of water (1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 and 0) and 1 mL of DNS solution at all points of the curve. The results were expressed in µmol glucose/g bread.

Determination of total phenolic compounds (TPC)

TPC was performed by the Folin-Ciocalteau method according method used by CHÁVEZ; ASCHERI; CARVALHO *et al.* (2017) with some modifications. In the extraction, 1 g of ground sample was added to 25 mL of acetone (70%), left under magnetic stirring for 30 min. The extracts were then cleaned in OASIS HLB cartridges to remove interferents (sugars) from the ingredients. For the reaction, 250 µL of clean extract was taken and 1.25 mL of 10% Folin-Ciocalteau solution was added, followed by 1 mL of 7.5% sodium carbonate solution and vortexed. The tubes were then placed in a water bath at 50 °C for 15 min, placed in an ice bath for 30 s and read at an absorbance of 760 nm. The standard was gallic acid (5-100 mg/L), prepared with aqueous acetone solution (7%) and the results were expressed as mg gallic acid equivalents (GAE)/g.

2,2'-Azinobis-3-ethylbenzotiazilone-6-sulphonic acid (ABTS$^+$) assay

Antioxidant capacity was determined by ABTS$^+$ radical scavenging capacity according to RE; PELLEGRINI; PROTEGGENTE *et al.* (1999), the extraction was performed using 2 g of GFBs in consecutive steps with two extraction solutions (50% methanol and 70% acetone, respectively). Both extractions it was vortexed for 60 s and allowed to stand for 60 min, then centrifuged at 2000 rpm for 15 min and the supernatants were separated and pooled. $ABTS^+$ solution was prepared by dissolving 0.1920 g $ABTS^+$ salt in 10 mL and 0.3784 g potassium persulphate in 5 mL distilled water (under refrigeration, in the dark and 16 hours in advance), The $ABTS^+$ radical was prepared by adding 10 mL of $ABTS^+$ solution with 0.176 mL of potassium persulphate. The $ABTS^+$ solution was diluted with 95% ethanol to obtain an initial absorbance of ~0.800 at 734 nm in a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). In the reaction, 30 µL of extract (sample) and 3 mL of $ABTS^+$ solution was taken, vortexed and read at a wavelength of 734 nm. The Trolox calibration curve was 100-1500 µmol/L and the results are expressed in µmol Trolox equivalente/100g.

Determination of total condensed tannin (TCT)

TCT was quantified using the vanillin acidified method described by BROADHURST e JONES (1978). Extracts were obtained from 1 g of bread sample previously ground in 25 mL of methanol solution with 10 % hydrochloric acid (37 % concentration), shaken vigorously for 1 minute and left to macerate under refrigeration for 8 hours. The extracts were then filtered with high-speed filter paper and extracts were obtained and kept in the dark. The reaction was carried out by taking 1 mL of extract with 5 mL of 4% vanillin solution (4 g vanillin in 100 mL of 10 % methanol solution at 10 % hydrochloric acid with 37 % concentration), shaking, left to react for 20 min and readings were taken at 500 nm absorbance, discounting a blank (1 mL extract of 10 % methanol solution at 10 % hydrochloric acid). A catechin standard curve (0.1 g catechin in 100 mL 80% methanol) with concentrations (0.1-1 mg/mL) was used to calculate the TCT concentration. Results were expressed in mg of catechin equivalent (mg CE/g).

Quantification of phenolic acids (PAs) and flavonoids by HPLC-DAD

The analysis of free phenolic compounds was carried out according to the method described by PÉREZ-JIMÉNEZ; ARRANZ; TABERNERO *et al.* (2008). Bread samples were dehydrated, ground and extracted with 4 mL of 50% (v/v, pH 2) aqueous methanol solution under mechanical agitation for 1 h and subsequent centrifugation (6000 g) for 5 min. The supernatant (extract 1) was recovered and 4 mL of 70% (v/v) acetone aqueous solution was added to the residue and the mechanical agitation and centrifugation steps were repeated to obtain the new supernatant (extract 2). Extracts 1 and 2 were pooled and mixed to a total of 8 mL and transferred to a 1.5 mL vial for chromatographic injection.

Solid samples residues were subjected to extraction of hydrolyzed phenolic compounds according to the method described by FRIGHETTO e BACCAN (2012). Alkaline hydrolysis was performed with 5 mL of a 2 M NaOH aqueous solution containing 1% ascorbic acid and 10 mM EDTA. This solution was added to the samples, followed by heating at 60° for 60 minutes. Then, 1.5 mL of 6 M aqueous HCl solution was added for acid hydrolysis. This solution was vortexed for 10 s, cooled to room temperature and centrifuged at 2700 rpm for 10 min. The supernatant was collected and partitioned with 6.5 mL ethyl acetate. The extraction was repeated with ethyl acetate. The organic fraction was dried under nitrogen gas (N_2) and diluted in 80% (v/v) methanol aqueous solution for chromatographic analysis under the conditions mentioned in the previous section. Flavonoids and phenolic acids (PAs) were quantified by external standardization with commercial analytical standards. In contrast, digested bread extracts were injected directly into the HPLC-DAD system, hence prior extraction steps were not applied as in the case of solid bread samples.

Phenolic compounds were analyzed by the method of SANTIAGO; GALHARDO BORGUINI; DA SILVA DE MATTOS DO NASCIMENTO *et al.* (2018) using a Waters® Alliance e2695 liquid chromatography system, with two reversed-phase BDS Hypersil C_{18}Thermo Scientific columns (50 mm×4. 6 mm; 100 mm×4.6 mm; both 2.4 µm) in series, gradient elution with a 0.15% (v/v) aqueous solution of phosphoric acid and acetonitrile, and a diode array detector (range: 200-600 nm). The injection volume and flow rate were 5 µL and 1.2 mL/min, respectively. The column temperature operated at 40 °C. Flavonoids and phenolic acids were identified by comparison of retention time and UV/VIS spectra with commercial laboratory analytical standards.

Statistical analysis

It was used one way ANOVA depend on the necessity following by Tukey's multiple range tests where differences were detected. Paired t-test was used to study the *in vitro* digestibility effect on starch and bioaccessibility of bioactive in breads and after they were digested. A significance level of 5% was used for all bivariate statistics. Principal component analysis (PCA) was used to search possible relationships between variable-variable and sample-variable, the PCA was performed after variables standardization to avoid the effect of the different variable magnitudes. The hierarchical clustering of Principal Components (HCPC) was performed to confirm possible groups in PCA, HCPC was performed using Euclidian distances and Ward method. Finally, Person's correlation was performed to evidence and strongest quantification of variable-variable correlations, for the correlation strongest scale, it was used a scale proposed by TELES; CHÁVEZ; OLIVEIRA; BON *et al.* (2019). Statistical analysis of the data carrier out using R software version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria) and the packages “ExpeDes”, “FactoMineR” and“factoextra”.

RESULTS AND DISCUSSION

Chemical composition of gluten-free and wheat breads

The moisture of whole grain gluten-free breads (39.25-41.25 g/100g) were higher than white and whole grain wheat breads (28.24-29.52 g/100g) (Table 1). Then, it could be attributed to the effect of extrusion on break starch leading to an increasing of water absorption and retention, which was verified by the increase of water absorption addressed in the work of COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021). The samples with the highest ash within the GFBs were SB and BB ($p<0.05$), while WWB content the highest among all breads, this demonstrated that breads made with whole grain flours contain higher mineral content than those made of refined flours as expected. However, in the MB sample the lower ash values are attributed to the presence of parboiled rice and depending on the degree of flour milling, it contains less bran and consequently significant reductions in ash (~ 40%) (MONKS; VANIER; CASARIL *et al.*, 2013).

The protein content of gluten-free breads was significantly lower than whole and refined wheat breads, which could be is attributed to the high-protein of wheat due to gluten, which is a protein with a higher degree of molecular organization and mass compared to other prolamins present in gluten-free cereals, which increases its molecular density and its quantification at the nitrogen level for total protein conversion (ANJUM; KHAN; DIN *et al.*, 2007). Regarding lipids, it was observed that the BB and WWB had the highest amount without differences among them ($p>0.05$), indicating that whole corn contributed to significant increase of this component. On the other hand, MB showed the lowest amounts of lipids, similar ($p>0.05$)to the refined wheat bread (WB) , due to the low lipid content of parboiled brown rice as reported by COMETTANT-RABANAL; CARVALHO; ASCHERI; CHÁVEZ *et al.* (2021).

The highest total dietary fibre was found in the SB and BB samples (5.99 and 6.05 g/100 g, respectively) among the GFBs, being 40% lower than that of gluten-free breads made from whole grain sorghum as was reported by TORBICA; BELOVIC e TOMIC (2019). while the WWBs stood out among the wheat samples, significantly outperforming the GFBs ($p<0.05$). These results confirm the high fiber amounts of corn and sorghum wholemeal flours (2.3 times) compared to refined flour-based breads ($p<0.05$) and even to GFBs containing parboiled rice flour, as in the case of multigrain bread (MB). GFBs had the lowest amounts of carbohydrate compared to wheat, showing significant reductions between 8.8-26.5% compared to whole and refined wheat breads respectively. Furthermore, these carbohydrate reductions were consistent with the lower release of reducing sugars from hydrolyzed starch during in vitro digestion of GFBs (Fig. 1a), despite being subjected to additional heat treatments such as extrusion cooking and rice parboiling compared to their wheat counterparts.

Table 1. Chemical composition of gluten-free and wheat breads.

Component (g/100g)	Gluten-free			Wheat	
	SB	BB	MB	WWB	WB
Moisture	40.2 ± 0.11^{d}	39.25 ± 0.02^{c}	41.25 ± 0.00^{e}	29.52 ± 0.01^{b}	28.24 ± 0.09^{a}
Ash	1.50 ± 0.03^{b}	1.45 ± 0.04^{b}	1.38 ± 0.01^{a}	1.72 ± 1.72^{c}	1.34 ± 0.01^{a}
Protein	7.71 ± 0.08^{b}	7.41 ± 0.04^{b}	6.33 ± 0.08^{a}	10.09 ± 0.12^{d}	8.71 ± 0.04^{c}
Lipids	4.86 ± 0.01^{b}	5.04 ± 0.07^{c}	4.22 ± 0.03^{a}	5.09 ± 0.00^{c}	4.93 ± 0.04^{b}
Total dietary fibre	5.99 ± 0.01^{c}	6.05 ± 0.04^{c}	5.21 ± 0.03^{b}	7.97 ± 0.04^{d}	2.65 ± 0.03^{a}
Carbohydrates	39.76 ± 0.02^{a}	40.81 ± 0.09^{b}	41.62 ± 0.00^{c}	45.62 ± 0.07^{d}	54.12 ± 0.1^{e}
Total calories	233.54 ± 0.33^{b}	238.24 ± 0.47^{c}	229.76 ± 0.06^{a}	268.65 ± 0.23^{d}	295.67 ± 0.57^{e}

Results are expressed as mean ± SD. Different letters in the same row indicated differences among samples ($p<0.05$) by Tukey test.SB: sorghum bread, BB: binary bread (corn/sorghum 1:1), MB: multigrain bread (parboiled rice/corn/sorghum 1:1:1), WWB: whole wheat bread and WB: white wheat bread.

Reducing sugars (RS) and bioactive compounds of gluten-free, wheat breads and their digested extracts.

The GFBs showed 83-89% fewer reducing sugars (RS) than whole wheat (WWB) and refined wheat (WB)($p<0.05$) (Fig. 1.a). This may be due to the lower amount of carbohydrates present in the GFBs (Table 1) and to evidence that the combined effect of bland extrusion and baking did not profoundly modify the starch structure, as high amounts of reducing sugars were not observed. Furthermore, all GFBs after *in vitro* digestion increased in reducing sugars, evidencing a lower release compared to whole (WWB) and refined (WB) wheat and a possible reduction of the blood glycemic index.

The GFBs, mainly SB and BB, despite being obtained by extrusion cooking and baking treatments, showed good retention of bioactive compounds such as TPC (6.60 to 23.13mg GA eq/100g) and TCT (0.65 to 1.56 mg catechin eq/100g) similar to WWB that was only baked ($p>0.05$), but with higher antioxidant capacities (283.30 to 595.82 µmol Trolox eq/100g) (Fig. 1b-d). These indicate a high retention of bioactive compounds with scavenging capacity of GFBs made from extrusion-processed flours. MB had the lowest TPC content against with WB ($p<0.05$), due parboiled brown rice contains lower dietary fiber and phytochemicals bound to it than other wholegrain cereals (GUO; BETA, 2013). In addition, parboiling reduces the TPC from 35-38% by the mechanism of thermal degradation in parboiled rice, as reported by MIR; BOSCO; SHAH e MIR (2016).

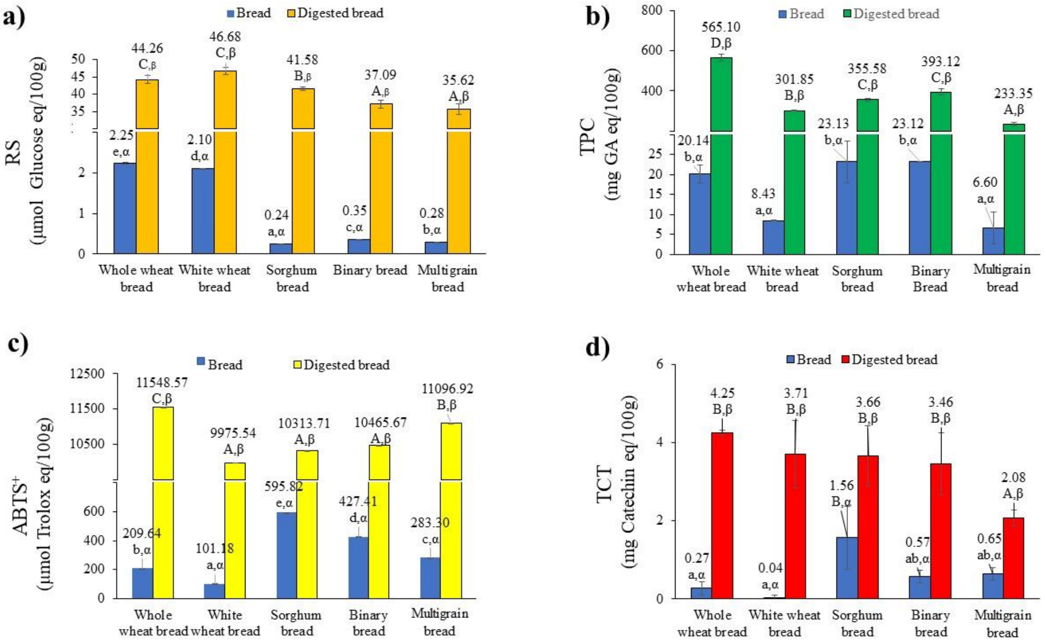

Fig. 1. Starch digestibility and bioactive compounds of gluten-free and wheat breads. a) release of reducing sugars (RS), b) total phenolic compounds (TPC), c) antioxidant capacity by ABTS+ radical scavenging and d) total condensed tannin (TCT). GA: gallic acid equivalent. Results represent the mean ± SD (n=3). Lower case letters indicate differences between bread samples ($p<0.05$). Capital letters indicate differences between digested extracts ($p<0.05$). Greek letters indicate differences between each bread sample and its digested extract.

TCT in cereals is found in very small amounts, sorghum being the one that stands out especially in high-tannin genotypes (1.72-4.48 mg CE/100 g) (COLLANTES; CARVALHO; ASCHERI *et al.*, 2022). However, in GFBs good TCT retentions (0.65-

1.66 EC/100 g) were found in spite of the heat treatments undergone, also to compared with results reported by CHÁVEZ; ASCHERI; CARVALHO *et al.* (2021) in extrudates . Furthermore, large increases in TPC (233.35 to 565.10 mg GA eq/100g), antioxidant capacity (9975.54 to 11548.11μmol Trolox eq/100g) and TCT (2.08 to 4.25 mg catechin eq/100g) were evident for all samples after *in vitro* digestion, standing outSB, BB among the GFBs and WWB These increases in TPC and TCT may be due to the release of membrane-trapped/bound phenols found in the bran (GUO; BETA, 2013) and proteins (D'ALMEIDA; MAMERI; MENEZES *et al.*, 2021), as well as in the aromatic amino acids (phenylalanine) released by enzymatic hydrolysis from proteins. While the high increase of ABTS$^+$ could be related to the formation of bioactive peptides of known antioxidant capacity during digestion with pepsin and pancreatin (CIAN; CABALLERO; SABBAG *et al.*, 2014).

It is possible that the higher TPC, ABTS$^+$ and TCT values in WB compared to GFBs could be due to the Maillard reaction formation of aromatic structures (phenolic groups) with recognized antioxidant capacity during baking (RÓŻAŃSKA; SIGER; SZWENGIEL *et al.*, 2021; SASANAM; RUNGSARDTHONG; THUMTHANARUK *et al.*, 2021), which could also contribute to the increase in TPC and TCT levels by spectrophotometric methods.

Phenolic acids (PAs) and flavonoids of gluten-free, wheat breads and their digested extracts

Ferulic acid was the most abundant phenolic acid in all samples for both wheat bread (GFB 127.39-345.38 μg/g) and WWB (143.81 μg/g), and GFB breads, where binary (BB) and multigrain (MB) breads had the highest values ($p<0.05$), due to the contribution of ferulic acid from yellow corn, which accounts for 85% of the total phenolics (SEVILLA; MARTÍNEZ; CARDADOR-MARTÍNEZ, 2018). GFBs presented the highest concentrations of five of the eight PAs compared to whole and white wheat breads (Table 2), which were ferulic, 4-hydroxybenzoic (12.91-36.41μg/g), *p*-coumaric (14.14-25.99μg/g), caffeic (4.80-11.89 μg/g) and chlorogenic (8.59-21.30 μg/g). SB, despite the process to produce these GFBs involved processes (sheared and heated during extrusion and baking) that usually cause damage on the components, SB showed the highest retention of most phenolic acids (protocatechuic, 4-hydroxybenzoic, vanillic, caffeic and chlorogenic) among all GFBs, while BB was excelled in *p*-coumaric and ferulic acids and multigrain bread in synaptic acid. Regarding the free phenolic acids present in the GFBs, 4-hydroxybenzoic, *p*-coumaric, ferulic, caffeic and chlorogenic acids were identified and quantified, while bound phenolic acids were predominantly constituted by *p*-coumaric and caffeic.

Only bound phenolic acids such as vanillic, *p*-coumaric and ferulic acids were found in WWB and GFBs. As it is known, this could indicate these phenolic acids are present in the pericarp. Wheat breads (WWB and WB) were characterized by the highest concentrations of flavonoid such as catechin (385.05 and 97.8 µg/g, respectively) compared to GFBs. Only in WWB the bound form of synaptic acid (6.19 µg/g) was found in higher concentrations than others, indicating that this phenolic acid is found in the wheat bran (LU; LUTHRIA; FUERST *et al.*, 2014). Similarly, these typical phenolic compounds found in wheat bread were also found but in lower concentrations in GFBs, with BB and MB having higher concentrations than sorghum bread, suggesting that both catechin (flavonoid) and synaptic acid (phenolic acid) were possibly degraded due to the thermal treatments of extrusion, baking and parboiling (only applied to rice).

After simulated *in vitro* digestion, liquid extracts were obtained from the bread samples which could only be analyzed as free PAs and flavonoids (catechin), as they lacked a solid fraction. The digestion resulted in increases of PAs such as protocatechuic and 4-hydroxybenzoic acids and very large amounts of catechin (as a flavonoid it has OH groups O-glycosides) which facilitates its solubility and bioaccessibility compared to the bound phenolics by breaking the glycosidic bonds of the phenolic compounds during the gastric phase under acidic conditions (HCl) and releasing bound phenolics (MOUSSA-AYOUB; EL-SAMAHY; KROH; ROHN, 2011). The same behavior was found by WU; LIU; LU *et al.* (2022) in roasted coffee, where it reported that heat treatment also favors the bioaccessibility of phenolic acids.

On the other hand, a degradative effect was observed on vanillic, p-coumaric, ferulic, caffeic, chlorogenic and synaptic acids after digestion, possibly caused by changes in pH that under epimerization and isomerization reactions can change the spatial configuration of the PAs or by enzymatic and microbial degradation that could generate minor phenolic metabolites, as found by WU; LIU; LU; BARROW *et al.* (2022) for bioaccessibility of roasted coffee phenolics.

Table 2. Phenolic acids and flavonoid (catechin) in wheat breads and gluten-free brads from extruded flours.

Phenolic acid/flavonoids (μg/g)	λ_{max} (nm)	Retention time (min)	Bread sample	Free	Bound	Total	Digested bread	*Total After digested
Protocatechuic	270	4.0	WWB	0.62 ± 0.01^{ab}	0.32 ± 0.02^{a}	0.94 ± 0.01$^{ab, \alpha}$	D-WWB	5.02 ± 2.53$^{b, \beta}$
			WB	ND	ND	ND$^{\alpha}$	D-WB	2.34 ± 1.27$^{ab, \beta}$
			SB	1.08 ± 0.04^{c}	0.42 ± 0.02^{b}	1.49 ± 0.06$^{c, \alpha}$	D-SB	5.39 ± 2.09$^{b, \beta}$
			BB	0.5 ± 0.03^{a}	0.26 ± 0.01^{a}	0.76 ± 0.04$^{a, \alpha}$	D-BB	1.08 ± 0.51$^{a, \beta}$
			MB	0.66 ± 0.05^{b}	0.30 ± 0.01^{a}	0.96 ± 0.06^{b}	D-MB	3.05 ± 1.08$^{ab, \beta}$
4-hydroxybenzoic	270	6.7	WWB	0.34 ± 0.02^{a}	0.31 ± 0.01^{a}	0.65 ± 0.04$^{a, \alpha}$	D-WWB	4.12 ± 1.77$^{a, \beta}$
			WB	0.28 ± 0.01^{a}	ND	0.28 ± 0.01$^{a, \alpha}$	D-WB	ND$^{\beta}$
			SB	32.6 ± 0.37^{d}	3.82 ± 0.06^{d}	36.41 ± 0.31$^{d, \alpha}$	D-SB	31.58 ± 9.87$^{c, \alpha}$
			BB	16.92 ± 1.22^{c}	2.63 ± 0.01^{c}	19.55 ± 1.23$^{c, \alpha}$	D-BB	14.36 ± 1.48$^{b, \beta}$
			MB	11.44 ± 0.16^{b}	1.47 ± 0.01^{b}	12.91 ± 0.15$^{b, \alpha}$	D-MB	5.93 ± 1.78$^{a, \beta}$
Vanillic	270	9.6	WWB	2.90 ± 0.14^{bc}	0.93 ± 0.04^{ab}	3.83 ± 0.11$^{c, \alpha}$	D-WWB	ND$^{\beta}$
			WB	0.94 ± 0.01^{a}	0.27 ± 0.01^{a}	1.20 ± 0.00$^{a, \alpha}$	D-WB	ND$^{\beta}$
			SB	3.63 ± 0.04^{d}	0.84 ± 0.06^{ab}	4.47 ± 0.10$^{d, \alpha}$	D-SB	ND$^{\beta}$
			BB	2.85 ± 0.04^{b}	1.17 ± 0.04^{b}	4.01 ± 0.00$^{c, \alpha}$	D-BB	ND$^{\beta}$
			MB	3.16 ± 0.01^{c}	0.31 ± 0.44^{a}	3.47 ± 0.45$^{b, \alpha}$	D-MB	ND$^{\beta}$
p-coumaric	310	12.4	WWB	0.51 ± 0.01^{a}	3.19 ± 0.01^{b}	3.7 ± 0.01$^{b, \alpha}$	D-WWB	ND$^{\beta}$

			WB	0.25 ± 0.01^{a}	0.33 ± 0.02^{a}	0.58 ± 0.01$^{a, \alpha}$	D-WB	ND$^{\beta}$
			SB	6.21 ± 0.13^{c}	7.94 ± 0.43^{c}	14.14 ± 0.3$^{c, \alpha}$	D-SB	23.45 ± 6.65$^{b, \beta}$
			BB	4.83 ± 0.08^{b}	21.16 ± 0.05^{e}	25.99 ± 0.13$^{e, \alpha}$	D-BB	13.99 ± 4.7$^{a, \beta}$
			MB	6.21 ± 0.13^{c}	12.34 ± 0.42^{d}	18.54 ± 0.28$^{d, \alpha}$	D-MB	11.38 ± 5.03$^{a, \beta}$
Ferulic	325	14.8	WWB	7.42 ± 0.22^{b}	136.4 ± 9.02^{b}	143.81 ± 9.23$^{b, \alpha}$	D-WWB	7.71 ± 0.75$^{b, \beta}$
			WB	1.87 ± 0.00^{a}	22.48 ± 1.58^{a}	24.35 ± 1.58$^{a, \alpha}$	D-WB	1.62 ± 0.25$^{a, \beta}$
			SB	10.13 ± 0.27^{c}	117.26 ± 7.84^{b}	127.39 ± 7.57$^{b, \alpha}$	D-SB	24.76 ± 1.49$^{d, \beta}$
			BB	12.02 ± 0.04^{c}	333.37 ± 0.93^{d}	345.38 ± 0.89$^{d, \alpha}$	D-BB	18.67 ± 4.01$^{c, \beta}$
			MB	24.21 ± 1.06^{d}	172.14 ± 12.04^{c}	196.35 ± 10.98$^{c, \alpha}$	D-MB	17.24 ± 1.52$^{c, \beta}$
Caffeic	325	10.1	WWB	ND	0.6 ± 0.03^{a}	0.6 ± 0.03$^{a, \alpha}$	D-WWB	ND$^{\beta}$
			WB	ND	ND	ND	D-WB	ND
			SB	6.83 ± 0.11^{c}	5.07 ± 0.18^{d}	11.89 ± 0.07$^{d, \alpha}$	D-SB	3.95 ± 0.64^{b}
			BB	3.86 ± 0.10^{b}	3.53 ± 0.10^{c}	7.39 ± 0.2$^{c, \alpha}$	D-BB	1.43 ± 0.51^{a}
			MB	3.13 ± 0.05^{a}	1.68 ± 0.08^{b}	4.80 ± 0.03$^{b, \alpha}$	D-MB	ND$^{\beta}$
Chlorogenic	325	9.9	WWB	ND	ND	ND	D-WWB	ND
			WB	ND	ND	ND	D-WB	ND
			SB	21.30 ± 0.04^{c}	ND	21.30 ± 0.04$^{c, \alpha}$	D-SB	ND$^{\beta}$
			BB	12.68 ± 0.17^{b}	ND	12.68 ± 0.17$^{b, \alpha}$	D-BB	ND$^{\beta}$
			MB	8.59 ± 0.19^{a}	ND	8.59 ± 0.19$^{a, \alpha}$	D-MB	ND$^{\beta}$

Synaptic	325	15.7	WWB	ND	6.19 ± 0.42^{d}	$6.19 \pm 0.42^{c, \alpha}$	D-WWB	ND^{β}
			WB	ND	ND	ND	D-WB	ND
			SB	ND	0.91 ± 0.04^{a}	$0.91 \pm 0.04^{a, \alpha}$	D-SB	ND^{β}
			BB	ND	2.93 ± 0.01^{c}	$2.93 \pm 0.01^{b, \alpha}$	D-BB	ND^{β}
			MB	1.00 ± 0.01^{a}	2.02 ± 0.11^{b}	$3.02 \pm 0.12^{b, \alpha}$	D-MB	ND^{β}
Catechin (Flavonoid)	270	9.3	WWB	383.05 ± 1.7^{c}	ND	$383.05 \pm 1.7^{c, \alpha}$	D-WWB	$9514.37 \pm 507.36^{c, \beta}$
			WB	97.8 ± 2.19^{b}	ND	$97.8 \pm 2.19^{b, \alpha}$	D-WB	$6748.74 \pm 1038.85^{ab, \beta}$
			SB	19.81 ± 0.22^{a}	ND	$19.81 \pm 0.22^{a, \alpha}$	D-SB	$8969.51 \pm 914.97^{bc, \beta}$
			BB	18.57 ± 0.38^{a}	ND	$18.57 \pm 0.38^{a, \alpha}$	D-BB	$5612.29 \pm 1149.26^{a, \beta}$
			MB	20.53 ± 0.47^{a}	ND	$20.53 \pm 0.47^{a, \alpha}$	D-MB	$9403.48 \pm 1285.32^{c, \beta}$

*The digested breads were liquid extracts, so it was only possible to quantify free phenolic acids = total

Results represent the mean ± SD (n=3), Lowercase letters in the same column compare the concentration of phenolic acids or flavonoids (catechins) between samples. Greek letters in the same row.

Principal component analysis for breads and their digested extracts

For bread after digestion samplesPC1 and PC2 explained 83.5% of the total variance (Fig. 2a) for all 13 response variables in five samples (three GFBs and two wheat breads). PCA showed that wheat breads were characterized by the highest amounts of reducing sugars among all samples.

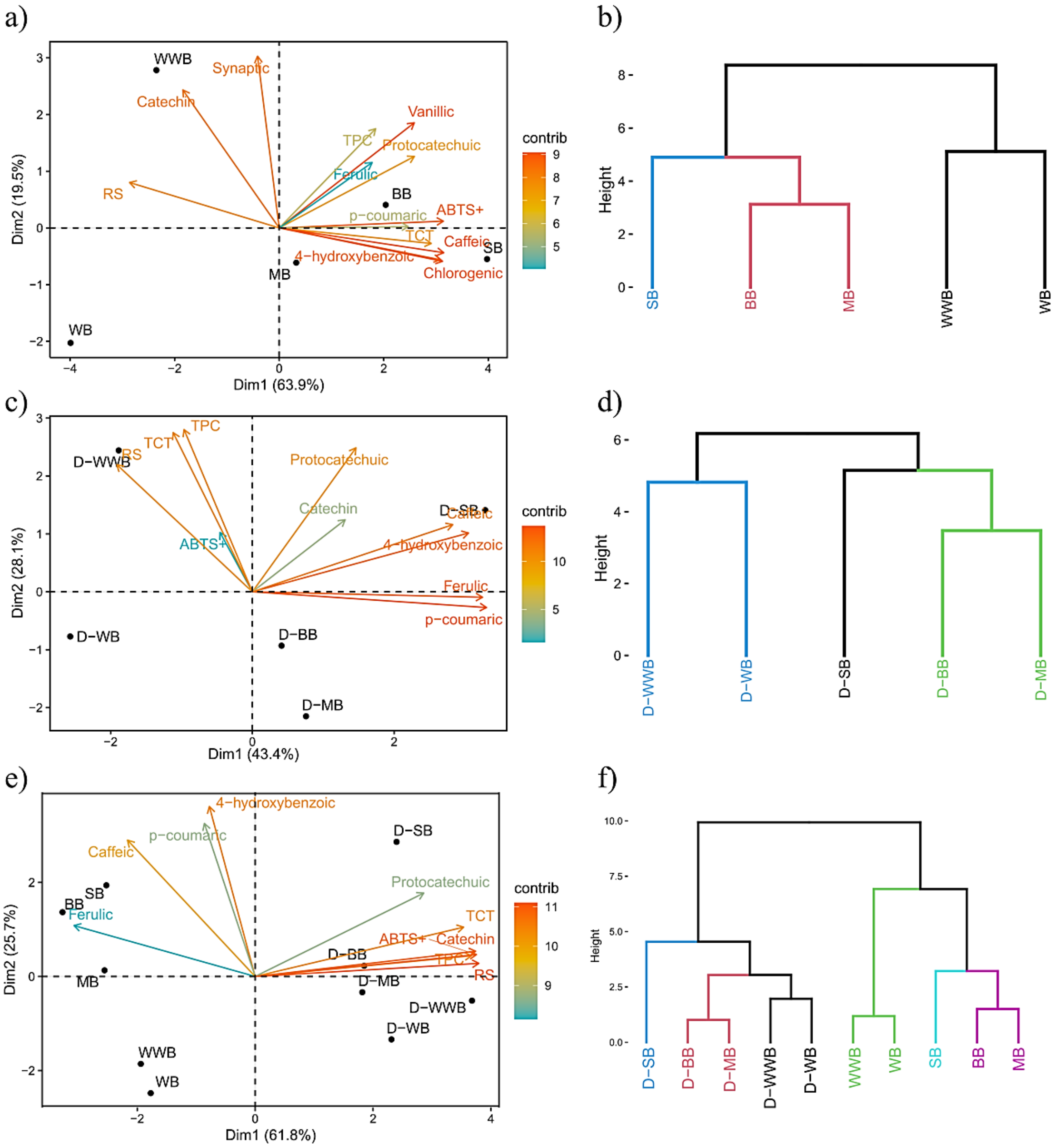

Fig. 2. Principal component analysis (PCA) of gluten-free and wheat breads. a-b) bit-plot and hierarchical clustering on principal components (HCPC) of breads, c-d) bit-plot and HCPC for digested breads and e-f) bit-plot and HCPC for bioaccessibility of breads and digested breads.

SB and BB showed the best PAs profiles, characterized by the highest amounts of TPC, TCT and high antioxidant capacity by ABTS$^+$ radical scavenging. On the other hand, WWB was characterized to have the highest amount of synaptic acid and catechin, while WB have the lest values of compounds. These findings were also confirmed by HCPC in Figure 2b, where it can be seen that SB stands out for having the highest values of some PAs, followed by samples BB, MB and WWB.

PCA for the samples after *in vitro* digestion (Fig. 2c), the first two principal components accounted for 71.5% of the variability of the data (10 variables for 5 samples). D-SB presented the highest retentions of protocatechuic, 4-hydroxybenzoic, caffeic and chlorogenic acids which could be corroborate in Table. 2. On the other hand, D-WWB presented the highest values in RS, TPC and TCT, since it was only subjected to baking while GFBs were subjected to extrusion and baking. Similarly, BB (corn-sorghum bread) showed high retentions *p*-coumaric and ferulic acids. While MB had the lowest retentions of most PAs among all whole grain breads. The 3 groups formed by HCPC confirmed the PCA results. Among the GFBs, D-BB and D-MB were close to each other, but D-SB was the most distinct and prominent sample. While the group formed by wheat breads (D-WWB and D-WB) were clustered together, differing from the GFBs (Fig. 2d).

Regarding the bioaccessibility experiment (Fig. 2e and f), the PC1 and PC2 explained 87.5% of the total variance (considering 10 variables and 5 samples). PCA (Fig. 2e) clearly separate the undigested and digested breads (with prefix D-), thus, digested samples have more amounts of protocatechuic, 4-hydroxybenzoic, ferulic, caffeic and *p*-coumaric acids, corroborating the results of Fig. 1 and Table 2. The D-SB (following for D-BB and D-MB) was who presented the highest values of most the above-mentioned compounds. Whereas D-MB and D-WB (against of digested samples) showed the lowest amounts of total bioactives (TPC, $ABTS^+$ and TCT) with a poor PA profile. On the other hand, D-WWB stood out and was characterized by having the highest TPC, antioxidant capacity by $ABTS^+$ radical scavenging and TCT among the samples, as well as having together with D-WB, the highest amounts of RS among the digested samples.

Regarding undigested samples, SB and BB have the greater values of ferulic acids and caffeic acids, while WB and WWB were the samples with lowest amount of most response variables used for the PCA. In is very important to point that these two samples (WB and WWB) where who more easily release RS after digestion as is clearly observed in the figure 2b due undigested and digested wheat breads were placed diametrically opposite, showing that the extracts from GFBs released lower amounts of reducing sugars between 11-23.7%, while in the whole wheat breads (D-WWB) it was only 5.7%.

Additionally, in this way, PCA evidenced release of high concentrations of catechin (as flavonoid) and protocatechuic acid intermediates after digestion for all samples and mainly the digested extracts of sorghum (D-SB) and whole wheat (D-WWB) had substantial increases in TPC, antioxidative capacity, TCT. In addition, slight losses of 4-hydroxybenzoic acid were observed in the extracts derived from the gluten-free breads but increased in the digested whole wheat bread (D-WWB).

The HCPC of bioaccessibility experiment (Fig. 2f), confirm the separation of the undigested and digested samples, which prove the effect of *in vitro* digestion and confirm the great differences between bread made from wheat (WB, WWB, D-WB and D-WWB) and from GF grains (SB, BB, MB, D-SB, D-BB and D-MB), these results are very important to demonstrate the benefits of the consumption of whole grains with high phytochemical concentration.

Regarding correlations, it was detected moderate and strong correlations (Fig. 3) of TPC, ABTS$^+$, TCT and catechin (flavonoid) with ferulic acid (-065, -0.71, -0.63 and -0.71 respectively), and between 4-hidroxybenzoic, *p*-coumaric and caffeic acid with RS with values of -0.66, -0.64 and -0.79 respectively, indicating that the release of total bioactives and reducing sugars (RS) occur simultaneously with significant reductions in ferulic acid during digestion. This behaviour was studied by BARROS; AWIKA e ROONEY (2012) who demonstrated a strong phenolic interaction with starches and its impact on decreasing starch digestibility.

From strong to very strong positive correlations were found between catechin (flavonoid) with TPC, ABTS$^+$ and TCT (0.77>r<0.99), as well as among PAs – PAs and some PAs – antioxidant capacity. Thus, it was demonstrated the results a strong relationship between the antioxidant capacity measured by ABTS$^+$ radical scavenging, total phenolic compounds (TPC) and total condensed tannins (TCT). Other authors also demonstrated these founds by DYKES; ROONEY e ROONEY (2013), LUZARDO-OCAMPO; RAMÍREZ-JIMÉNEZ; CABRERA-RAMÍREZ *et al.* (2020) and CHÁVEZ; ASCHERI; CARVALHO; BERNARDO *et al.* (2021) who worked with sorghum grains, cooked sorghum and extruded sorghum, respectively.

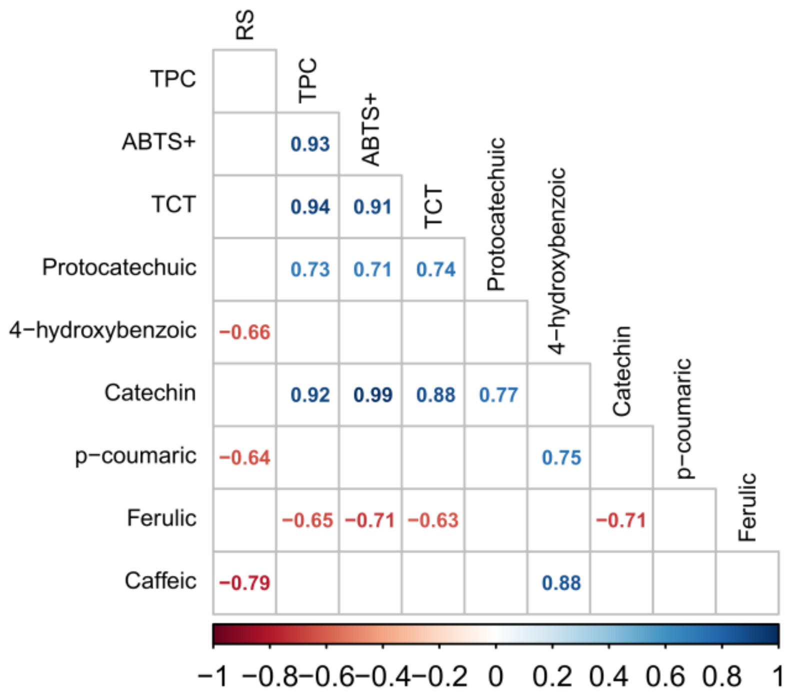

Fig. 3. Correlogram from Pearson´s correlations of the phenolic acids, catechin TPC, TCT and ABTS$^+$ quantified from bread samples (gluten-free and wheat), before and after digested.

Furthermore, high positive correlations were found between protocatechuic acid and catechin, also with TCP, antioxidant capacity and TCT ($0.71 > r \leq 0.75$), indicating a strong relationship between both phenolic compounds and the preponderant influence of protocatechuic acid on the increase of total phenolics and condensed tannins, as well as antioxidant capacity, findings that were similar to those found by CHÁVEZ; ASCHERI; CARVALHO; GODOY *et al.* (2017) in extruded blends of whole grain sorghum flour and toasted coffee powder.

CONCLUSIONS

Gluten-free breads (GFBs) showed lower amounts of reducing sugars, indicating lower starch digestibility than their wheat counterparts. GFBs obtained by extrusion and baking showed good retention of total phenolic compounds (TPC) with high antioxidant activity by ABTS$^+$ and similar amounts of total condensed tannins (TCT) as wheat breads, with sorghum bread (SB) having the best phenolic acid profile. After digestion, a high release of the catechin (flavonoid) and protocatechuic acid (phenolic acid) was observed for all samples, as well as *p*-coumaric acid only in D-SB. In addition, total degradation of vanillic, chlorogenic and synaptic acids was evident in all samples. As well as partial degradation of ferulic acid, 4-hydroxybenzoic acid (all samples), *p*-coumaric acid in corn/sorghum bread (D-BB) and multigrain bread (D-MB), as well as caffeic acid only in wheat (D-WWB) and multigrain bread (D-MB) after in vitro digestion, due to the degradative effect of hydrochloric acid and the pH-sensitivity of these phenolic acids during in vitro digestion.

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CONCLUSÃO GERAL

A tecnologia de extrusão termoplástica permitiu desenvolver propriedades funcionais das farinhas integrais sem glúten e avaliá-las utilizando propriedades de pasta e técnicas farinográficas e mecânico-dinâmicas aplicadas às massas para evidenciar suas propriedades viscoelásticas e potenciais aplicações e/ou contribuições para a produção de pães ou massas alimentícias sem glúten e sem aditivos. Durante as avaliações reológicas, a técnica farinográfica (exclusiva para trigo) foi adaptada para analisar farinhas sem glúten tratadas termicamente e foi utilizada como ferramenta na avaliação das características farinográficas das massas (absorção e consistência da massa, tempo de desenvolvimento da massa, tempo de estabilidade, entre outros). Além disso, foi realizada a caracterização mecânica, física e estrutural dos pães sem glúten, onde foi observado um progresso aceitável na formação da estrutura e do volume, enquanto a adição de 5% de germinado de milheto permitiu o amolecimento do miolo de pão. As interações entre milho, arroz parboilizado e farinhas de sorgo pré-tratadas por extrusão permitiram o estabelecimento de modelos de regressão matemática significativos sem falta de ajuste para todas as propriedades reológicas (pasta, farinográficas e mecânico-dinâmicas) das massas, e foi estabelecido que a farinha de sorgo extrudada tinha as melhores características de qualidade (maior volume, menor perda no cozimento e dureza do miolo) dos pães. Finalmente, com a realização da determinação da digestibilidade *in vitro*, verificou-se que pães isentos de glúten obtidos de farinhas integrais extrudadas mostraram boa retenção de bioativos quando expressos como compostos fenólicos totais e taninos totais com alta capacidade antioxidante por captura do radical $ABTS^+$ com alta liberação do flavonoide (catequina), demonstrando elevada bioacessibilidade dos fitoquímicos. Entretanto, observou-se a degradação total (vanílico, clorogênico e sináptico) e parcial (ferúlico, 4-hidroxibenzóico, cafeico e *p*-cumárico) de alguns ácidos fenólicos. Além disso, após a digestão, os pães sem glúten tiveram menor liberação de açúcares redutores em comparação com seus equivalentes de trigo. Entre os pães sem glúten, o pão de sorgo apresentou melhor retenção e perfil de ácidos fenólicos.

SUGESTÕES PARA FUTUROS TRABALHOS

Realização de estudos sensoriais de pão integral isento de glúten com uso de farinha pré-cozida por extrusão de forma a se avaliar sua aceitação.

Estudar o efeito da substituição de diferentes níveis (30-90%) de farinhas integrais pré-cozidas por farinhas cruas refinadas para optimização das formulações de pão sem glúten já existentes.

Estudar diferentes graus de cozimento por extrusão, de forma a avaliar sua funcionalidade e aplicações em pães isentos de glúten.

Explorar outras técnicas de cozimento e germinação das farinhas integrais cruas e estudar sua funcionalidade e aplicabilidade de forma a melhorar suas propriedades viscoelásticas em misturas prontas para pão integral isento de glúten.

Uso de outros grãos isentos de glúten, como por exemplo, os pulses (grãos secos de leguminosas) e outros cereais e/ou pseudocereais.