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Maria Eugenia Araujo Silva Oliveira

Germinated rice: technological production routes, physical and chemical characteristics, metabolomics approaches and metagenomics aspect

November

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PhD thesis presented to the Graduate Program in Food and Nutrition, Federal University of the State of Rio de Janeiro as partial requirement

for the title of PhD in Food and Nutrition.

Supervisor: Cristina Yoshie Takeiti

Co-supervisor: Carlos Wanderlei Piler de Carvalho and Dirce Yorika Kabuki

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JURY

Dra. Cristina Yoshie Takeiti
President (Supervisor- PPGAN UNIRIO)

Profa. Dra. Maria Teresa Pedrosa Silva Clerici
University of Campinas (UNICAMP)

Profa. Dra. Eveline Lopes Almeida
Federal University of Rio de Janeiro (UFRJ)

Dra. Fabíola Helena dos Santos Fogaça
Empresa Brasileira de Pesquisa Agropecuária (Embrapa)

Dr. Otniel Freitas Silva
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"Deixar que os fatos sejam fatos naturalmente, sem que sejam forjados para acontecer.
Deixar que os olhos vejam pequenos detalhes lentamente.
Deixar que as coisas que lhe circundam estejam sempre inertes, como móveis inofensivos, pra lhe servir quando for preciso, e nunca lhe causar danos morais, físicos ou psicológicos".

Chico Science

ABSTRACT

Rice (*Oryza* spp.) is not just a staple food; it is a versatile product that plays an important role in terms of interactions between food, economy and social development in many parts of the world, specialty in Asia and Latin America. Germination is a process that modifies the starchy matrix and increases bioactive compounds especially phenolic acids, and γ-aminobutyric acid (GABA) may deliver important nutritional and technological characteristics. The aim of this study was to evaluate the effect of short germination and polishing processes in different rice cultivars, *Japonica* (low amylose) and *Indica* (high amylose) on thermal, cooking qualities, metabolomics aspects and metagenomics approach. The results showed that short germination and polishing affect the cultivars in different ways in terms of α-amylase activity, cooking time, carbohydrate chemistry, bioactive compounds and diversity in fungal and bacterial profile. The findings of this study can be useful for producing new rice products by the food industry that can meet consumers needs with high levels of GABA, dietary fiber content, increase of umami amino acids (L-glu and proline) and reduced cooking time.

Keywords: rice, germination, γ-aminobutyric acid, starch.

RESUMO

O arroz (*Oryza* spp.) não é apenas um alimento de subsistência, é um produto indispensável que desempenha um papel importante em termos das interações entre a alimentação, a economia e o desenvolvimento social em muitas partes do mundo, especialmente na Ásia e América Latina. A germinação é um processo que modifica a matriz amilácea e aumenta os compostos bioativos, especialmente os ácidos fenólicos e o ácido γ-aminobutírico (GABA), que podem conferir características nutricionais e tecnológicas importantes. O objetivo deste estudo foi avaliar o efeito de processos de curto tempo de germinação associados ao polimento em cultivares de arroz *Japonica* (baixa amilose) e *Indica* (alta amilose), nas propriedades físicas, na qualidade tecnológica, numa abordagem metabolômica e em aspectos metagenômicos. Os resultados mostraram que a germinação curta e o polimento afetam as cultivares de diferentes formas em termos de atividade da α-amilase, tempo de cozimento, química dos carboidratos complexos, compostos bioativos e diversidade do perfil fúngico e bacteriano. Os resultados deste estudo podem ser úteis para a produção de novos produtos à base de arroz pela indústria alimentícia com o intuito de satisfazer as necessidades dos consumidores com níveis elevados de GABA, no teor de fibra alimentar, no aumento de aminoácidos umami (L-glu e prolina) e com redução do tempo de cozimento.

Palavras-chave: arroz, germinação, ácido γ-aminobutírico, amido.

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proportional to correlation coefficient (*r*) based on the scale display (lower side); where: FA: ferulic acid, ORY: γ-oryzanol, gamma-TE: γ-tocotrienol, L-glu: L-glutamic acid, GABA: γ-aminobutiric acid, FN: falling number, PT: pasting temperature, PV: peak viscosity, MV: medium viscosity, BDV: breakdown viscosity, FV: final viscosity, S: setback.

Figure 5. Biplot from principle component analysis (PCA) (A) and hierarchical cluster dendogram (B) of rice. Where: FA: ferulic acid, ORY: γ-oryzanol, gamma-TE: γ-tocotrienol, L-glu: L-glutamic acid, GABA: γ-aminobutiric acid, FN: falling number, PT: pasting temperature, PV: peak viscosity, MV: medium viscosity, BDV: breakdown viscosity, FV: final viscosity, S: setback, F=BRS Formoso; G= Guaporé; E= Empasc 104; M=Mochi; NG = non-germinated; G = germinated; B= brown rice; P=polished rice.

Chapter V

Figure 1. Viscoamylographic properties (RVA) of rice before and after germination and polishing.

Figure 2. Metabolomic analysis: (A) number of identification of each sample; (B) total relative ion abundance of phenolic compounds; (C) distribution of phenolic classes in free (FPC) and bound (BPC) extracts. F: flavonoids; L: lignans; OP: other polyphenols; PA: phenolic acids; S: stilbenes; NI: non-identified. Different lowercase and uppercase letters mean a significant difference ($p < 0.05$) between free and bound extract samples, respectively. Error bars represent standard deviation (n=3).

Figure 3. Principal component analysis (PCA) biplot of rice samples in free, bound and total extracts. The samples (symbols) are distributed according to relative intensity of identified phenolic compounds (red circles). Where: t= total phenolic compunds; b= bound phenolic compounds and f= free phenolic compounds.

Chapter VI

Figure 1. Scanning electron microscopy (SEM) of non-germinated (A, B), soaked (C, D) and germinated (E, F) rice starch.

Figure 2. Pasting curves of starches from non-germinated, soaked and germinated rice starch.

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List of abbreviations

ANOVA- Analysis of variance

DNA- Deoxyribonucleic Acid

DRX- X-ray Diffraction

DSC- Differential Scanning Calorimetry

FAA- Free Amino Acids

FN- Falling Number

GABA- Gamma-aminobutyric acid

HCA - Hierarchical Cluster Analysis

HPLC – High-Performance Liquid Chromatography

L-glu- L-glutamic acid

PC - Phenolic compounds

PCA - Principal Component Analysis

PCoA- Principal Coordinate Analysis

RVA- Rapid Visco Analyzer

SD- Standard Deviation

TAA- Total Amino Acid

UPLC-MS/MS - Ultra Performance Liquid Chromatography coupled to tandem Mass Spectrometry

ΔH- Enthalpy

Summary

General introduction, objectives, and strategy

Rice (*Oryza* spp.) is one of the most widely consumed cereal in the world and provides over 20% of the global daily energy intake, especially grown and consumed in Asia, playing an important role in terms of interactions between food, economy and social development (FAOSTAT, 2020). In Brazil, rice represents the food identity, in combination with beans that leads to improved the amino acid balance being consumed by all socioeconomic classes and regions (Brasil, 2014).

Despite the production of rice, people are still affected by hunger that has increased from 618 million in 2019 to 768 million in 2021 since the Covid-19 pandemic (Development Initiatives, 2022). Additional to this scenario, the Ukraine x Russia war and climate changes have affected the state of malnutrition that was ongoing. Almost a third (29.3%) of the world's population, that represents 2.3 billion people were moderately or severely food insecure in 2021. Paradoxically, people continue to consume inadequate diets in terms of fiber content, bioactive compounds and daily energy intake resulting in obesity and non-communicable diseases (NCDs) that lead to overweight and obese epidemic levels around 40% of adults and 20% of children (Development Initiatives, 2022).

Climate change has added a new dimension of uncertainty to world rice production. The projections about climate changes impact adversely on crop productivity and food availability (FAO, 2018). Therefore, the development of different genotypes of rice can be constitute a way of boosting the nutritional improvement and economic development of the population. Created in 1975, the Brazilian Germplasm Active Bank (BAG-ARROZ) has 20,791 accessions of rice used to develop the Brazilian varieties, cultivars and strains for breeding programs in Brazil and other parts of the world. The conservation and sustainable use of these genetic resources are fundamental to the future of rice research and cultivation, representing the long-term competitiveness and sustainability of Brazilian agriculture (Embrapa, 2023).

Although the consumption of brown rice has increased in the last decade due to the massive efforts on pursuing foods with greater nutritional value, a large majority of consumers reject this product due to sensory characteristics (especially in regards to texture)

and food preparation techniques (longer cooking time compared to polished rice). Few processes can be used to improve the quality of brown rice such as germination. The literature reports that germination of rice leads to higher levels of phytochemicals such as gamma-aminobutyric acid (GABA), gamma-oryzanol, tocotrienols and ferulic acid, improving its digestibility and reducing the preparation time (Zhang et al. 2014; Kamara et. al, 2010). Several health benefits of GABA have been reported, including lowering blood pressure and cholesterol plasmatic levels (Nishimura et al. 2014). In this scenario, germinated grains are excellent examples of functional foods, because in addition to their energy-nutritional value, they can also reduce the risk of NCDs incidence and can stimulate the germinated cereals market.

Recently, based on factors such as demand, population growth, increase in purchasing power, urbanization rate, level of education and the number of people working outside home and living alone, there has been a major change in food consumption profile. Consumers are demanding formulations that help optimize their mental performance, immune support and are made with natural ingredients. Furthermore, climate changes have increased interest in energy-saving and energy efficient products. Another important aspect is the media's emphasis on themes such as health and nutrition, which is a pillar for the development of new products in the area of healthy eating and food supplementation (Mintel, 2023).

Since in the last decade rice consumption is stagnant (IBGE, 2018), germinated rice can be of nutritional appeal and an alternative to increase the interest of rice. The food industry is constantly seeking to diversify the production line in order to meet the new market trends as higher quality, nutritious and sensorial interesting products combined with convenience. Products based on GABA rice are already a reality, mainly in the Asian market, and consisting of a market niche (OLIVEIRA et al., 2023).

This PhD thesis was conducted in Programa de Pós-Graduação em Alimentos e Nutrição (PPGAN-UNIRIO) following the research line "Processing, quality, valorization of food, coproducts and residues". The experimental part of this study was carried out at Empresa Brasileira de Pesquisa Agropecuária (Embrapa Agroindústria de Alimentos) entitled “Germinated brown rice: combined technologies to increase phytochemical

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In order to contribute to this field of research, the main objective of this study was to investigate the effect of germination processes to obtain rice of increased content of phytochemicals (GABA, ferulic acid, gamma oryzanol) and to evaluate their physical, chemical and metagenomics aspect. Therefore, five hypotheses guided this work: (i) how the rice genotype was affected by germination? (ii) is starch structure affected by germination? (iii) is the bioactive compounds profile changed after germination and polishing? (iv) carbohydrate chemistry and texture are affected by the culinary preparation? and (v) is the microbial population influenced by the germination process?

This manuscript consists of nine chapters divided into three main parts (Figure 1). The first part (Chapter II) focused on the literature overview about the production, consumption and market of rice and the grain structure. The results obtained during this thesis are structured in six scientific papers, three of them are already published in peer reviewed scientific journals, one is under review, and the others are in preparation for submission.

The second part consists of scientific production data obtained during the thesis development. In this sense, five original articles were structured: (i) Chapter III "How does germinated rice impact starch structure, products and nutritional evidence? – A review" published in Trends in Food Science & Technology (I.F. 15.3; Qualis CAPES A1), (ii) Chapter IV "Short germination and debranning affect bioactive compounds and pasting properties of rice genotypes" published in Journal of Food Processing and Preservation (I.F. 2.609, Qualis CAPES A4); (iii) Chapter V is entitled "Role of short germination and milling on physical properties, amino acid and metabolomic profiles of high amylose rice fractions" published in Food Research International (I.F. 8.1, Qualis CAPES A1); (iii) Chapter VI is

entitled “Role of short soaking/germination on rice starch characteristics” submitted to Journal of Cereal Science (I.F. 3.8, Qualis CAPES A2) ; (iv) Chapter VII How culinary techniques affect different types of rice products during storage? in preparation to submit to Carbohydrate Polymers (I.F 11.2, Qualis CAPES A1) and (v) Chapter VIII is entitled “The metagenomics approach reveals different microbial profile in high and low amylose” submitted to International Journal of Food Microbiology (I.F. 5.4, Qualis CAPES A1). Finally, in order to articulate the data presented in the chapters covered by the scientific production, a conclusion chapter (Chapter IX) presents the main findings to better understand the effect of germination on cultivars.

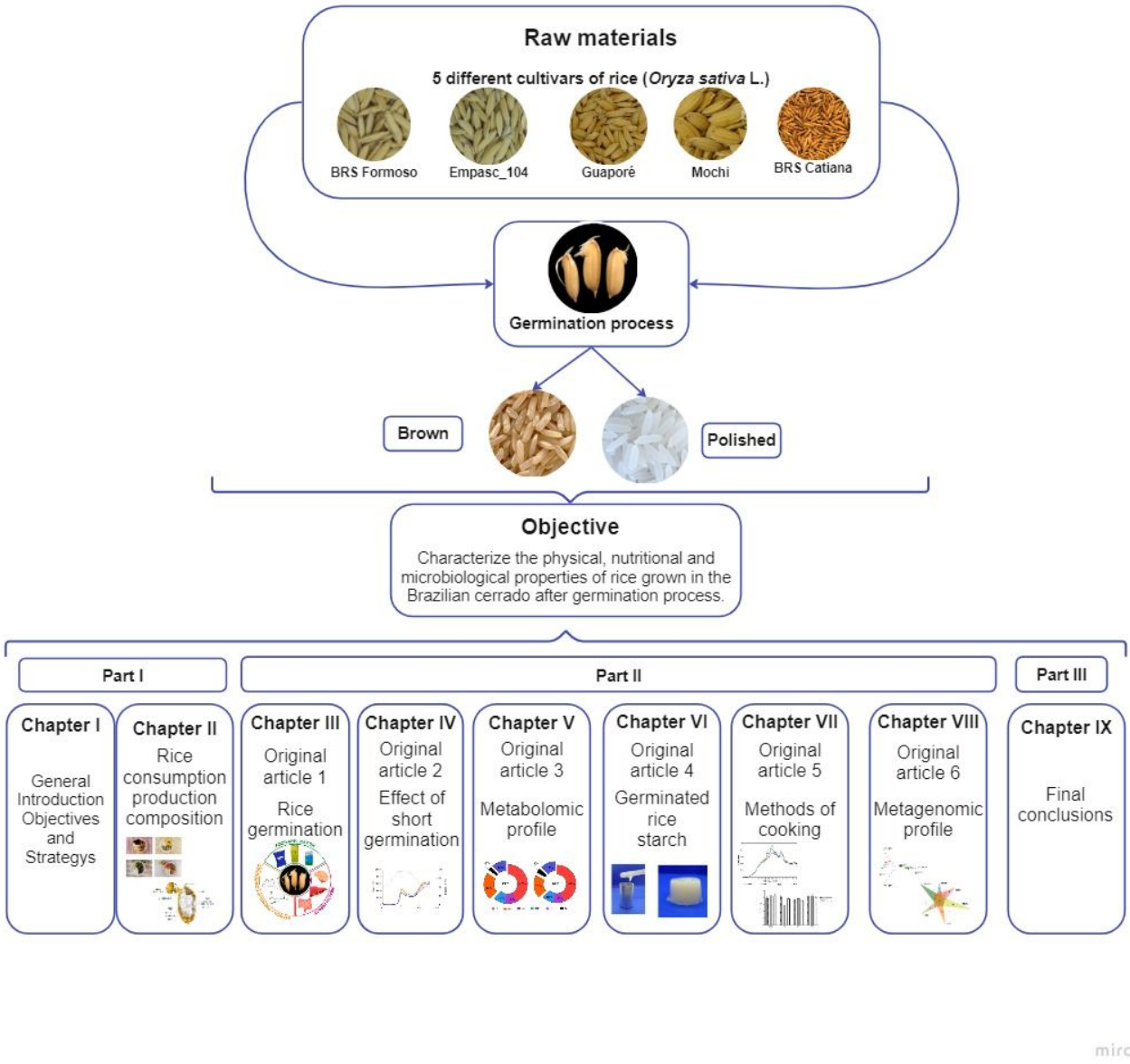

Figure 1. Thesis design.

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PART I

Literature overview

Chapter I- Rice production and consumption

Chapter II- Structure and composition of rice grain

Chapter I

1. Rice- production and consumption

Rice (*Oryza sativa* L.) is a major staple food in Asia, Latin America and the Caribbean (Fig. 2), and an important source of carbohydrates, minerals, vitamins, fiber and bioactive compounds (Sen et al., 2020). Rice contributes to food security, since most poor people in all regions and countries of the world can buy it to meet their daily energy needs (FAO, 2022).

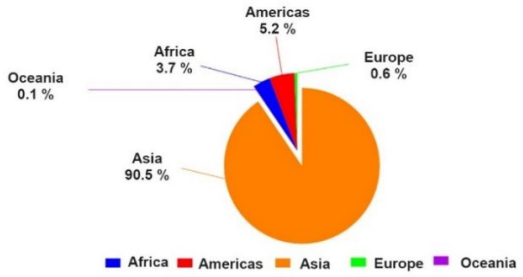

Figure 2. Production share of rice by region. Source: FAOSTAT (2023).

According to the latest projections by the OECD-FAO Agricultural Outlook, global rice production (The majority of the projected production increase (52 Mt, i.e. 89.7%) is expected to occur in Asia where India (+20 Mt), China (+6 Mt), Vietnam (+4.5 Mt) and Thailand (+2.5Mt). At the global level, the average *per capita* food use of rice is projected to maintain a similar level as in the base period at around 55 kg per year (Table 1) (OECD-FAO, 2021a).

Table 1. Rice per capita consumption (kg/person/year).

	2018-20	**2030**	**Growth rate (% p.a.)**
Africa	27.4	31.5	1.2
Oceania	13.5	14.2	0.44
North America	6.3	6.6	0.42
Europe	20.7	25.6	-0.08
Latin America and Caribbean	**28.0**	**28.1**	**-0.14**
Asia	77.2	77.5	-0.15

Source: OECD/FAO (2021a)

In Brazil, the rice production is most concentrated in the Southern region, responsible for more than 70 % of the national supply. In the 2022/2023 harvest, crop performance was very satisfactory, with yields above 8,000 kg/ha and 10,033.6 Mt of production. In recent harvests, the area under rice has been decreasing, both for irrigated and non-irrigated rice, mainly due to high production costs and crop substitution, such as corn and soybeans. The area of irrigated rice was estimated at 1,177.2 thousand hectares. As for non-irrigated land, there was a 4 % reduction in area compared to the 2021/22 harvest, estimated at 303.3 thousand hectares (CONAB, 2023).

In Brazil, rice ranks 7th among the product that arouse interest considering the greatest consumer desire once it is launched on the market (Table 2) and it is the food category in which the brand exerts the greatest influence at the time of purchase (44%) (Table 3) (FIESP, 2010). There are currently 41 members, including private companies and unions associated with the Brazilian Association of Rice Industries (ABIARROZ), which monitors the main events related to industrial rice production, taking advantage of its proximity to the public bodies responsible for managing the interests of the segment, increasing the loyalty of rice growing in the country (ABIARROZ, 2023).

Table 2. Ranking of arouse interests in consumer desire of Brazilian market (percentage).

Product	**%**
Yogurt	32
Crackers and cookies	28
Ready-to-drink juices	27
Chocolates and candies	25
Cheese	24
Frozen or semi-ready meals	21
Rice	**19**

Source: Fiesp/Ibope (2010).

Table 3. Top of mind brands ranking at the moment of purchase (percentage) in different food categories.

Product	%
Rice	**44**
Bean	36
Coffee	32
Milk	24
Yogurt	19
Chocolates and candies	14
Frozen or semi-ready meals	13

Source: Fiesp/Ibope (2010).

Rice combined with beans score for almost a quarter of the daily meal of the Brazilian population's daily diet. This situation reflects the dietary reality of the vast majority of Brazilians whom prefer fresh or minimally processed foods. Rice is an extremely versatile food, and is consumed with legumes, vegetables, eggs and meat using various culinary techniques (Fig. 3) such as risottos, *arroz à grega*, *arroz de cuxá*, *arroz carreteiro*, *galinhada* and *Maria Izabel*. Rice is also an ingredient in traditional Brazilian desserts called *arroz-doce* or *arroz de leite* (BRASIL, 2014).

Figure 3. Dietary composition of the lunches of Brazilians. Source: Guia Alimentar para a População Brasileira (2014).

According to the latest Brazilian Consumer Expenditure Survey (POF-IBGE), rice is one of the foods with the highest average daily consumption *per capita* (131.4 g/day) and is the *in natura* food that provides the highest energy intake, 11.1% of total energy, especially for the lower income population (IBGE, 2020). Rice consumption has decreased in Brazil to 84.0 % (2008) to 76.1 % (2018), in all income quarters, but the reduction was more pronounced in the last income quarter, with a reduction of 79.9% to 67.1%. Brown rice consumption also fell in the first three income quarters, but increased from 4.7% to 5.2% in the last income quarter. As for the variations by Brazilian regions, in the Midwest, South and Southeast there was a reduction in the frequency of rice consumption of approximately 8 percentage points, with an increase in rice-based preparations from 3.3% (2008) to 6.1% (2018) in the South and from 1.5% (2008) to 4.0% (2018) in the Midwest (IBGE, 2020).

Chapter II

1.2 Structure and chemistry composition of rice grain

Rice is a member of the family *Poaceae* (formerly *Gramineae* or grass). Rice is harvested as paddy rice, or, in botanical terms, “spikelets”. Spikelets of different cultivars may vary in proportion but have the same basic structure (Figure 4.). Rice is composed basically for starch, with lower amounts of proteins, lipids, fiber and ash (Juliano, B. O., & Bechtel, 1985).

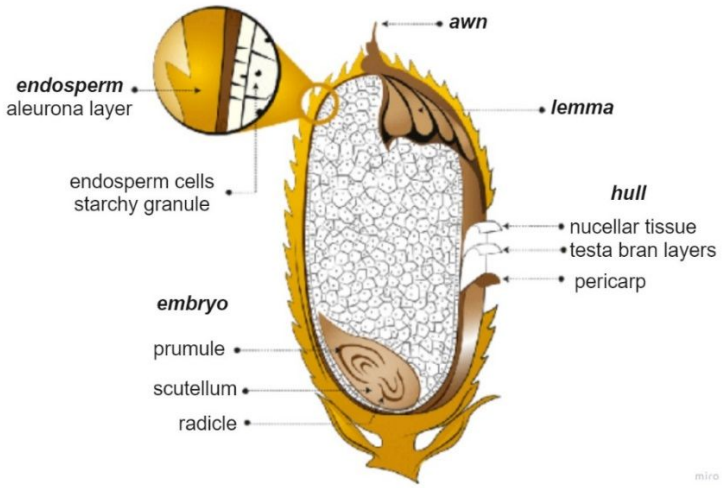

Figure 4. Rice grain morphological structure (Shakri et al. 2021).

In fact, rice is not only a staple food, it is also a source of fiber, carbohydrate, minerals, vitamins, and other biomolecules such as phytosterols, vitamin E, γ-oryzanol, phenolic acids (i.e. ferulic acid, gallic acid, syringic acid), anthocyanin and proanthocyanidins (i.e. epicatechin, cyanidin-3-O-glucoside), flavonoids (i.e. quercetin, apigenin, kaempferol, tricin), carotenoids (i.e. lutein, zeaxanthin, β-carotene) and phytosterols (i.e. stigmasterol, β-sitosterol) (Sen et al., 2020).

There are 23 species of rice, but only two of them are cultivated commercially: (i) *Oryza sativa* L. (Figure 5A), which originates from the tropics of Asia and (ii) *Oryza glaberrima* Steud. (Figure 5B), originating from West Africa (FAO, 2004). Many *Oryza sativa* varieties are cultivated commercially throughout the world, and are subdivided in two major subspecies (i) *Indica*, mainly long-grain rice that grows in tropical, subtropical, and partly temperate zones; and (ii) *Japonica*, a round-grain rice grown in temperate zones. Basmatic and fragrant rice are categorized under *Indica* rice type (OECD, 2021b). The genetic variation in the *Indica* group is much larger than in the *Japonica* group. The *Indica* has an elongated caryopsis with a ratio of 4:5, examples consisting of basmati rice and jasmine rice. The *japonica* species is opaque and sticky and is used in sushi, risotto and Asian dishes that require stickiness characteristics. The length:width ratio is 2:3 (Oka, 1988).

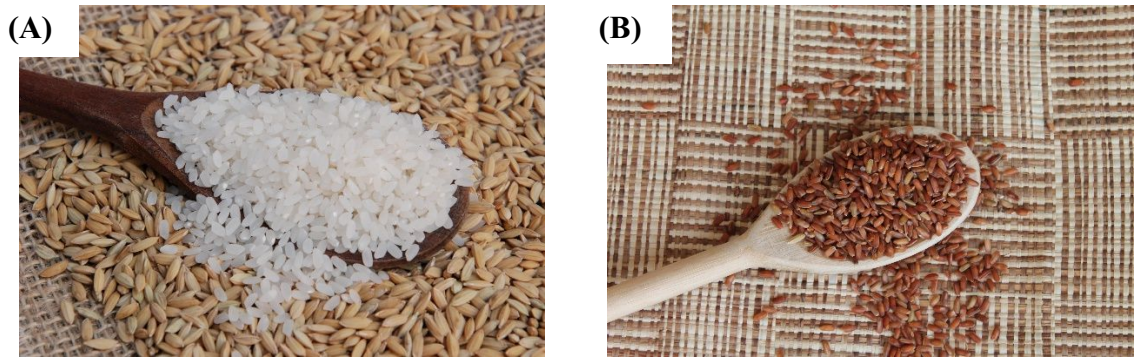

Figure 5. Visual aspect of *Oryza sativa* L. (A) and *Oryza glaberrima* Steud. (B).
Credits: Maria Eugênia Ribeiro (Embrapa)

The amylose and amylopectin contents are crucial factors that determine the taste and texture differences in rice varieties. The amylose content of *Indica* (26-31%) is usually higher than that of *Japonica* rice (17.3-19.7%). Besides, *Japonica* has a more glutinous texture than *Indica* rice. Cooking techniques may also affect the taste of rice based on its amylose content; *Indica* rice is boiled, meanwhile *Japonica* rice can be boiled and steamed. However, in many cases, boiled *Indica* rice is then cooked and fried as main or side dishes (meats, fish, and vegetables) or soused with curry, gambo, and other types of soup. The high amylopectin content of *Japonica* rice contributes to a higher thickness and sweetness in taste. In general, and contrary to *Indica* rice, *Japonica* rice maintains this texture after cooking, depending on the keep-warm function of the rice cooker used. This makes *Japonica* rice popular in cooking preparations such as sushi and rice balls. Consequently, cooking techniques reinforce the differences in taste between *Indica* and *Japonica* rice (OECD, 2021b).

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PART II

Based on following publications

Chapter III- How does germinated rice impact starch structure, products and nutritional evidences? – A review

Chapter IV- Short germination and debranning affect bioactive compounds and pasting properties of rice genotypes

Chapter V- Role of short germination and milling on physical properties, amino acid and metabolomic profiles of high amylose rice fractions

Chapter VI- Role of short soaking/germination on rice starch structure

Chapter VII- How culinary techniques affect different types of rice products during storage?

Chapter VIII- The metagenomics approach reveals different microbial profiles in high and low-amylose rice after germination and polishing processes

Chapter III

How does germinated rice impact starch structure, products and nutritional evidences? – A review

Maria Eugenia Araujo Silva Oliveira[1], Pedro Paulo Saldanha Coimbra[1], Melicia Cintia Galdeano[2], Carlos Wanderlei Piler de Carvalho[2], Cristina Yoshie Takeiti[2]*

[1]Food and Nutrition Graduate Program, Nutrition School, Federal University of the State of Rio de Janeiro, UNIRIO. Av. Pasteur, 296, Urca, 22290-240, Rio de Janeiro, RJ, Brazil.

[2]Embrapa Agroindústria de Alimentos, Avenida das Américas, 29501, 23020-470, Rio de Janeiro, RJ, Brazil.

*Corresponding author: cristina.takeiti@embrapa.br

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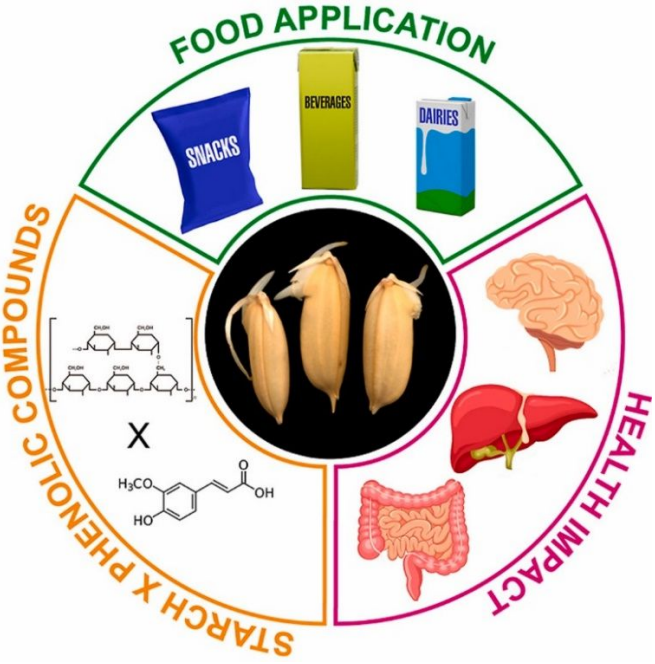


Highlights

- Rice is an important staple food crop to deliver nutrients broadly.
- Germination improves phenolic acids, lipophilic antioxidants and neurotransmitter.
- Starch-polyphenols interaction play an important role in starch digestibility.
- Rice metabolites exert hypoglycemic, anti-inflammatory, and anticancer activity.
- Food processing is an efficient tool in order to delay starch digestibility.

ABSTRACT

Background: In recent years, germination is used as a tool that improves nutritional quality of cereals by increasing digestibility and concentration of bioactive compounds, and thereby, gaining visibility concerning to reducing of risk of no communicable diseases (NCDs). Germination promotes the enzymatic modification of carbohydrates especially relating to starch-polyphenol interactions that could be used in glycaemia control.

Scope and approach: The Web of Science database was searched for studies published between 1992 and 2021 inorder to investigate the relationship among germination, starch and health benefits. The aim of this review is to reinforce and summarize the impact of germination on starch modifications, technological approaches, niche products and evidence of nutritional benefits.

Key findings and conclusions: Germination is a process that modifies starch digestibility and improves bioactive compounds especially phenolic acids, may delivering important nutritional and technological characteristics (starch-phenolic interactions) that impact on physiological performance (delayed digestibility and glycaemia control). In addition, germinated rice shows GABAergic effects and anti-inflammatory activity and it can mediate oxidative stress-related pathogenesis and carcinogenesis. In light of this evidence, the role of food technology is to develop processes in order to obtain products that deliver low glycaemic index (GI) as well as high phenolic content with good sensory attributes.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most produced crops around the world. Approximately 755,473,800 tonnes of rice are produced in 2019, and Asia is the world largest producer and the consumer region (90.6%) (FAOSTAT, 2019). The global rice production is expected to grow by 58 Mt to reach 567 Mt by 2030 and world rice consumption is expected to increase by 0.9% p.a. over the next ten years, compared with 1.1% p.a. in the last decade (OECD-FAO, 2021). Rice is an important cereal due to its nutritional value. Primarily, it is a source of carbohydrates and minerals such as calcium (Ca), magnesium (Mg), phosphorus (P), and traces of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (Verma & Srivastav, 2020).

One of the industry's strategies to add value to a product that is already widely consumed, such as rice, is an innovative focus on nutritional benefits or consumption options. Small or individual portions, ready-to-eat or express products meet the desires of consumers that seek convenience and exclusive foods (EUROMONITOR, 2019). Moreover, the Covid-19 era has changed the pattern of food consumption, i.e., families started to prepare most of their meals at home using fresh and clean label ingredients, and consuming more artisanal products out of their concerns with healthiness, natural life style and vegan mood.

In recent years, the consumption of germinated brown rice has been increasing due to its high nutritional value and content of bioactive compounds, which can help to reduce the risk of diseases (Wang, Xiao, et al., 2020). Germination is a technique that improves the sensory and nutritional quality of cereals by increasing their digestibility, since it activates the dormant enzymatic system of the grain, inducing the hydrolysis of high molecular weight compounds. Biological and technological tools have showed that rice germination promotes an increase in bioactive compounds such as gamma-aminobutyric acid (GABA), phenolic compounds (ferulic, syringic, p-cumaric, caffeic and vanillic acids), lipophilic antioxidants (γ-oryzanol and tocopherol), soluble antioxidants (anthocyanins and anthocyanidins), B vitamins, prebiotic compounds and dietary fibers (arabinoxylans and resistant starch) (Cho & Lim, 2016; Cornejo et al., 2015; Songsamoe et al., 2021).

Types of rice (whole grain, polished, germinated or other processed product) can affect the bioavailability of nutrients and impact human health (Guzman-Ortiz et al., 2019;

Kongkachuichai et al., 2020). Although there are several studies in the literature reporting changes and modifications that occur during rice germination (Kongkachuichai et al., 2020; Li et al., 2017; Wang, Xiao, et al., 2020). The literature does not record any studies elucidating the rice starch-phenolic compounds interaction during germination process, but some authors has observed interactions between starch-phenolic compounds in wheat and maize (Chen et al., 2020; He et al., 2019). Thus, this review aims to emphasize the importance of nutritional and technological aspects that could affect the physiological effects of germinated rice in order to shown some new insights in this field.

2. Data search strategy

The studies that were included on this narrative review were found on the Web of Science and PubMed databases as well as organizational sites with publication date from January of 1992 to March of 2021. The searched keywords included "germination", "rice", "starch", "phenolic compounds", "ferulic acid", "GABA", "germination process", "sprouted grains", "products", "health benefits", "cancer" or a combination of them with the boolean operator "and". Products made with germinated rice were found on food and beverage sales websites until December 2021 (Table 3).

3. Germination process

Germination is a very simple, inexpensive, environmentally-friendly and biologically safe process induced by the action of enzymes, external stimuli and internal phytohormones. It is a post-harvest rice processing method widely used in order to be naturally fortified with minerals (Fe, Zn, Ca, Se, I), vitamins and to enhance the levels of γ-aminobutyric acid (GABA) and other bioactive components such as γ-oryzanol and phenolic compounds (Saha & Roy, 2020).

During this process, some seed tissue reserves are degraded to be used during respiration and synthesis of new cellular constituents for embryo development (Nkhata et al., 2018). In addition to improve the nutritional quality of cereals, germination can also decrease phytic acid content, an antinutrient with negative effects on mineral bioavailability and protein digestibility (Albarracín et al., 2019).

Germination itself begins when grains are soaked in water and ends with the protrusion of the coleoptile, followed by the formation of the radicle. The dormant seed rapidly resumes its metabolic activity after imbibition, resulting in the partial degradation of the macromolecules stored in the endosperm. In turn, such degradation provides the energy and nutrients required by the embryo development. Imbibition of rice seeds is a three-phase process: (i) the first phase is characterized by rapid water uptake and DNA restoration (about 0 to 24 h after imbibition), followed by a plateau phase; (ii) the second phase is characterized by the synthesis of mitochondria and by the translation of the stored mRNA, and (iii) a final increase in water uptake, that occurs after the embryonic axes leave the surrounding structures (Fig. 1) (Damaris et al., 2019).

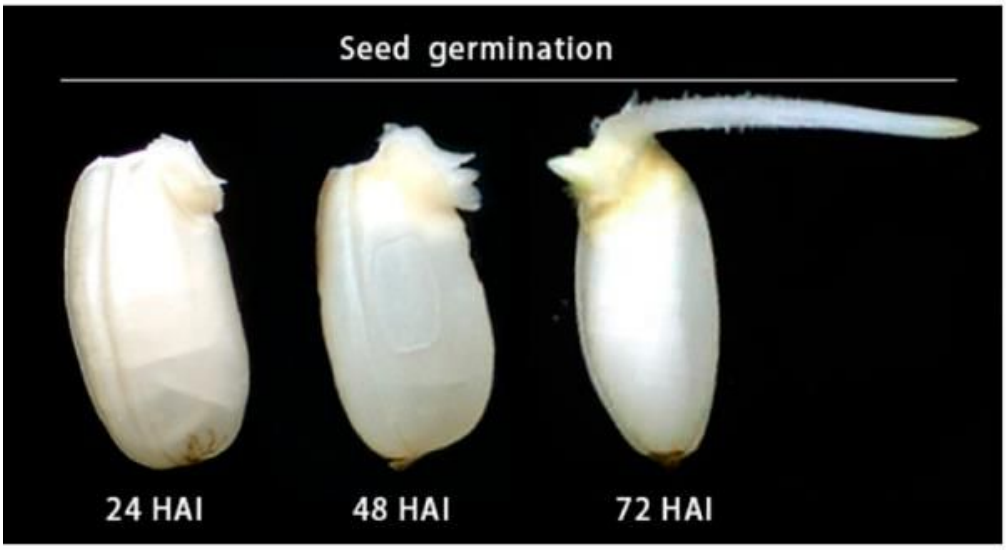


Fig. 1. Morphological changes occuring during germination of rice grain.

Where: HAI = Hours After Imbibition (Damaris et al., 2019).

There are many factors that, individually or in combination (e.g., cultivar, genetic variation, enviromental conditions, maceration and time, germination temperature and time, ionic strength and moisture content), affect rice germination (Table 1), interfer in the increase of phenolic compounds and in the synthesis of other compounds, for example GABA (Hussain et al., 115 2020).

Table 1. Processing conditions for rice grain germination.

Cultivar	Germination methods		Treatment used	Reference
	Soaking	*Germination*		
Koshihikari	35 °C, 3 h	35°C, 21h	Gaseous treatment in soaking	Komatsuzaki et al. (2007)**
Jing 305 Guichao 2	25-35 °C, 24 h	20-40°C, 48h	Acidic soaking conditions, adition exogenous L-glutamic acid and gibberellin in soaking water	Zhang et al. (2014)**
Ilpum	30 °C, 24 h	30 °C, 48h	Heat-moisture	Cho & Lim (2018)
Gangteyou 37	26-28 °C, 12 h	28°C, 72h	Ultrasound application on soaked rice (12h) and germinated rice (66h) for 5 min.	Ding et al. (2018)
Brown rice	-	28°C, 48h	Electrolyzed oxidizing water in soaking	Zhang et al. (2018)
Hashemy	25ºC, 48h	30ºC, 48h	Low-pressure plasma	Zargarchi & Saremnezhad (2019)
Dongnong 429	30°C, 12h	15 -40°C, 40h	Cyclic celulase	Zhang et al. (2019)
6 cultivars*	25–28°C, 96h	-	Cold plasma	Yodpitak et al. (2019)
Pampeira	40°C	30°C, 36h	Abiotic stress	Nascimento et al. (2020)
Suijing 18	-	30°C, 40h	Autoclaving	Ren et al. (2020)
HeituXZ	-	37°C, 32h	High-intensity ultrasound (HIU) stress to pre-germination	Xia et al. (2020)

* In this study six rice cultivars were used (Sanpatong 1, Khao Jow Hawm Phitsanulok 1, Khao Dawk Mali 105, Niaw Sanpatong, Rice Department 6 and Luem Pua).

** Most cited articles in the literature. 420 citations and 108 respectively.

4. Starch modifications during rice germination

Starch structure consists of two types of molecules formed by branched amylopectin and linear and slightly branched amylose. The building block of both molecules is an α-D-glucopyranose residue, forming α-1,4-glucosidic bonds in linear structure of amylose and

additional α-1,6-glycosydic branches in amylopectin molecules (Fredriksson et al., 1998; Takeda et al., 1990). Starch content in rice grains ranges from 75 to 80% (Juliano, 1993). Although the different types of grains (long, medium and short) have different starch levels that affect their technological properties, amylose content is the main component responsible for the different functionalities observed among rice varieties. Basic types of grains are divided into common and waxy rices. The amylose content of waxy rice ranges from 0 to 2%, while the amylose content of common or regular rice varieties is classified as low (9–20%), medium (20–25%) or high (>25%). The texture of cooked waxy rice starch tends to be stickier, and paste viscosity readings shown lower peak and setback viscosities than in common rice starch (J. N. BeMiller & Whistler, 2009).

Starch in non-germinated material has insoluble and semi-crystalline granules composed of amylose and amylopectin chains (Wang & Copeland, 2013). The degradation of starch molecular chain is the main source of energy used in the germination process. Complete hydrolysis of starch occurs by the combined action of α-amylase, β-amylase, starch debranching enzyme and α-glucosidase. The action of α-amylase reaches maximum peak after 48 h of germination and shows an important role in the modification of starch granules in germinated rice (Damaris et al., 2019).

Many studies have reported that several factors influence enzymatic activation, such as temperature, steeping time, and variety/cultivar. At high temperatures (35 °C), enzymatic activation is faster, and in most cereals, enzymatic activation reaches its peak on the 4th day after germination begins. The enzyme activity occurs differently in the grain tissues, and indeed the α-amylase is mainly found in the aleurone and scutellum whereas it is present in the pericarp and in the endosperm of immature grains (Guzmán-Ortiz et al., 2019).

During rice germination, several changes occur in the starch structure **(Table 2)**. Hydrolysis of amylose and amylopectin occurs by cleaving glycosidic linkages (Kalita et al., 2018). The activated α-amylase and debranching enzymes that convert starch into small molecules, play a critical role in starch hydrolysis and also the ratio of amylose and amylopectin content during germination. Higher degradation of starch may be attributed to the presence of higher amylose content, since rice varieties with higher level of amylose are more rapidly degraded by hydrolytic enzymes than by waxy varieties (Kalita et al., 2017).

Table 2. Modifications in rice starch during germination processes.

Rice cultivars	Processing conditions	Main conclusions	Reference
Two commercial varieties of long grain de-hulled rice (Oryza sativa L.)	– Soaked in distilled water at 26 ± 2°C for 12 h; – Germination at 28 ± 2°C with moisture (>95%) supplied by an ultrasonic humidifier for 8, 24, and 36h	– Ultrasonication enhanced starch hydrolysis and decreased falling number and viscosities values; – Different responses of starch hydrolysis after germination can be caused by the starch content of each cultivar.	Ding, Hou, et al. (2018b)
Chokuwa (low amylose rice) Aijong (normal amylose rice)	– Soaked in distilled water at 24h at 30°C and 35°C for 5 days	– The germination time had a significant effect on pasting properties as they decreased with germination time. – Thermal behavior germinated rice was affected by germination time.	Kalita et al. (2018)
Paddy rice (PR 123)	– Soaked in distilled water at 25ºC for 10 h; – Germination for 12, 24, 36, and 48h at temperatures of 25, 30, and 35C, in an incubator (85% relative humidity)	– During germination, hydrolytic enzymes act on starch, reducing its concentration and resulting in higher total and reducing sugar amounts.	Singh et al. (2018)
Chuchung	– Soaked in distilled water in at 25°C for 24h; – Germination at 25ºC for 48h	– Increased α-amylase and β-amylase activities occurred in the coarse fraction of the jet-milled Germinated Brow Rice (GBR) flour; – Compared with the hammer-milled flour, jet milling increased *in vitro* starch hydrolysis.	Lee et al. (2019)
Yongyou 15	– Soaked in distilled water for 12h; – Sprouted for 48h at 30°C	– Germinated rice demonstrated changes of microstructure, and distribution of molecular water may be attributed to softness; – Degradation of starch and protein can result in evenly distribution of molecular water in rice kernel during germination treatment	Hu et al. (2019)

Table 2. Modifications in rice starch during germination processes. (Continuation)

Rice cultivars	Processing conditions	Main conclusions	Reference
Japonica hybrid rice, "Chunyou 84"	– Soaked in water for 30 min at 30°C; – Germination for two periods (5 and 9 h) at 37°C; – High pressure treatment after germination (150 MPa/min and the depressurization time was less than 10s)	– Values of setback and breakdown pasting properties decreased after germination and high-pressure treatment; – Enzymes were activated for starch hydrolysis by breaking connection points and making them smaller and more dispersed; – The amount of connecting parts decreased with increased germination time	Wang et al. (2020)
Rough rice of a long-grain cultivar (Roy J)	– Aerobic germination, grains were placed on top of two layers of cotton cloth with 700 mL of deionized water and water was added every 6 to 12h; – Anaerobic germination, steeped rice was placed in a stainless-steel tray with 2,000 mL of deionized water, and water was changed every 6 to 12h. The trays were placed in an incubator at 30°C for 2 or 4 days.	– Bread prepared from 4-day aerobic GBR showed less hardness and starch retrogradation (66 to 90%).	Wunthunyarat et al. (2020)
HomChaiya	– Germination for three periods (3, 5 and 7 days) and three temperatures (40, 50 and 60°C).	– Total starch decreased and reducing sugar increased with prolonged germination of rice; – Starch granules are more vulnerable to enzymatic attack upon extended germination time and at higher temperatures.	Lekjing & Venkatachalam (2020)
Waxy brown rice grain	– Germinated in an incubation chamber for 48 h with 80% humidity at different temperature parameters (25, 30 and 35 °C).	– Germination altered structures and pasting properties of starch in brown rice; – Germinated rice starches (especially at high germination temperature) displayed pits and pores on granular surface, decreased particle size, lessened amounts of crystallites and degree of molecular ordered structure	Wang et al. (2020)

Table 2. Modifications in rice starch during germination processes. (Continuation)

Rice cultivars	– Processing conditions	– Main conclusions	Reference
Glutinous brown rice	– Soaking in distilled water (pH 7, grain to water ratio 1:2 w/v) at 25 °C for 12 h – Germinated at a constant temperature for 72 h at 35 °C and 65% humidity.	– The effect of germination on the distribution of amylopectin length resulted in a decrease in relative crystallinity, gelatinization temperature, gelatinization enthalpy and pasting viscosities;	He et al. (2020)
PR-123	– Steeped for 12 h at 25 °C – Germinated for 24, 48 and 72 h at 25 °C.	– Germination significantly decreased total starch content and caused depolymerization of starch molecules; – Continuous reduction of viscosity occurred as germination progressed. – Germinated flours accelerated molecular interactions between starch and proteins and decreased starch hydrolysis; – Germination induced changes in starch and protein and infuenced the structural and functional properties of cereal flours.	Kaur & Gill (2020)

Changes in the crystalline pattern after germination indicate that there is a lack of the ordered structure of the amylopectin, resulting in an ordered base in the organization of amylose. During the malting process, generally there is no enzymatic action in V- and B-type starch that could modify the starch diffraction pattern (Claver et al., 2010). Once germination is one of the malting steps, it is relevant to study the diffraction pattern of starch in this process.

As X-diffraction pattern changes, modification on starch microstructure occurs simultaneously. The non-germinated rice starch granule is surrounded by well-defined protein bodies and incorporated into a matrix. This structure is destroyed during germination, especially in prolonged germinations. Up to two days of germination, starch granules become polyhedral and irregular, and they remain intact or slightly modified. After three days of germination, protein bodies begin to break down due to enzymatic action, and pits and holes can be seen on the surface of some starch granules indicating the formation of internal channels. Starch granules lose their smooth surface, becoming rougher and slightly corroded. After four days of germination, this structure is seriously damaged. Protein bodies are broken, and microstructures are fragmented. This fragmentation of the starch granules increases the area of the contact surface, making it available for chemical and enzymatic reactions (Wu et al., 2013).

In fact, there is a correlation between the distribution and frequency of the pores with the enzymatic digestion of the starch granules after germination. The openings affect the action pattern of amylolytic enzymes. Pores can be formed in four different ways: (i) they are dried in the kernel or after isolation; (ii) they are produced by *in situ* amylases or by amylases produced during wet milling; (iii) they are artifacts of preparation techniques; (iv) they are a natural feature of granule structure (Fannon et al., 1992). Enzymatic patterns carried out on the starch structure include pin-holes, sponge-like erosion, numerous medium-sized holes, distinct *loci* leading to single holes in individual granules, and surface erosion. Enzymes that are activated during germination penetrate into the starch granule, resulting in degraded granules. Enzymes can corrode the surface of the starch granule (exo-corrosion) and/or act along the channels from selected points on the surface towards the center of the granule (endo-corrosion), by which the hydrolysis process begins (Claver et al., 2010).

Germination also reduces the double helical ordered structure in external regions of the starch granules, causing a decrease in crystallinity, gelatinization temperatures, and enthalpies (Pinkaew et al., 2017; Zhang et al., 2020). The alternations in hierarchical structures are impacted by α-amylase activities in germinated rice starches. Especially at high germination temperatures (around 35°C), a high alpha-amylase activity results in the appearance of pits and pores on the granular surface. This causes changes in the surface of starch granules, which become gradually rough; therefore the pores in the granules are enlarged leading to a reduction in particle size, in the amounts of crystallites and in the degree of molecular ordered structure. As a result, smaller and more heterogeneous particles are formed (Wang et al., 2020).

5. Interaction between phenolic compounds and starch during germination

Consumption of phenolic acids associated with healthy lifestyle can help to reduce the risk of various diseases such as stroke, type 2 diabetes mellitus, cancer and cardiovascular diseases (Rashmi & Negi, 2020). In addition to improving health, phenolic compounds can contribute to sensorial characteristics of foods, e.g., enhancing taste and color perceptions. The bioavailability will depend if these compounds are present in free or in conjugated form in food matrices as consequence of the applied food process (Silva et al., 2019).

During seed germination, the metabolism of phenolic compounds occurs as following: (i) synthesis of natural phenolic compounds starts from glucose or aromatic amino acids (Herrmann & Weaver, 1999); (ii) macromolecular nutrients are decomposed by enzymes action (Paucar-Menacho et al., 2017); and (iii) phenolic compounds are consumed as they scavenge free radicals or work as intermediates of signal compounds (Minwei et al., 2020).

Although numerous articles in the literature have reported that there is an increase in phenolic compounds during rice germination, there is a lack of information concerning the understanding of metabolic dynamic during this process. Up to date, there is no record of studies relating the interaction between phenolic compounds and starch during germination process. Once germination is a very complex process that involves several procedures **(Table 1)**, the content of these compounds is expected to be affected regarding the reactive nature

of theses molecules as presented in some articles involving simple addition of polyphenols and hydrothermal treatment.

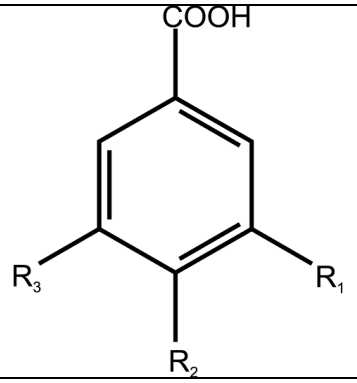
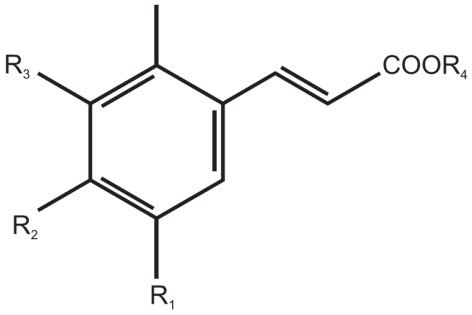
Molecular Structure	R_1	R_2	R_3	R_4	Compound
	H	OH	OH	-	Protocatechuic acid
	H	OH	H	-	Hydroxybenzoic acid
	H	OH	OCH_3	-	Vanillic acid
	OCH_3	OH	OCH_3	-	Syringic acid
	OH	OH	OH	H	Caffeic acid
	H	OH	H	H	p-Coumaric acid
	OCH_3	OH	H	H	Ferulic acid
	OCH_3	OH	OCH_3	H	Sinapinic acid
	OH	OH	H	quinate	Chlorogenic acid

Fig. 2. Chemical structures of major biofunctional components in brown rice and germinated brown rice adapted from (Cho & Lim, 2016).

Phenolic compounds can interact with amylose and amylopectin in different ways (Li et al., 2018), in order to form a short-range order structure of crystalline regions in resistant starch (Han et al., 2020). This happens when amylose interacts by covalent bonds with small molecules and forms inclusion complexes in the shape of left-handed helices named V-type amylose (Obiro et al., 2012). In other words, part of polyphenols could be embedded into the starch structure. The formation of these interactions depends on the chemical structure of starch, the concentration and type of phenolic compound, and food processing (F. Zhu, 2015). Besides, other authors (Han et al., 2020; Karunaratne & Zhu, 2016) stated that polyphenol solubility plays important role in polyphenol-starch interactions. These interactions can affect the physical, rheological, chemical and nutritional properties of

matrix formed between starches and phenolic compounds from different sources. The addition of ferulic acid into heated and sheared starch in excess of water can reduce the hardness, adhesiveness, cohesiveness, and springiness of the formed gel, however, enzyme susceptibility of granular starch by α-amylase was not affected (Karunaratne & Zhu, 2016).

Changes in the heat stability measured by DSC were observed after addition of caffeic acid to rice starch at various mass ratios. These changes was corroborated by ^{1}H NMR data that supported the attachtment of caffeic acid molecules in the starch-water matrix via hydrogen bonding rather than inclusion complex interaction (Igoumenidis et al., 2018). Ferruzzi et al. (2020) incorporated phenolic compounds (gallic acid, catechin and epigallocatechin gallate) in porridge matrices model. They reported less degradation of phenolics in the presence of starch than in the presence of starch/protein that was attributed to the ability of phenolic compounds to form V-type starch inclusion complexes.

6. Food application of germinated rice

Sprouted rice can already be found in several countries and it is used in different applications in products such as rice grains, flours, snacks, dairy products, pasta, drinks and baby foods **(Table 3).** The demand for this type of product is very common in Asian countries, because rice is part of the population's eating habits as the consumers prefer more natural, healthier and pollution-free foods. In this scenario, sprouted grains emerge as an interesting alternative to develop new products for new market niches (J. Ding & Feng, 2019) especially the gluten-free market. Germinated rice has a neutral flavor compared with other cereals, and can be incorporated into different preparations, improving their nutritional quality while providing a good sensory experience (Finnie et al., 2019).

Table 3. Commercially available germinated rice products.

Category	Brand	Product	Country
Rice	Ottogi	Cooked germinated brown rice	Japan
	Koshihikari	Sprouted GABA brown rice	
	Eden	Organic sprouted mochi	
	Asahi	GABA Brown Rice Diet Risotto	
	Cheil Jedang	Cooked Sprouted Brown Rice Cooked budding sprouted brown rice	South Korea
	MRM BSCM foods	Sprouted black rice powder Germinated Brown Rice	Thailand
	Minvita	Organic sprouted GABA black rice	USA
	To Your Health	Sprouted brown rice	
	Macro Vegetarian	Sprouted brown rice risotto	
	Lundberg	Vegetable fried germinated rice	
Flour and crispy	NAGO	Germinated brown rice crispy rice crust	South Korea
	Salud viva	Germinated rice flour	Spain
	To Your Health	Organic Sprouted Brown Rice Flour	USA
	Azure		
	Blue Mountain		
	One Degree Organic Foods		
	Pureliving		
	One Degree Organic Foods	Sprouted brown rice crisps	
	Now Sports	Sprouted brown rice protein	
Drink	Fabala	Sprouted brown rice sake	Japan
	Dutch Mill	UHT soy milk with Japanese rice	Thailand
	V-Fit	Germinated Brown Rice Milk	
	Thaiflowin	Ready-to-Drink Germinated Brown Rice	
	Nestlé	Germinated Riceberry Instant Cereal	
	Botanica Kao Kum	Instant cereal drink powder with purple rice, brown rice, corn and wheat	

	Doi Kham	Germinated Brown Hom Nin Rice with cereal	
	Dream	Sprouted rice drink	USA

Table 3. Commercially available germinated rice products. (Continuation)

Category	Brand	Product	Country
Pasta	Akitakomachi	Germinated Brown Rice Pasta	Japan
	Lohas	Organic GABA brown rice ramen	Taiwan
	Supreme Thai	Brown rice GABA pasta	Thailand
Snack	DHC	Germinated brown rice chocolate bar	Japan
	Apple Monkey	Organic germinated brown rice puff	USA
	Little Quacker	Organic brown sprouted rice puff	
Baby Food	Love Earth	Organic sprouted brown rice baby meal	Malaysia
	Xongdur	Baby sprouted brown rice banana & pumpkin Instant Germinated Brown Organic Rice	Thailand
Dairy	Mozzarisella	Sprouted brown rice yogurt Sprouted brown rice vegan cheese Sprout brown rice mascarpone Sprouted brown rice with Nori and Ulva	Italy

Note: Searches were carried out on websites until 2021, December.

The combination of germinated rice flour and other flours result in important nutritional/technological gains besides competitive market advantages. Mathilde et al. (2020) reported that, as a result of its high amylase content, germinated rice flour was able to hydrolyze the large starch molecules of cassava flour in smaller molecules as maltose and dextrins.

Both dairy products and nondairy substitutes can be formulated with germinated rice flour resulting in a good sensory acceptance with high GABA content (Cáceres et al., 2019) consisting in natural and healthy choice for vegetarians, vegans and consumers interested in low-carbon diets contributing to achieve some of the 17 sustainable development goals (SDGs) as a part of 2030 Agenda declared by United Nations (United Nations, 2015). Germinated rice can be used in value-added rice-based beverages mainly to improve solubilization that can be attributed due to natural enzymatic hydrolysis of starch. After activation of endogenous enzyme systems during germination, germinated rice drinks showed significantly fewer processing losses using wet milling when compared to drinks produced from non-germinated grains (Beaulieu et al., 2020).

As discussed earlier, soaking and germination processes increase antioxidant capacity and phenolic content along with the increasing of protein digestibility compared to raw material (20% higher) (Albarracín et al., 2019). With the increase in germination time, pasting viscosity parameters decreased. Thus, germinated grains can be used to formulate different products, such as beverages, and create several new food formulas for celiac disease patients (Kaur & Gill, 2020).

Although gluten-free bread still remains as major technological challenge, germination is an interesting tool able to turn hard into soft texture of rice-based cookies. Compared to bread, gluten plays a minor role in biscuits allowing the use of a wide variety of flours. Thus, malting, fermentation and germination are the processes that can improve the overall quality of biscuits without affecting negatively their technological characteristics. In addition to the technological aspect, germination also provides greater total phenolic content, DPPH radical scavenging activity and total dietary fiber content, improving the nutritional characteristics of cookies (Di Cairano et al., 2018). Replacing rice flour with germinated rice-potato starch blend lead to better quality cookies in terms of physical properties such as textural and color properties (Bolarinwa et al., 2018).

7. Health impacts of the consumption of germinated rice

7.1. Starch digestibility and glycaemic control

During the early stages of germination, starch is hydrolyzed by α-amylase releasing simple sugars, especially glucose and fructose, that will be used in order to synthetize cell structural polymers, such as cellulose, hemicellulose, and lignin (Nkhata et al., 2018). Depletion of starch by α-amylase associated with structural polymers (cellulose, hemicellulose and lignin) produced by rice cells, increases the resistant starch and dietary fiber contents, promoting a desirable reduction of the glycemic index (GI) (Kongkachuichai et al., 2020; Nkhata et al., 2018).

Digesting starch process begins in the mouth caused by the presence of oral alpha-amylase and mechanical compression during mastication. After swallowing, the predigested starch goes to the stomach, where it is exposed to acidic pH and goes to the small intestine. In the duodenum, pancreatic alpha-amylase and alpha-glucosity will continue the hydrolysis of starch, releasing simple sugars in the intestine lumen (L. Sun & Miao, 2019). The starch digestibillity is severe affected by intrinsic food characteristics as starch granule organization (Toutounji et al., 2019), starch-lipid or starch-protein interactions (Wang et al., 2020), starch chain size (Zhang et al., 2008), amylose-amylopectin ratio (Toutounji et al., 2019), starch-polyphenols interaction (L. Sun & Miao, 2019). Concerning extrinsic factors, it is also affected by storage time, starch gelatinization, starch retrogradation (Toutounji et al., 2019), and enzymatic activity on digestion (X. Lu et al., 2021).

There are two common structural characteristics that modify the digestibillity in a slowly digestible starch (SDS-State): (i) starch with a higher proportion of short chains known as A-type starch and (ii) starch with higher proportion of long chains as known as B-type starch (Dhital et al., 2017; Zhang et al., 2008). Guo (2018) demonstrated that waxy maze amylopectin starch composed by short-chain starch with high branching density, had lower hydrolysis rate which implies in lower absorption and higher formation of resistant starch. Regarding B-type starch that is found in potatoes, the lenght of the amylopectin is short, but the amylose chains are longer and can extend into multiple crystals providing stabilization for the starch granule. In both cases, the starch structure is a barrier for the enzymatic activity and favors a slower digestion, representing a physical entity proposed by Zhang et al. (2008).

Therefore, starch microstructure alteration tends to be an effective alternative to delay digestibility (Tian et al., 2018) and to develop rice cultivars with low GI with acceptable cooking quality, thus providing opportunities in mitigating the global rise in type II diabetes and related to non-communicable diseases (Guzman et al., 2017). These authors reported that cooked rice grain digestibility is mainly due to ratio and structure of amylose/amylopectin chains besides interaction between starch with proteins, lipids, cell wall polysaccharides and isoflavonoid α-amylase inhibitors during germination. In fact, both amylose and resistant starch (RS) affect the rate of digestion during the course of germination, in which high levels of amylose and RS lead to lower GI value. In contrast, rice varieties with high GI have the least amylose and RS levels showing the fastest starch hydrolysis rate due to their higher short-chain amylopectin content, that is the most preferred substrate in germinating seedlings followed by long-chain amylose.

There are scarce data in the literature reporting *in vivo* GI of germinated rice. Kongkachuichai et al. (2020) investigated the effect of germination, parboiling and polishing on the total polyphenols, ferulic acid and GABA levels of seven traditional varieties of Thai rice. Germination followed by parboiling had less effects on the bioactive compounds, increased dietary fiber contents and produced low-medium GI rice, which could be beneficial for health promotion, especially in pre-diabetic subjects.

7.2. Gluten-related disorders and GABAergic effects

Rice is a gluten-free cereal with lysine as a limiting amino acid. For this reason, it is frequently studied as a raw material for the production of foods aimed to the celiac patients or those with wheat-related disorders (Bolarinwa et al., 2018; Nitisuk et al., 2019). During the germination process, protein and non-protein amino acids are produced and GABA emerge as an interesting bioactive compound for health of people with these pathologies. It has been demonstrated that GABA consumption can offer different health benefits, e.g., immune system modulation (Wu et al., 2017), motor learning and antidepressant effects (Teng et al., 2017). Moreover, it may be related to a positive prognostic in breast cancer (Brzozowska et al., 2017).

7.3. Anti-inflammatory activity

Germination process promotes the activation of lipases in different parts of rice grain that may favor the formation of linoleic acid over saturated fatty acids such as palmitic acid (Guzmán-Ortiz et al., 2019). Linoleic acid is a polyunsaturated fatty acid, also known as Omega 6 (ω-6), which has been related to an increase of inflammatory response. Therefore, studies have demonstrated that the dietary recommendations of this fat acid (2-10% of total energy intake) are sufficient to achieve the recommended daily intake (RDI) and provide cardiovascular benefits (Jandacek, 2017).

Vitamin E is a major lipid-soluble antioxidant against cell oxidative metabolism and its total content is also increased after the germination process. As the cellular organization of rice changes during germination, vitamin E homologues can be released from the cell membranes of rice bran (consisting of pericarp, seed coat, nucleus and aleurone) (Idowu et al., 2020) and become available in the form of tocopherol and tocotrienol homologues, especially α and γ-tocopherols which are mostly present on rice germ (Phuong et al., 2021). α-tocopherol is a lipophilic vitamin that can be found on cell membranes and acts as a powerful antioxidant against lipid peroxidation and free radical scavenging, thus maintaining cellular health (Bruinen et al., 2018).

Germination is also related to the increase of the concentration of γ-oryzanol, ferulic acid and polyphenols (Kongkachuichai et al., 2020). Upon consumption, γ-oryzanol is mostly converted into ferulic acid, and they are both related to various health effects (Kokumai et al., 2019), such as anti-cancer properties (Panyathep & Chewonarin, 2020), improvement of cognitive skills (Mhillaj et al., 2018) and neuronal protection against inflammation (Mastinu et al., 2019). Conversely, polyphenols may act on chronic diseases in many ways. They can act on the endothelium, improving the effectiveness of vessel smooth muscle function and reducing oxidative stress of vascular microenvironment. They can also act on glucose homeostasis, providing antioxidant, antiproliferative and apoptotic effects (Costa et al., 2017). Polyphenols may be able to modulate starch digestion and glycaemic level (Sun & Miao, 2019); to regulate cardiovascular parameters, serum lipids profile, blood pressure and oxidative stress (Bahramsoltani et al., 2019). Recently, polyphenols are related to the

reduction of symptoms of autism spectrum disorders by modulating key pro-oxidative pathways on the brain (Castelli et al., 2017; Serra et al., 2019).

7.4. Oxidative stress-related pathogenesis and carcinogenesis

Table 4 summarizes some cellular, animal assays, and clinical trials involving germinated rice and several health disorders. The most cited benefits were the adjustment of metabolic syndrome biomarkers, such as serum lipid adjustment (Nhung et al., 2016), hyperglycaemia control (Hao et al., 2019), as well as the reduction of hepatic lipid accumulation (Lee et al., 2019). Other effects such as mood modulation (Felice et al., 2020), neuroprotective (Oo et al., 2020) and motor learning effects (Chompoopong et al., 2016) have also been reported. In cellular assays, most of the studies found that germinated samples were able to act during the cellular cycle in different metabolic paths simulating the disease models. In this way, Sun et al. (2020) suggested that germinated rice extract was able to upregulate apoptosis in their oxidative stress model whereas Petchdee et al. (Petchdee et al., 2020) found that germinated rice extract prevented apoptosis in cardyomioblast myocardial schemia model. These data suggest that cells are able to use germinated rice bioactives efficiently according to the tissue in order to promote health. In regarding to the animal models, the rice consumption as food or as in extract form could reduce body weight, body fat, seric glucose, and seric insulin levels (Chung et al., 2019; Lee et al., 2019) as well as exhibit a protective effect in cancer models (Li et al., 2019). Oo et al., (2020) have also demonstrated that chronic consumption (at least 5 weeks of continuous consumption) was able to positively affect brain health. Finally, clinical trial studies have shown a reduction of biomarkers of dislipidaemya, diabetes and atherosclerosis, body weight, and mood disturbance.

Table 4. Cellular, animal and clinical trials on the effect of germinated rice or derived products on human health.

Cellular assays

Sample	Cell type	Intervention	Main findings	Reference
Germinated brown rice extract	Rat basophilic leukemia cells (RBL-2H3)	Evaluation of the anti-allergic activity of a butanolic extract in comparison with *Phellinus linteus* extract	Cells treated with germinated brown rice extract presented lesser degranulation, calcium influx and reduced the TNFα and IL-4 mRNA expression in a dose dependent manner.	Kwon & Park (2019)
Germinated brown rice extract	Rat cardiomyoblasts (H9c2 cells) myocardial ischemia reperfusion injury model	Different concentrations of extract as pretreatment for ischemic buffer.	Germinated brown rice was able to prevent apoptosis owing to suppression of the production of caspase 3 and p38 MAPK.	Petchdee et al. (2020)
Germinated brown rice extract	H9c2 cardiomyocytes as ischemic-reperfusion insury model	Incubation with germinated brown rice extract for 24 h and simulated ischemic/reperfusion for 40 min	Administration of the extract significantly reduced the cell death and apoptosis. In addition, the extract was able to maintain the mitochondrial membrane potential and respiration. However, the exact mechanism is still unknown.	Demeekul et al. (2021a)
Germinated brown rice extract	Isolated porcine cardiomyocites for ischemic-reperfusion injury model	Cells were divided in cultured 3 groups: germinated brown rice pretreated, cardioplegic solution or inchemic-reperfusion solution	Germinated brown rice group presented improved cell bioavailability agains ischemic-reperfusion near the non-injured group.	Demeekul et al. (2021b)

Animal models

Sample	Animal model	Intervention	Main findings	Reference
Germinated brown rice	24 female Sprague-Dawley rats and 6 male offsprings per intervention group	Normal diet, high fat diet, high fat diet + 50% germinated brown rice diet, high fat diet + high dose GABA or high fat diet + low dose GABA.	Intrauterine exposure to germinated brown rice or GABA triggered effects that favored energy balance and body weight homeostasis as well as improved insulin sensibility and downregulated pro-inflammatory biomarkers.	Adamu et al. (2017)
Germinated brown rice	38 male 5-week old Sprague Dawley rats as	Control group, depression induced w/ no intervention, depression	The ingestion of germinated brown rice protected the animals from depression-induced changes on sperm quality and	Roboon et al. (2017)

	induced depression model	induced + antidepressant, depression induced + GABA or depression induced + germinated rice.	morphology of testicular structure and number of androgen receptors.	
Germinated pigmented rice "Superjami"	30 female Sprague Dawley rats menopausal model (ovariectomized, 3 months old)	8 weeks with normal diet, or normal diet with 20% of germinated or non-germinated rice flour.	Reduction of body weight gain, body fat, glucose and insulin levels during the experiment. In addition, the animals exhibited higher antioxidant enzymes activity and bone metabolism preservation effects.	Chung et al. (2019)
Pre-germinated brown rice extract	40 6-week old mice C57BL/6 as induced metabolic syndrome model	18 weeks with normal diet, high fat diet (HFD) or HFD with oral extract supplementation.	The extract, which contains GABA, γ-oryzanol, flavonoids and anthocianidins was proved to ameliorate metabolic syndrome symptoms such as hyperglycaemia owing to improvement of cellular mechanisms.	Hao et al. (2019)
Germinated brown rice extract	6-week old female BALB/c mices housed in pathogen free conditions allergic model	Oral administration of butanolic germinated brown rice extract (25 mg/kg) or cetirizine (20 mg/kg)	Reduced allergic reactions as extravasion of Evans blue dye, infiltration of immune cells in animals with passive cutaneous anaphylaxis and ear swelling.	Kwon & Park (2019)
Rough rice hydric extract	5-week old C57BLKS/J-db/db mice diabetic model	8 weeks with normal diet or water substitution by rice extract.	Diabetic animals reduced food intake, reflecting an inhibition of hepatic fat accumulation. In addition, there may be a protective effect over β-pancreatic cells, with a serum glycaemic reduction.	Lee et al. (2019)
Fermented germinated brown rice	66 male F344 rats (3 or 5 weeks old) colorectal carcinogenesis model	10 weeks with normal diet, 10% dry matter of germinated rice and 90% of normal diet or different concentrations of fermented germinated rice.	The germinated rice and fermented germinated rice diets exhibited a protective effect against induced colorectal carcinogenesis, possibly owing to activation of apoptotic mechanisms.	Li et al. (2019)
Germinated brown rice	66 male F344 rats as colorectal carcinogenesis model	10 weeks of normal diet, 10% of germinated brown rice diet alone or with *L. acidophilus* and/or *B. animalis* subsp. *lactis*.	Consumption of germinated rice alone or in combination with the probiotic cultures was able to promote a protective effect over the colon cells owing to regulation of antioxidative and apoptosis mechanisms.	Lin et al. (2019)

Germinated brown rice	Winstar rats in a Hypercholesterolemic model.	Four groups: normocholesterolemic, high cholesterol-fed (HC), HC + white rice (WR), HC + germinated brown rice (GBR)	In comparison with WR group, GBR group presented a significative reduction of hypercholesterolemia biomarkers as well as increased the fecal excretion of cholesterol and increased the concentration of high density lipoprotein in blood, which is desirable.	Sarkar et al. (2019)
Germinated brown rice extract	8-week old male spontaneous hypertensive rats in type 2 diabettes model	Control group; intervention group: type 2 diabettes groups treated with 30 or 300 mg/kg of germinated brown rice extract	Improvement of hypertension and tachycardia. Reduction of hyperlipidemia and inflammation. The effects were probably due to an improvement of insulin and AMPK pathways in liver and muscles as well as a possible promotion of a reduction of heart hypertrophy biomarkers.	Liang et al. (2020)
Germinated brown rice	72 ICR male 5-week old mice vascular cognitive impairment model	5 weeks with normal diet, germinated brown rice gelatin or GABA gelatin.	The germinated brown rice was able to promote a neuroprotective effect on the animals inhibiting neuronal apoptosis. However, the mechanisms remain unclear.	Oo et al. (2020)
Germinated brown rice extract	18 New Zealand White rabbits myocardial ischemia reperfusion injury model	1 μg/kg/day of extract during 120 days.	Chronic intake of germinated brown rice could reduce the frequency of myocardial arrhythmias. In addition, animals presented reduced myocardial infarcted area after reperfusion.	Petchdee et al. (2020)
Germinated brown rice extract	6 pigs as model for cardiac open-heart surgical model	Two types of cardioplegic solutions were applied: 1) St. Thomas cardioplegic solution, and 2) St. Thomas cardioplegic solution + germinated brown rice (1 mg/kg)	Maintainance of the blood pressure and heart rate in germinated brown rice group. Germinated brown rice group also presented a reduction of sodium, potassium and lactase blood concentration suggesting a better cardioprotective potential.	Demeekul et al. (2021b)

Clinical trials

Sample	Population	Intervention	Main findings	Reference
Pre-germinated brown rice bran encapsulated extract	60 post-menopausal Vietnamese women (45–65 years)	300 mg of encapsulated extract daily or placebo for 6 months.	Improvement of atherosclerosis biomarkers such as serum lipids, reduction of body fat, and reduction of TNF-α associated with an increase of adiponectin.	Nhung et al. (2016)

GBR mix (germinated brown rice, salt and micronutrient)	124 orthopaedic and gastrointestinal surgical patients (45-65 years)	20 g of GBR mix in bouts of 3 meals (20 g x 3meals) 8 h before the surgery	Improvement of blood sugar, food and fluid intake post-surgery in the patients, reduction of inflammatory markers, length of hospital stay, nausea and vomiting.	Thacker et al. (2021)

8. Conclusions and outlooks

Rice is one of the most consumed cereal around the world. Although Asia is the largest producer and consumer worldwide, Latin America rises as an important producer driven mainly by plant-based market. On the other hand, the Covid-19 era accelerated the demand for natural products, no additives and artisanal practices, besides clean label requirements. In this scenario, germination process emerges as a simple and natural technology carried out since ancient times that promotes multiple changes to the rice delivering metabolites that impact both technological characteristics and human health. Among metabolites, phenolic compounds play an important role against some chronic diseases, especially in the regulation of blood glucose level. The interactions between phenolic compounds-starch that occur during rice germination and their impact on technological, nutritional, physical and sensory properties are neglected in the literature. Phenolic compounds can interact with amylose and amylopectin in different ways, in order to form a short-range order structure of crystalline regions in resistant starch, i.e., part of polyphenols is embedded into this starch structure. The formation of these interactions depends on the starch structure, the concentration and type of phenolic compound, the type of food processing, but nevertheless, polyphenol solubility seems to play a main role in polyphenol-starch interactions. Sprouted rice is especially found in Asian market in products that include predominantly rice grains, pasta, and drinks. Moreover, the plant-based market is a new trend of sudden growth, therefore, it is expected that germinated rice will be used as an ingredient in those formulations. Regarding the impact on health, germinated rice is mainly related to starch digestibility and glycaemia control. Structure of amylose/amylopectin chains besides their interactions with proteins, lipids, cell wall non-starch polyssacharides and isoflavonoid α-amylase inhibitors formed during germination tends to be an effective alternative to delay digestibility. Thus, the development of rice cultivars with low GI with acceptable cooking quality provides health benefits that could help people with type II diabetes, obesity and other glycaemic-related disorders manage their diets.

Declaration of interest

The authors have no relevant interests to declare.

CRediT authorship contribution statement

Maria Eugenia Araujo Silva Oliveira and Pedro Paulo Saldanha Coimbra: Writing, literature search, and drafted the manuscript. Melicia Cintia Galdeano, Carlos Wanderlei Piler de Carvalho and Cristina Yoshie Takeiti: Supervision and writing- review & editing. All authors read and approved the final manuscript.

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Chapter IV

Short germination and debranning affect bioactive compounds and pasting properties of rice genotypes

Maria Eugenia Araujo Silva Oliveira[1]*, Rosemar Antoniassi[2], Allan Eduardo Wilhelm[2], Manuela Cristina Pessanha de Araujo Santiago[2], Sidney Pacheco[2], Priscila Zaczuk Bassinello[3], José Manoel Colombari Filho[3], Carlos Wanderlei Piler de Carvalho[2], Cristina Yoshie Takeiti[1,2]

[1] Food and Nutrition Graduate Program, Nutrition School, Federal University of the State of Rio de Janeiro, UNIRIO. Av. Pasteur, 296, Urca, 22290-240, Rio de Janeiro, RJ, Brazil.
[2] Embrapa Agroindústria de Alimentos, Avenida das Américas, 29501, 23020-470, Rio de Janeiro, RJ, Brazil.
[3]Embrapa Arroz e Feijão, km 12 - Zona Rural, GO-462, 75375-000, Santo Antônio de Goiás-GO, Brazil
*Corresponding author: mariaeugenia@edu.unirio.br

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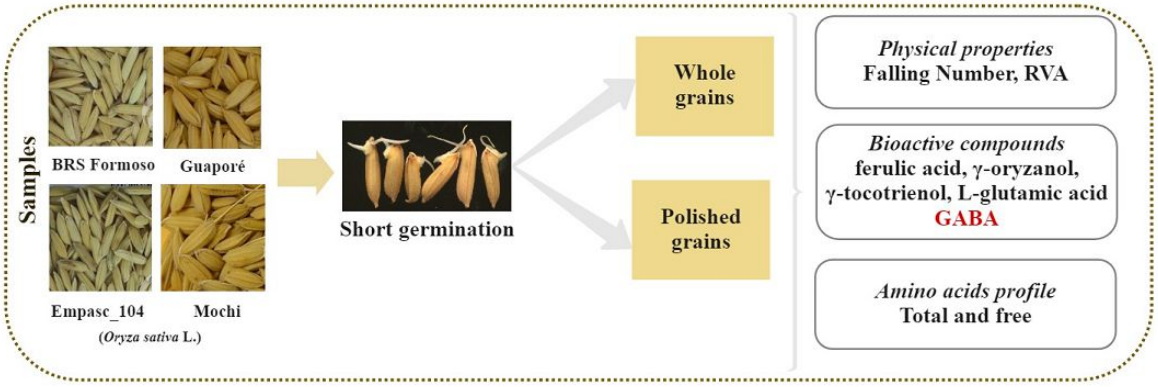


Highlights

- Short germination (< 24 h) did not increased the α-amylase activity
- High amylose content impacted pasting properties and GABA synthesis
- Polishing reduced of γ-oryzanol is more pronounced in non-germinated rice

ABSTRACT

Germination is a natural metabolic process widely used to modify sensory properties and nutritional aspects. Rice containing high amylose content has a larger germ and, consequently, could accumulate higher γ-aminobutyric acid (GABA) levels in this portion. This study aimed to evaluate the effect of short germination and debranning on α-amylase activity, pasting properties, phytochemicals, and amino acid profile of genotypes with distinct amylose content. The results of the falling number showed that *BRS Formoso* (high amylose) presented the highest value (>1000 s), indicating no activity of α-amylase and higher retrogradation (3793–3593 cP), whereas *Mochi* (waxy genotype) demonstrated the lowest retrogradation (maximum of 241 cP). Short germination increased L-glutamic acid and GABA contents in all genotypes, but debranning decreased these compounds. Short-time germination led to an expressive increase in umami amino acids, mainly in *BRS Guaporé* and *Mochi*, which also showed high levels of proline in both brown and polished grains.

1. Introduction

Rice (*Oryza sativa* L.) is a crucial global staple food. In the year of 2020 the world area harvested was 164,192,164 M tones and production was 756,743,722 M tones (FAOSTAT, 2020). From the nutritional point of view, brown rice contains more nutritional components in terms of ferulic acid, γ –oryzanol, and gamma-aminobutyric acid (GABA) than polished rice (white rice). However, the consumption is still not expressive due to its dark appearance, hard texture, and longer cooking time (Wu et al., 2011) but considering the nutritional value and potential health benefits, its consumption should be encouraged.

In addition, brown rice (BR) presented short shelf-life due to rancidity tendency that difficult commercialization, product development, and consumption of BR-based products (Saleh et al., 2019). Then, polished rice is still the largest form of consumption, especially in Asia and South America, representing the world's major consumer markets (van Dam, 2020).

Germination is a simple process that can be used for increase the nutritional quality and expand rice consumption. Rice is deficient in amino acids which expected to increase after germination (Kamjijam et al., 2020). The increment of total free amino acids, after germination, has been related to the degradation of protein by protease and also by the synthesis of new enzymes, which helps to liberate free amino acids (Moongngarm & Saetung, 2010).

Recently, researchers have shown an increased interest in germinated brown rice (GBR) due to the phenolic content, vitamin E, and GABA (Cho & Lim, 2016), besides several products (dairy, beverages, pasta, snacks, ready to eat, flour) made from GBR were developed especially in the Asian market.

Despite germination consisting of a simple process, the choice of a rice genotype is essential to achieve adequate technological properties, good cooking quality, and increments in bioactive compounds mainly GABA and phenolic acids. Rice that presents high amylose content has a larger germ and, consequently, accumulate higher GABA levels due to the storage of this portion in the grain (Chungcharoen et al., 2015b; Varanyanond et al., 2005).

Germination treatment seems to be a solution to this dilemma between nutrition and sensory qualities (Zhao et al., 2020). A moderate germination process ranging from one to three days may improve the flavor of brown rice by increasing phenolic compounds.

Nevertheless, a longer germination time produces an excess of aldehydes, phenolic compounds (sulfide-4-vinylguaiacol and 4-vinylphenol), and dimethyl sulfide that can affect the palatability and rice quality (F. Wu et al., 2011), impact energy efficiency, operating costs, and food safety.

There is a lack of information about physical properties, bioactive compounds, GABA, and amino acids profiles in short germination, especially considering polished rice, which is the most preferable choice of consumption. Therefore, the present study evaluates the impact of short germination associated with the debranning process on the physicochemical quality of rice genotypes with different amylose in to order to provide useful information for the development of germinated brown rice (GBR) and polished rice-based (PR) products.

2. Materials and methods

2.1. Material

Four rice (*Oryza sativa* L.) genotypes were selected from Active Germplasm Bank of *Embrapa Rice and Beans* (Santo Antônio de Goiás-GO, Brazil) belonging to two rice ecotypes: BRS Formoso (*O. sativa* subsp. *indica* (F), Guaporé (*O. sativa* subsp. *indica*) (G), Empasc_104 (*O. sativa* subsp. *indica*) (E) and Mochi (M) (*O. sativa* subsp. *japonica*). The materials were chosen according to the apparent amylose content (high, medium, and low contents) (Table S1) determined according to the method ISO 664 (ISO, 2007). All genotypes were multiplied in the 2018/2019 harvest using a flood-irrigated system in the farm experimental field of *Embrapa Rice and Beans* (6° 29' 8" S, 49° 18' 32" W). After harvest, the rice grains were naturally dried. .

2.2. Germination process

Germination was performed according to the methodology described by Zhang et al. (2014) with some modifications. The seeds (500 g) of paddy rice were soaked in deionized water (1 L) at pH 5.6 by adding L-glutamic acid (L-Glu) at 1.0 g L^{-1} (Sigma Aldrich, Ref. RES5063G-A701X, St. Louis, USA) and gibberellic acid (GA3) (Sigma Aldrich, Ref. G7645-5G, St. Louis, USA) at 0.25 mg L^{-1} for 4 h in a fan oven at 30 °C. After this step, the

grains were drained and allowed to germinate in a fermentation cabinet (National Mfg. Co., Lincoln, USA) at a controlled temperature of 35 °C and relative humidity of 95% for 24 h. The germinated paddy rice grains were dried in a circulated air oven at 50 ºC overnight, then husk and pericarp (10%) were removed with help of a rice polisher machine Suzuki (Santa Cruz do Rio Pardo-SP, Brazil) for 2 min and then ground in an M 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) fit with a 0.8 mm sieve aperture obtaining a flour that was stored in a freezer until further analyses.

2.3. Analysis of ferulic acid

The sample extraction was performed according to Pérez-Jiménez et al. (2008). Chromatographic analysis was performed according to Nascimento et al. (2017) with quantification by external standardization with *trans* ferulic acid (Ref.128708, Sigma Aldrich, St. Louis, USA) and identification through comparison of retention time and UV/VIS spectra in a Waters® Alliance model 2690/5 high-performance liquid chromatographic system (Waters Corporation, Milford, USA), with a Waters® diode array detector model 2996 (quantification at 325 nm) and Empower® software (2002) (Waters Corporation, Milford, USA), with two Thermo® BDS HYPERSIL C_{18} columns (Thermo Fisher Scientific, Waltham, USA) in series (50x4.6 mm, 2.4 µm; and 100x4.6 mm, 2.4 µm). Columns temperature at 30ºC. The elution was performed in gradient mode with phosphoric acid 1.5 mL/L in water (Phase A) and acetonitrile (Phase B) with a flow of 1.2 mL.min $^{-1}$. The injection volume was 15 µL and the running time was 30 min.

2.4. Analysis of γ-oryzanol and γ-tocotrienol

The extraction was performed with isopropanol by vortex stirring for 1 min (two steps) followed by ultrasound (5 min) and the procedure was repeated twice; the centrifugation was carried out at 10.000 rpm/10 min. The extract was filtered with Chromafil XTRA-PTFE 0.2 µm (Macherey-Nagel, Düren, Germany). The simultaneous analysis was performed by using a C18 column XBridge (150 x 3 mm, 3.5µm) (Waters Corporation, Milford, USA) and Alliance model 2695 with a fluorescence detector (FLR) model 474 (λexc=290 nm and λem=330 nm), and 2998 Photodiode array (PDA) Waters® (Waters Corporation, Milford, USA). The separation was achieved at 30 ºC and elution in gradient mode with methanol and acetonitrile with a flow of 0.5 mL.min^{-1}. The running time was 20

min. The quantification of the gamma-oryzanol mixture was a sum of cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and sitosteryl ferulate (Ref. ASB 000153395, Chromadex, Longmont, USA) based on the response of the PDA detector set at a wavelength of 325 nm. The quantification of gamma-tocotrienol (Ref. 1.0852413, Merck, Darmstadt, Germany) was obtained by FLR detector (Waters Corporation, Milford, USA) and external standardization and the purity of the standard was checked by UV spectrophotometry model 8453 (Agilent Technologies, Santa Clara, USA) at 298nm.

2.5. Amino acid composition analysis of rice

2.5.1. Separation and quntification of amino acids by acid hydrolysis

For the separation and quantification of the main amino acids contained in food proteins, it was used the methodology described by Pacheco (2014) and acid hydrolysis step by method 994.12 (AOAC, 2000). Then, a 50 µL aliquot was removed and placed in a vial to be dried in a vacuum desiccator for 24 h with subsequent derivatization reaction (Cohen & Michaud, 1993). Chromatographic analysis was performed by using a Waters® Alliance model 2690/5 high performance liquid chromatographic system, with a fluorescence detector (λexc=250 nm and λem=395 nm) and Empower® software (2002) (Waters Corporation, Milford, USA), Thermo BDS HYPERSIL C18 column (100x4.6 mm; 2.4 µm) (Thermo Fisher Scientific, Waltham, USA) at 37 °C, elution in gradient mode with AccQ Tag® (Waters Corporation, Milford, USA): water (1:10; v/v) (Phase A), acetonitrile (Phase B) and water (Phase C) with a flow of 1.0 $mL.min^{-1}$.The injection volume was 5 µL and the running time was 45 minutes with a delay of 10 min. Quantification was performed by external standardization with the elaboration of an analytical curve by using standards (Amino Acid Standard H, Ref. WAT088122, Waters Corporation, Milford, USA) also submitted to the derivatization step with 6-aminoquinolyl-succimidyl-carbamate (AQC) (Waters Corporation, Milford, USA).

2.5.2. Separation and quantification of free amino acids and GABA

For the separation and quantification of the free amino acids, in addition to γ-aminobutyric acid (GABA) (Ref. 03835, Sigma Aldrich, St. Louis, USA), contained in rice in free form, it was performed an acid extraction step and subsequent derivatization with 6-

aminoquinolyl-succimidyl-carbamate (AQC) (Waters Corporation, Milford, USA). For the acid extraction step, it was weighed 1 g of the sample into a 50 mL screw-cap tube, added 10 mL of 0.1 M HCl, with vortex for 1 min, and then extraction in ultrasound for 10 min, with subsequent centrifugation at 6000 rpm for 10 min and filtration through a 0.45 μm filter. After that, it was performed the derivatization reaction, where 60 μL of borate buffer (AccQ-Fluor Borate Buffer) (Waters Corporation, Milford, USA) was added to 20 μL of extract, being the rest of the reaction performed as already described above. Chromatographic analysis was also performed in the same way that was described before, with a flow of 0.8 $mL.min^{-1}$ and a shorter elution gradient with a final run time of 41 min.

2.6. Physical properties

The falling number (FN) was determined according to method 56-81.04 (AACC, 2010) for indirect measurement of the α-amylase activity. Viscosity properties were determined using a Rapid Viscosity Analyzer series 4 (RVA) (Newport Scientific, Warriewood, Australia) according to method 76-21.01 (AACC, 2010).

2.7. Statistical analysis

Physical analysis, ferulic acid determination and amino acids profiles were conducted in duplicate or quadruplicate. Analysis of γ-oryzanol and γ-tocotrienol were conducted in quadruplicate. All results were expressed as mean ± standard deviation (SD). The statistical examination, heat map and Principal Component Analysis (PCA) plot were generated for quality parameters using XLSTAT version 2021.4.1 and GraphPad Prism version 8.0.1. The difference in mean of the same result was tested by *t*-test ($p < 0.05$). Correlogram for correlation analysis and their significant test was generated in R package 'corrplot' in R-version 1.2.5042.

3. Results and discussion

3.1. Bioactive compounds

Alterations on ferulic acid, γ-oryzanol, γ-tocotrienol, L-glutamic acid, and GABA contents after germination and polishing are shown in Table 1. Short germination caused a decrease in bioactive compounds, except L-Glu and GABA. Concerning ferulic acid content,

all genotypes showed a significant statistical reduction ($p<0.05$) of values (*BRS Formoso* from 4.70 to 2.55 µg/g, *Empasc_104* from 104 3.10 to 2.10 µg/g and *Mochi* from 2.95 to 2.50 µg/g), except observed for *Guaporé* genotype (2.65 to 3.10 µg/g).

The content of phenolic compounds (PC) is strongly correlated to rice genotypes. For instance, *Japonica* rice has significant higher PC than *Indica* rice (Ding et al., 2019). Changes in phenolic compounds occur not only during germination but also in the soaking step, thus washing and soaking operations can cause leaching of soluble phenolic compounds (Owolabi et al., 2018). It should be also mentioned that PC can be converted into flavonoids, tannins, lignins, and other compounds (Zhang et al., 2015). In our study, *Indica* ecotype (*BRS Formoso*) showed the highest values of ferulic acid (Table 1) prior germination but also the highest loss after germination (45% of reduction).

Table 1. Dynamic changes of bioactive compounds, L-glutamic acid (g/100g) and GABA (g/100g) of rice after germination and polishing.

Sample	Ferulic acid (µg/g)	γ-oryzanol (µg/g)	γ-tocotrienol (µg/g)	L-glutamic acid (g/100g)	γ-aminobutiric acid (mg/100g)
FNGB	4.70±0.00^{a}	250.23±5.19^{b}	16.99±0.56^{ab}	12.66±0.73^{fg}	0.28±0.07^{fg}
FNGP	2.80±0.00^{bcd}	61.32±15.72^{g}	10.10±0.39^{k}	7.13±0.04^{g}	0.20±0.00^{g}
GNGB	2.65±0.00^{cde}	313.82±3.91^{a}	15.00±0.23^{efg}	13.96±0.72^{efg}	0.35±0.28^{ef}
GNGP	2.40±0.00^{ef}	130.63±11.66^{ef}	11.22±0.16^{ijk}	10.57±0.40^{g}	0.28±0.07^{fg}
ENGB	3.10±0.00^{b}	279.02±73.98^{ab}	17.65±0.45^{ab}	10.44±1.30^{g}	0.22±0.14^{g}
ENGP	2.15±0.00^{fg}	118.27±12.17^{f}	13.86±0.32^{gh}	6.68±0.09^{g}	0.19±0.00^{g}
MNGB	2.95±0.00^{bc}	183.92±22.23^{cd}	12.12±0.21^{i}	43.06±2.82^{b}	0.39±0.15^{de}
MNGP	2.05±0.00^{g}	103.60±9.27^{fg}	10.60±0.21^{jk}	20.04±3.91^{de}	0.25±0.42^{fg}
FGB	2.55±0.00^{de}	263.08±4.05^{ab}	17.70±0.61^{a}	20.56±1.81^{de}	0.50±0.07^{d}
FGP	2.40±0.00^{ef}	112.98±13.79^{fg}	13.49±1.09^{h}	24.29±1.28^{cd}	0.49±0.37^{d}
GGB	3.10±0.00^{b}	305.57±4.14^{a}	16.61±0.63^{abc}	63.67±4.18^{a}	1.26±0.21^{a}
GGP	2.90±0.00^{bc}	248.56±3.41^{b}	14.18±0.36^{fgh}	29.74±0.82^{c}	0.82±0.35^{bc}
EGB	2.10±0.00^{fg}	195.43±6.55^{c}	15.65±0.36^{cde}	30.33±0.69^{c}	0.76±0.35^{c}
EGP	1.70±0.00^{h}	133.50±11.00^{def}	16.45±0.42^{bcd}	18.98±2.04^{def}	0.43±0.49^{de}
MGB	2.50±0.00^{de}	181.90±1.99^{cde}	15.39±0.46^{def}	38.12±0.19^{b}	0.90±0.42^{b}
MGP	2.35±0.00^{efg}	82.13±1.55^{fg}	11.79±0.11^{ij}	30.32±0.05^{c}	0.72±0.56^{c}

Values are the mean ± SD Different letters mean a significant difference ($p < 0.05$) in the same column.

As expected, rice polishing caused a decrease in the levels of ferulic acid in the non-germinated rice ($p < 0.05$), but there was no statistical difference between brown and polished germinated rice, except for *Empasc_104* (reduced from 2.1 µg/g to 1.7 µg/g). Although polishing leads to a decrease in ferulic acid levels, no statistical difference between brown and polished germinated rice was observed, which is an interesting advantage since most Latin-American and Asian consumers prefer white rice. This finding could be considered an interesting characteristic, especially for markets in which white rice is the predominant consumed product.

According to Ding et al., (2019), who worked with *Japonica* and *Indica* ecotypes, they found that ninety percent of phenolic compounds were abundant in the husk and in the bran fractions and the former presented significant higher phenolic compounds and antioxidant activity (C. Ding, Liu, Li, et al., 2018). Regarding γ-oryzanol and γ-tocotrienol contents, short germination did not significantly increase these compounds. There was no significant difference comparing germinated and non-germinated brown rice ($p < 0.05$). *Empasc_104* genotype presented a reduction of γ-oryzanol from 279.02 to 195.43 μg/g. In our work, debranning effect was more pronounced in non-germinated rice than germinated rice, indicating that type of genotype (*Guapore*) acts as a preponderant influence on the γ-oryzanol content.

Short germination showed a significant increase in L-glutamic acid and GABA in all genotypes, but polishing cause a decrease, except for *BRS Formoso*. There was no significant statistical difference ($p < 0.05$) between the germinated and polished germinated samples for this genotype. Our findings do not corroborate with the release of free glutamic acid, a substrate for GABA synthesis, that can influence the formation of GABA as observed by Chaijan & Panpipat (2020). In our study, polishing of germinated rice caused a decrease in GABA, but polished samples showed higher levels of GABA than non-germinated brown samples, indicating that this phytochemical may have been transferred to the endosperm during the germination process due to the dilution effect.

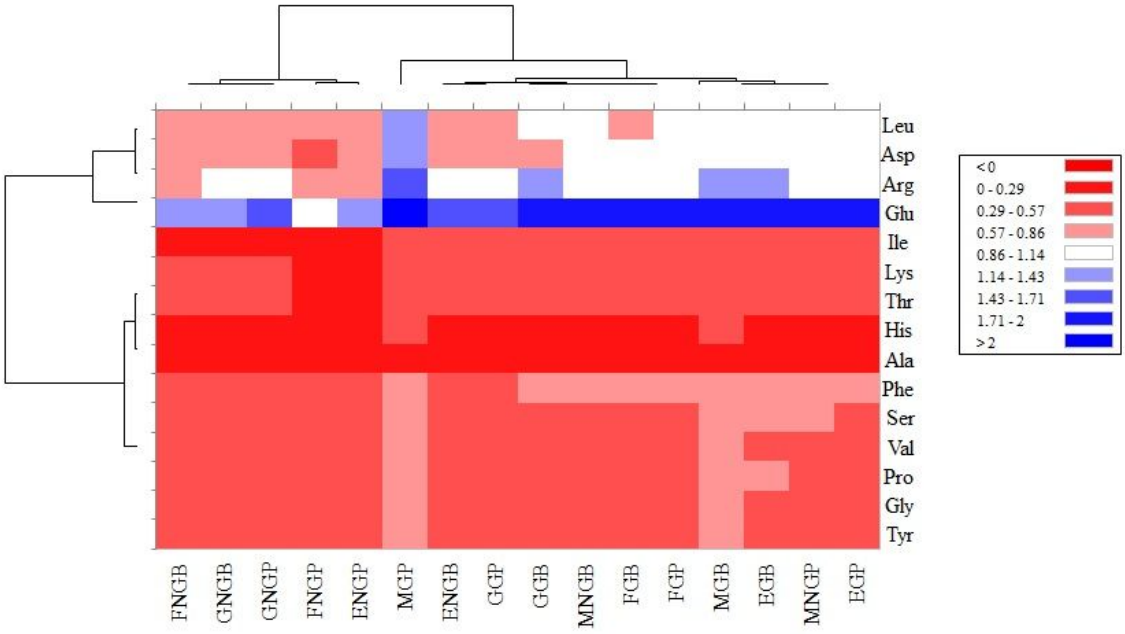
There was no significant statistical difference ($p<0.05$) between the brown (0.50 mg/100 g) and polished (0.49 mg/100 g) BRS *Formoso* germinated rice. Mochi genotype, a *Japonica* variety that was cultivated in Brazilian Savanah (*Cerrado*) region, presented the second biggest increment of GABA after germination (Fig. 3). This genotype showed a greater increase than *BRS Formoso* and *Empasc_104* genotypes. This result was different from the one reported by Zhang et al. (2014). This study concluded that the *Indica* rice showed higher GABA levels than the *Japonica* rice.

3.2. Amino acid profiles

Total amino acids (TAA) and free amino acids (FAA) contents are presented in Table S2 and Table S3. Heat map analysis was used for clustering rice samples based on similarities of amino acid concentrations (Figure 1A) and free amino acid (Figure 1B)

concentrations for each genotype after germination and polishing. As expected in rice, glutamic acid was the predominant amino acid. Our results showed values ranging between 1.12 to 2.97 g/100g (Table S4). There was a little increase in all total amino acid (TAA) contents of all genotypes after short soaking (4 h/30 °C) and short germination time (24 h/35 °C) (Figure 1A). Several essential amino acids were found in low levels after a short germination process (His, Lys, Ile, Thr) (Table S2), contrary to the *Mochi* genotype that showed the highest values of total amino acids profile in both non-germinated and germinated grains. In addition, no statistical difference ($p<0.05$) was observed between TAA values after germination and polishing step for aspartic acid, glutamic acid, arginine, alanine, and serine levels.

(A)



(B)

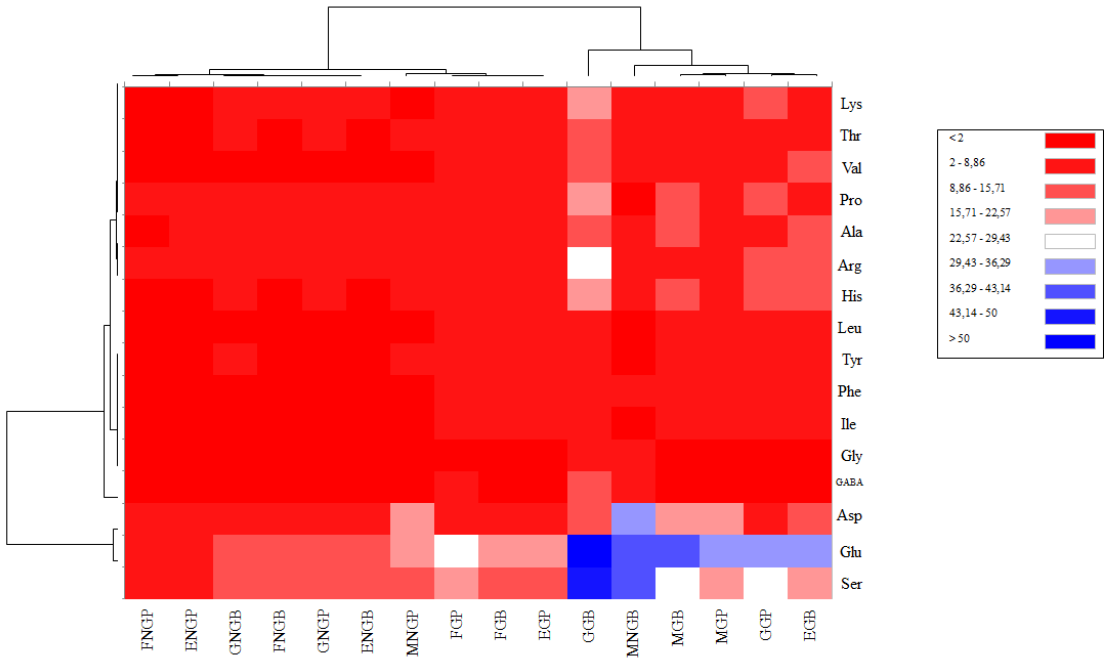


Figure 1. Heat map analysis by clustering groups of GABA and amino acids based on amino acid profiles of (A) total (g/100g) and (B) free amino acids (mg/100g). Where: F= BRS Formoso; G= Guaporé; E= Empasc 104; M= Mochi; NG = non-germinated; G = germinated; B= brown rice; P= polished rice.

Concerning free amino acids (FAA) there was a significant increase ($p<0.05$), especially in *Guaporé* genotype (Figure 1B). Brown germinated *Mochi* showed reduced levels of aspartic acid, serine, glutamic acid, and glycine than the non-germinated brown sample. But, *Mochi* was the genotype that presented the highest levels of all amino acids (Table S3). The accumulation of free amino acids (FAAs) in rice grains is a complex process that involves different biochemical networks and control mechanisms, most of which remains undisclosed until now.

In the work of Kamara et al (2010) there were significant differences between *Indica* and *Japonica* genotype in the concentrations of some FAAs and rice from *Japonica* ecotype tend to accumulate more total glutamate derived (Glu, Gln, His, Arg, Pro, and GABA) than total aspartate-derived (Asp, Asn, Thr, Lys, Ile, and Met) FAAs (Kamara et al., 2010). In addition to the type of genotype, differences in the environmental conditions such as total

rainfall and average of minimum and maximum temperature can impact the amino acids composition in seeds (Liyanaarachchi et al., 2021).

Polishing after germination caused a great decrease in FAA levels, but *BRS Formoso* showed no statistical difference ($p<0.05$) between brown and polished germinated samples. The impact of polishing in GABA results was more evident in non-germinated *Mochi* (36% of reduction) and germinated *Empasc_104* (43% of reduction) rice. In a period of short germination (0-24 h), most of the amino acids are concentrated in the embryo (prior debranning). After this time, during longer germination periods (72-96 h), they tend to increase and migrate into the endosperm and aleurone layer away from the embryo, until the storage proteins were degraded into amino acids and reduced nitrogen to support seed germination (Kamjijam et al., 2020). Lower values of FAA in white rice could be due to the removal of significant amounts of amino acids present in the pericarp during polishing (debranning) (Liyanaarachchi et al., 2020). This may explain the decrease in GABA levels after polishing (debranning) in our results (Table 1), as this process removes the external portions of the grain (pericarp and embryo).

Kamjijam et al. (2020) investigated the localization of amino acids in two genotypes of germinated rice grain in white rice variety and in colored rice variety during 0, 24, 48, 72, 96, 120, and 144 h. The highest rates of GABA and glutamic acid were observed within 48 h germination of both varieties (26.12 and 34.28 mg/100g, respectively). GABA and essential amino acids such as alanine, arginine, glycine, methionine, proline, serine, tyrosine, tryptophan, and valine were more concentrated during the period of 72–96 h than 0–24 h. Although there were a greater content of GABA and essential amino acids during prolonged germination, longer time affected the palatability, from a sensory perspective, which is not interesting attribute.

After germination, there was an expressive increase in umami amino acids (Glu, Asp), mainly in *Guaporé* and *Mochi* genotypes (Table S3). In addition, it was noted that these two genotypes showed levels of Pro in both brown grains (*Guapore*, 2.92 to 18.40 mg/100 g; *Mochi*, 1.54 to 9.73 mg/100g) and polished grains (*Guapore*, 3.19 to 8.87 mg/100 g; *Mochi*, 3.02 to 6.13 mg/100g). Since Pro is the precursor for the synthesis of 2-acetypyrrolysinene (2-AP), which contributes to the popular popcorn aroma in aromatic rice

(Kamara et al., 2010), this characteristic is useful in sensory study to describe qualityand/or quantitative sensations.

3.3. Physical properties

3.3.1. Falling Number test (FN)

FN values of the non-germinated and germinated samples were significantly different for the *BRS Formoso* (brown rice) and *Guaporé* (Figure 2). However, only the germinated samples of Guaporé showed value of FN that indicated some alpha-amylase activity. Conversely, *Empasc_104* (intermediate amylose content) and *Mochi* (waxy rice) showed no statistical difference ($p < 0.05$) in values between germinated and non-germinated samples, and the later showed the lowest FN (approximately 62 s), indicating the highest enzyme activity as a result of breaking starch granules therefore the viscosity. Regarding the debranning process, there was no statistical difference ($p < 0.05$) between brown and polished materials before and after germination using *BRS Formoso* (high amylose content) (FN = 820-908 s), whose values indicate no relevant enzyme activity.

Short germination did not appear to activate the α-amylase enzyme, particularly in the rice with high amylose content. The binomial time *versus* temperature and the starch content is preponderant for the α-amylase activity. Wang et al (2020) concluded that the germination temperature strongly affects the α-amylase activity, which in turn has a prominent impact on the hierarchical structures of rice starch, alternations of hierarchical structures, and modulation of pasting properties during the germination process. These authors reported that levels of α-amylase activity were significantly strengthened with temperature increasing (25°C to 35°C), ranging from 0.04 U/g to 3.19 U/g. Li et al. (2020)(C. Li, Jeong, Lee, et al., 2020) observed that the activated amylases preferentially act on the amorphous regions and on the degraded fragments during prolonged germination (> 60 h).

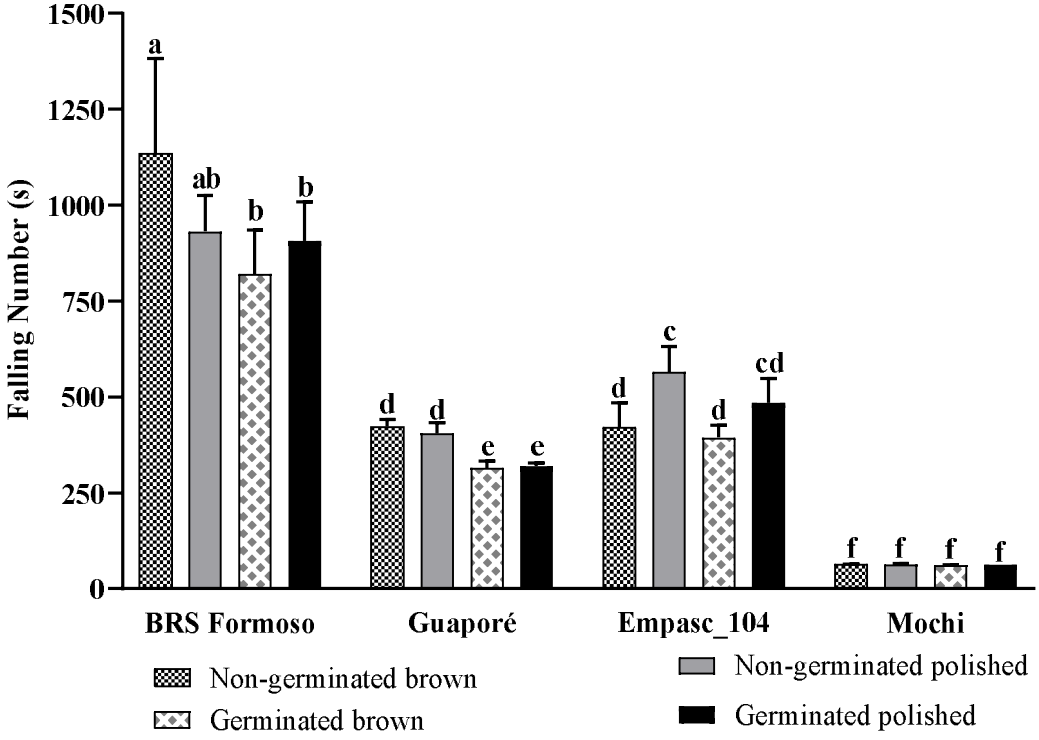


Figure 2. Falling Number (s) values of germinated, non-germinated, brown and polished rice. Different letters mean a significant difference (p <0.05). Error bars represent standard deviation (n = 3).

3.3.2. Pasting properties

The pasting curves of non-germinated and germinated rice flours are presented in Fig. 3 and pasting parameters are shown in Table S3 (Supplementary material). The pasting temperature ranged from 62.85 to 76.22 °C. As shown in Fig. 2, pasting curves of intermediate amylose content rice exhibited similar profiles (Figures 2b and 2c), while *Mochi* profile was distinguished among all of them (Figure 2d); *BRS Formoso* showed the higher retrogradation tendency (3793-3593 cP), whereas *Mochi* demonstrated the lowest retrogradation independently of germination or polishing (maximum of 241 cP).

Rice starches with higher amylose content are more sensitive to polishing than waxy rice starch (Xu et al., 2021). These authors presumed that polishing would damage short-chain amylopectin to protect amylose in high amylose starches, promoting the swelling of granules and, consequently, increasing viscosity (similar to *BRS Formoso*). In contrast, waxy

starch rice, the amylopectin would degrade severely, leading to a decrease of viscosity (similar *Mochi*). In addition, after the polishing step, most of the samples showed an increase in pasting properties values (Table S2).

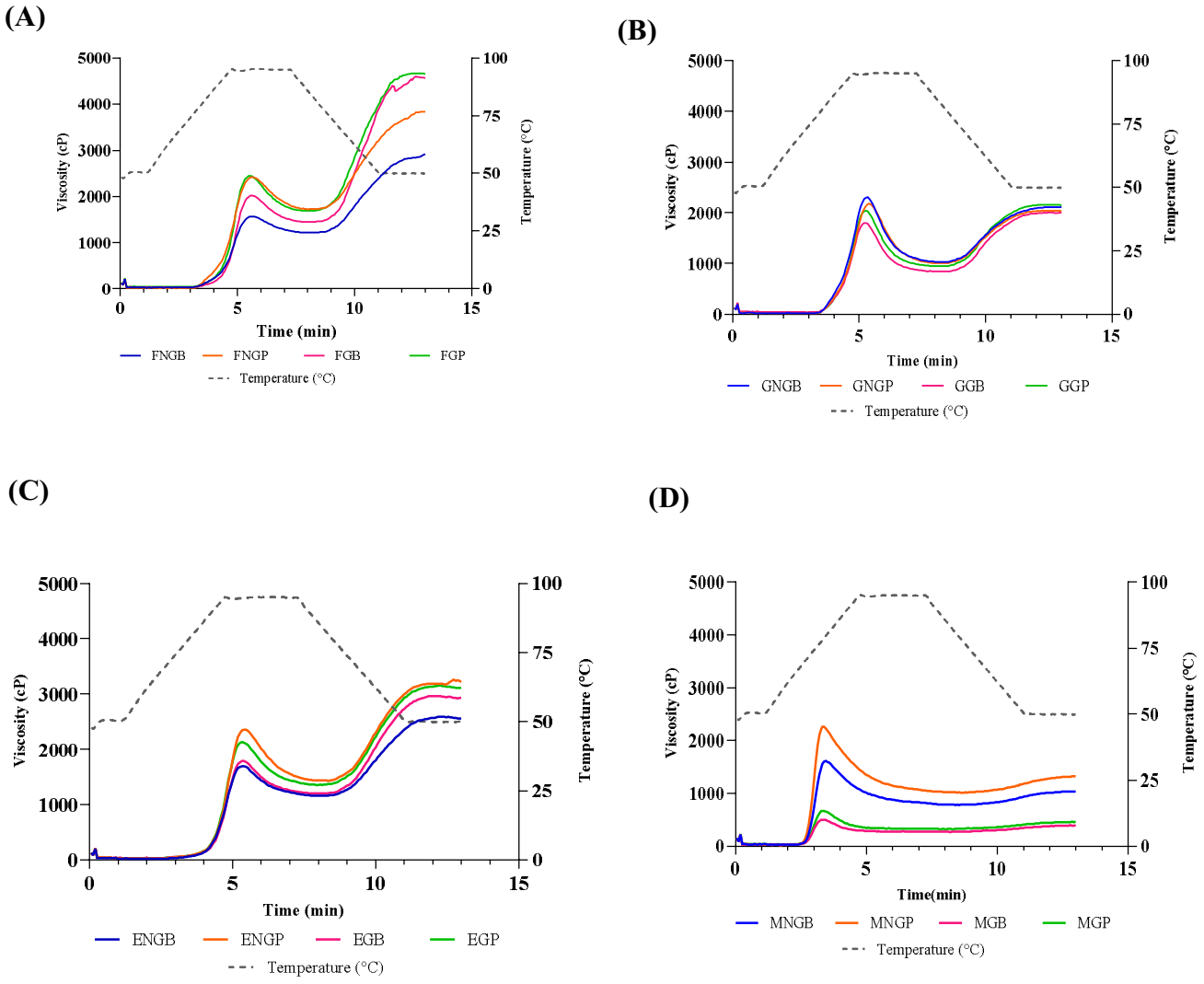


Figure 3. Pasting curves of non-germinated and germinated brown and polished rice, where: (a) BRS Formoso, (b) BRS Guaporé, (c) Empasc_104 and (d) Mochi. Where: F=BRS Formoso; G= Guaporé; E=Empasc 104; M=Mochi; NG = non-germinated; G = germinated; B= brown rice; P= polished rice.

Xu et al (2011) reported great changes of pasting properties in bronw rice after 24 h of germination, especially in terms of peak viscosity which decreased, contradicting our

findings except for Mochi genotype, i.e. there was an increase of peak viscosities observed both for *BRS Formoso* (high amylose) and *Empasc 104* (intermediate amylose) genotypes. In this sense, it is expected that germination can efficiently regulate the pasting properties of starch, but the mechanism of its action remains unknown (influence of genotype, amylose content, phenolic profile, etc.). Wang et al. (2020) reported that the enhanced enzyme activities during germination promoted the erosion of starch granules, the decrease of relative crystallinity and short-range ordered degree, and lengthening of double helix structure. These structural disorganizations of germinated rice starch resulted in reduced swelling degree and water-holding capacity, inducing the lower paste viscosity (e.g., peak and final viscosity), breakdown, and setback values.

3.4 Pairwise correlation coefficient (r), principal component analysis (PCA), and clustering of different genotypes of rice parameters

A correlation test was performed to understand the relationships between bioactive compounds, physical properties, and amino acids profile of rice genotypes (Fig. 4). There is a positive correlation between FA and FN, that can be explained by the phenolic compounds and enzyme or/and starch form non-covalent interactions that lead to physical changes (F. Zhu, 2015), especially in high-amylose starch (*BRS Formoso*) L-Glu and GABA demonstrated a very positive correlation, as shown in several previous works. The correlogram also indicates a positive relationship between ORY, gamma TE, and PT; and, FN and all pasting properties values (PT, BV, MV, BDV, FV, S) as expected since the α-amylase activation changes viscosity properties by cleavage amylose chains.

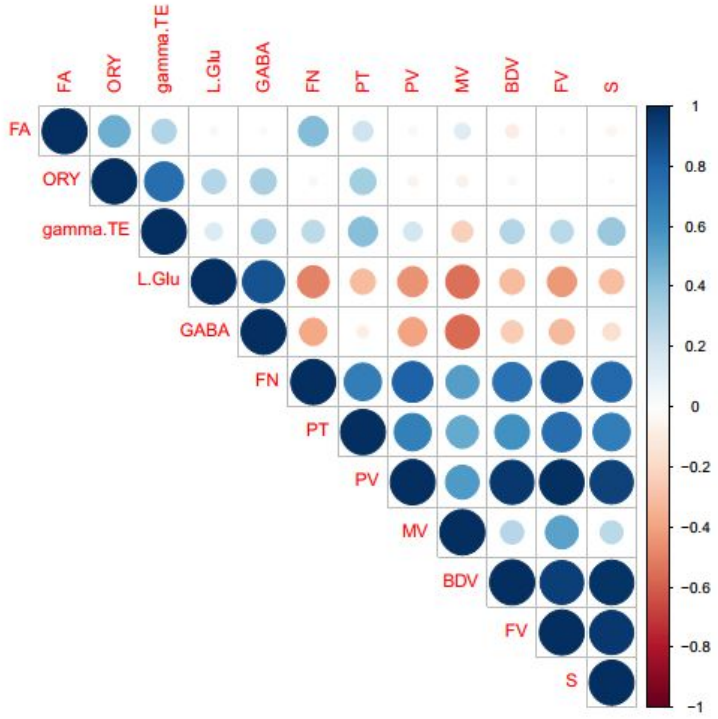


Figure 4. Correlogram for bioactive compounds and physical properties of rice. Blue indicates positive correlation, red negative correlation, color intensity and circle size are proportional to correlation coefficient (*r*) based on the scale display (lower side); where: FA: ferulic acid, ORY: γ-oryzanol, gamma-TE: γ-tocotrienol, L-glu: L-glutamic acid, GABA: γ-aminobutiric acid, FN: falling number, PT: pasting temperature, PV: peak viscosity, MV: medium viscosity, BDV: breakdown viscosity, FV: final viscosity, S: setback.

A negative correlation between L-Glu, GABA, and pasting properties values was observed. L-Glu and others AAs can impact pasting properties due to interactions occurred between AAs-starch. According to Xu et al (2022), these interactions between AAs and water significantly decreased the number of starch-water hydrogen bonds, maintaining a higher number of starch-starch intra hydrogen bonds, improving the intra-hydrogen bonds within starch chains, resulting in more stable structures and thus, inhibited starch gelatinization as well as its swell capacity. A negative correlation between GABA and FN was also observed. This finding is contrary to previous studies which have suggested that some hydrolytic enzymes such as amylases, proteases, phytase, and β-glucanase hydrolyzed larger molecules, forming precursors and increasing GABA and other bioactive compounds (H. Chung et al., 2014; Svihus et al., 2007).

PCA was used to evaluate the relationship among 12 variables related to rice genotypes. PCA plot was generated by two components, PC1, and PC2. Variability explained by PC1, PC2 are 49.04% and 22.01%, respectively. Thus, total variability in the two components accounted for 71.05%. Parameters of the vector which were close together are positive and highly correlated. Most variables are described by PC2 (FA, GABA, L-glu, gamma TE, ORY, PT, FT, FV, S, and BDV) (Fig. 5A). PCA 1 is described only by PV and MV.

The *Mochi* genotype (waxy rice) was the most discrepant among all, as shown in Fig. 5A and one unique cluster in Fig. 5B. As mentioned above, *Mochi* has lower amylose content and the most discrepant results for the physical analyses. In this genotype, the effect of germination and polishing was greater, as we can see in the Fig. 5B. The impact of polishing on *BRS Formoso* and *Empasc_104* genotypes can be seen in the PCA plot (Fig. 5A) and in the cluster (Fig. 5B). The FNGB, FGB, ENGB and EGB samples have a higher correlation with the results of physical (PT, S, FN, FV, BDV) and bioactive properties (ferulic acid). This results can be explained because the amylose content. The GNGB showed the major correlation with GABA indicates the difference between brown and polished rice.

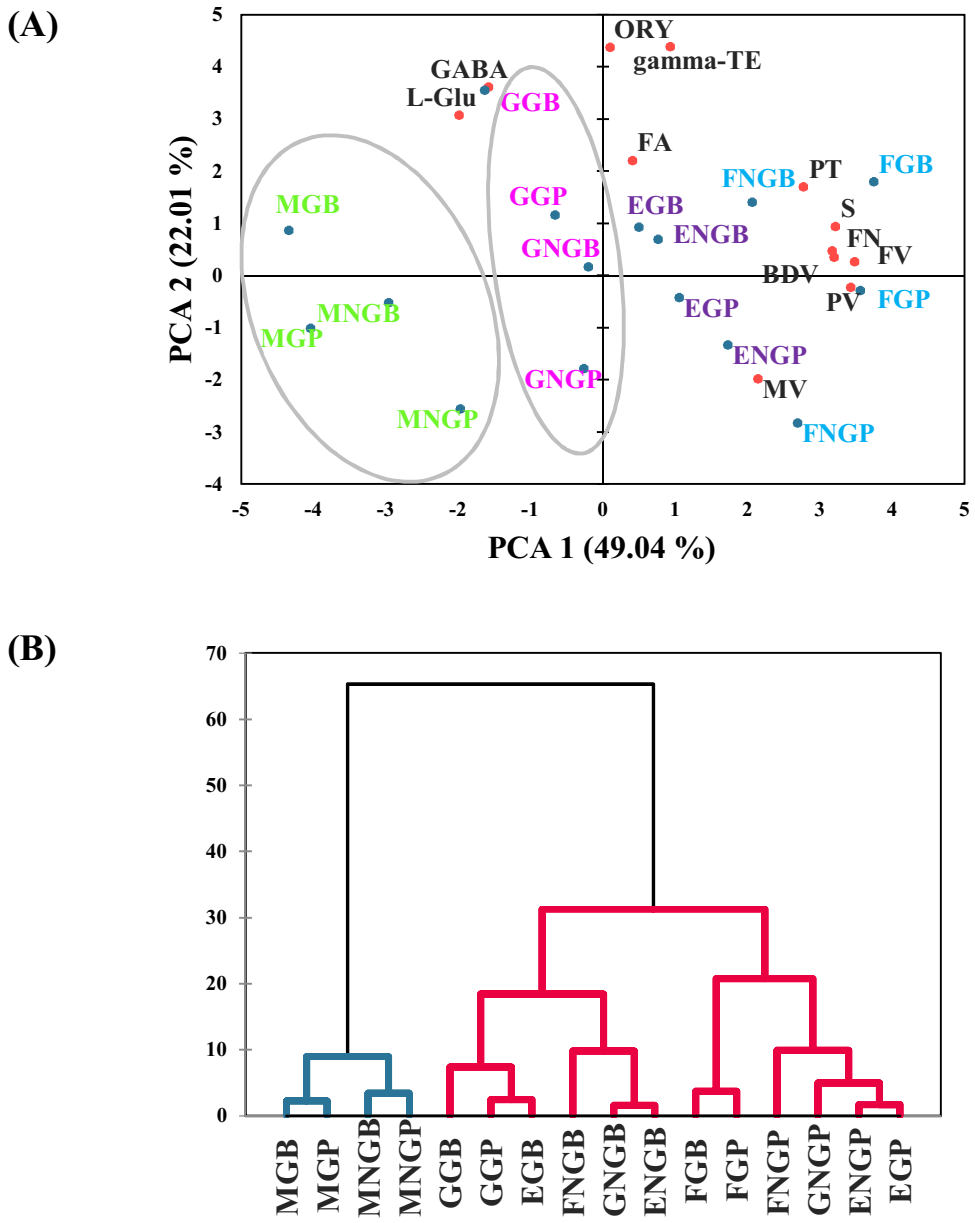


Figure 5. Biplot from principle component analysis (PCA) (A) and hierarchical cluster dendogram (B) of rice. Where: FA: ferulic acid, ORY: γ-oryzanol, gamma-TE: γ-tocotrienol, L-glu: L-glutamic acid, GABA: γ-aminobutiric acid, FN: falling number, PT: pasting temperature, PV: peak viscosity, MV: medium viscosity, BDV: breakdown viscosity, FV: final viscosity, S: setback, F=BRS Formoso; G= Guaporé; E= Empasc 104; M=Mochi; NG = non-germinated; G = germinated; B= brown rice; P=polished rice.

4. Conclusion

Short germination associated with debranning affected differently bioactive compounds, amino acid profiles and pasting properties of rice genotypes containing different amylose levels. Short germination caused a decrease in bioactive compounds, however, there was a significant increase in FAA, especially in *Guaporé* (intermediate amylose content) that demonstrated an expressive increase in umami amino acids (Glu and Asp) as well as in *Mochi* (waxy starch) rice. These two rice genotypes also showed very high levels of proline, a precursor for the synthesis of 2-acetyl proline that provide popcorn aroma in *Jasmine* or *Thai* rice. *BRS Formoso* (high amylose content) lead to the highest FN value, suggesting that germination conditions were insufficient to activate the α-amylase. *Mochi* presented a distinct viscosity profile between germinated and non-germinated rice, with a 5-fold reduction in PV and a lower tendency to retrogradation compared to rice with higher amylose content. Despite the global food crisis, our findings can help the food industry which in turn is constantly pursuing diversification of product niches that combine higher quality, nutritious properties, sensory experience and convenience foods. Indeed, it is a competitive advantage to have genotypes capable of developing products with differentiated characteristics and future works are necessary to explore possible applications of these rice cultivars in different GABA rice-based products.

Conflict of interest

None

Acknowledgments

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CRediT authorship contribution statement

Maria Eugenia Araujo Silva Oliveira: Investigation, Conceptualization, Formal analysis, Methodology, Writing - original draft. **Rosemar Antoniassi:** Formal analysis, Methodology,

Data curation, Writing - review & editing. **Allan Eduardo Wilhelm:** Formal analysis, Methodology, Data curation. **Manuela Cristina Pessanha de Araujo Santiago:** Formal analysis, Methodology, Data curation. **Sidney Pacheco:** Formal analysis, Methodology, Data curation. **Priscila Zaczuk Bassinello:** Project administration, Resources, Writing - review & editing. **José Manoel Colombari Filho:** Project administration, Resources, Writing - review & editing. **Carlos Wanderlei Piler de Carvalho:** Conceptualization, Supervision, Resources, Writing - review & editing. **Cristina Yoshie Takeiti**: Conceptualization, Supervision, Project administration, Resources, Writing - review & editing.

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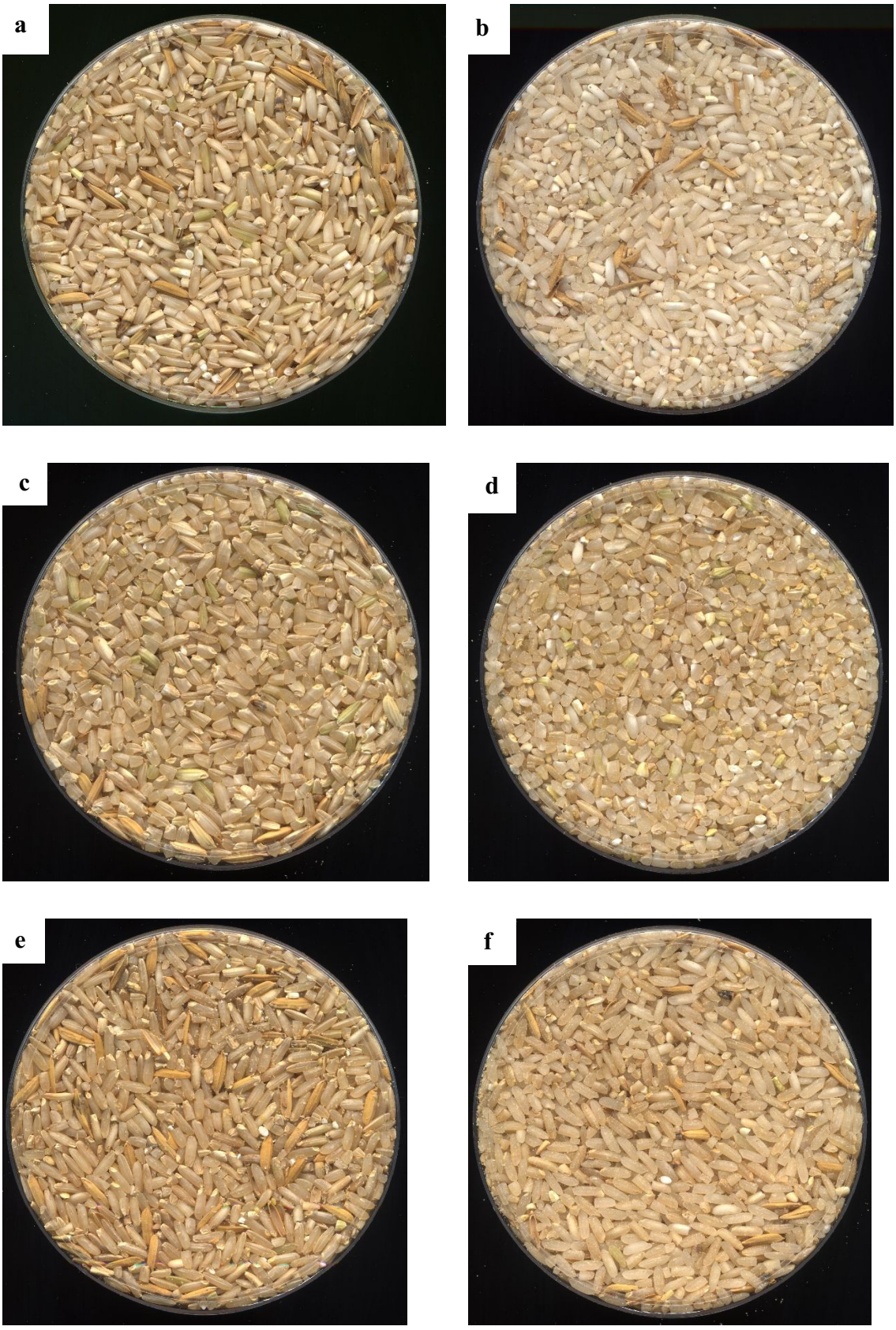
Supplementary Material

Table S1. Analysis of amylose apparent content

Cultivars	Apparent amylose content (%) (ISO 6647)	Accession number	Ecotype	Appearence of polished grain
BRS Formoso	25.60±0.64[a]	BRA 00006250-5	*Indica*	White core
Guaporé	14.15±0.24[b]	BRA 00002909-0	*Indica*	Light brown
Empasc_104	15.15±0.32[b]	BRA 00004842-1	*Indica*	Light brown
Mochi	1.86±0.24[c]	BRA 00011224-3	*Japonica*	Waxy

Each value is the mean of two replicates. For the same column, data with same letters do not differ significantly from each other whereas data with different superscripts differ significantly at the probability level $p < 0.05$

Figure S1. Visual aspect of rice samples: BRS FORMOSO (1), Guaporé (2), EMPASC_104 (3) and Mochi (4). Credits: Banco Ativo de Germoplasma do Arroz –BAG, Embrapa Arroz e Feijão.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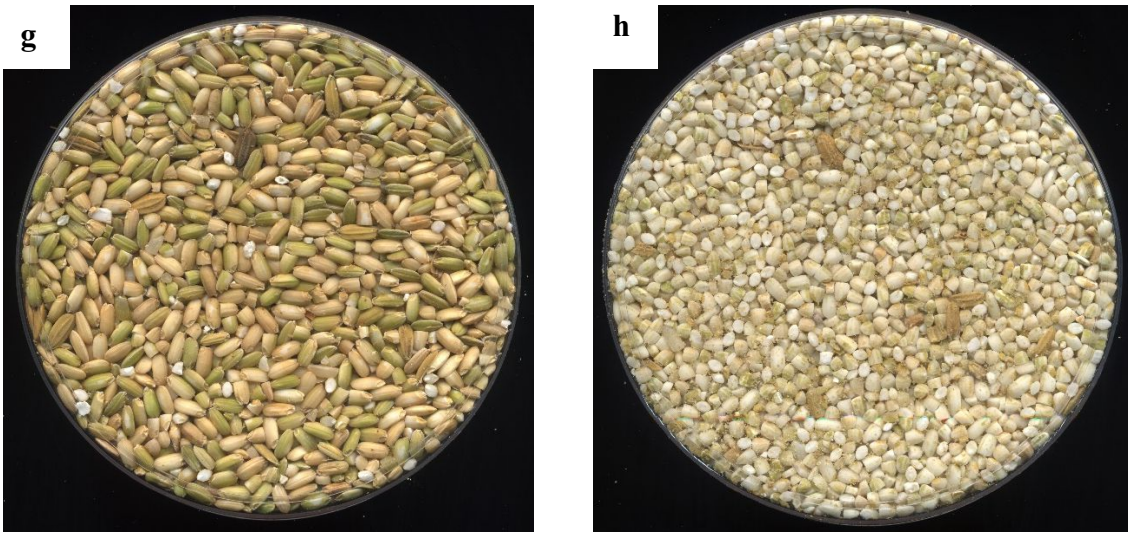

Figure S3. Germinated rice after germination process: a) BRS Formoso brown; b) BRS Formoso polished; c) Guaporé brown; d) Guaporé polished; e) EMPASC_104 brown; f) EMPASC_104 polished; g) Mochi brown, h) Mochi polished.

.

Table S2. Physical properties of germinated and non-germinated polished and brown rice

Samples	RVA Parameters					
	Pasting Temperature (°C)	**Peak viscosity (cP)**	**Minimun viscosity (cP)**	**Breakdown viscosity (cP)**	**Final Viscosity (cP)**	**Setback (cP)**
FNGB	74.97±3.42^{a}	2916.00±82.02^{e}	1074.50±468^{abcd}	1841.50±385.37^{bcdef}	2916.00±82.02^{e}	1759.50±501.33^{bcdefg}
FNGP	73.77±1.73^{ab}	3839.00±41.00^{b}	1945.00±108.89^{a}	1894.00±67.88^{bcde}	3836.00±36.76^{b}	1891.00±72.12^{bcdef}
GNGB	71.87±1.09^{ab}	2310.00±8.48^{gh}	1353.00±185.96^{ab}	956.50±194.45^{ghijk}	2114.50±36.06^{gh}	761.00±149.90^{hijkl}
GNGP	75.05±0.07^{a}	2179.50±58.68^{hi}	1129.50±55.86^{abcd}	1050.00±2.82^{fghij}	2033.50±6.36^{gh}	904.00±49.49^{ghijkl}
ENGB	74.55±0.56^{a}	2593.00±56.56^{f}	1201.00±329.51^{abc}	1392.00±272.94^{defg}	2556.50±65.76^{f}	1355.50±263.75^{cdefgh}
ENGP	75.37±2.86^{a}	3258.50±36.06^{cd}	1036.50±106.77^{abcde}	2222.00±142.83^{bc}	3221.00±1.41^{d}	2184.50±105.35^{bc}
MNGB	63.62±0.03^{c}	1610.00±18.38^{l}	837.50±9.19^{bcdef}	772.50±27.57^{ghijk}	1040.50±10.60^{k}	203.00±19.79^{jkl}
MNGP	64.05±0.53^{c}	2266.50±2.12^{h}	1060.00±9.89^{abcd}	1206.50±7.77^{efg}	1324.00±0.00^{j}	264.00±9.89^{ijkl}
FGB	75.82±1.16^{a}	4606.50±253.85^{a}	774.50±10.59^{bcdef}	3832.00±806.10^{a}	4568.00±271.52^{a}	3793.50±788.42^{a}
FGP	74.20±0.00^{a}	4670.70±31.81^{a}	1063.00±19.79^{abcd}	3607.50±51.61^{a}	4656.00±31.11^{a}	3593.00±50.91^{a}
GGB	75.02±0.03^{a}	2007.50±14.14^{ij}	903.50±20.50^{bcdef}	1104.00±11.31^{efghi}	1997.00±25.45^{gh}	1093.50±4.94^{fghij}
GGP	76.22±0.60^{a}	2163.00±8.48^{hi}	996.00±2.82^{abcdef}	1167.00±11.31^{efgh}	2149.50±12.02^{g}	1153.50±9.12^{defghi}
EGB	75.42±0.61^{a}	2968.00±31.81^{e}	895.50±23.33^{bcdef}	2072.50±14.84^{bcd}	2926.00±5.65^{e}	2030.50±17.67^{bcd}
EGP	72.60±1.09^{b}	3161.50±0.70^{de}	1092.50±53.03^{abcd}	2069.00±21.21^{bcd}	3109.50±4.94^{de}	2017.00±57.98^{bcde}
MGB	62.85±1.20^{c}	507.50±7.07^{m}	272.00±7.07^{cdef}	230.50±6.36^{jk}	391.50±6.36^{l}	119.50±0.70^{kl}
MGP	63.67±0.03^{c}	671.00±12.72^{m}	327.00±4.24^{bcdef}	344.00±2.82^{ijk}	464.50±7.77^{l}	241.00±142.83^{ijkl}

Each value is the mean of two replicates. For the same column, data with same letters do not differ significantly from each other whereas data with different superscripts differ significantly at the probability level $p < 0.05$. (Where: F=BRS Formoso; G=BRS Guaporé; E=Empasc 104; M=Mochi; NG = non-germinated; G = germinated; W=brown rice; P=polished rice).

Table S3. Total amino acids profile (g/100g) of germinated and non-germinated polished and brown rice

Samples	Asp	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Lys	Ile	Leu	Phe
FNGB	0.70±0.01^{efg}	0.44±0.00^{cde}	1.41±0.02^{f}	0.42±0.00^{d}	0.21±0.00^{e}	0.82±0.02^{cde}	0.32±0.00^{e}	0.08±0.00^{d}	0.45±0.00^{g}	0.43±0.00^{bc}	0.40±0.01^{e}	0.30±0.00^{bcd}	0.29±0.00^{cdef}	0.70±0.01^{cde}	0.51±0.01^{f}
FNGP	0.55±0.08^{g}	0.36±0.03^{e}	1.12±0.14^{h}	0.33±0.02^{e}	0.17±0.01^{f}	0.71±0.07^{e}	0.25±0.01^{g}	0.06±0.00^{e}	0.35±0.02^{h}	0.36±0.01^{c}	0.32±0.02^{f}	0.23±0.03^{d}	0.23±0.01^{f}	0.58±0.04^{e}	0.44±0.00^{h}
GNGB	0.69±0.12^{efg}	0.41±0.05^{cde}	1.38±0.23^{fg}	0.41±0.03^{d}	0.22±0.01^{def}	0.90±0.15^{bcde}	0.30±0.02^{ef}	0.09±0.02^{cd}	0.41±0.04f^{g}	0.43±0.06^{bc}	0.38±0.04^{e}	0.30±0.06^{bcd}	0.27±0.04^{def}	0.69±0.09^{cde}	0.49±0.03^{g}
GNGP	0.72±0.06^{def}	0.44±0.03^{cde}	1.45±0.12^{f}	0.39±0.04^{de}	0.20±0.02^{e}	0.86±0.04^{cde}	0.29±0.04^{f}	0.09±0.00^{cd}	0.41±0.05^{fg}	0.43±0.05^{bc}	0.38±0.03^{e}	0.30±0.01^{bcd}	0.27±0.04^{def}	0.71±0.07^{bcde}	0.46±0.05^{gh}
ENGB	0.84±0.1^{bcde}	0.53±0.04^{bcd}	1.70±0.26^{e}	0.47±0.02^{bc}	0.24±0.02^{d}	1.04±0.02^{bcde}	0.35±0.04^{e}	0.11±0.00^{c}	0.49±0.08^{f}	0.50±0.07^{c}	0.47±0.07^{d}	0.35±0.02^{bcd}	0.34±0.02^{bcdef}	0.84±0.11^{bcde}	0.57±0.08^{e}
ENGP	0.61±0.02^{fg}	0.41±0.02^{de}	1.31±0.07^{g}	0.37±0.01^{de}	0.18±0.00^{ef}	0.73±0.01^{de}	0.28±0.00^{f}	0.07±0.00^{de}	0.37±0.00^{h}	0.43±0.01^{bc}	0.35±0.00^{ef}	0.24±0.00^{cd}	0.25±0.02^{ef}	0.63±0.02^{de}	0.48±0.01^{e}
MNGB	0.91±0.00^{bc}	0.56±0.02^{bcd}	1.81±0.00^{d}	0.51±0.02^{bc}	0.27±0.01^{c}	1.09±0.03^{abcd}	0.40±0.02^{c}	0.11±0.00^{c}	0.54±0.02^{d}	0.52±0.00^{abc}	0.50±0.00^{cd}	0.39±0.00^{ab}	0.36±0.04^{bcde}	0.89±0.02^{abcd}	0.61±0.05^{de}
MNGP	0.94±0.07^{bc}	0.58±0.04^{abc}	1.88±0.14^{d}	0.49±0.04^{c}	0.27±0.02^{c}	1.10±0.09^{abc}	0.40±0.03^{c}	0.11±0.00^{c}	0.55±0.06^{d}	0.55±0.03^{abc}	0.52±0.02^{cd}	0.38±0.02^{abc}	0.37±0.04^{bcde}	0.91±0.02^{abcd}	0.66±0.04^{c}
FGB	0.87±0.03^{bcd}	0.56±0.04^{bcd}	1.77±0.08^{de}	0.52±0.05^{b}	0.26±0.02^{c}	1.11±0.10^{abc}	0.39±0.04^{cd}	0.11±0.00^{c}	0.54±0.07^{d}	0.49±0.06^{c}	0.52±0.04^{cd}	0.38±0.02^{abc}	0.37±0.02^{bcde}	0.84±0.05^{bcde}	0.60±0.10^{de}
FGP	0.87±0.04^{bcd}	0.54±0.02^{bcd}	1.81±0.11^{d}	0.48±0.02^{c}	0.24±0.02^{d}	1.09±0.04^{abcd}	0.38±0.02^{d}	0.11±0.00^{c}	0.52±0.02^{e}	0.49±0.03^{c}	0.53±0.04^{cd}	0.38±0.02^{abc}	0.39±0.04^{abcd}	0.86±0.05^{bcde}	0.59±0.03^{e}
GGB	0.85±0.07^{bcde}	0.56±0.03^{bcd}	1.73±0.13^{e}	0.53±0.02^{b}	0.28±0.01^{bc}	1.15±0.06^{abc}	0.39±0.03^{cd}	0.14±0.05^{bc}	0.55±0.04^{d}	0.54±0.02^{abc}	0.51±0.02^{cd}	0.35±0.03^{bcd}	0.37±0.02^{abcdefg}	0.87±0.08^{bcde}	0.63±0.03^{d}
GGP	0.82±0.09^{cde}	0.53±0.04^{bcd}	1.67±0.18^{e}	0.49±0.03^{c}	0.26±0.02^{c}	1.09±0.11^{abcd}	0.36±0.02^{de}	0.10±0.00^{c}	0.51±0.04^{ef}	0.50±0.04^{c}	0.48±0.05^{d}	0.32±0.06^{bcd}	0.35±0.03^{bcdef}	0.81±0.05^{bcde}	0.57±0.02^{e}
EGB	0.93±0.08^{abcd}	0.60±0.02^{abc}	1.96±0.16^{c}	0.52±0.00^{b}	0.27±0.00^{c}	1.15±0.04^{abc}	0.41±0.07^{bc}	0.15±0.00^{bc}	0.58±0.06^{c}	0.57±0.01^{b}	0.57±0.02^{bc}	0.36±0.00^{bcd}	0.41±0.02^{abc}	0.97±0.05^{abc}	0.67±0.04^{c}
EGP	0.87±0.07^{bcd}	0.56±0.04^{bcd}	1.89±0.16^{d}	0.48±0.04^{c}	0.26±0.02^{c}	1.10±0.14^{abc}	0.38±0.03^{d}	0.11±0.00^{c}	0.54±0.04^{d}	0.55±0.05^{abc}	0.54±0.02^{c}	0.36±0.00^{bcd}	0.40±0.02^{abcd}	0.92±0.07^{abcd}	0.63±0.04^{d}
MGB	0.98±0.06^{b}	0.66±0.04^{ab}	2.97±0.13^{a}	0.61±0.04^{a}	0.31±0.01^{b}	1.25±0.14^{ab}	0.46±0.03^{b}	0.16±0.04^{b}	0.64±0.04^{b}	0.61±0.03^{a}	0.59±0.06^{b}	0.43±0.04^{ab}	0.42±0.02^{ab}	1.10±0.05^{ab}	0.75±0.01^{b}
MGP	1.17±0.07^{a}	0.75±0.07^{a}	2.38±0.13^{b}	0.64±0.02^{a}	0.35±0.03^{a}	1.44±0.14^{a}	0.49±0.02^{a}	0.18±0.00^{a}	0.71±0.02^{a}	0.62±0.03^{a}	0.71±0.06^{a}	0.52±0.06^{a}	0.51±0.05^{a}	1.18±0.12^{a}	0.81±0.07^{a}

Each value is the mean of two replicates. For the same column, data with same letters do not differ significantly from each other whereas data with different superscripts differ significantly at the probability level $p < 0.05$. (Where: F=BRS Formoso; G= Guaporé; E=Empasc 104; M=Mochi; NG = non-germinated; G = germinated; B= brown rice; P=polished rice).

Table S4. Free amino acid profile (mg/100g) of germinated and non-germinated polished and brown rice

Samples	Asp	Ser	Glu	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Lys	Ile	Leu	Phe	GABA
FNGB	6.49±0.62^{fghi}	10.40±0.79^{ghij}	12.66±0.73^{ghi}	1.25±0.07^{de}	1.94±0.02^{hi}	3.29±0.04^{ijk}	1.78±0.08 ^{kl}	2.27±0.16^{ij}	3.22±0.04^{de}	1.94±0.01^{e}	1.61±0.06^{h}	2.37±0.26^{jk}	1.43±0.02^{gh}	1.47±0.02^{f}	1.66±0.00^{gh}	0.28±0.07^{fg}
FNGP	3.36±0.04^{i}	5.00±0.11^{jk}	7.14±0.04^{i}	0.98±0.00^{e}	1.57±0.01^{i}	2.36±0.01^{k}	1.39±0.01^{l}	1.44±0.02^{m}	3.59±0.18^{de}	1.75±0.00^{e}	1.28±±0.00^{h}	1.58±0.02^{k}	1.26±0.00^{h}	1.31±0.00^{f}	1.54±0.00^{h}	0.20±0.00^{g}
GNGB	6.53±0.27^{fghi}	15.14±0.12^{efgh}	13.96±0.72^{fgh}	1.65±0.03^{bc}	2.39±0.00^{ghi}	3.86±0.02^{hijk}	4.67±0.03^{defg}	3.39±0.04^{ghi}	2.92±0.37^{de}	2.15±0.00^{e}	1.71±0.03^{gh}	2.69±0.02^{ijk}	1.50±0.02^{gh}	1.49±0.02^{k}	1.71±0.01^{gh}	0.35±0.28^{ef}
GNGP	4.97±0.21^{hi}	10.01±0.33^{hij}	10.58±0.40^{hi}	1.36±0.00^{bcde}	2.01±0.02^{hi}	3.03±0.00^{ijk}	3.29±0.02^{hij}	2.53±0.12^{hij}	3.19±0.04^{de}	1.91±0.01^{e}	1.43±0.00^{h}	2.49±0.10^{ijk}	1.31±0.04^{h}	1.34±0.01^{f}	1.59±0.01^{h}	0.28±0.07^{fg}
ENGB	6.17±0.20^{ghi}	8.89±1.38^{ijk}	10.45±1.30^{hi}	1.60±0.24^{bcd}	1.97±0.19^{hi}	3.06±0.44^{ijk}	1.77±0.18 ^{kl}	2.61±0.24^{hij}	2.94±0.20^{de}	1,99±0.04^{e}	1.59±0.09^{h}	2.09±0.41^{k}	1.36±0.04^{h}	1.55±0.12^{f}	1.60±0.00^{h}	0.22±0.14^{g}
ENGP	4.29±0.12^{hi}	5.53±0.36^{jk}	6.68±0.09^{i}	1.29±0.08^{cde}	1.58±0.07^{i}	2.46±0.22^{k}	1.36±0.00^{l}	2.02±0.21^{ij}	3.12±0.01^{de}	1.79±0.04^{e}	1.29±0.03^{h}	1.44±0.41^{k}	1.25±0.02^{h}	1.39±0.02^{f}	1.47±0.07^{h}	0.19±0.00^{g}
MNGB	31.75±1.93^{a}	39.45±0.60^{a}	43.06±2.82^{b}	2.28±0.01^{a}	4.13±0.10^{fgh}	4.29±0.00^{ghijk}	3.33±0.04^{hij}	7.73±0.16^{cd}	1.54±0.22^{e}	1.86±0.62^{e}	2.96±0.00^{efg}	3.84±0.06^{hijk}	1.97±0.00^{fg}	1.91±0.00^{f}	2.03±0.00^{g}	0.39±0.15^{de}
MNGP	17.09±3.28^{b}	15.61±1.38^{defg}	20.03±3.91^{def}	1.36±0.20^{bcde}	2.66±0.36^{ghi}	2.74±0.27^{k}	2.08±0.41^{jkl}	4.06±0.77^{fgh}	3.02±0.23^{de}	2.06±0.10^{e}	1.92±0.26^{gh}	1.82±0.45^{k}	1.57±0.20^{gh}	1.73±0.16^{f}	1.75±0.06^{gh}	0.25±0.42^{fg}
FGB	6.19±0.65^{ghi}	12.35±1.86^{fghi}	20.56±1.81^{def}	1.27±0.08^{cde}	4.38±0.19^{fg}	5.90±0.74^{efghij}	3.48±0.48^{ghi}	5.14±0.02^{ef}	4.17±0.08^{de}	3.26±0.07^{d}	3.61±0.27^{ef}	5.02±0.31^{fghi}	2.28±0.10^{f}	3.12±0.30^{e}	2.70±0.01^{ef}	0.55±0.07^{d}
FGP	6.12±0.43^{ghi}	16.92±0.12^{def}	24.29±1.27^{cde}	1.73±0.70^{b}	5.37±0.03^{f}	7.13±0.02^{efg}	4.05±0.03^{efgh}	6.13±0.15^{ij}	5.58±0.07^{cd}	3.35±0.07^{d}	4.01±0.10^{e}	6.95±0.31^{defg}	2.48±0.11^{ef}	3.19±0.12^{e}	2.78±0.07^{ef}	0.49±0.38^{d}
GGB	13,09±0.82^{cd}	44.99±3.52^{a}	63.67±4.17^{a}	2.29±0.10^{a}	19.60±1.49^{a}	24.49±0.02^{a}	15.37±0.93^{a}	11.82±0.79^{a}	18.40±1.42^{a}	7.24±0.50^{a}	12.79±0.95^{a}	17.98±0.72^{a}	6.21±0.28^{a}	7.37±0.51^{a}	5.37±0.34^{a}	1.26±0.21^{a}
GGP	7.13±0.19^{fgh}	23.94±0.04^{bc}	29.75±0.82^{c}	1.45±0.01^{bcd}	11.26±0.01^{c}	13.42±1.47^{bc}	8.53±0.07^{b}	6.22±0.09^{de}	8.87±0.04^{bc}	4.85±0.01^{b}	6.87±0.09^{cd}	9.50±0.05^{bc}	3.56±0.01^{c}	4.29±0.02^{d}	3.69±0.00^{c}	0.83±0.21^{bc}
EGB	9,88±0.59^{def}	16.96±0.03^{def}	30.33±0.69^{c}	1.46±0.09^{bcd}	14.15±1.58^{b}	13.67±0.07^{b}	4.96±0.20^{cdef}	9.00±0.77^{bc}	2,02±0.40^{e}	6.41±0.45^{a}	8.93±0.44^{b}	6.58±0.09^{efg}	4.95±0.28^{b}	6.60±0.00^{b}	4.73±0.10^{b}	0.77±0.35^{c}
EGP	5.25±0.67^{hi}	14.03±0.09^{fghi}	18.98±2.04^{efg}	1.47±0.04^{bcd}	5.98±0.04^{ef}	6.18±2.02^{efghi}	3.54±0.05^{ghi}	4.52±0.42^{fg}	4.79±1.42^{de}	3.22±0.11^{d}	3.71±0.20^{ef}	4.76±0.46^{ghij}	2.24±0.10^{f}	3.12±0.23^{e}	2.47±0.05^{f}	0.44±0.49^{de}
MGB	19.20±0.00^{b}	27.63±0.82^{b}	38.12±0.19^{b}	1.48±0.00^{bcd}	9.78±0.10^{cd}	8.58±0.22^{de}	5.03±0.16^{cdef}	10.14±0.04^{b}	9.73±0.04^{b}	4.55±0.00^{bc}	6.87±0.16^{cd}	5.98±0.60^{fgh}	3.32±0.04^{cd}	5.04±0.02^{c}	3.46±0.00^{cd}	0.90±0.42^{b}
MGP	16.01±0.29^{bc}	20.48±1,20^{cde}	30.33±0.57^{c}	1.48±0.05^{de}	7.72±0.01^{de}	6.90±0.53^{efgh}	4.16±0.12^{efgh}	8.62±0.43^{bc}	6.13±1.42^{cd}	3.74±0.07^{cd}	5.99±0.27^{d}	7.50±0.19^{cdef}	2.93±0.07^{de}	4.03±0.18^{d}	3.06±0.02^{de}	0.72±0.56^{c}

Each value is the mean of two replicates. For the same column, data with same letters do not differ significantly from each other whereas data with different superscripts differ significantly at the probability level $p < 0.05$. (Where: F=BRS Formoso; G= Guaporé; E=Empasc 104; M=Mochi; NG = non-germinated; G = germinated; B= brown rice; P=polished rice).

Chapter V

Role of short germination and milling on physical properties, amino acid and metabolomic profiles of high amylose rice fractions

Maria Eugenia Araujo Silva Oliveira[a], Luciana Ribeiro da Silva Lima[a,b,c], Millena Cristina Barros Santos[a,b,c], Nathalia Ferrari Fonseca de Sales[c], Renata Marenda Ferreira[c], Luiz Claudio Cameron[b], José Manoel Colombari Filho[e], Priscila Zaczuk Bassinello[e], Carlos Wanderlei Piler de Carvalho[d], Mariana Simões Larraz Ferreira[a,b,c], Cristina Yoshie Takeiti[a,d]

[a]Food and Nutrition Graduate Program, Federal University of State of Rio de Janeiro, UNIRIO, Brazil

[b]Center of Innovation in Mass Spectrometry, Laboratory of Protein Biochemistry (IMasS-LBP), UNIRIO,Brazil

[c]Laboratory of Bioactives, Food and Nutrition Graduate Program, Federal University of State of Rio de Janeiro, UNIRIO, Brazil

[d]Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil

[e]Embrapa Agroindústria de Alimentos, Rio de Janeiro, RJ, Brazil

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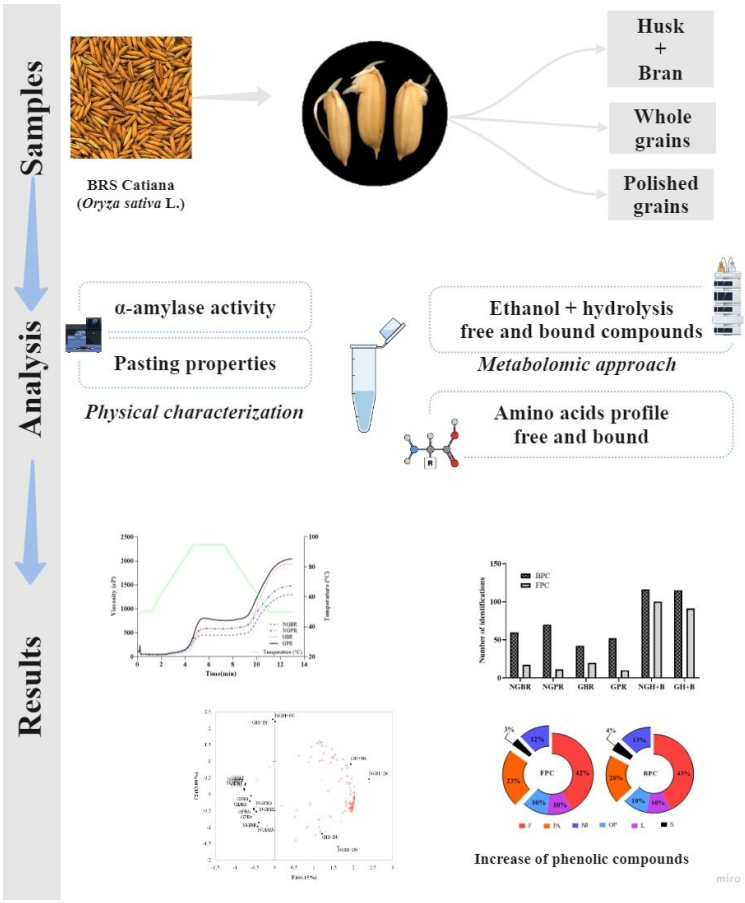


Highlights

- Thermal and pasting viscosity are not change by short germination.
- Milling has little effect on phenolic compounds and GABA of germinated rice.
- Metabolomics unveil distinguished phenolic profile in high amylose rice fractions.
- *trans*-Ferulic acid is the major bound phenolic compound in rice husk.

ABSTRACT

Short germination is a process that can improve bioactive compounds in rice. This work aimed investigate the physical properties, phenolic compounds (PC), antioxidant activity and amino acids composition of husk + bran, brown and milled rice with high amylose content after short germination (16 h). α-amylase activity (Falling Number, FN) and enthalpy (ΔH**)** were unchanged ($p < 0.05$). RVA curve profiles were similar, even though after short germination and milling. Globally, metabolomics analysis identified 117 PC, in which 111 (bound), 104 (free) and 21 revealed in both extracts. *p*-Coumaric, *trans*-ferulic and ferulic acids were the most abundant PC revealed in all fractions. The portion husk + bran showed the highest level of total antioxidant activity (709.90 μmol TE) in both free and bound fractions. In terms of total amino acids, there was no statistical difference ($p < 0.05$) among non-germinated and germinated samples, contrary to free amino acids content. Glutamic acid (Glu) presented the highest values combining short germination and milling (1725-1900 mg/100g) consequently, leads to higher value of GABA (12.21 mg/100g). The combination of short germination and milling demonstrated a good strategy to improve the nutritional quality of rice, unless the thermal and pasting properties have been altered, contribute to potential health benefits on human nutrition.

Keywords: Short germination, milling, phenolic compounds, HPLC, LC-MS-MS

1. Introduction

Rice (*Oryza sativa* L.) is a staple food and the main cereal consumed in Asia and Brazil (FAOSTAT, 2023). In 2021, the world production was 787,293,867.41 M tonnes. The production of rice is concentrated in Asians countries and Brazil is the tenth largest producer in the world (FAOSTAT, 2023).

In Brazil, the production and consumption of rice is in decline in the last decade (FAOSTAT, 2023) and thus, new forms of rice-based product presentations must be designed in order to dynamize the rice production chain. Brazilian market prefer fine long class grains, with a loose and soft consistence pattern after cooking, which is characterize by high amylose content (25-30%) (Rangel et al., 2019), in contrast to Asian countries that prefer glutinous rice (waxy starch). In addition to market factors, consumers seek out 'energy-saving' products, i.e. how food and drink use less energy to produce, to store and/or prepare (Mintel, 2023).

Rice grains is composed by (i) husk, rich in cellulose and silica; (ii) bran which is composed of pericarp (high in cellulose), (iii) seed coat and aleurone layers (high in oil, protein, vitamins and minerals); (iv) endosperm high in starch and poor in proteins, fat and minerals and (v) embryo, high in protein and minerals (Juliano, 1972). Milling is a process that makes rice consumption possible, in which removal and discarded of husk and bran may be used in various applications, improving the functionality and the utilization of PC of rice by-products (Ding et al., 2018a).

In this context, germination is an alternative of bioprocess used for improving the nutritional quality of rice with minimal energy inputs. The process consists of three steps: (i) sanitization; (ii) soaking and (iii) germination. There are intrinsic (cultivar, amylose content) and extrinsic (time, temperature, pH) factors that should be considered during germination process (Gan et al., 2016; Qian Zhang et al., 2014). Starch is the major component of rice, and the amylose/amylopectin ratio play an important role, because during germination, there is an increase of α-amylase activity, which can lead to changes in the starch matrix (Kalita et al., 2017; Wang et al., 2020).

Longer process of germination (> 24h) improves bioactive compounds but affect the sensorial and microbiological quality of rice. Therefore, modification of the process conditions e.g. short process (< 24h) is necessary to maintain the grain quality (Kamjijam et al., 2020) and having energy-efficient foods and drinks. Many studies have reported positive effects of germination in terms of bioactive compounds, although a few studies have shown that time influences germination process that affect antioxidant activity, physical properties and metabolic profiles along with rice processing steps such as dehusking. Among bioactive compounds generated during rice germination, γ-aminobutyric acid (GABA) is one the most studied due to their effects on insomnia (Byun et al., 2018), prolongation of sleep (Wu et al., 2014), blood pressure (Nishimura et al., 2014) and anti-inflammatory (Ali et al., 2021; Cataldo et al., 2020). Besides, other phytochemicals related to germinated rice are the phenolic compounds such as ferulic acid, flavonoids, and *trans*-ferulic acid due to the antioxidants effects (Kasote et al., 2021).

Metabolomics analysis is a rapid, accurate and environmentally friendly method for identifying the metabolic profiles of foods in order to understand the potential usage of the technology and their relationship to processing, composition and function (Utpott et al., 2022). This technique can be applied to investigate foods in terms of (i) food composition analysis, (ii) food quality safety, (iii) food traceability and (iv) food properties (Li et al., 2021; Wu et al., 2022). Few studies are related to the use of metabolomics approach either on grain germination or on rice quality (Ding et al., 2018b; Kim et al., 2020). Therefore, the aim of this study was to investigate the role of short germination and milling combination on physical properties, PC profiles, antioxidant capacity and amino acid contents in different fractions of high amylose rice.

2. Material and methods

2.1. Chemicals

The following reference standards, as well as MS-grade acetonitrile and methanol, were purchased from Sigma-Aldrich (St. Louis, USA): vanillic acid, *p*-coumaric acid, catechin, caffeic acid, ellagic acid, *trans*-ferulic acid, kaempferol, myricetin, pyrogallol, flavanone, quercetin, gallic acid, epicatechin, 4-hydroxybenzylalcohol, 4-hydroxybenzaldehyde acid, 4-hydroxybenzoic acid, 4-hydroxybenzoic acid, 4-phenylacetic

acid, sinapic acid, benzoic acid, quercetin-3-*O*-glucoside, 3,4-dihydroxy phenylacetic acid, epigallocatechin, epicatechingallate, chlorogenic acid, 2,5-dihydroxybenzoic acid, 4-methoxycinnamic acid, 2-hydroxycinnamic acid, 3-hydroxy-4-methoxycinnamic acid, *trans*-cinnamic acid, 3-methoxycinnamic acid, and L-(-)-3-phenylacetic acid. Formic acid was purchased from Fluka (Buchs, Switzerland). Ultrapure water was obtained through the Barnstead™ Smart2Pure™ (Thermo Fisher Scientific, Massachusetts, USA) purification system. Other unmarked reagents were of analytical grade.

2.2. Samples

BRS Catiana (*Oryza sativa* L.) was selected by lowland rice breeding program of *Embrapa Arroz e Feijão* (Santo Antônio de Goiás-GO, Brazil) because it is the cultivar that best represented the genetic background of brazilian rice (Rangel et al., 2019). The material was chosen according to the apparent amylose content (Table S1) determined according to the method ISO 664 (ISO, 2007). The cultivar was multiplied in 2018/2019 harvest using a flood-irrigated system in the farm experimental field of *Embrapa Arroz e Feijão* (6° 29' 8" S, 49° 18' 32" W), following the crop-management practices adopted for rice cultivation in Brazil.

2.3. Germination process

Germination was performed according to the methodology described by Zhang et al. (2014) with some modifications (7h/30 °C of soaking and 16h/35°C of germination). After this step, the grains were drained and allowed to germinate in a fermentation cabinet (National Mfg. Co., Lincoln, USA) at a controlled temperature of 35 °C and relative humidity of 95% for 16 h. The germinated paddy rice grains were dried in a circulated air oven at 50 ºC overnight, then husk and pericarp were removed with help of a rice polisher machine model MT-97 n° 3788-5 (Suzuki, Santa Cruz do Rio Pardo-SP, Brazil) for 2 min and then ground in an M 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) fit with a 0.8 mm sieve aperture obtaining a flour that was stored in a freezer until further analyses. Thus, the samples were denominated as non-germinated brown rice (NGBR); non-germinated polished rice (NGPR); non-germinated husk + bran (NGH+B), germinated brown rice (GBR), germinated polished rice (GPR) and germinated husk + bran (GH+B).

2.4. Physical characterization

2.4.1. Physical properties

The falling number (FN) was determined according to method 56-81.04 (AACC, 2010) and paste viscosity properties were carried out using a Rapid Visco Analyzer series 4 (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia) according to method 76-21.01 (AACC, 2010).

2.3.2. Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analyzes was conducted using a Q200 (TA Instruments, New Castle, USA) according to Bernardo et al. (2018). The samples (~2 mg), dry basis, were accurately weighed and transferred to hermetic aluminum pans added of deionized water (2:1). Then, the pans were sealed and allowed to rest for 18 h at room temperature before performing the experiments. Scan was done from 5 to 120 °C at a rate of 10 °C/min. An empty pan was used as reference. DSC data were analyzed to calculate onset (To), peak (Tp), conclusion (Tc) and enthalpy of gelatinization (ΔH).

2.5. Free and bound phenolic compounds extraction

Phenolic compounds (PC) extraction was performed in triplicate according to Santos et al. (2019) with modifications. Free PC (FPC, soluble) was extracted using 70 mg of rice flour added of 50 mg of celite and 1 mL of 80% ethanol. Samples were stirred in a shaker at 25 °C (200 rpm, 10 min) (TE420, Tecnal, Brazil) and centrifuged (5,000 × *g*, 25 °C, 10 min) on a Heraeus Megafuge 16R (Thermo Fisher Scientific, Karlsruhe, Germany). The supernatant was removed and placed in a new falcon flask. Extraction was performed twice and the supernatants extracts obtained were pooled together. The pellets resulted were submitted to a sequential hydrolysis (alkaline and acid) according to the protocol to extraction of bound PC (BPC, insoluble) established by Santos et al. (2022). Both extracts obtained (free and bound) were evaporated without temperature (SpeedVac Savant, ThermoFisher Scientific, USA) and reconstituted in 1.5 mL of methanol, acetonitrile, and ultrapure water (MilliQ, Millipore, Darmstadt, Germany) (2:5:93, v/v/v). The reconstituted extracts were filtered (13 mm, 0.22 μm, hydrophilic PTFE) (Analítica) and stored in vials at − 80 °C.

2.6. Folin–Ciocalteu reducing capacity and determination of antioxidant activity

The total reducing capacity of Folin-Ciocalteu reagent was determined according to Singleton et al. (1999) adapted for 96-well microplates Santos et al. (2019). The absorbance readings at 750 nm were performed on a microplate reader Flex Station III (Molecular Devices, USA). Gallic acid was used as standard for the calibration curve (5–130 μg/mL; R^2 = 0.9953) and results were expressed as mg gallic acid equivalents (mg GAE/100 g of sample, in dry basis).

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the method reported by Brand-Williams et al., (1995) The absorbance was measured using a microplate reader at 517 nm.Trolox was used as a standard for the calibration curve (30-150 μg/mL; R^2 = 0.9951) and results were expressed as μmol of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (μmol TE/100 g of sample, dry basis). All samples were analyzed in triplicate.

2.7 HPLC analysis for total and free amino acid and phenolic profile quantification

2.7.1.Separation and quantification of amino acids by acid hydrolysis

For the separation and quantification of the main amino acids in food proteins, the methodology described by Pacheco(2014) and the acid hydrolysis step by method 994.12 (AOAC, 2000) were used. Then, a 50 μl aliquot was removed and placed in a vialtobedried in a vacuum desiccator for 24 h with subsequent derivatization reaction (Cohen & Michaud, 1993). Chromatographic analysis was performed by using a Waters® Alliance model 2690/5 high-performance liquid chromatographic system, with a fluorescence detector (λexc = 250 nm and λem = 395 nm) and Empower® software (2002) (Waters Corporation, Milford, USA), Thermo BDS HYPERSIL C18 column (100 × 4.6 mm; 2.4 μm) (Thermo Fisher Scientific, Waltham, USA) at 37 °C, and elution in gradient mode with AccQTag® (Waters Corporation, Milford, USA); water (1:10; v/v) (Phase A), acetonitrile (Phase B), and water (Phase C) with a flow of 1.0 mL/min. The injection volume was 5 μl and the running time was 45 min with a delay of 10 min. Quantification was performed by external standardization with the elaboration of an analytical curve by using standards (Amino Acid Standard H, Ref.

WAT088122, Waters Corporation, Milford, USA) also submitted to the derivatization step with 6-amino quinolyl-succimidyl-carbamate (AQC) (Waters Corporation, Milford, USA).

2.7.2. Separation and quantification of free amino acids and GABA

In order to determine free amino acids and γ-aminobutyric acid (GABA) (Ref. 03835, Sigma Aldrich, St. Louis, USA), contained in rice, an acid extraction step and subsequent derivatization with 6- aminoquinolyl-succimidyl-carbamate (AQC) (Waters Corporation, Milford, USA) were performed according to Oliveira et al. (2022). For the acid extraction step, 1 g of the sample was weighed into a 50 ml screw-cap tube, 10 ml of 0.1 M HCl was added, with vortex for 1 min, and then extracted in ultrasound for 10 min, with subsequent centrifugation at 6000 rpm for 10 min and filtration through a 0.45 μm filter. After that, it was performed the derivatization reaction, where 60 μl of borate buffer (AccQ-Fluor Borate Buffer) (Waters Corporation, Milford, USA) was added to 20 μl of extract and there action was performed as already described above. Chromatographic analysis was also performed likewise earlier, with a flow of 0.8 ml/min and a shorter elution gradient with a final run time of 41 min.

2.7.3 Identification and quantification of phenolic compounds by HPLC-DAD

PCs were quantified in a HPLC system Flexar (Perkin Elmer, Massachusetts, USA) equipped with an automatic injector and analyzed through a PDA detector. The profile analysis of PC was performed according to Gomes & Torres (2016) with modifications. Separation of compounds was performed with a 100-5-C18 reverse phase column 4.6 × 250 mm (Kromasil), held at 40 °C. Sample injection volume was ram 20 μL, with a flow rate of 0.8 mL/min, using the mobile phase gradient A (water ultrapure containing 0.3% formic acid), B (100% methanol) and C (100% acetonitrile): 0.0 min -85% A; 14.5% B; 0.5% C; 7.0 min – 55% A; 43.5% B; 1.5% C; 14.0 min – 5% A; 93% B; 2% C; 20 min – 1% A; 97% B; 2% C; 23 min – 15% A; 83% B; 2% C; 23 to 33 min - 85% A; 14.5% B; 0.5% C. The HPLC analysis was performed at 260, 280 and 320 nm and the determination of the PC was according to the retention times and PDA spectra (λ from 230 to 350 nm) compared to commercial standards. Quantitative analysis was performed according to external calibration curves with each corresponding standard at 1.0; 5.0; 7.5; 10.0 and 20.0 ppm. In total, 23

standards were used to identify and quantify the phenolic compounds in the samples, gallic acid, pyrogallol, 4-hydroxybenzyl alcohol, catechin, chlorogenic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, caffeic acid, epicatechin, vanillic acid, syringic acid, vanillin, *p*-coumaric acid, *trans*-ferulic acid, rutin, 4-methoxycinnamic acid, myricetin, 2-hydroxycinnamic acid, *p*-anisic acid, quercetin, trans-cinnamic acid, 3-methoxycinnamic acid and flavanone. All analyses were performed in triplicate, and concentrations of individual phenolic compounds were expressed in mg kg^{-1}.

2.9. Metabolomic analysis of rice phenolic profile by UHPLC-QTOF-MS

The analysis of rice phenolic profile was performed on an UPLC Acquity system (Waters Co., Milford, MA) coupled to XEVO G2S Q-TOF (Waters Co., Manchester, UK) equipped with an ionization source electrospray using the analytical conditions previously described (Maia et al., 2020). The chromatographic separation was performed using an UPLC HSS T3 C18 column (100 × 2.1 mm, 1.8 µm particle diameter; Waters) set at 30 °C and flow rate of 0.5 L/min of the mobile phases (A: ultrapure water containing 0.3% formic acid and 5 mM ammonium formate, and B: acetonitrile containing 0.3% formic acid). The injection volume was 2 µL. Data were acquired in MS^E negative and centroid mode between *m/z* 50 and 1200 and collision energy ramp of 20-45 V. In addition, pooled quality control samples (QC-samples) containing an aliquot of all extracts were injected at every 9 sample injections. The QC-samples and mixed solution of 33 standards of PC were analyzed at the same conditions of the samples. The raw data processing was performed using the software Progenesis QI following parameters such as: isotopic distribution of neutral mass, exact mass, retention time and MS/MS fragments spectra. The annotation was carried out with a custom data bank built from PubChem and Phenol-Explorer 3.6 (http://phenol-explorer.eu/) databases. The following parameters were also applied: exact mass error (<10 ppm); isotopic similarity (>80%); score (>30) and highest fragmentation score, all generated by the software. In addition, only PC present in the three technical replicates (3/3) and presenting CV < 30% were considered as tentatively identified. Comparison of MS/MS experimental spectra with data found in NORMAN MassBank (https://massbank.eu/MassBank) and MassBank of North America (https://mona.fiehnlab.ucdavis.edu), Phenol-Explorer database, data from the literature, and chemical characteristicswere also used to help the

annotation of PC, following the levels of identification described by (Schrimpe-Rutledge, 2016).

2.10. Statistical analysis

Statistical analysis was performed with Tukey's test ($p < 0.05$) and one-way ANOVA, using XLSTAT software (Addinsoft, Paris, France). Metabolomic data generated were exported to perform the multivariate statistical analysis; principal component analysis (PCA) by XLSTAT (Addinsoft, Paris, France); the orthogonal partial least-squares discriminate analysis (OPLS-DA) and S-plot by EZinfo 3.0 (Waters Corporation, Milford, USA).

3. Results and discussion

3.1. Physical properties

3.1.1. α-amylase activity

The falling number (FN) value can be used as an indicator of α-amylase activity during germination time that decreases caused by an increase in α-amylase activity (Feng et al., 2019). According to the results (Table 1), FN values ranged from 371s (NGPR) to 327s (GBR) and did not shown a statistical difference between all samples ($p> 0.05$). Wang et al. (2020) reported that levels of α-amylase after 48h of germination with different temperature (25°C to 35°C), ranging from 0.04 U/g to 3.19 U/g. In our study, short germination time (< 24h) did not induce the α-amylase activation in the *BRS Catiana* cultivar.

Table 1. Physical properties of rice before and after germination and polishing.

Sample	Falling Number (s)	DSC			
		T_o	T_p	T_c	ΔH (J/g)
NGBR	369.00±27.87[a]	58.20±0.82[a]	65.66±0.39[a]	78.45±1.06[a]	8.66±0.71[a]
NGPR	371.00±34.93[a]	57.61±0.75[a]	64.83±0.08[a]	78.04±1.09[a]	9.19±2.4[a]
GBR	327.00±3.00[a]	57.97±0.55[a]	65.05±0.09[a]	78.62±0.59[a]	7.97±0.55[a]
GPR	357.00±1.73[a]	58.30±1.71[a]	65.46±0.57[a]	81.15±0.05[a]	9.72±0.06[a]

T_o= Temperature onset, T_p= Peak temperature, T_c= Conclusion temperature, ΔH= Enthalpy. Data are means ± SD (n = 3). Where: NGBR= Non-germinated brown rice; NGPR= Non-germinated polished rice; GBR= Germinated brown rice and GPR= Germinated polished rice.

The α-amylase is not activated in the beginning of the germination process and increases progressively. In the initial of germination, β-amylases provide the energy for the plants by hydrolysis of sucrose and maltose (Palmiano & Juliano, 1972). Some parameters such as rice cultivar, protein, lipid and ash content, starch gelatinization temperatures, endogenous gibberellin, time (> 96 h), temperature (30-35°C), and amylose-amylopectin ratio (A-AR) can affect the α-amylase activity under germination conditions (Kalita et al., 2017; Wunthunyarat et al., 2019).

Another hypothesis that can explain our result is the inhibitory activity of a different polyphenols (mainly flavonoids, phenolic acids and tannins) against α-amylase (Giuberti et al., 2020). The little/no activation of α-amylase during the germination process can be interesting for the processing and technological development of some products with better nutritional and sensory characteristics, such as gluten-free pasta and breads. For pasta, limiting amylases activity may be helpful for cooking-up quality and proper dough structure of pasta after extrusion (Finnie et al., 2019) and for bread, the intense action of α amylase (long process > 48 h) could result in excessive liquefaction and dextrinization, causing inferior bread quality (Cornejo & Rosell, 2015).

3.1.2. Pasting properties

Germination can affect the pasting viscosity of rice. The process induces disruption of the starch granules, leading to the appearance of pores and pits on the granule surface, disrupting crystallites and reducing molecular ordered degree (Xu et al., 2021). The pasting

curves are presented in Fig. 1 and pasting parameters are shown in Table S2. The initial pasting temperature (PT) ranged from 72.9 to 77.4°C. There was no difference ($p > 0.05$) in PT between the samples, which is in agreement with the DSC results (Table 1). As shown in Fig. 1, pasting profiles of non-germinated, germinated and polished rice exhibited a similar form. The short germination process caused a little effect in rice pasting properties.

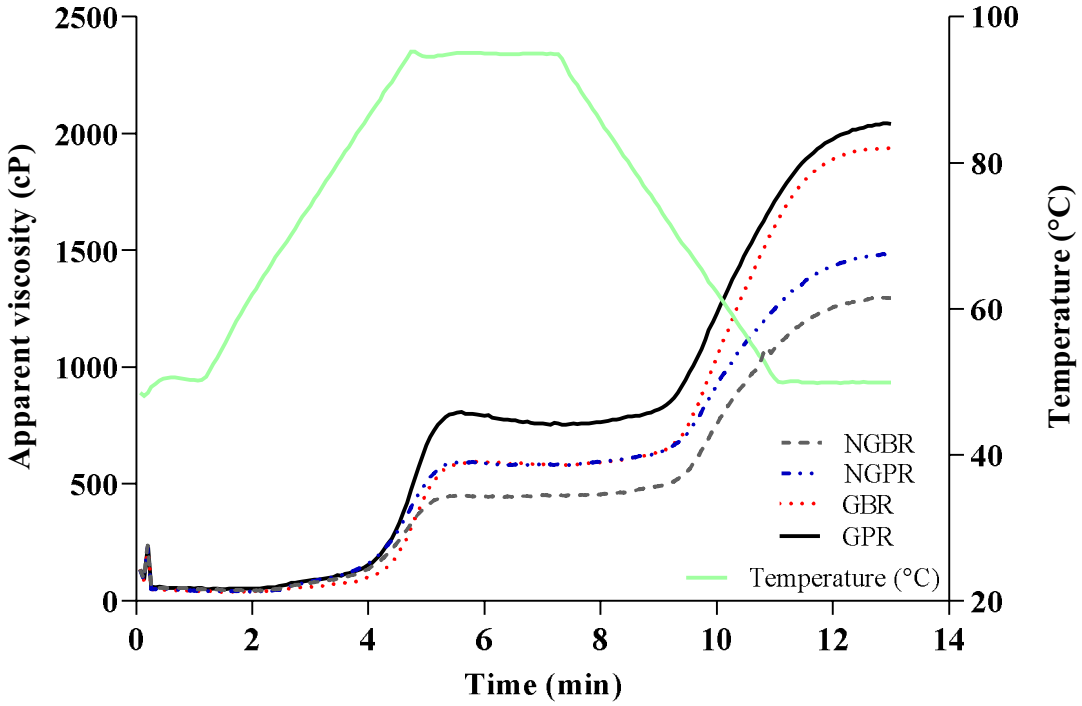


Figure 1. Viscoamylographic properties (RVA) of rice before and after germination and polishing.

The germinated samples had higher values of peak viscosity (808.5 cP), breakdown (334.5 cP), final viscosity (2028.0 cP) and setback (1554.0 cP). Our results are different from the ones found by Li et al.(2020) and Xu et al. (2021) that observed a decrement trend in viscosity after germination which may be attributed to higher synthesis of amylases that break down the starch producing smaller portions as dextrins, maltose and glucose and decrease of protein content by proteases (Capanzana & Buckle, 1997).

Polishing affected the pasting values of germinated rice more than non-germinated rice and increased the viscosity of GPR and NGPR. The effect of polishing in the grains

depends of rice variety and starch composition. Polishing can decrease the viscosity of waxy rice and increase high amylose rice (Xu et al., 2021). Rice with high amylose content (such as *BRS Catiana*) was more sensitive to polishing. Polishing decrease the crystallinity causing changes in physicochemical, rheological and pasting properties (Xu et al., 2021). There was no statistical difference ($p< 0.05$) between brown and polished germinated rice excepted for BDV (Table S2).

3.1.3. Gelatinization properties

The values obtained by DSC are presented in Table 1. Unexpected, the gelatinization temperatures (T_0, T_p, T_c) and enthalpy were not affected either by germination or polishing, as well the falling number values ($p < 0.05$). Significant changes in gelatinization properties occurred 24h after germination and intensify with the extension of the process. T_0, T_p, and T_c increase and enthalpy decreases slightly (Wu et al., 2013). Our results of gelatinization properties corroborate to those found in FN and in the pasting properties by RVA, in which both germination and polishing not affected the viscosity profile (Fig. 1).

The enzymatic activity of α-amylase during germination is responsible for the erosion of starch granules, the decreases the relative crystallinity and the short-range ordered degree, and the unfolding of the double helix structure (Wang et al. 2020), consequently, less energy is required to unravel and melt double helices of starch in germinated flours (Li et al., 2020). According to Kalita et al. (2017), amylose-amylopectin range (A-AR) plays an important role during germination. Rice genotype with intermediate amylose (20.2%) showed higher enzyme activity, starch degradation and loss percent of crystallinity when compared to low amylose ones (12.5%).

The first hypothesis for the findings in DSC results is the interaction between starch and PC, although this phenomenon is little explored in the literature. PC are increased during the germination process, forming specific non-covalent bonds via hydrogen bonds and hydrophobic interactions that can modify the gelatinization properties and digestibility of starch (Giuberti et al., 2020) due to the development of type 5 resistant starch (RS5) (Qadir & Wani, 2022). The complex formed by the interaction with the starch-polyphenol have a similar crystalline starch-lipid-like arrangement forming V-type complexes, which are well

recognized as type 5 resistant starch (RS5) (Romero Hernández et al., 2022). RS5 is produced by the intentional rearrangement of starch molecules e.g. starch-lipid complex by gut microbiota fermentation that promoting the formation of short-chain fatty acids (SCFA) (Bojarczuk, Sk, et al., 2022).

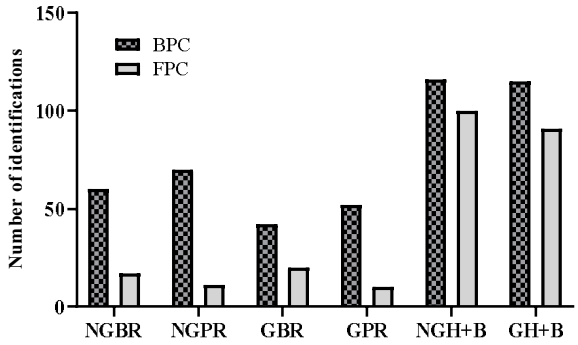
The other hypothesis is the interactions between starch and protein/lipid during germination. The lipids and proteins present in the endosperm forming a coating of the starch surface, resulting in restriction of granular swelling (Li et al., 2020; Wu et al., 2013) that reduces the rice starch digestion. Since rice is basically composed by starch, which is transformed into glucose that is quickly absorbed during digestion (Jukanti et al., 2020), germination is an alternative to change the starch digestibility due to modifications in the starch matrix that could help the food industry to develop rice products with desirable starch digestibility for control the blood glucose level (Cheng et al., 2022).

3.2 Phenolic profile of rice samples

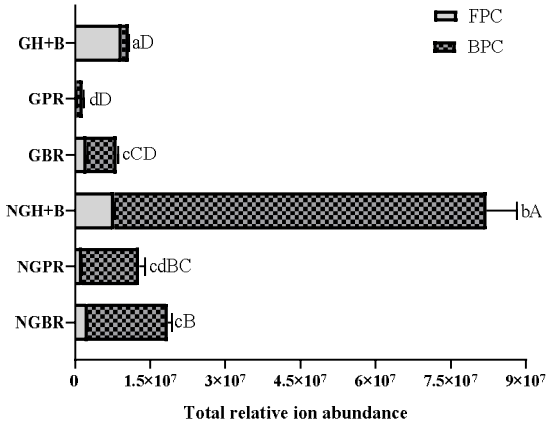
To obtain a comprehensive characterization of the phenolic profile for rice samples, as well as to better understand the effect of germination and polishing on this profile, samples were submitted to an untargeted metabolomics analysis by UHPLC-QTOF approach. Globally, 117 PC were tentatively identified in this work, including isomers, most of them belonged to bound (111) than free (104) extract (Fig. 2A). All of these compounds as well as the parameters used for annotation are showed in Table S3 (Supplementary Material). Twenty-one PC were commonly identified in both extracts, such as *trans*-ferulic and *p*-coumaric acids. The PC in the bound extract were more abundant than in the free PC (Fig. 2B).

Six PC were fully confirmed by reference standards (Table S3, compounds in bold), including gallic, sinapic and vanillic acids. In addition, 13 compounds were detected presenting the same identification parameters such as molecular weight, chemical formula and MS/MS fragments, but eluted at different retention times, suggesting the existence of isomers. This result shows the ability of metabolomic tools to detect and identify compounds with the same *m/z* but which interact in different ways with the stationary and mobile phases.

(A)



(B)



(C)

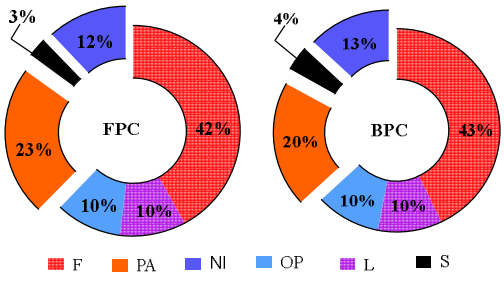


Figure 2. Metabolomic analysis: (A) number of identification of each sample; (B) total relative ion abundance of phenolic compounds; (C) distribution of phenolic classes in free (FPC) and bound (BPC) extracts. F: flavonoids; L: lignans; OP: other polyphenols; PA: phenolic acids; S: stilbenes; NI: non-identified. Different lowercase and uppercase letters mean a significant difference ($p < 0.05$) between free and bound extract samples, respectively. Error bars represent standard deviation (n=3).

The compounds belonging to the flavonoid subclass were identified in highest number (42%), followed by phenolic acids (20%), other polyphenols (11%), lignans (10%), and stilbenes (4%). Also, flavonoids were identified in higher number for both extracts, as shown in Fig. 2C. Although it was not possible to confirm the identity of 14% of these compound, it was possible to classify these unknown compounds into flavonoids and phenolic acids derivatives based on their MS/MS profile (Table S3). The abundance of these subclasses also changes according to the extract, as shown in Fig. 2C.

Flavonoids are a class of phenolic compounds responsible for various metabolic functions in plant tissues, generally classified into seven subclasses: flavonols, flavones, isoflavones, anthocyanidins, flavanones, flavanols and chalcones. They have three rings (C6-C3-C6) and their bioactivity depend on the arrangement of hydroxyl, methoxy, and glycosidic side groups on the basic skeleton (Shen et al., 2022). Flavonoids are important for human health due to their well-known antioxidant effects. However, in recent years, scientific literature has shown that in addition to this action, these compounds also have antimicrobial effects and interactions with human gut microbiota (Wang et al., 2019).

The gut microbiota plays an important role in the human immune system. It is influenced by intrinsic factors such as genetics and by extrinsic factors such as lifestyle and diet. Changes in its composition can lead to various health problems such as gastrointestinal, hepatic, metabolic, immune-related, oncologic, neurological and psychiatric diseases (Bié et al., 2023). More studies are needed to understand how flavonoids interact with the gut microbiota, nevertheless, studies suggest that flavonoids participate in the catabolism of the gut microbiota (Li et al., 2023).

The digestion and absorption of flavonoids in the human body starts in the mouth, in which are partly deconjugated followed by a digestion and absorption in stomach. In the small intestine and colon occur the degradation to small phenolic compounds, absorption and enzymatic reactions (deglycosylation, methylation, glucoronidation, demethylation and conjugation) and transported to the liver. After that, the metabolites are transported to target cells and tissues, as well as excreted to bile for enterohepatic re-circulation (Shen et al., 2022). The mechanism of action is not yet known, but flavonoids have a prebiotic effect since

they may help the growth of *Bifidobacterium* and *Lactobacillus*, decreasing the pathogenic groups such as *Clostridium/Enterobacter* and *Bacteroides* (Owolabi et al., 2020).

Phenolic acids were the main compounds identified in samples, representing, in average, 81% of total relative abundance. Among them, the phenolic acids *p*-coumaric, ferulic and *trans*-ferulic made up the list of the major compounds in all samples (Table S3). Also, the bound fraction was more abundant than the free ones in all samples, with phenolic acids as the majority (Fig 2B). The germination process resulted in the 55% reduction of the total relative abundance of PC when the germinated sample (GBR) is compared to its control (NGBR).

Short time germination (< 24 h) did not increase the total relative abundance of PC in rice samples. However, despite the short time, some changes in the abundance of some compounds, for example, slightly reducing the abundance of flavonoids and bound lignans, as well as appear to have influenced the increase in the abundance of some free flavonoids. After polishing, the samples showed a decrease in free and bound PC (Fig. 2B) corroborating previous works that demonstrated that most PC are located in the outer portion (bran) of the rice grain, rarely distributed in rice endosperm (Ding et al., 2018a).

Multivariate analyses were performed to verify the similarities and differences among samples (Fig 3). For both analyses were considered all compounds tentatively identified as well as all extracts of samples. PC1 and PC2 for the free and bound extracts explained more than 79% of variance, suggesting that the content of phenolic compounds is significantly different between the rice samples. OPLS-DA (Fig. S1) was performed to identify the compounds that could distinguish samples, where the S-plot format was chosen to shown the results. Five models were built to compare the polishing and the germination effects.

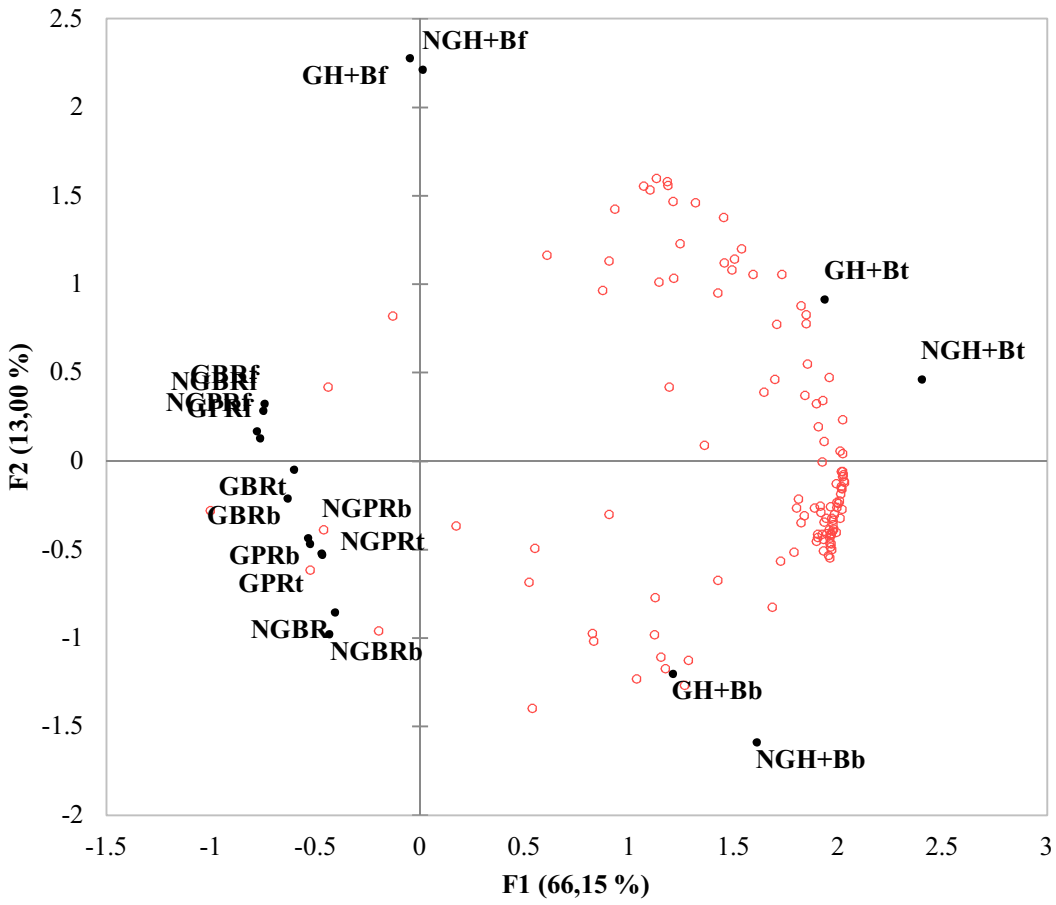


Figure 3. Principal component analysis (PCA) biplot of rice samples in free, bound and total extracts. The samples (symbols) are distributed according to relative intensity of identified phenolic compounds (red circles). Where: t= total phenolic compunds; b= bound phenolic compounds and f= free phenolic compounds

Knowing that there are many parameters that can influence the phenolic composition, such as the enzymes activity, genotype, different soaking, temperature, adjuvants process and germination time some studies have shown that the synthesis/hydrolysis of PC begins to increase 24 hours after the beginning of germination (long process) (Chu et al., 2020; Yodpitak et al., 2019). Changes in the composition of the phenolic profiles do not only occur during germination, but also during soaking. So, one hypothesis to explain the decrease of these compounds after germination on our work is the leaching during the rice soaking phase (Owolabi et al., 2018) and the conversion into other phenolics by polyphenol oxidase and complex with proteins (Rousseau et al., 2020).

3.3. Total phenolic content and antioxidant activity

The total phenolic content (TPC) was estimated by measuring the reduction capacity of the extracts. The results are shown in Table 2. There were significant differences ($p < 0.05$) for TPC content between samples. The averaged TPC was 262.69 mg GAE/100 g and ranged from 68.66 ± 3.50 to 530.58 mg GAE/100 while the antioxidant activity against DPPH ranged from 434.84 ± 25.73 to 631.82 ± 60.43 μmol TE/100g of sample. Analyzing free and bound extracts showed that bound extract generally had 38% more phenolic content than free extract ($p < 0.05$). In rice, the distribution and concentration of PC depends on several factors, such as (i) ecotype: *Japonica* rice has significant higher PC and antioxidant activity than *Indica* rice; (ii) genotype; (iii) portion of the grain: bran and husk provided more than 90% of PC and (iv) process: polishing cause a decrease in PC (Ding, et al., 2018a; Pang et al., 2018).

Table 2. Phenolic content and antioxidant activities determined in rice extracts.

Sample	**Folin-Ciocalteu (mg GAE/100 g db)**			**DPPH (μmol TE/100 g db)**		
	Free	Bound	Total	Free	Bound	Total
NGBR	76.86±2.08^{a}	90.37±1.08^{bc}	167.22±3.17^{b}	233.50±35.21^{a}	249.91±22.24^{c}	483.42±47.47^{c}
NGPR	48.16±1.29^{b}	53.06±1.71^{cd}	100.31±2.99^{c}	203.52±14.52^{ab}	277.84±14.15^{bc}	481.36±28.66^{c}
NGH+B	78.03±6.13^{a}	452.55±22.50^{a}	530.58±28.40^{a}	265.39±11.43^{a}	349.91±31.71^{b}	629.12±27.79^{ab}
GBR	68.65±1.86^{a}	110.92±2.45^{b}	179.57±4.45^{b}	229.28±13.89^{a}	308.26±41.22^{bc}	537.55±55.12^{bc}
GPR	38.74±0.74^{b}	29.92±2.22^{d}	68.66±3.50^{c}	151.04±21.02^{b}	283.80±4.65^{bc}	434.84±25.73^{c}
GH+B	73.36±6.80^{a}	464.67±33.10^{a}	529.80±29.09^{a}	265.06±38.63^{a}	444.84±29.26^{a}	709.90±67.89^{a}

Different letters mean a significant difference ($p < 0.05$) in the same column between the same extracts. db (dry basis), GAE (gallic acid equivalent), DPPH (2-diphenyl-1-picrylhydrazyl), TE (Trolox equivalent). Where: NGBR= Non-germinated brown rice; NGPR= Non-germinated polished rice; NGH+B= Non-germinated husk and bran; GBR= Germinated brown rice and GPR= Germinated polished rice.

The increase in TPC during germination may be related to the activation of two enzymes as (i) PAL (phenylalanine ammonia-lyase) and (ii) CW-PRX (cell wall peroxidase). PAL is responsible for catalyzing free phenolic acids, mainly in the shoot fraction, after 2 days after germination and CW-PRX induced the formation of bound phenolic acids and lignin, notably in soaking (Cho & Lim, 2018).

In this work, germination caused a 10% decrease in FPC and 18% in BPC. The reduction in free and bound contents was greater in the GPR (44%) than in the NGPR (20%) sample. But, there was no statistical difference between NGH+B and GH+B ($P< 0.05$). This decrease can be explained due to (i) polishing process and (ii) leaching of free compounds during the immersion step as mentioned above. Contrary to these findings, Munarko and Sitanggang (2021) reported that TPC decreased after soaking (24 h) but increased after 24 h of germination. The TPC significantly increased reaching higher values between 36 and 72 h (longer germination). In glutinous rice (> 98% of amylopectin of total starch), the increase of TPC occurred after 48 h of germination from 3430 mg/kg to 5370 mg/kg (Wang et al., 2022).

According to Ti et al. (2014) the increase of FPC content coincided with a decline in BPC, although not up to the same extension, which may indicate that the increased FPC may be due to (i) the liberation of the bound phenolics (some enzymes that are synthesized to degrade storage macromolecules could liberate bound phenolics); and (ii) the synthesis of new phenolics in response to the germination treatment. During germination process, a direct opposite trend detected in both free and bound forms. The increase of the bound phenolics may be explained by the polymerization and oxidation of phenolics, and by certain changes in enzymes involved in the synthesis and degradation of FPC or BPC.

3.6 Identification and quantification of phenolic compounds by HPLC-DAD

This work identified and quantified 17 PC through commercial standards, which are shown in the Table 3. 9 PC were identified in NGBR with catechin (1.19 mg/g) being the compound with the highest content in the free extract and *trans*-ferulic acid (1.33 mg/g) in the bound. *Trans*-ferulic acid was identified in all samples in both the free and bound fractions. NGH+B showed the major PC on bound form (10 PC).

GBR showed 59% more free PC and reduced bound PC by 5%. There was a loss of two compounds in the free fraction after germination: 4-methoxycinnamic acid and *p*-coumaric acid, both from the hydroxycinnamic acid class. Also, there was the synthesis of a flavonoid (flavonone) after germination. In the bound fraction, in addition to flavonone, there was also synthesis of quercetin and vanillin. The hypothesis is that germination seems to promote greater degradation of phenolic acids and flavonoid synthesis.

Table 3. Individual phenolic compounds identified in the ethanol extracts of rice by HPLC-DAD.

Compound identified	RT (min)	UV λmax (nm)	Molecular formula	*m*	ID PubChem	Free (mg/g)						Bound (mg/g)					
						NGBR	NGPR	NGH+B	GBR	GPR	GH+B	NGBR	NGPR	NGH+B	GBR	GPR	GH+B
3-methoxycinnamic acid	15.88	280	$C_{10}H_{10}O_3$	178.18	637668	0.08±0.02	-	-	1.58±2.21	0.41±0.28	-	0.71±0.53	-	-	0.24±0.07	-	-
4-hydroxybenzoic acid	6.612	320	$C_7H_8O_2$	124.14	125	-		-	-		-	-	-	0.05±0.01	-	-	0.05±0.03
4-methoxycinnamic acid	12.88	320	$C_{10}H_{10}O_3$	178.18	699414	0.22±0.17	-	-	-	-	-	0.60±0.41	0.47±0.37	0.86±0.18	0.26±0.19	0.21±0.17	0.83±0.49
Caffeic acid	9.817	320	$C_9H_8O_4$	180.16	689043	0.14±0.19	-	-	0.03±0.01	-	-	-	-	0.07±0.05	-	-	0.09±0.07
Gallic acid	3.5	260	$C_7H_6O_5$	170.12	370	-	-	0.05±0.02	-	-	-	-	-	-	-	-	-
p-Coumaric acid	11.694	320	$C_9H_8O_3$	164.16	637542	0.08±0.02	-	0.33±0.01	-	0.02±0.02	0.01±0.00	0.18±0.11	0.09±0.01	7.30±038	0.13±0.04	0.03±0.00	7.16±2.15
Syringic acid	10.367	280	$C_9H_{10}O_5$	198.17	10742	-	-	0.04±0.06	-	-	-	-	-	1.84±0.23	-	-	1.70±0.70
trans-Cinnamic acid	15.794	280	$C_9H_8O_2$	148.16	444539	-	0.27±0.33	-	-	-	-	-	-	-	-	-	-
trans-Ferulic acid	12.323	320	$C_{10}H_{10}O_4$	194.18	445858	0.20±0.14	0.14±0.04	0.19±0.12	0.18±0.08	0.07±0.00	0.14±0.00	1.33±0.44	1.04±0.01	2.12±0.30	0.89±0.32	0.57±0.02	2.08±0.78
Vanillic acid	9,986	280	$C_8H_8O_4$	168.15	8468	-	-	-	-	-	-	-	-	0.10±0.01	-	-	0.11±0.05
4-hydroxybenzyl alcohol	6.577	280	$C_7H_8O_2$	124.12	125	-	-	-	-	-	1.25±0.09	-	-	0.28±0.16	-	-	0.12±0.06
Catechin	7.576	280	$C_{15}H_{14}O_6$	290.27	9064	1.19±0.48	-	-	0.91±0.44	0.56±0.43	-	-	-	-	-	-	-
Flavanone	18.39	260	$C_{15}H_{12}O_2$	224.25	10251	-	-	-	0.38±0.32	-	0.14±0.02	-	-	.-	0.25±0.07	-	-
Pyrogallol	4.3	260	$C_6H_6O_3$	126.11	1057	-	-	-	-	-	-	-	-	0.23±0.05	-	-	-
Quercetin	15.08	260	$C_{15}H_{10}O_7$	302.23	5280343	-	-	-	-	-	-	-	0.27±0.19	-	0.63±0.45	-	-
Rutin	12.506	260	$C_{27}H_{30}O_{16}$	610.5	5280805	-	-	0.22±0.01	-		-	-	-	-		-	-
Vanillin	11.041	280	$C_8H_8O_3$	152.15	1183	-	-	0.17±0.15	-	-	0.01±0.00	-	-	0.65±0.24	0.25±0.19	-	0.54±0.25

RT = retention time; *m*= molecular mass. Identification of the compound was confirmed by the authentic standard. All other compounds were tentatively identified by comparing their UV and mass spectral characteristics with those reported in literature.

Table 4. Most abundant phenolic compounds identified.

Putative identification	Molecular formula	*m/z*	RT (min)	Score (%)	FS (%)	Main fragments (% relative intensity)	Mass Error (ppm)	IS (%)	Class
***p*-Coumaric acid**	**$C_9H_8O_3$**	**163.0387**	**7.44**	**53.5**	**78.2**	**119.0502 (100); 93.0345 (7.40); 117.0346 (3.27)**	**-8,28**	**98.69**	**PA**
***trans*-Ferulic acid**	**$C_{10}H_{10}O_4$**	**193.0491**	**8.00**	**46**	**40.7**	**134.0373 (100), 109.0295 (2.15); 106.0424 (10)**	**-7.95**	**97.97**	**PA**
Tricin/ Jaceosidin/ 3,7 Dimethylquercetin	$C_{17}H_{14}O_7$	329.0652	11.03	47.3	45.7	314.0431 (89.62); 161.0244 (20.78); 227.0350 (19.18); 243.0299 (7.62)	-4.37	95.87	F
Ferulic acid	$C_{10}H_{10}O_4$	193.0490	8.23	53.6	79.1	165.0557 (100); 149.0608 (23.30)	-8.27	98.00	PA
Phlorizine	$C_{21}H_{24}O_{10}$	435.1287	8.28	41.2	13.2	149.0608 (23.30)	-2.20	95.53	F
Rosmanol isomer	$C_{20}H_{26}O_5$	345.1700	12.09	49.7	55.3	345.1707 (70.86); 331.1914 (70.76); 268.1469 (41.81); 283.1703 (12.32); 301.1809 (7.29)	-2.13	95.95	OP
Caffeic acid	**$C_9H_8O_4$**	**179.0336**	**6.21**	**56.7**	**93.8**	**135.0451 (100)**	**-7.94**	**98.51**	**PA**
Eupatorin isomer I	$C_{18}H_{16}O_7$	343.0814	9.05	50.4	58.7	269.0455 (79.67); 181.0506 (68.46); 241.0506 (62.31); 284.0690 (48.06); 255.0663 (20.49); 299.0925 (19.24); 343.0823 (19.11)	-2.68	96.65	F
Resveratrol 3-*O*-glucoside	$C_{20}H_{22}O_8$	389.1236	8.73	51	63.6	93.0345 (60.86); 269.0819 (53.64)	-1.58	93.57	S
Pebrellin	$C_{19}H_{18}O_8$	373.0919	8.12	45.7	35.6	167.0350 (67.36); 357.0979 (32.36)	-2.52	95.81	F

m/z = mass/charge; RT = retention time; FS = fragmentation score; IS = isotope similarity; F = flavonoids; PA = phenolic acids; OP = other polyphenols; S = stilbenes. Bold represent reference standard

Some food processing as germination could increase the bioavailability and/or bioaccessibility of PC, specialty PC in the bound form that can be released by endogenous enzymes from the cereal matrices during the process (Wang et al., 2014).

Polishing caused a decrease in both FPC and BPC fractions and greater reduction of compounds in non-germinated than in germinated samples, indicating that these compounds are mostly located in the bran layer, as mentioned above. In polished rice, 5 PC were identified and quantified. The *p*-coumaric acid (0.02 mg/g) and *trans*-cinnamic acid (0.27 mg/g) were the only compounds found in the polished samples in FPC, showing that the polishing was able to release CF in the free fractions, by breaking chemical bonds between these metabolites and the cell wall (Cho & Lim, 2018).

3.7 Changes of amino acids profile after germination and polishing

Total amino acids (TAA) and free amino acids including GABA contents are presented in Table 5. A little increase in all contents of TAA after short germination was observed. In addition, no statistical difference ($p < 0.05$) was observed on TAA values after germination and polishing step. The TAA levels observed in rice ranged between 260-1900 mg/100g. Glutamine and arginine were the highest non-essential amino acids present in TAA (1865-1340 mg/100g) after germination, respectively, and leucine the highest essential amino acid (1005 mg/100g).

Both TAA and FAA presented a little increase in concentration, except for asparagine, glutamine and serine and alanine showed the highest value after germination. This result concurred with Komatsuzaki et al. (2007) that found a decrease in aspartic, serine, asparagine, and glutamic acid. These amino acids can be modified during soaking, in which glutamate decarboxylase (GAD) is activated and glutamic acid is converted to GABA. During plant metabolism of growing, a large amounts of metabolites are involved in amino acid pathways such as pantothenic acid, L-serine, L-proline, L-aspartic acid, L-glutamate, L-asparagine, glutathione (J. Zhu et al., 2022), the decarboxylation of glutamate (GABA shunt), the degradation of polyamine and the non-enzymatic conversion of proline into GABA (Lee et al., 2022).

Table 5. Amino acids profile (mg/100g) of rice before and after germination and polishing.

Amino Acids	Type	NGBR	NGPR	GBR	GPR
Asp	Total	875.00±63.63^{a}	870.00±35.35^{a}	925.00±148.49^{a}	925.00±84.85^{a}
	Free	17.29±0.62^{A}	7.27±0.33^{B}	7.02±0.17^{BC}	6.15±0.07^{C}
Ser	Total	555.00±63.63^{a}	580.00±49.49^{a}	655.00±120.20^{a}	615.00±91.92^{a}
	Free	46.93±2.16^{A}	14.13±0.25^{C}	23.13±2.56^{B}	27.37±1.16^{B}
Glu	Total	1725.00±120.20^{a}	1760.00±63.63^{a}	1865.00±304.05^{a}	1900.00±183.84^{a}
	Free	19.94±0.19^{A}	7.23±0.81^{C}	11.94±0.36^{B}	9.21±0.52^{BC}
Gly	Total	520.00±70.71^{a}	540.00±49.49^{a}	635.00±106.06^{a}	560.00±113.13^{a}
	Free	1.99±0.09^{AB}	1.10±0.00^{B}	2.77±0.42^{A}	3.21±0.33^{A}
His	Total	260.00±28.28^{a}	260.00±14.14^{a}	315.00±63.63^{a}	275.00±49.49^{a}
	Free	4.43±0.29^{BC}	2.18±0.03^{C}	7.27±0.60^{A}	4.43±0.36^{B}
Arg	Total	1140.00±98.99^{a}	1170.00±63.63^{a}	1340.00±254.55^{a}	1265.00±275.77^{a}
	Free	7.27±0.34^{A}	3.31±0.04^{B}	8.77±0.34^{A}	8.43±0.52^{A}
Thr	Total	385.00±63.63^{a}	400.00±42.42^{a}	455.00±77.78^{a}	410.00±70.71^{a}
	Free	2.62±0.16^{BC}	1.50±0.02^{C}	4.83±0.50^{A}	4.42±0.26^{A}
Ala	Total	135.00±35.35^{a}	160.00±42.42^{a}	180.00±28.28^{a}	150.00±56.56^{a}
	Free	5.49±0.02^{AB}	2.79±0.14^{B}	10.35±0.65^{A}	9.54±1.27^{A}
Pro	Total	500.00±28.28^{a}	510.00±14.14^{a}	640.00±113.13^{a}	560.00±113.13^{a}
	Free	2.40±0.31^{BC}	3.33±0.04^{A}	2.47±0.02^{B}	1.82±0.21^{C}
Tyr	Total	540.00±70.71^{a}	550.00±42.42^{a}	640.00±98.99^{a}	585.00±134.35^{a}
	Free	3.00±0.12^{B}	2.12±0.01^{C}	3.92±0.19^{A}	3.31±0.14^{AB}
Val	Total	510.00±42.42^{a}	510.00±21.21^{a}	600.00±113.13^{a}	550.00±70.71^{a}
	Free	3.12±0.10^{AB}	1.70±0.01^{B}	5.40±0.42^{A}	4.42±0.52^{A}
Lys	Total	375.00±21.21^{a}	370.00±14.14^{a}	390.00±127.27^{a}	400.00±28.28^{a}
	Free	5.72±0.00^{AB}	2.01±0.25^{B}	8.17±0.55^{A}	8.59±0.98^{A}
Ile	Total	360.00±28.28^{a}	360.00±7.07^{a}	430.00±84.85^{a}	400.00±42.42^{a}
	Free	2.13±0.04^{AB}	1.49±0.01^{B}	3.26±0.19^{A}	2.73±0.28^{A}
Leu	Total	855.00±63.63^{a}	870.00±35.35^{a}	1005.00±205.06^{a}	950.00±113.13^{a}
	Free	2.54±0.07^{BC}	1.99±0.03^{C}	4.80±0.04^{A}	3.80±0.41^{AB}
Phe	Total	630.00±84.85^{a}	660.00±56.56^{a}	760.00±127.27^{a}	690.00±141.42^{a}
	Free	2.33±0.11^{BC}	1.73±0.01^{C}	3.44±0.16^{A}	2.85±0.18^{AB}
GABA		4.14±0.09^{BC}	2.80±0.14^{C}	10.44±0.98^{AB}	12.21±1.95^{A}

Different lower case and uppercase letters mean a significant difference ($p < 0.05$) between total and free amino acid profile, respectively Data are means ± SD (n = 3).

Despite the short germination time, there was an increase in GABA by 60% (4.14 to 10.44 mg/100g) in GBR and 77% (2.80 to 12.21 mg/100g) in GPR. The impact of polishing was more pronounced in non-germinated than germinated samples. There was no statistical difference between GBR and GPR samples ($p > 0.05$) but a decrease by 32% in non-germinated samples was noticed. Longer germination time (72-96 h) produced higher amino acid content. The concentration of TAA, FAA and GABA increased between 24–48

h as much as the enzyme activity increased breakdowns and hydrolyzed endosperm. The amino acids were concentrated in the embryo region at the beginning of germination (0–24 h), and increased and spread into the endosperm and aleurone layer away from the germ after 72–96 h (Kamjijam et al., 2020).

Due to the short period of time, the non-activation of the enzyme may be related to the small increase in these amino acids, it seems to be the same trend as observed by FN values. The accumulation of amino acids in rice is a complicated process involving a complex of biochemical networks and control mechanisms, most of which remain unclear (Kamara et al., 2010). Most studies involving rice germination and amino acids profile are in long processes (> 24 h). But, longer germination time obtained higher GABA content but also affected the palatability of rice. Our findings on GABA content were different from those found by Kamjijam et al. (2020) of 31.36 and 38.75 mg/100g of GABA in KDML 105 and Riceberry varieties, respectively and higher than Müller et al. (2021) (1.86 mg/100 g) after 40 h germination.

4. Conclusion

The present research showed that short germination time (< 24 h) did not cause enzyme activation or changes in physical properties of high amylose rice. Despite the short time, the process was effective in improving the phenolic compounds, antioxidant capacity and amino acids profile of the studied cultivar. Polishing caused a decrease in phenolic compounds, amino acids and GABA, but this reduction was more pronounced in non-germinated rice than in germinated rice. These results may contribute to the understanding the short germination process and provide the information for the utilization of husk, GBR and GPR as functional food ingredients development for promoting better health.

CRediT authorship contribution statement

Maria Eugenia Araujo Silva Oliveira: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing **Luciana Ribeiro da Silva Lima:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Millena Cristina Barros Santos:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Nathalia Ferrari Fonseca de Sales:** Conceptualization, Formal analysis, Investigation,

Writing – original draft. **Renata Marenda Ferreira:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Luiz Claudio Cameron**: Resources, Supervision. **José Manoel Colombari Filho**: Project administration, Resources. **Priscila Zaczuk Bassinello:** Project administration, Resources, Writing – review & editing. **Carlos Wanderlei Piler de Carvalho:** Conceptualization, Writing – review & editing, Supervision, Project administration, Resources. **Mariana Simões Larraz Ferreira:** Conceptualization, Writing – review & editing, Supervision, Project administration, Resources. **Cristina Yoshie Takeiti:** Conceptualization, Writing – review & editing, Supervision, Project administration, Resources.

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Supplementary material

Table S1. Analysis of amylose apparent content of BRS Catiana.

Apparent amylos content (%) (ISO 6647)	**Accession number**	**Ecotype**	**Appearence of polished grain**
24,24	BRA 00073457-4	*Indica*	White core

Table S2. Physical properties of germinated and non-germinated polished and brown rice.

Sample	RVA Parameters					
	Pasting Temperature (°C)	**Peak viscosity (cP)**	**Minimun viscosity (cP)**	**Breakdown viscosity (cP)**	**Final Viscosity (cP)**	**Setback (cP)**
NGBR	74.21±3.28[a]	418.00±0.18[c]	327.50±3.40[b]	90.50±4.24[b]	1296.50±6.76[b]	969.00±35.35 [b]
NGPR	72.91±1.66[a]	593.50±2.41[b]	398.50±16.26[b]	195.00±14.15[c]	1484.00±7.07[b]	1085.50±9.19 [b]
GBR	76.23±0.07[a]	596.50±1.85[b]	312.50±9.15[b]	284.00±1.14[c]	1936.50±6.26[a]	1624.00±7.07 [a]
GPR	77.37±0.42[a]	808.5±4.63[a]	474.00±9.89[a]	334.5±6.36[a]	2028.00±8.32[a]	1554.00±8.29 [a]

Each value is the mean of two replicates. For the same column, data with same letters do not differ significantly from each other whereas data with different superscripts differ significantly at the probability level ($p < 0.05$).

Table S3. All phenolic compounds tentatively identified by metabolomic analysis.

Number	Putative identification	Molecular formula	*m/z*(exp.)	RT (min)	Score (%)	FS (%)	Main fragments (relative intensity %)	ME (ppm)	IS	Samples											
										Free						Bound					
										GBR	NGBR	GPR	NGPR	GH+B	NGH+B	GBR	NGBR	GPR	NGPR	NGH+B	GH+B
FLAVONOIDS																					
1	Eupatorin	C18H16O7	343.0794	6.23	39.8	20.7	135.0541 (100)	-8.65	87.89								x	x	x		
2	(-)-Epicatechin	C15H14O6	289.0708	6.44	40.8	14.4	125.0244 (31.47)	-3.30	93.61											x	x
3	Myricetin	C15H10O8	317.0295	7.12	37.7	5.17	99.0087 (100)	-2.51	86.31	x	x	x	x	x	x			x	x	x	x
4	Pelargonidin 3-*O*-rutinoside	C27H31O14+	578.1654	7.34	39.1	6.79	206.0795 (11.56), 253.0506 (5.01)	2.24	91.62											x	x
5	Apigenin 7-*O*-apiosyl-glucoside	C26H28O14	563.1404	7.44	48.4	47.2	119.0502 (100), 93.0345 (7.40), 117.0346 (3.27)	-0.42	95.33	x	x		x	x	x						
6	Kaempferol 3-*O*-sophoroside	C27H30O16	609.1455	7.48	42.8	29.9	327.0510 (75.95), 109.0295 (68.19), 357.0616 (37.50), 429.0827 (18.71)	-0.92	85.17					x	x						
7	Luteolin 7-glucoside / Luteolin-6-*C*-glucoside /Quercetin 3-*O*-rhamnoside	C21H20O11	447.0932	7.51	41.9	14.4	137.0244 (100)	-0.22	95.64						x						
8	Phloretin	C15H14O5	273.0761	7.66	44.7	29.9	207.0662 (79.69)	-2.85	97.18					x	x		x	x		x	x
9	Chrysoeriol 7-*O*-apiosyl-glucoside	C27H30O15	593.1505	7.77	48.8	51.6	93.0345 (100), 243.0455 (22.52), 412.0878 (16.25), 593.1512 (15.33)	-1.20	93.84	x				x	x						
10	Naringenin-7-*O*-glucoside	C21H22O10	433.1124	7.84	37.4	1.08	107.0502 (59.13)	-3.82	90.59						x		x			x	x
11	Naringenin4'-*O*-glucoside	C21H22O10	433.1142	8.01	45.6	38.7	243.0662 (100), 229.0506 (81.54), 375.1085 (46.72), 207.0662 (13.86)	0.49	89.66					x	x					x	x
12	Pebrellin	C19H18O8	373.0919	8.12	45.7	35.6	167.0350 (67.36), 357.0979 (32.36)	-2.52	95.81					x	x		x		x	x	x
13	Phlorizine	C21H24O10	435.1287	8.28	41.2	13.2	149.0608 (23.30)	-2.20	95.53	x				x	x		x		x	x	x

14	Tetramethylscutellarein	C19H18O6	341.1025	8.30	44.7	31.5	109.0506 (100), 341.1030 (60.58), 326.0796 (27.70), 287.0924 (7.91)	-1.57	93.69					x	x	x	x				x
15	Glycitin	C22H22O10	445.1136	8.30	42.8	30.9	267.0662 (52.15), 326.0795 (27.70), 385.0929 (8.03)	-1.01	84.07		x		x	x	x		x	x	x	x	x
16	Narirutin	C27H32O14	579.1707	8.32	37.4	7.17	221.0445 (100)	-2.18	82.40					x	x			x		x	x
17	Tectoridin	C22H22O11	461.1097	8.59	47.6	49.9	413.0878 (35.77), 293.0455 (25.09), 166.0478 (7.48)	1.67	90.32					x	x					x	x
18	Tetramethyl scutellarein isomer I	C19H18O6	341.1043	8.66	40.5	10.8	326.0796 (16.49)	3.54	95.66					x	x				x		x
19	Apigeninglucoside	C21H24O9	419.1338	8.80	43.4	29.8	255.0663 (20.75), 329.1030 (5.06)	-2.17	89.87					x	x		x		x	x	x
20	Procyanidindimertype-B	C30H26O12	577.1351	8.80	40.2	15.6	315.0874 (100), 219.0662 (88.45)	-0.08	85.64					x	x		x	x	x	x	x
21	Sativanone	C17H16O5	299.0921	8.82	52.3	67.3	255.0663 (100), 217.0506 (27.65)	-1.29	95.50					x	x				x	x	x
22	Eupatorin isomer I	C18H16O7	343.0814	9.05	50.4	58.7	269.0455 (79.67), 181.0506 (68.46), 241.0506 (62.31), 284.0690 (48.06), 255.0663 (20.49), 299.0925 (19.24), 343.0823 (19.11)	-2.68	96.65		x			x	x	x	x	x	x	x	x
23	Stevenin	C16H12O5	283.0599	9.12	44.7	31.7	221.0608 (37.42), 97.0295 (32.65), 239.0713 (21.56), 258.0533 (11.54), 215.0349 (10.66)	-4.63	97.06					x	x				x	x	x
24	Hesperetin	C16H14O6	301.0710	9.39	39.1	2.96	99.0451 (100)	-2.53	95.71					x	x					x	x
25	Gardenin B	C19H18O7	357.0977	9.52	40.8	9.34	313.0717 (3.25)	-0.84	95.89					x	x		x	x	x	x	x
26	Puerarin	C21H20O9	415.1017	9.57	43.5	35.4	385.0928 (79.98), 325.0717 (59.73)	-4.24	86.98					x	x					x	x
27	Vestitone	C16H14O5	285.0755	9.71	43.4	24.8	228.0428 (22.33), 171.0451 (21.28), 199.0400 (12.95)	-4.63	97.66					x	x		x		x	x	x
28	Tectorigenin	C16H12O6	299.0552	9.71	47.6	45.6	256.0377 (39.30), 228.0428 (22.33), 199.0400 (12.95)	-2.88	95.68										x	x	x
29	Isosakuranetin	C16H14O5	285.0754	9.86	45.6	37.9	177.0557 (100), 251.0713 (19.06), 148.0529 (9.615), 225.0557 (7.79)	-5.03	95.69					x	x				x	x	x
30	O-Desmethylangolensin	C15H14O4	257.0809	9.87	42.7	21	225.0557 (32.30), 221.0608 (17.26)	-4.00	96.93											x	x
31	3'-O-Methylviolanone	C18H18O6	329.1033	9.89	42.7	33.9	301.1081 (81.32), 225.0557 (25.12)	0.77	80.44					x	x			x	x	x	x
32	Cirsimaritin	C17H14O6	313.0702	9.91	51.6	69.5	181.0506 (100), 283.0611 (70.28), 135.0451 (51.72), 255.0662 (21.62), 299.0561 (6.88)	-5.04	94.45					x			x		x	x	x

33	Eupatorin isomer II	C18H16O7	343.0811	9.93	48.2	47.2	181.0506 (100), 283.0611 (70.28), 135.0451 (51.72), 255.0662 (21.62), 299.0561 (6.88)	-3.59	98.09					x	x	x	x	x	x	x	x
34	Orobol / Kaempferol / Luteolin	C15H10O6	285.0398	10.00	41.5	14.5	178.0271 (18.89)	-2.21	95.85										x	x	x
35	Apigenin 6-C-glucoside	C21H20O10	431.0967	10.02	38.1	1.96	164.0690 (54.72)	-3.91	93.21					x	x	x	x	x	x	x	x
36	Isorhamnetin	C16H12O7	315.0500	10.07	38.4	2.38	109.0295 (29.03), 111.0451 (12.07)	-3.11	93.33						x					x	x
37	Tetramethylscutellarein isomer II	C19H18O6	341.1024	10.10	42.5	17.1	341.1030 (100), 326.0796 (83.76)	-2.07	97.71					x	x	x	x	x	x	x	x
38	Orobol / Kaempferol / Luteolin isomer	C15H10O6	285.0400	10.16	40.8	11.6	151.0400 (100), 241.0506 (11.49)	-1.46	93.99		x			x	x						
39	Quercetin / 6-Hydroxyluteolin / Morin	C15H10O7	301.0345	10.18	39.7	8.31	151.0400 (100)	-3.00	93.49						x	x	x		x	x	
40	Nepetin	C16H12O7	315.0506	10.23	52.7	70.1	285.0404 (100), 269.0455 (64.43), 239.0350 (44.00), 215.0350 (38.06), 257.0455 (23.94), 243.0299 (22.73), 267.0298 (14.83), 301.0701 (14.97), 300.0275 (14.36)	-1.47	95.14					x	x				x	x	x
41	Eriodictyol	C15H12O6	287.0561	10.25	45.5	39.3	239.0504 (44.00), 215.0350 (38.06), 257.0455 (23.91), 212.0478 (23.09), 243.0284 (22.73), 254.0220 (11.23), 229.0506 (10.34)	-0.10	88.22		x			x	x		x		x	x	
42	Procyanidin dimer type-B isomer	C30H26O12	577.1371	10.26	40.1	17.5	269.0455 (64.43), 215.0350 (38.06), 187.0400 (24.98), 301.0717 (14.97), 201.0557 (12.75), 199.0383 (10.59), 229.0506 (10.34)	3.45	87.09								x	x		x	x
43	4'-*O*-Methylepigallocatechin	C16H16O7	319.0808	10.32	40.2	10.3	207.0662 (100)	-4.81	96.36	x				x	x					x	x
44	Violanone	C17H16O6	315.0866	10.34	50.1	59.4	271.0975 (57.76), 99.0451 (14.51), 282.0533 (12.27), 299.0561 (6.52), 225.0557 (6.50)	-2.65	94.21					x	x		x	x	x	x	x
45	3,7-Dimethylquercetin	C17H14O7	329.0659	10.39	41.3	15	285.0404 (52.48)	-2.35	94.23											x	x
46	Dihydroquercetin 3-*O*-rhamnoside	C21H22O11	449.1107	10.41	41.4	19.4	403.1034 (22.71), 285.0404 (23.34)	3.98	92.38					x	x					x	x
47	Nobiletin	C21H22O8	401.1235	10.46	40.2	8.77	207.0662 (61.15), 97.0295 (25.00), 99.0451 (14.51)	-1.60	94.02					x	x		x	x	x	x	x
48	Sativanone isomer	C17H16O5	299.0907	10.48	41.1	17.4	255.0663 (44.36), 92.0267 (17.67)	-6.12	95.20						x			x	x	x	x

49	Cirsilineol	C18H16O7	343.0813	10.59	39.4	3.69	131.0502 (19.33), 219.0299 (12.69)	-3.04	96.84					x		x	x	x	x	x	x
50	Dihydroformononetin	C16H14O4	269.0805	10.60	42.1	20.3	223.0764 (22.82)	-5.23	96.22					x	x	x			x	x	x
51	3'-Hydroxydaidzein / 8-Hydroxydaidzein / 6-Hydroxydaidzein / Genistein / Galangin / Apigenin / 7,3',4'-Trihydroxyflavone	C15H10O5	269.0450	10.91	39.5	7.55	197.0244 (14.19)	-2.04	92.37					x	x				x	x	x
52	Tricin / Jaceosidin / 3,7-Dimethylquercetin	C17H14O7	329.0652	11.03	47.3	45.7	314.0431 (89.62), 161.0244 (20.78), 227.0350 (19.18), 243.0299 (7.62)	-4.37	95.87	x	x	x	x	x	x	x	x	x	x	x	x
LIGNANS																					
53	1-Acetoxypinoresinol	C22H24O8	415.1399	8.83	40.3	8.11	319.1030 (5.06)	0.14	93.62					x	x					x	x
54	Sesaminol	C20H18O7	369.0967	9.48	42.9	22.7	267.0663 (61.47), 207.0662 (53.37), 325.1081 (28.26)	-3.40	95.70					x	x	x		x	x	x	x
55	Lariciresinol / Isolariciresinol	C20H24O6	359.1525	9.78	41.1	26.5	297.1132 (100), 359.1500 (31.43)	7.05	86.90	x				x	x	x	x	x	x	x	x
56	Sesamolinol	C20H20O7	371.1142	9.87	47	40.5	301.1081 (25.23), 299.0925 (22.41), 338.0796 (19.17), 315.0874 (12.95)	1.57	96.36					x	x		x	x	x	x	x
57	Syringaresinol	C22H26O8	417.1559	9.89	42.3	19.8	301.1081 (81.32), 403.1762 (5.40)	0.88	92.81					x	x		x	x	x	x	x
58	Conidendrin	C20H20O6	355.1180	9.96	42.4	19.9	265.0870 (22.01), 355.1187 (7.50)	-1.94	94.20					x	x	x	x		x	x	x
59	Sesamin	C20H18O6	353.1032	10.16	40.1	7.93	151.0400 (100)	0.35	92.81									x	x	x	x
60	Matairesinol	C20H22O6	357.1342	10.44	40	9.78	325.1081 (29.83), 331.1550 (6.07)	-0.34	90.83					x	x	x	x	x	x	x	x
61	Conidendrin isomer	C20H20O6	355.1179	10.46	48.1	48.2	355.1187 (100), 310.0846 (100), 265.0870 (43.89), 325.1081 (32.94), 322.0846 (28.86), 337.1081 (22.42), 92.0267 (9.13), 306.0897 (6.01)	-2.25	94.80					x	x	x	x	x		x	x
62	Nortrachelogenin / 7-Hydroxymatairesinol / Isohydroxymatairesinol	C20H22O7	373.1305	10.84	38.3	0.179	85.0295 (69.50)	3.35	95.05						x		x		x	x	x
63	Sesamolin	C20H18O7	369.0969	11.53	43.5	25.9	299.0925 (14.28)	-3.01	95.18					x						x	x
OTHER POLYPHENOLS																					
64	Phlorin	C12H16O8	287.0768	5.25	55.7	94.3	144.0428 (100)	-1.66	86.06					x	x						
65	Esculetin	C9H6O4	177.0179	6.08	50.9	65.8	121.0295 (100)	-8.17	97.94		x			x	x		x	x	x	x	x

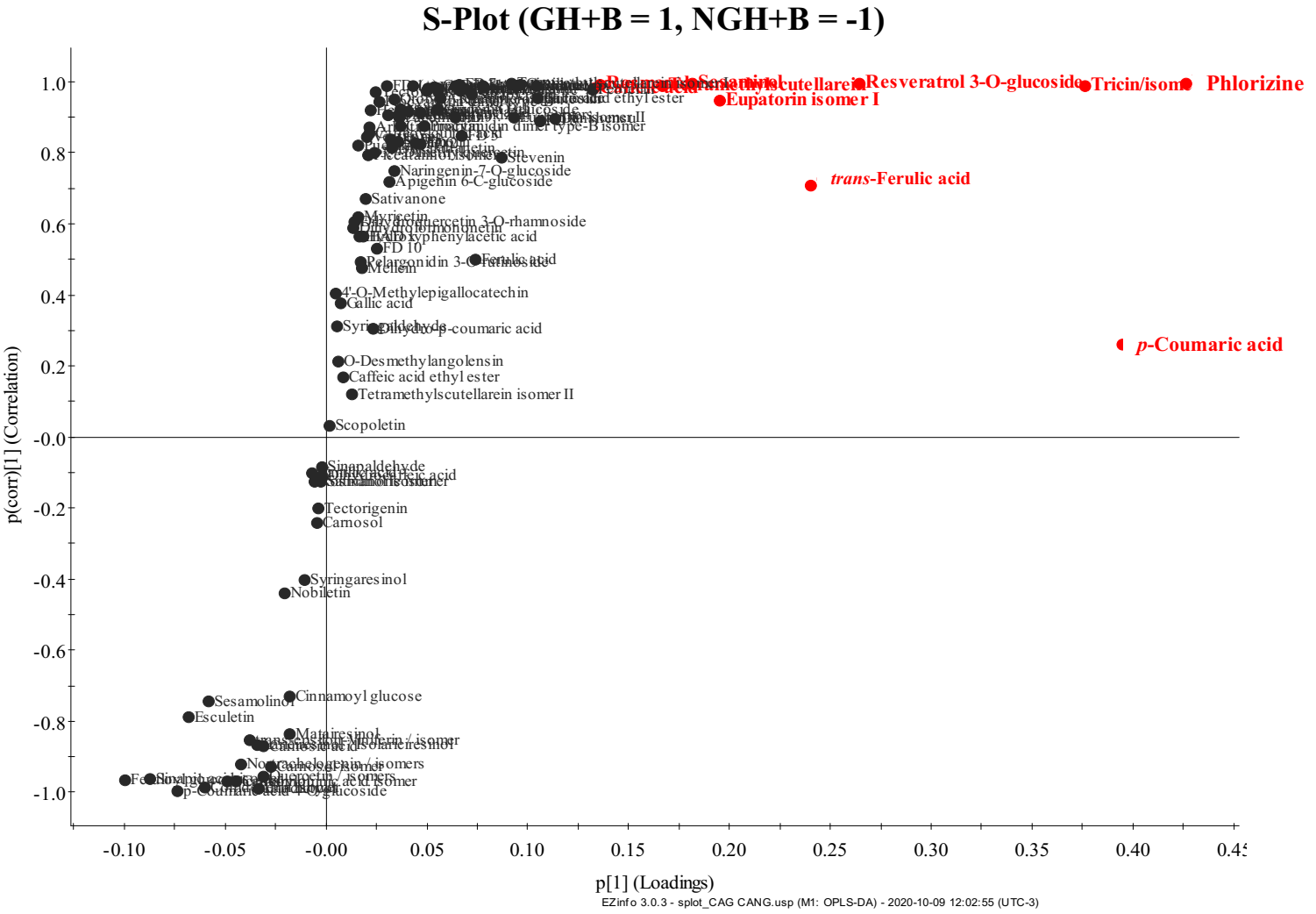
66	Arbutin	C12H16O7	271.0813	6.53	52.5	72.9	132.0428 (100)	-3.77	94.18					x	x					x	x
67	Mellein	C10H10O3	177.0542	7.17	42.2	24.1	133.0659 (63.59)	-8.45	96.46								x			x	x
68	Scopoletin	C10H8O4	191.0335	7.73	47.3	51.6	167.0349 (100)	-7.62	93.59	x					x	x		x	x	x	x
69	Sinapaldehyde	C11H12O4	207.0648	7.85	52	73	135.0451 (100)	-7.13	95.21	x		x		x	x	x	x		x	x	x
70	Syringaldehyde	C9H10O4	181.0491	9.10	38.6	4.44	97.0295 (22.44)	-8.34	97.87				x	x	x					x	x
71	Rosmanol	C20H26O5	345.1694	10.10	46.3	39.5	287.1652 (33.80), 327.1602 (30.35), 269.1547 (14.30), 111.0451 (13.22), 297.1496 (9.06)	-3.98	96.82					x	x	x	x	x	x	x	x
72	Rosmanol isomer	C20H26O5	345.1700	12.09	49.7	55.3	345.1707 (70.86), 331.1914 (70.76), 268.1469 (41.81), 283.1703 (12.32), 301.1809 (7.29)	-2.13	95.95	x	x		x	x	x	x	x	x	x	x	x
73	Carnosol	C20H26O4	329.1744	11.69	43.1	25.1	299.1652 (30.12)	-4.19	95.37					x	x		x			x	x
74	Carnosol isomer	C20H26O4	329.1741	11.95	43	24.8	313.1445 (9.66)	-5.25	96.05					x	x	x	x			x	x
75	Carnosic acid	C20H28O4	331.1907	12.07	38.9	5.41	331.1915 (70.76), 301.1809 (7.29)	-2.28	91.92	x				x	x				x	x	x
PHENOLIC ACIDS																					
76	**Gallic acid**	**C7H6O5**	**169.0132**	**1.85**	**50.3**	**60.3**	**169.0142 (50.72), 125.0244 (100)**	**-6.11**	**98.34**						**x**					**x**	**x**
77	Danshensu	C9H10O5	197.0438	2.00	55.5	88.5	138.0322 (100)	-8.60	98.55					x	x					x	x
78	2,3,4-Trihydroxybenzoic acid	C7H6O5	169.0126	3.09	36.9	1.17	71.0138 (7.10)	-9.75	93.87					x	x						
79	Gallic acid ethylester	C9H10O5	197.0441	4.21	49.9	61.1	151.0400 (100), 164.0115 (71.52)	-7.35	96.42					x	x				x	x	x
80	Hydroxyphenylacetic acid	C8H8O3	151.0390	4.75	54	91.8	125.0244 (100)	-7.27	86.44					x	x			x		x	x
81	**Vanillic acid**	**C8H8O4**	**167.0335**	**6.02**	**37.1**	**0**	**0**	**-8.95**	**95.58**	**x**					**x**		**x**		**x**	**x**	**x**
82	**Caffeic acid**	**C9H8O4**	**179.0336**	**6.21**	**56.7**	**93.8**	**135.0451 (100)**	**-7.94**	**98.51**		**x**	**x**		**x**	**x**		**x**	**x**	**x**	**x**	**x**
83	Dihydroferulic acid	C10H12O4	195.0646	6.28	37.2	2.39	117.0346 (2.94)	-8.58	93.32	x				x	x					x	x
84	Dihydrocaffeic acid	C9H10O4	181.0492	6.46	50.3	66	99.0451 (100), 125.0244 (31.47)	-7.72	93.98						x			x		x	x
85	Dihydro-*p*-coumaric acid	C9H10O3	165.0547	7.32	51.5	65.8	103.0553 (100), 101.0397 (37.84)	-6.31	98.62	x	x	x		x	x		x	x	x	x	x
86	Caffeoylquinic acid	C16H18O9	353.0885	7.40	39.9	10	99.0087 (35.73), 108.0217 (34.01), 232.0377 (14.62), 230.0584 (4.96)	2.05	91.91						x					x	x

87	***p*-Coumaric acid**	**C9H8O3**	**163.0387**	**7.44**	**53.5**	**78.2**	**119.0502 (100), 93.0345 (7.40), 117.0346 (3.27)**	**-8.28**	**98.69**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
88	Caffeic acid ethyl ester	C11H12O4	207.0644	7.44	39.6	14.6	117.0346 (3.27), 173.0608 (0.32)	-9.02	93.61	x		x	x	x	x	x		x	x	x
89	Sinapic acid isomer	C11H12O5	223.0602	7.84	48.6	52.4	135.0451 (100)	-4.53	95.96					x	x	x	x			x	x
90	***trans*-Ferulic acid**	**C10H10O4**	**193.0491**	**8.00**	**46**	**40.7**	**134.0373 (100), 109.0295 (2.15), 106.0424 (10)**	**-7.95**	**97.97**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
91	**Sinapic acid**	**C11H12O5**	**223.0598**	**8.11**	**51**	**64.5**	**181.0506 (100), 94.0275 (6.42)**	**-6.31**	**97.62**		**x**			**x**	**x**		**x**	**x**	**x**	**x**	**x**
92	**Ferulic acid**	**C10H10O4**	**193.0490**	**8.23**	**53.6**	**79.1**	**165.0557 (100), 149.0608 (23.30)**	-8.27	98.00	x	x	x	x	x	x	x	x	x	x	x	x
93	Cinnamoyl glucose	C15H18O7	309.0977	8.43	52.1	69	144.0428 (100), 146.0584 (53.78)	-0.88	92.49					x	x			x		x	x
94	Paeoniflorin	C23H28O11	479.1571	8.73	40.2	9.34	403.134 (11.95)	2.59	94.65					x	x	x	x	x	x	x	x
95	*p*-Coumaricacid 4-*O*-glucoside	C15H18O8	325.0918	8.78	46.7	41	219.0662 (74.76), 200.0690 (29.86)	-3.29	96.11					x	x			x	x	x	x
96	Rosmarinicacid	C18H16O8	359.0772	8.82	44	23.5	315.0874 (37.53), 303.0510 (6.11)	-0.06	96.79					x	x	x	x			x	x
97	Feruloyl glucose	C16H20O9	355.1020	8.89	38.7	7.44	180.0639 (100)	-4.19	90.94	x				x	x		x	x		x	x
98	Caffeoylquinic acid isomer	C16H18O9	353.0873	9.16	41.6	14.3	187.0400 (61.71)	-1.39	95.30						x					x	x
STILBENES																					
99	Resveratrol 3-*O*-glucoside	C20H22O8	389.1236	8.73	51	63.6	93.0345 (60.86), 269.0819 (53.64)	-1.58	93.57					x	x	x	x	x		x	x
100	Piceatannol	C14H12O4	243.0647	9.52	40.2	9.04	108.0217 (7.25), 225.0557 (3.79)	-6.51	99.37					x	x	x			x	x	x
101	Piceatannol isomer	C14H12O4	243.0654	9.82	41.4	16.2	213.0557 (39.45)	-3.61	94.95									x	x	x	x
102	trans-epsilon-Viniferin / epsilon-Viniferin	C28H22O6	453.1338	10.57	39.8	11.8	282.0897 (100), 131.0502 (19.33)	-1.28	88.98						x			x		x	x
UNKNOWN COMPOUNDS																					
Phenolic acids derivatives																					
103	HAD1	C9H6O4	177.0176	5.84	52.8	76.2	148.0166 (92.56)	-9.50	98.20					x	x					x	x
104	HAD2	C18H16O8	359.0779	7.43	41	11.4	117.0346 (3.27)	1.85	95.75					x	x					x	x
105	HAD3	C18H16O8	359.0762	9.87	41.1	12.9	323.0561 (6.74), 311.0561 (2.74)	-3.03	96.34					x	x					x	x
106	HAD4	C20H18O6	353.1019	10.86	40.1	9.53	338.0796 (33.11)	-3.32	94.85									x	x	x	x
Flavonoids derivatives																					

107	FD1	C26H28O14	563.1396	7.64	43.9	27.2	163.0400 (100), 353.0667 (5.64), 101.0244 (4.81), 473.1079 (0.70)	-1.76	94.44	x	x	x	x	x	x						x
108	FD2	C22H22O10	445.1132	8.09	47.4	46.5	327.0874 (16.21), 312.0639 (3.24), 401.1241 (2.44)	-1.81	92.90					x	x	x	x	x	x	x	x
109	FD3	C21H24O9	419.1334	8.19	42.5	21.1	167.0350 (57.66), 389.1241 (4.57)	-3.16	95.26					x	x		x		x	x	x
110	FD4	C21H20O9	415.1041	8.46	40.8	14	146.0584 (100)	1.65	92.02					x	x	x	x	x	x	x	x
111	FD5	C17H14O6	313.0707	8.69	50.2	58.8	137.0244 (100), 93.0346 (60.86)	-3.54	96.34							x			x	x	x
112	FD6	C19H18O8	373.0921	8.83	44.5	30.6	177.0557 (100), 257.0455 (6.13)	-2.04	94.48					x	x		x		x	x	x
113	FD7	C15H12O6	287.0548	9.25	39.9	9.3	123.0451 (64.46), 109.0295 (38.67)	-4.65	95.82		x			x	x		x		x	x	x
114	FD8	C17H14O6	313.0705	9.54	41.2	14	254.0584 (6.10), 256.0741 (3.42)	-3.91	96.46					x	x	x	x	x	x	x	x
115	FD9	C21H22O8	401.1240	9.96	38.8	7.04	149.0608 (100)	-0.38	87.63					x	x		x	x	x	x	x
116	FD10	C17H16O5	299.0918	10.18	44.4	28	151.0400 (100), 299.0925 (41.52), 284.0690 (35.87), 219.0662 (34.65)	-2.40	96.79					x	x					x	x
117	FD11	C18H16O7	343.0810	12.37	39	7.14	99.0451 (15.38), 328.0558 (6.01)	-3.97	92.45					x	x						

m/z = mass/charge ratio; RT = retention time; FS = fragmentation score; IS = isotopic similarity; Bold represent reference standard

(A)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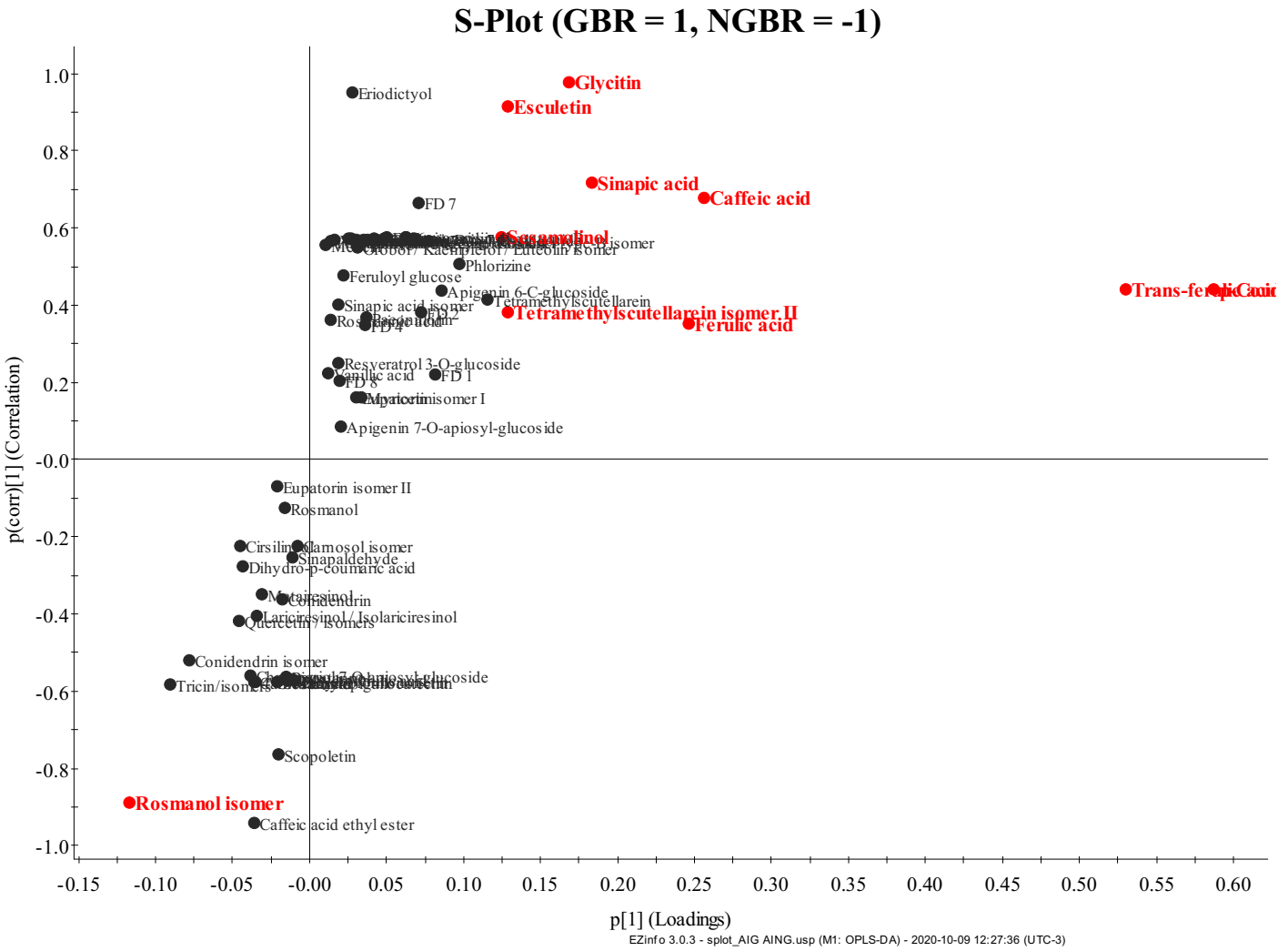
Resveratrol 3-O-glucoside
Tricin/isomer
Phlorizine
Eupatorin isomer I
trans-Ferulic acid
p-Coumaric acid
Stevenin
Naringenin-7-O-glucoside
Apigenin 6-C-glucoside
Sativanone
Myricetin
FD 10
Ferulic acid
4'-O-Methylepigallocatechin
Gallic acid
Dihydro-p-coumaric acid
O-Desmethylangolensin
Caffeic acid ethyl ester
Tetramethylscutellarein isomer II
Scopoletin
Tectorigenin
Carnosol
Syringaresinol
Nobiletin
Cinnamoyl glucose
Sesamolinol
Esculetin
Matairesinol
p[1] (Loadings)
p(corr)[1] (Correlation)
EZinfo 3.0.3 - splot_CAG CANG.usp (M1: OPLS-DA) - 2020-10-09 12:02:55 (UTC-3)

(B)


S-Plot (GBR = 1, NGBR = -1)
p(corr)[1] (Correlation)
p[1] (Loadings)
1.0
0.8
0.6
0.4
0.2
-0.0
-0.2
-0.4
-0.6
-0.8
-1.0
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Glycitin
Esculetin
Sinapic acid
Caffeic acid
Eriodictyol
FD 7
Phlorizine
Feruloyl glucose
Apigenin 6-C-glucoside
Tetramethylscutellarein
Sinapic acid isomer
Tetramethylscutellarein isomer II
Ferulic acid
FD 4
Resveratrol 3-O-glucoside
Vanillic acid
FD 1
FD 8
Apigenin 7-O-apiosyl-glucoside
Eupatorin isomer II
Rosmanol
Cirsiliol
Salvigenin
Sinapaldehyde
Dihydro-p-coumaric acid
Conidendrin
Quercetin 7 isomers
Conidendrin isomer
Scopoletin
Rosmanol isomer
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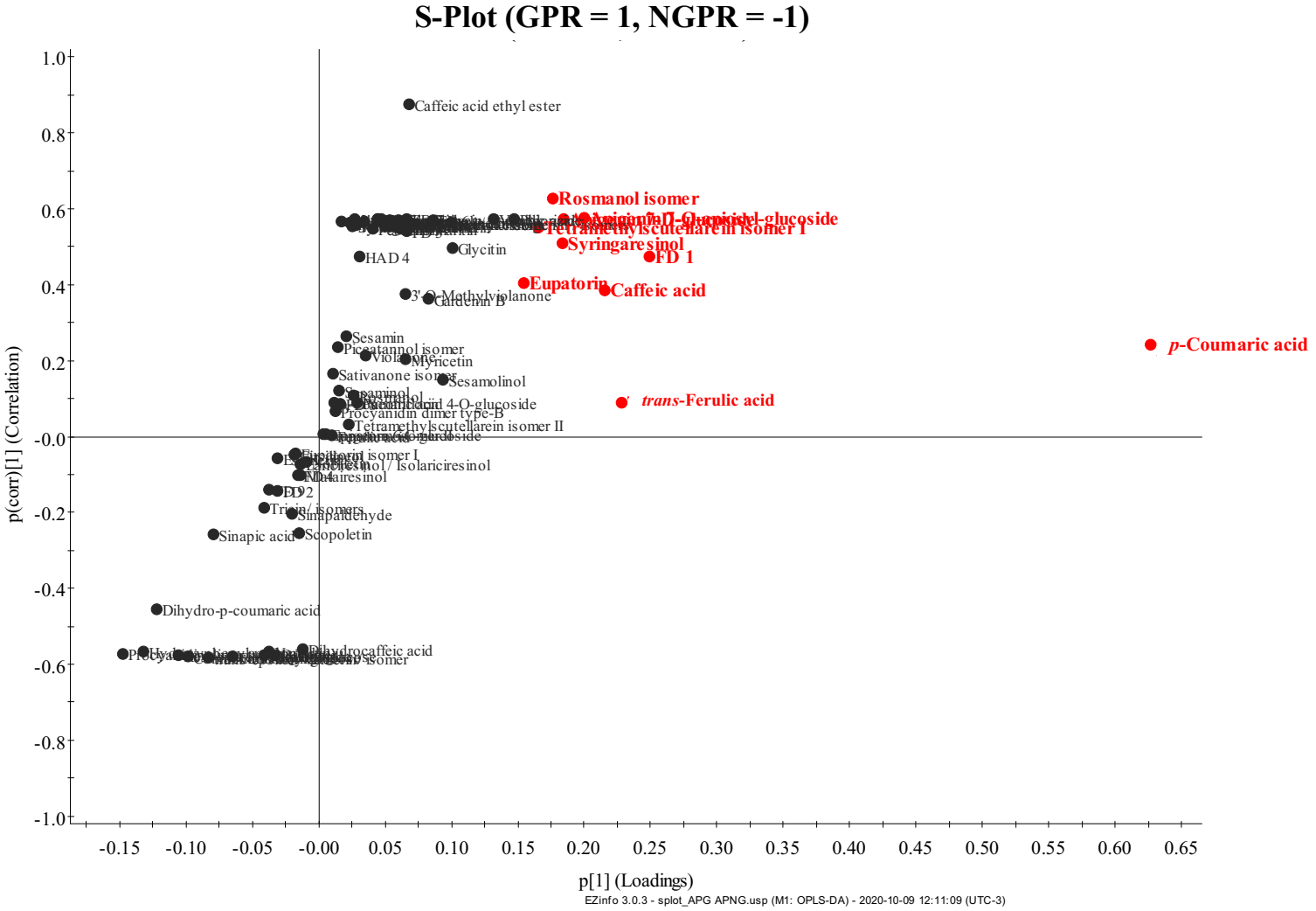
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Syringaresinol
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HAD 4
Eupatorin
Caffeic acid
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Gardenin B
Sesamin
Piceatannol isomer
Myricetin
Sesamolinol
Sativanone isomer
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Scopoletin
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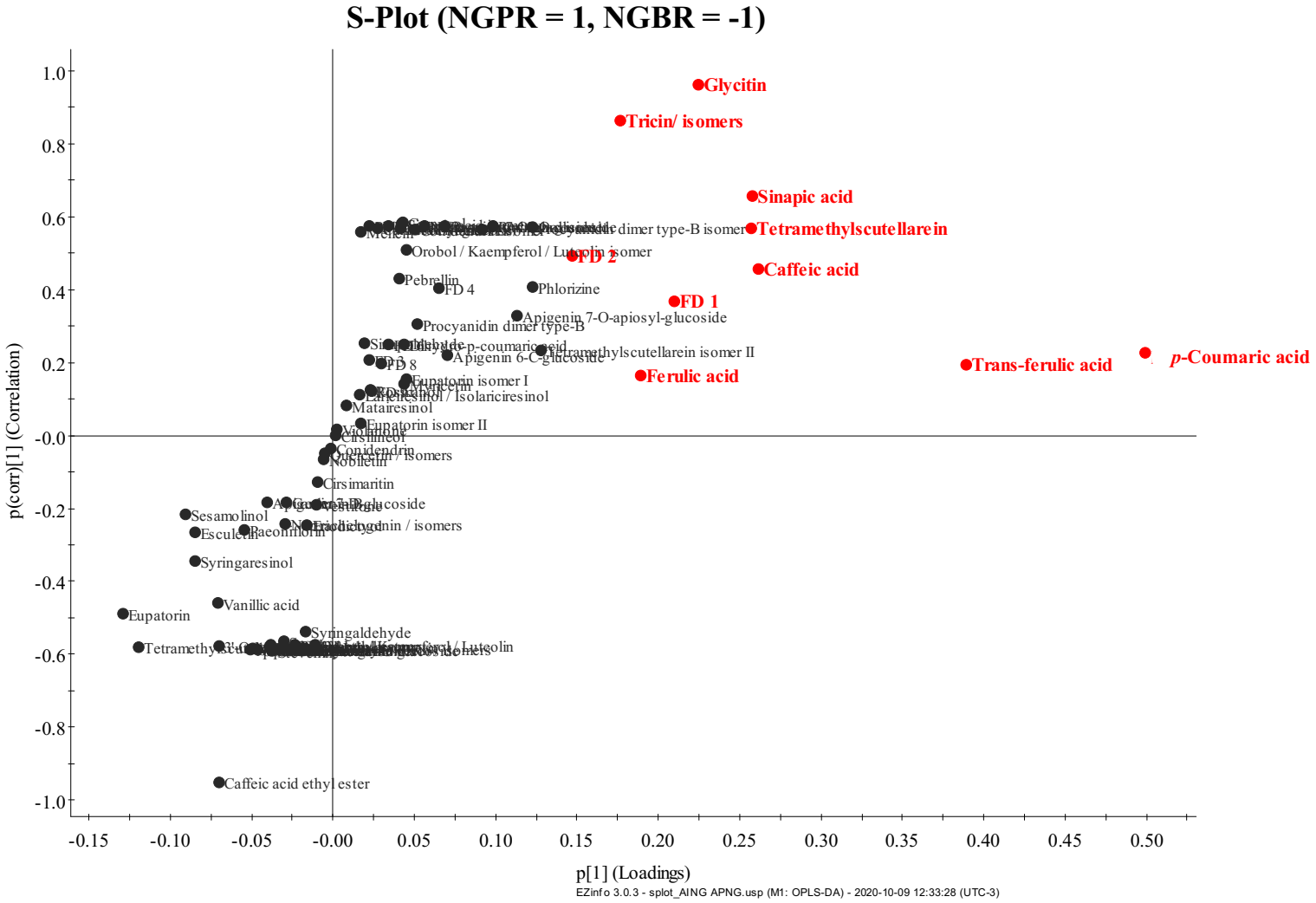
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Tricin/ isomers
Sinapic acid
Tetramethylscutellarein
Caffeic acid
FD 2
FD 1
Trans-ferulic acid
p-Coumaric acid
Ferulic acid
Orobol / Kaempferol / Luteolin isomer
Pebrellin
FD 4
Phlorizine
Apigenin 7-O-apiosyl-glucoside
Procyanidin dimer type-B
Apigenin 6-C-glucoside
Tetramethylscutellarein isomer II
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Eupatorin isomer I
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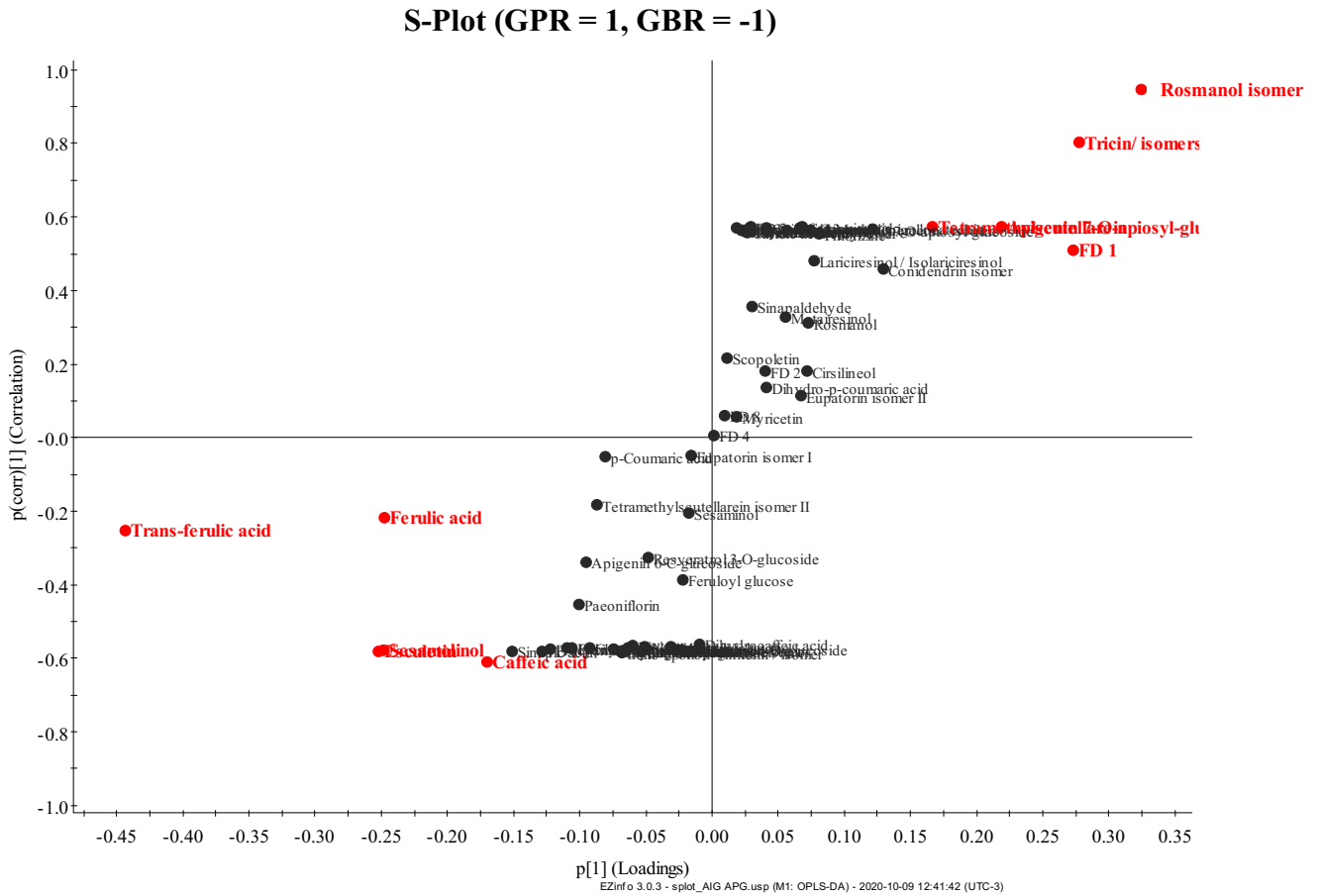


Figure S1. S-plots comparing husk, bran and rice before and after germination and polishing process. (A) NGH+B *vs* GH+B; (B) NGBR *vs* NGPR, (C) NGBR *vs* GBR, (D) NGPR *vs* GPR and (E) GBR *vs* GPR. Marked in red: the ten discriminants phenolic compounds - VIP (Variance Important Projection).

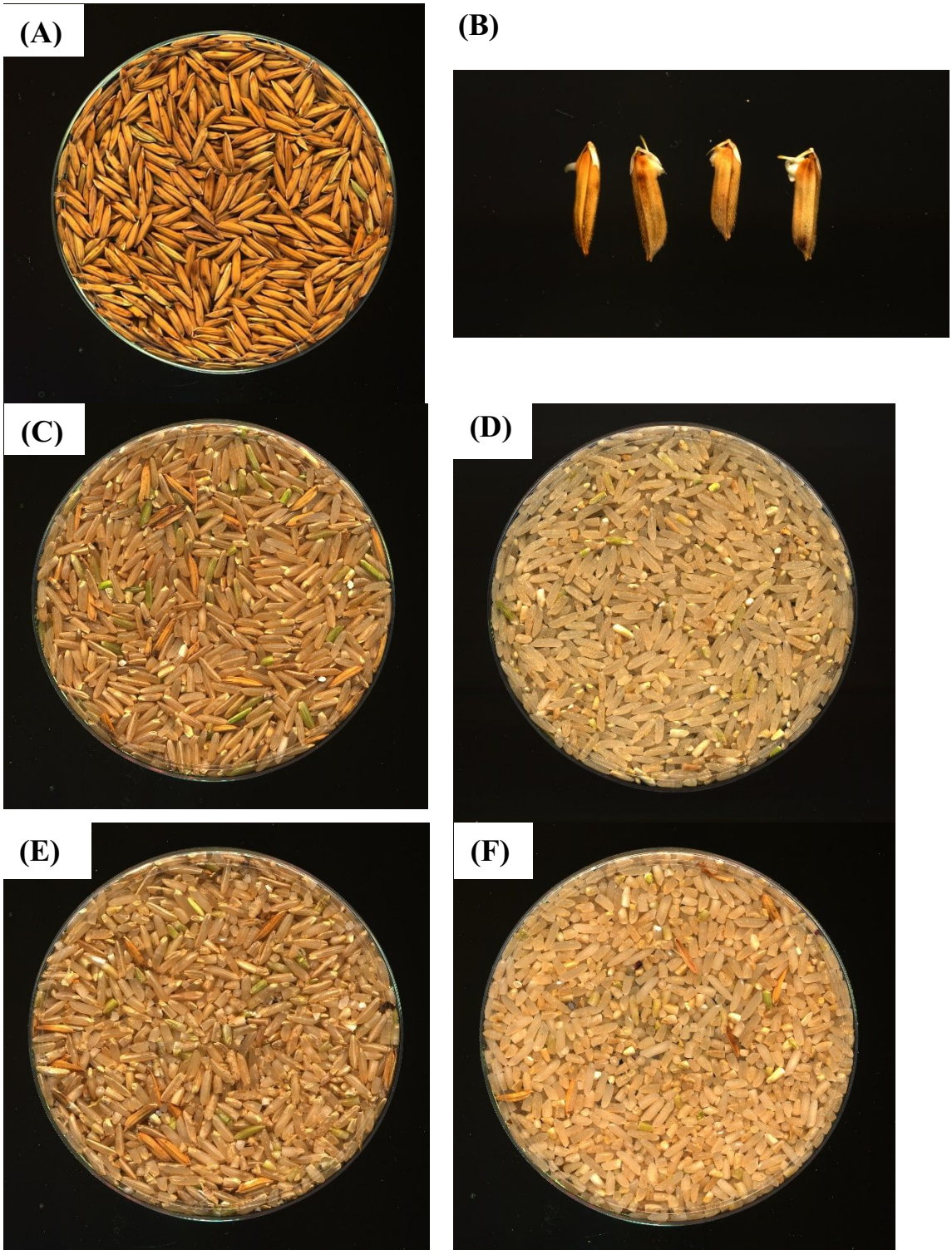


Figure S2. Visual aspect of BRS Catiana. (A) paddy rice, (B) germinated rice, (C) non-germinated brown rice, (D) non-germinated polished rice, (E) germinated brown rice, (F) germinated polished rice.

Chapter VI

Role of short soaking/germination on rice starch characteristics

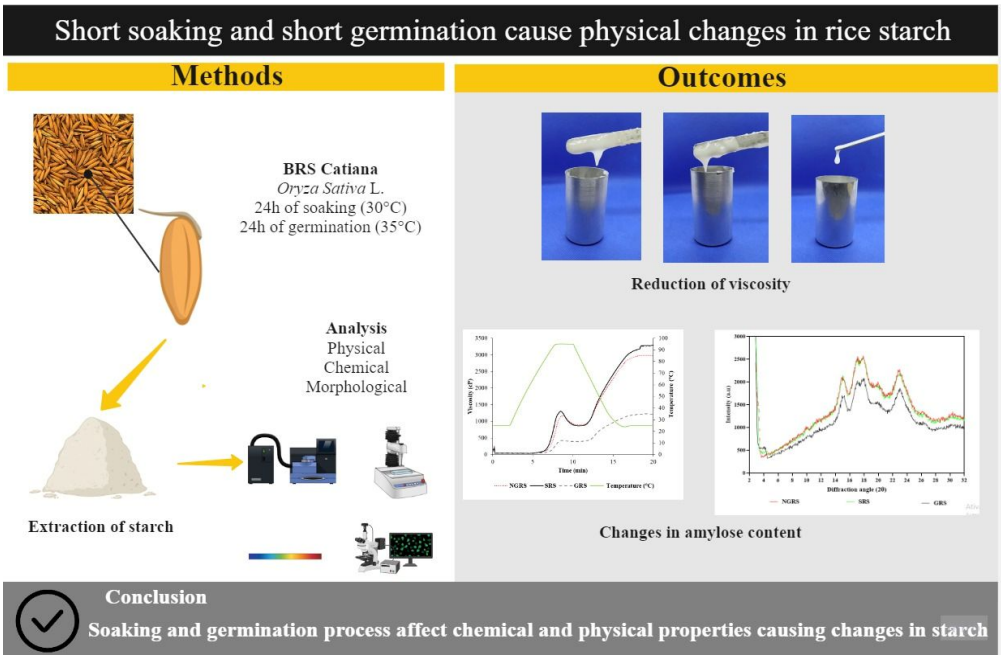
Maria Eugenia Araujo Silva Oliveira[1], Priscila Zaczuk Bassinello[2], José Manoel Colombari Filho[2], Carlos Wanderlei Piler de Carvalho[3], Cristina Yoshie Takeiti[1,3]

[1] Food and Nutrition Graduate Program, Nutrition School, Federal University of the State of Rio de Janeiro-UNIRIO, Av. Pasteur, 296, Urca, 22290-240, Rio de Janeiro-RJ, Brazil

[2] Embrapa Arroz e Feijão, km 12 - Zona Rural, GO-462, 75375-000, Santo Antônio de Goiás - GO, Brazil

[3] Embrapa Agroindústria de Alimentos, Avenida das Américas, 29501, 23020-470, Rio de Janeiro-RJ, Brazil

Paper submitted to Journal of Cereal Science



Highlights

- Germination cause a decrease in RVA viscosities whereas soaking enhance.
- No change in the colors of the materials was observed.
- The decrease in particle size was more pronounced in germinated starch than soaked.
- High gel strength after 3 days of refrigeration in germinated starch.

ABSTRACT

Soaking and germination are processes used to improve nutritional characteristics in rice. During these processes the activity of enzymes can affect the rice starch. Hence, the current investigation was aimed to understand the impact of soaking and germination process in a starch structure in terms of morphologic, texture, physicochemical, physical, color characteristics, gelatinization properties. It was observed that soaking and germination caused changes in granule surface and reduced relative crystallinity (4.81 and 1.60 %), enthalpy (9.08 and 5%) and peak viscosity (9.16 and 67.15%). The enhancement of enzymes during this process affected the gel strength during cooling. Our results are useful to provide bases for utilization of soaked and germinated rice starches in developing products for the food industry.

Keywords: Germination, α-amylase, viscosity, texture analysis

1. Introduction

Rice (*Oryza sativa* L.) is the ninth most produced commodity in the world, constituting an important staple food that provides food security primarily in Asian countries (FAOSTAT, 2021). Rice is an excellent source of carbohydrates, mostly composed of starch (~90%) in the endosperm, is hypoallergenic, presenting bland flavor and white color, which gives it several advantages compared to starches extracted from other vegetables sources such as sorghum and corn (J. BeMiller & Whistler, 2009; Juliano, 1972).

In recent years, the food market has been seeking for clean label products that also present aroma, flavor and texture of conventional products. Food consumers are demanding formulations that help them optimize their health performance, adapt to the realities of living with climate change and which have the appeal of 'energy-saving' (Mintel, 2023). In this point of view, germination is a naturally, eco-friendly, simple and efficient process used to improve the nutritional quality of rice in terms of phenolic compounds in addition to modify starch digestibility (Oliveira et al., 2022).

Germination can be divided in two steps: soaking and germination. Soaking is the first step of germination when rice grains are immersed in water for a period at constant temperature. This is a crucial step for radicle formation, changes in the physical and cooking properties of rice and the accumulation of bioactive compounds especially GABA (Gong et al., 2020; Munarko & Sitanggang, 2021; Patil & Khan, 2011). Several factors influence soaking and germination such as the time of both soaking and germination. Longer time (>72 h) increase GABA and other phytochemicals, but decrease the palatability of rice, in addition to, changes in starch during germination are time dependent, as some amylolytic enzymes such as alpha-amylase have higher activity after 24 h (Damaris et al., 2019; Kamjijam et al., 2020).

The increase of phenolic compounds (e.g. ferulic acid) during the process can promote interactions between amylose and these compounds causing the V-type binding complex which can alter the physical, rheological and nutritional properties of the starch (M. Li et al., 2018; Obiro et al., 2012). During germination, many changes occur in the plant tissue and causes a modifications in physical, chemical, sensory, functionality and nutritional characteristics of starch, making an impact on glycemic index, digestibility and resistant

starch (RS) content (T. Liu et al., 2022). Despite being recently studied, these alterations may help answer some questions such as when, why and how germination alters the starch structure. Recently, a lot of studies are showing the effects of soaking and germination in the nutritional properties of germinated brown rice (GBR) (J. Ding, Hou, et al., 2018a; Feng et al., 2019; Owolabi et al., 2019), however, a little information concerning the changes of physical, physicochemical and chemical in starch are reported in the literature. Therefore, the present work was aimed to evaluate changes in physicochemical, chemical, and physical properties of rice starch after 24 h of soaking and 24 h of germination process, to understand the behavior and provide reference information for rice starch product development that might be potent in the food industry.

2. Material and Methods

2.1. Material

BRS Catiana (*Oryza sativa* L.), selected from Active Germplasm Bank of Embrapa Rice and Beans (Santo Antônio de Goiás, Brazil), was multiplied in the 2018/2019 harvest using a flood-irrigated system in the farm experimental field of Embrapa Rice and Beans (6° 29′ 8″ S, 49° 18′ 32″ W).

2.2. Methods

2.1 Soaking and germination process

Soaking and germination was performed according to the methodology described by Zhang et al. (2014) with some modifications (24 h of soaking and 24 h of germination). After this process, the grains were shelled and polished (2 min) using a rice polisher machine (Suzuki, model BSV, Santa Cruz do Rio Pardo, São Paulo, Brazil). Non-germinated polished rice (NGBR) was used as control.

2.2. Starch extraction

The polished rice grains after soaking and germination were submitted to wet extraction, according to Silva et al. (2019). After the starch extraction the following samples were obtained: soaked Rice Starch (SRS) and Germinated Rice Starch (GRS).

2.3. Chemical characterization of starch

Moisture content was determined using an oven with air circulation at 105 °C until constant weight. Ash, lipid, protein and fiber content were determined according to methods n. 923.03, 945.38, 46.13 and 985.29, respectively (AOAC, 2020). The total carbohydrate content was calculated by difference. Total starch (TS), amylose (AM), resistant starch (RS) contents were determined using a K-TSA-100A, K-AMYL and K-TSTA-100 06/17 standard kits, respectively (Megazyme International, Bray, Ireland).

2.4. Pasting properties

Pasting properties were determined using a Rapid Viscosity Analyzer series 4 (RVA) (Newport Scientific, Warriewood, Australia) with the Thermocline for Windows software (version 3.0) according to method 76-21.01 (AACC, 2010).

2.5 Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) analyzes was conducted using a Q200 (TA Instruments, New Castle, USA) according to Bernardo et al. (2018) (Bernardo et al., 2018).

2.6. Particle size distribution (PSD)

The PSD of starch samples was determined using a laser diffraction particle size analyzer (SDC S3500, Microtrac, Montgomeryville, USA). The samples were dispersed in isopropyl alcohol (99%). The volume-based mean diameter (D[4,3]) and the area-based mean diameter (D[3,2]) were calculated using the Flex Software, version 11.0.0.3 (Microtrac, USA).

2.7. Starch microstructure

Starch microstructure was observed by Scanning Electron Microscopy (SEM) Leo 440i (LEO Electron Microscopy, Cambridge, UK). Dried samples were metalized with gold in a Sputter Coater K450 (EMITECH, Kent, UK). The image acquisition was performed by LEO software, 3.01 version (LEO Electron Microscopy, Cambridge, UK).

2.8. X-ray diffraction pattern

Crystalline structure was analyzed using an X-ray diffractometer D2 Phaser (Bruker, Rheinfelden, Germany), operating at Cu-K wavelength with 0.154 nm, 30 kV and 10 mA. Samples were scanned in duplicate from 2° to 32° (2θ) at the rate of 0.05°/min, with a step size of 0.02° and divergence slit width of 0.6 mm equipped with a Lynxeye detector (Bruker, Rheinfelden, Germany). Relative crystallinity (RC) was calculated as the ratio of area of crystalline region and total area, using software Diffrac Evaluation Eva v.3.0 (Bruker, Rheinfelden, Germany).

2.9. Color measurement

CIELab measurements (CIE, 1978) were carried out about Oliveira et al. (2019) using a Color Quest XE (Hunter Lab, Reston, USA) in reflectance and specular exclusion modes with observer/illuminant 10° and D65. Color parameters were expressed in terms of L^*, a^*, and b^* and White Index (WI) was calculated (Eq. (3)).

$$\text{WI}= 100- \sqrt{(100 -* L^2) +* a^2 +* b^2} \quad (3)$$

2.10. Gel strength

The strength of the starch gels was determined by a puncture assay using a Texture Analyser TA.XT Plus (Stable Micro Systems Ltd., Sussex, UK) with a load cell of 50 kgf (490.3 N). The gel obtained using the Rapid Visco Analyser (RVA) was stored for 3 days in a refrigerator (5 ± 2 °C). A 5 mm cylindrical probe (P/0.5R) was used to penetrate the samples until a distance of 6 mm at 1 mm·s^{-1}. Prior to analysis, the samples were removed from refrigerator and kept for 1 h at room temperature. The force measured by the function of the penetration depth was used to evaluate the sample strength using a software Exponent version 6.1.27.0 (Stable Micro Systems Ltd., Sussex, UK).

2.11 Statistical analysis

One-way ANOVA was carried out using XLSTAT software (Addinsoft, Paris, France) and GraphPad software (Dotmatics, San Diego, United States).

3. Results and discussion

3.4. Chemical characterization

Table 1 presents chemical properties of the starch samples of non-germinated rice starch (NGRS), soaked rice starch (SRS) and germinated rice starch (GRS). Moisture values ranged between 7.31 to 9.36 % and NGRS presented higher moisture content. All samples showed low values of ash, which indicates the good extraction and the purity of the material and SRS showed the smallest value (0.28 %).

As expected, rice starch should contain high levels of carbohydrates and fewer levels of protein and lipids content. In the present work, carbohydrates are higher than 87 % but total starch content did not present a statistical difference among samples ($p < 0.05$). Soaking and germination affect the protein content and SRS and GBR showed a little decrease in protein (10 % and 42 %), respectively. During grain germination, especially in the initial process (48 h), protein decreased by enzymatic activity and utilized for the embryonic tissue (Palmiano & Juliano, 1972).

Except protein reduction, no significant difference ($p < 0.05$) in the content of lipids, carbohydrates, total starch, resistant starch and amylose content of SRS decreased. However, Xu et al. (2012) studied the effect of 12 h of soaking at room temperature followed by 24 h of germination at 30 °C and observed an increase in moisture, ash and protein, and a decrease in lipids and amylose contents. The authors observed a decrease in amylose (173.9 to 121.3 $g \cdot kg^{-1}$) in GBR starch and total starch in GBR flour (798.9 to 721.1 $g \cdot kg^{-1}$). They attributed these results to the increase of amylolytic enzymes during the germination process.

Resistant starch (RS) can be defined as *"the portion of starch that is not digested in the small intestine and is fermented in the colon by microorganisms, resulting in the formation of short-chain fatty acids, which may be associated with some metabolic effects"* (Bojarczuk, Skąpska, et al., 2022). Results showed there was no statistical difference ($p < 0.05$) concerning RS content, indicating that both soaking and germination processes were not able to modify the starch probably due to the short time applied.

Table 1. Chemical characterization of rice starches

Sample	Constituents (%)							
	Moisture	Ash	Lipids	Proteins	Carbohydrate*	Total starch	Amylose	Resistant starch
NGRS	9.36±0.03[a]	0.36±0.02[a]	0.46±0.00[a]	5.12	88.69±0.01[a]	67.58±2.04 [a]	16.15±1.83[a]	0.50±0.08 [a]
SRS	7.31±0.02[b]	0.28±0.01[b]	0.43±0.01[a]	4.60	87.38±0.03[a]	69.62±0.45 [a]	14.26±1.73[b]	0.51± 0.01[a]
GRS	7.57±0.00[b]	0.36±0.00[a]	0.50±0.04[a]	2.93	88.63±0.04[a]	72.30±2.23 [a]	17.95±1.53[a]	0.48±0.03 [a]

Values are means ± standard deviation. *Carbohydrates are obtained by difference. Data with same letters in the same column do not differ significantly by Tukey test ($p < 0.05$).

3.1. Starch microstructure

Fig. 1 shows the SEM photographs of non-germinated (A, B), soaked (C, D) and germinated (E, F) rice starches. The non-germinated rice starch granules had an irregular, polyhedral and compact shape, small granules with diameters of 3 µm, as well as soaked and germinated rice starch. After soaking and germination, there were little changes in the morphology of the granules. Apparently, short soaking and short germination process (24 h) caused little changes in starch granules.

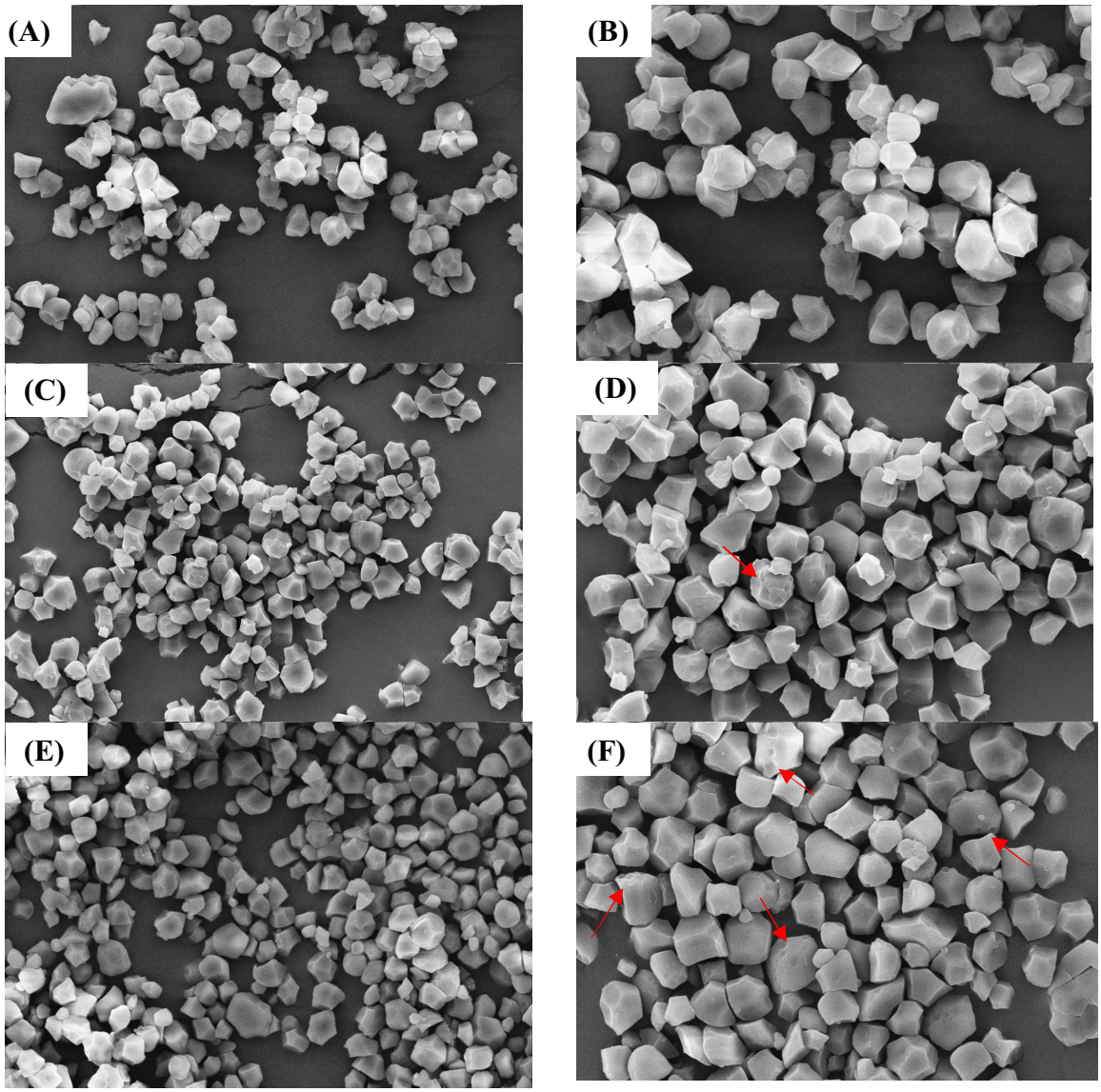


Figure 1. Scanning electron microscopy (SEM) of non-germinated (A, B), soaked (C, D) and germinated (E, F) rice starch. Red arrows represents changes in the starchy matrix.

These results are similar to those reported by Pal et al. (2023) that found changes in the starch granules after 24 h/28 °C of soaking followed by 48 h/28 °C of germination. In this study, starch from germinated brown rice showed that most granules continued intact but became rough and slightly eroded with pits in the surface, as pointed by the arrows (Fig. 1D, 1F). The authors related that this change in the granules can indicate the penetration of the enzymes activated during the process. These enzymes, including α-amylase, β-amylase, limit dextrinase and α-glucosidase, hydrolyzed the starch granules causing pores on the surface. On the other hand, Chungcharoen et al. (2015) did not observed changes in the granules of rice starches submitted to germination, even after 60 h of process at 35 °C.

3.2. Pasting properties

Pasting curves obtained from RVA of NGRS, SRS and GRS are shown in Figure 2 and the parameters are presented in Table S1. Pasting profile of SRS was similar with the profile for NGRS, but germination had an overall decrease in the all parameters, especially breakdown viscosity (BDV) (93 % of reduction, from 378 cP to 29 cP) and peak viscosity (PV) (67% of reduction, from 1270 cP to 425 cP). There was no difference ($p < 0.05$) on pasting temperature (PT) for all samples and final viscosity (FV) for NGRS and SRS, but, FV of GRS reduced 63% compared to NGRS (3300 cP to 1216 cP).

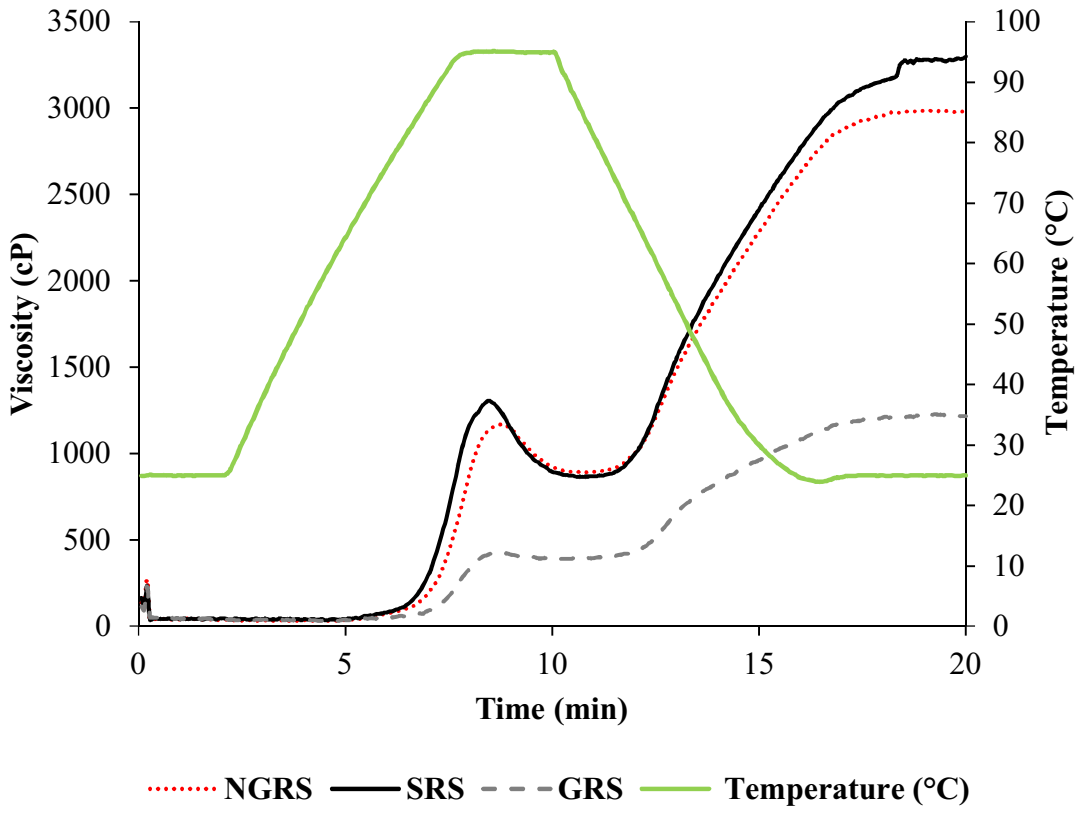


Figure 2. Pasting curves of starches from non-germinated, soaked and germinated rice starch.

Long soaking processes (4 days) at 25 °C facilitate the gelatinization of rice, decrease pasting temperature (PT) suggesting that rice is easy to cook after soaking and increased PV and BDV on glutinous rice. These changes can be attributed to the combination of starch granules with the free hydrophilic groups (Gong et al., 2020).

The reduction of paste properties caused by germination is now elucidated in the literature, and it is attributed to starch degradation by the increased α-amylase activity (Pal et al., 2016). The temperature of the germination process is a condition that most influences the viscosity. The reduction of PV and BDV is more pronounced in 35 °C. After the germination, the starch matrix shows a reduction in the size of the amylose chains leading to greater stability of the paste at high temperatures, reducing the PV and BDV (Wang et al., 2020b).

3.3. Thermal properties

The thermal properties of NGRS, SRS and GRS were shown in Table 2. T_o, T_p, and T_c of ranged between 61.9 to 59.0 °C, 63.8 to 66.7 °C and 73.73 to 78.2 °C (Table 1).

Table 2. DSC parameters and crystalline structure of non-germinated, soaked and germinated rice starch.

Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c - T_0 (°C)	ΔH (J/g)	Relative crystallinity (%)
NGRS	59.01±0.31^{b}	63.81±0.39^{b}	73.73±0.07^{c}	14.72±0.38^{b}	9.69±0.28^{b}	27.40±0.14^{a}
SRS	59.51±0.93^{ab}	64.29±0.61^{b}	75.56±0.07^{b}	16.05±1.00^{a}	8.81±0.22^{ab}	26.08±0.63^{a}
GRS	61.92±0.66^{a}	66.67±0.03^{a}	78.24±0.00^{a}	16.32±0.66^{a}	8.37±0.31^{a}	25.65±0.56^{b}

T_0 = Temperature onset, T_p = Peak temperature, T_c = Conclusion temperature, ΔH= Enthalpy. Values are (mean ± standard deviation). Data with the same letters in the same column do not differ significantly by Tukey test ($p < 0.05$).

NGRS showed higher enthalpy as compared to GBR (8.8 to 9.7 J/g) and as compared to SRS (8.8 to 8.4 J/g) although statistical difference was not observed in NGBR and SRS samples ($p < 0.05$). The increase of T_o, T_c under high temperature of germination (35 °C) can be explained by the amount of double helical structure and the increase of amylose. Starches from GBR need more thermal energy to disrupt the molecular structure (Hongwei Wang et al., 2020b).

Soaking process demonstrated similar behavior to germination, as both caused an increase in the range of T_c - T_0 and decrease in enthalpy (Table 2). This can be explained by the reduction in the amount of double helical structures activated by α-amylase during soaking and germination, hence causing a degradation in the starch matrix and a formation of carbohydrates with less polymerization degree (dextrin, fermentable sugars) (Pal et al., 2023a; Hongwei Wang et al., 2020a).

3.5 Color analysis

Visual aspect, white indexes and gel texture of starches are shown in Fig. 3. Visually, all starches presented white coloration and good flowability. No statistical differences were present in the samples ($p< 0.05$) concerning the whiteness indexes (W.I.) evidencing that soaking and germination process did not affect the color characteristics. Rice starch is naturally white and provides it commercial value advantages over other starches such as sorghum, wheat and rye (J. N. BeMiller & Whistler, 2009) in terms of wide range of applications.

3.6 Particle size distribution (PSD)

Figure 3 (E, F) showns the PSD of non-germinated, soaked and germinated rice starches. The non-germinated sample presented a higher value of D [4, 3] if compared to the SRS and GRS ($p < 0.05$). All samples showed a similar distribution of mean particle size, presenting a bimodal distribution curve and higher frequencies of particles with 7 μm (12, 13.69 and 13.15 % respectively). Soaking can affect the PSD by the softening effect during water immersion (Pal et al., 2023a) and germination caused a enzymatic activation of amylolytic enzymes leading to the formation of more heterogeneous pores and pits on the surface of the granule decreasing PSD , as shown by SEM micrographs (Fig. 1). Our results are similar to Li et al 2017, Wang et al 2020 and Pal et al 2023 that also observed this behavior more intensely in rice starches germinated at 35 °C.

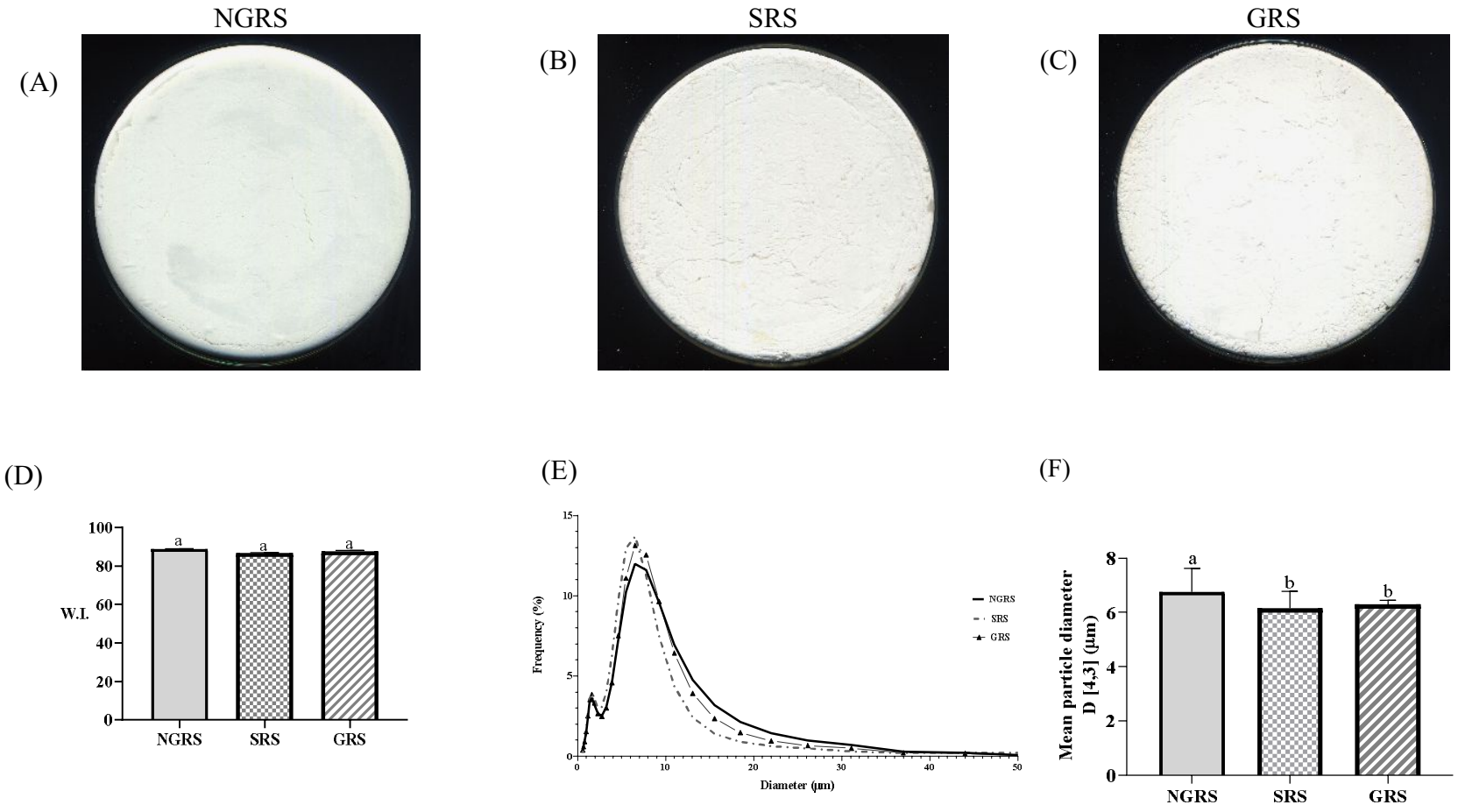


Figure 3. Images of the visual aspect (A, B, C), white indexes (D), particle size distribution (E) and mean particle diameter (F). Bars indicate standard deviations. Variations followed by the same letters do not differ significantly ($p < 0.05$). W.I.= White Index.

3.4 Crystalline structure

Fig. 4 showns the XRD patterns of NGBR, SRS and GBR. All samples exhibit well-defined diffraction peaks at 15°, 17°, 18° and 23,5° (2Θ) indicating a typical A-type polymorph form, and a slight decrease intensity of SRS and GBR peaks. The relative crystallinity (RC) calculated by the X-ray diffractograms of starches is shown in Table 2.

RC ranged from 25.65 to 27.40 %. Soaking and germination process slightly reduced RC (4.81 %, 6.38 %), respectively. Our results are in agreement with others reported in the literature (C. Li et al., 2017; Pal et al., 2023a; Wang et al., 2020a).

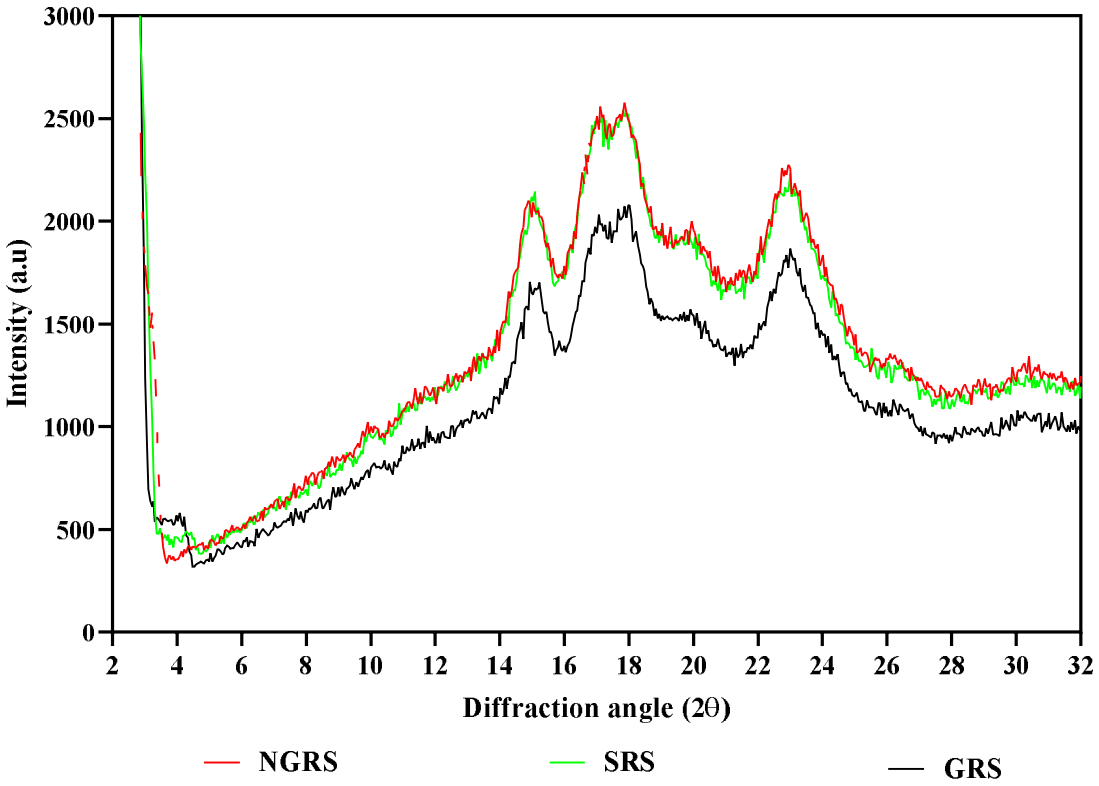


Figure 4. X-ray diffraction of non-germinated (NGRS), soaked (SRS) and germinated (GRS) rice starch.

3.5 Gel strength

The results of the penetration test are show in Figure 5. All samples formed a consistent gel (Figure 5) after refrigerator storage (5 °C) and similar gel characteristics were observed in NGRS, SRS and GRS. Unexpectedly, the maximum value of peak force of gel after cooling was obtained by GRS (0.29 N) followed by SRS (0.24 N) and NGRS (0.21 N). Under cooling, the starch chains (amylose and amylopectin) conduct to formation of a more ordered structure, meaning that this phase transition is a retrogradation process. During cooling, amylose molecules of long chain length would form extensive crosslinks and this involves the formation of ordered structures via hydrogen bonding and/or hydrophobic interactions. On the other hand, amylopectin *per se* could be responsible for the crystallinity in the starch gel that becomes firmer on storage over several weeks (Hoover, 1995; Miles et al., 1985).

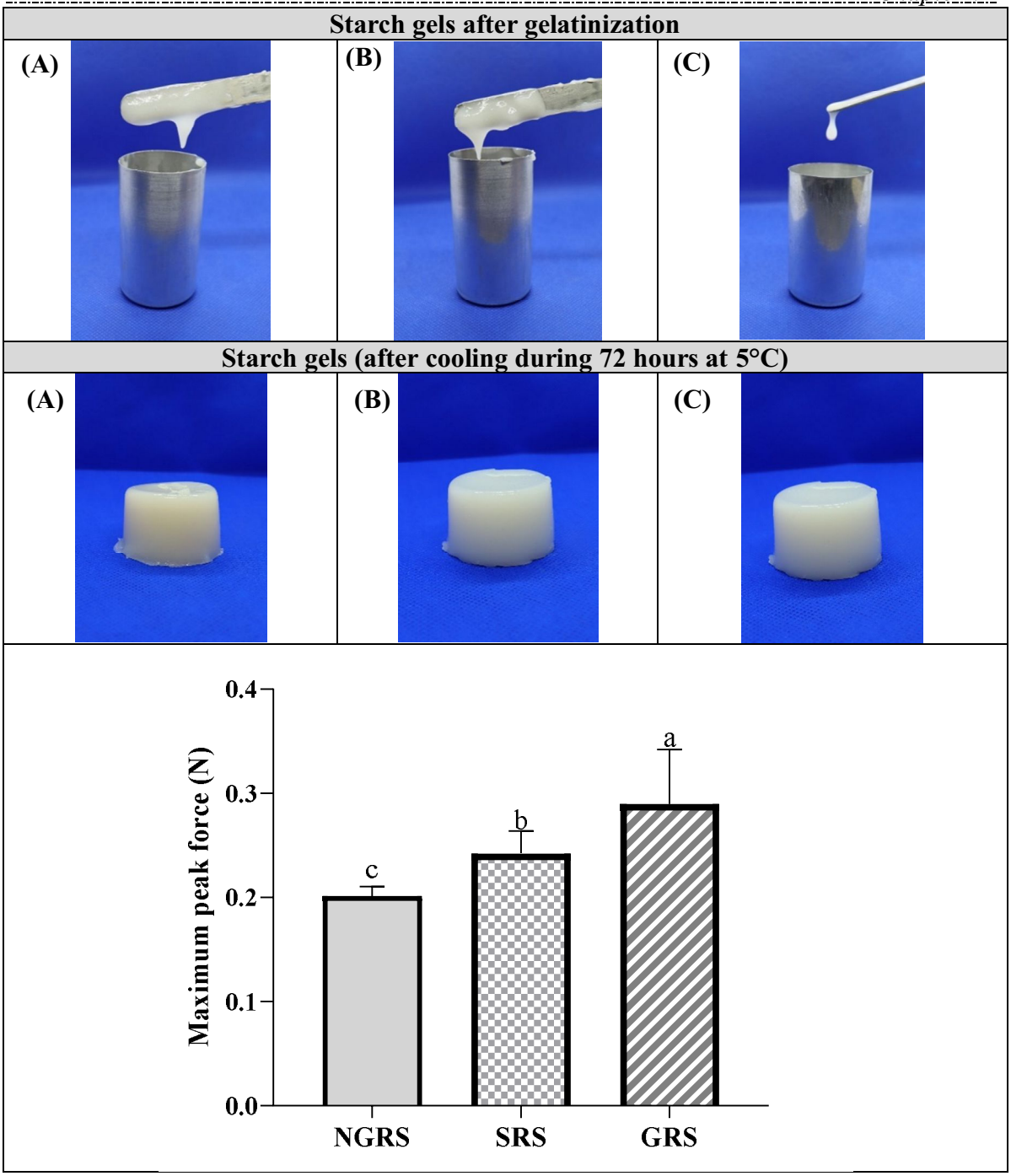


Figure 5. Visual aspect and gel texture profile (puncture test) obtained after RVA assays and cooled 72 h at 5°C: (A) NGRS, (B) SRS and (C) GRS.

This behavior could be explained by (i) the increase of α-amylase during soaking and germination process that debranching the amylose portion leads to reduction of peak viscosity and decrease of peak intensity (as showed in our results of RVA, RC and X-Ray diffraction) and (ii) the reorganization of amylopectin chains during cooling storage that help to promote gel firmness. However, this result has not been found in the literature. Wang et

al. 2020 observed that increasing proportions of B2 and B3 chains of amylopectin after germination process may facilitate the strengthened entanglement of glucan chains in the shell of "granule ghosts" and a rearrangement among the adjacent molecular chains following the paste cooling.

4. Conclusion

Soaked and germinated rice starches had pits and pores on the granule surface. Both processes decreased protein content, total starch, gelatinization enthalpy, and relative crystallinity. Differences in color characteristics and resistant starch comparing soaking and germinated starches were not found. Gel strength of the GRS were 16.37 % higher than the SRS and 28.58 % higher than NGRS. These findings help to understand how soaking and germination processes change the rice starch structure and functionality providing insights for food manufacturing uses in order to obtain natural ingredients for development of clean label products.

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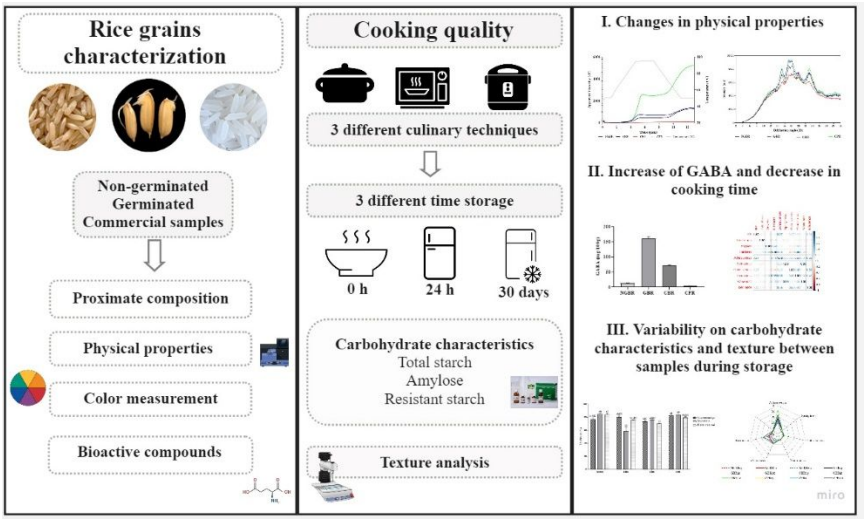
Chapter VII

How culinary techniques affect different types of rice products during storage? A comparative study

Maria Eugenia Araujo Silva Oliveira[1], Monique Santos Barreto[2], Leda Maria Fortes Gottschalk[3], Priscila Zaczuk Bassinello[4], José Manoel Colombari Filho[4], Carlos Wanderlei Piler de Carvalho[3], Cristina Yoshie Takeiti[1,3]

[1] Food and Nutrition Graduate Program (PPGAN), Federal University of the State of Rio de Janeiro, UNIRIO, 22290-240, Rio de Janeiro, RJ-Brazil
[2] Federal Rural University of Rio de Janeiro (UFRRJ), BR-465, Km 07, Zona Rural, Seropédica - RJ, 23890-000
[3] Embrapa Agroindústria de Alimentos, Avenida das Américas, 29501, 23020-470 - Rio de Janeiro, RJ – Brasil
[4] Embrapa Arroz e Feijão, Rodovia GO-462, Km 12, Zona Rural, 75375-000 - Santo Antônio de Goiás, GO-Brasil

Paper in process to submit to Carbohydrate Polymers



Highlights

- Short germination caused an increase on pasting properties
- GABA increase 103 % after germination
- Germination was effective in reduction of cooking time (31 to 22 min)

ABSTRACT

Rice is a cereal widely consumed throughout the world, mostly in its polished form (PR). Germination is a process used to improve the nutritional and sensory qualities of brown rice (BR), but consumers refuse this product due to its sensory perceptions such as texture and/or flavor and long preparation time. Therefore, the aim of this work was to evaluate the effect of germination on the proximate composition, physical properties, phenolic compounds and the effect of different cooking preparation and storage conditions on the carbohydrate characteristics. Germination was not able to cause an increase in dietary fiber (6.7 %) content. Falling Number (FN) ranged between 366 s (GBR) to > 400 s (commercial samples), showing statistical difference ($p < 0.05$). Germination did not cause a decrease in crystallinity and DSC parameters but showed peak viscosity (PV) 41% higher than non-germinated brown rice (NGBR) and the final viscosity (FV) 6.59 % higher than NGBR. γ-aminobutyric acid (GABA) had a higher increase after germination (12.3-160 μg/g) despite the short time. Regarding cooking properties, germination was effective in reducing cooking time (31 to 22 min) using a conventional pan that is the more common rice preparation. RS content was observed after 30 days under freezing, only in electric rice cookers showing a significant increase (100 %) demonstrating a time-dependent phenomenon. Concerning amylose (AM), germination leads to a decrease in contents regardless of the cooking method used. RS content was observed after 30 days under freezing, only in electric rice cooker showing a significant increase (100 %) demonstrating a time-dependent phenomenon. After cooking (t= 0 h) germination induced in GBR prepared by electric rice cooker, the highest values of hardness (19.7 N) and chewiness (24.6 N). Freezing (t= 30 days) caused a decrease in hardness (12.7-19.2 N) and chewiness (5.3-17.1 N) in comparison with commercial and non-germinated samples. Freezing caused an increase of RS (100 %) only in conventional rice cookers. It is concluded that GBR has potential to become innovative process to improve GABA and texture in order to create added-value of BR.

Keywords: Physical properties, GABA, texture profile

1. Introduction

In the last decade, lots of consumers are eating foods prepared or cooked away from home expecting to have safe and healthy meals with good taste and quality (Aguilera, 2018). More consumers are becoming aware of different levels of processing from media reports, regulations and voluntary on-pack labels. Frozen products are example of convenience minimally processed foods, which enhance nutrition and increase shelf life (Mintel, 2024).

Rice (*Oryza sativa* L.) is a staple food mostly produced and consumed in Asian countries (FAOSTAT, 2023). Rice is commonly consumed after polishing (PR) due to smaller cooking time and sensory characteristics such as taste, appearance, and texture (Saleh et al., 2019). Brown rice (BR) has superior nutritional quality to PR and an improved source of vitamins, minerals, fiber, γ-oryzanol, and phytochemical components which can support human health (Mir et al., 2020).

Germination is an alternative to improve the sensory properties of BR and preserve all nutrients in the rice grain for human consumption in order to create the highest value from rice. Germinated brown rice (GBR) contains several bioactive compounds such as phenolic acids, flavonoids, γ –oryzanol and GABA with different health benefits already elucidated in the literature such as antioxidant, antidiabetic, and anticancer activity (Saleh et al., 2019).

It is well known that rice varieties, processing conditions, and cooking methods affect the starch structure. However, non-studies have investigated these effects by combining type of cooking and storage time-temperature. Conventional pan, microwave and electric rice cookers were used to evaluate the effects of cooking type procedures on different rice types: non-germinated brown rice, germinated brown rice, commercial brown rice and commercial polished rice. The aim of this present study, therefore, was to investigate the effect of different cooking methods and storage on physical properties of grains, carbohydrate profile and texture characteristics of cooked rice.

2. Material and methods

2.1 Material

BRS Catiana (*Oryza sativa* L.) was selected by *Banco ativo de Germosplasma do Arroz* (BAG) of *Embrapa Arroz e Feijão* (Santo Antônio de Goiás-GO, Brazil), multiplied in 2020/2021 harvest using a flood-irrigated system in the farm experimental field of Embrapa Arroz e Feijão (6° 29′ 8″ S, 49° 18′ 32″ W). After harvest, the rice grains were naturally dried. Two commercial rice samples (fine long) were used as control (commercial brown rice, CBR and commercial polished rice, CPR).

2.2. Germination process

Germination process was performed according to the methodology described by Oliveira et al. (2023) with some modifications (4 h of soaking and 16 h of germination). Thus, the samples were denominated as non-germinated brown rice (NGBR) and germinated brown rice (GBR).

2.3 Proximate composition

Proximate compositions were determined according to AOAC standard methods: (i) protein content method n. 46-13 (AOAC, 1995), (ii) fat content method n. 945.38 (AOAC, 2005), (iii) dietary fiber content method n. 985.29 (AOAC, 2005), (iv) ash content method n. 923.03 (AOAC, 2005), (v) moisture content was determined in the oven at 105°C until constant weight and (vi) energy value was determined according RDC n.º 360 de 23 de Dezembro de 2003 (ANVISA, 2003).

2.4. Physical properties

2.4.1. Falling Number

The falling number (FN) was determined according to method 56–81.03 (AACC, 2010).

2.4.2. Pasting properties

Pasting properties were carried out using a Rapid Visco Analyzer series 4 (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia) according to method 76-21.01 (AACC, 2010).

2.4.3. X-ray diffraction pattern

Crystalline structure was analyzed using an X-ray diffractometer D2 Phaser (Bruker, Rheinfelden, Germany), operating at Cu-K wavelength with 0.154 nm, 30 kV and 10 mA. Samples were scanned in duplicate from 2° to 32° (2θ) at the rate of 0.05°/min, with a step size of 0.02° and divergence slit width of 0.6 mm equipped with a Lynxeye detector (Bruker, Rheinfelden, Germany).

2.4.4. Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analyzes was conducted using a Q200 (TA Instruments, New Castle, USA) according to Bernardo et al. (2018). The samples (~2 mg), dry basis, were accurately weighed and transferred to hermetic aluminum pans added of deionized water (2:1). Then, the pans were sealed and allowed to rest for 18 h at room temperature before performing the experiments. Scan was done from 5 to 120 °C at a rate of 10 °C/min. An empty pan was used as reference. DSC data were analyzed to calculate onset (T_o), peak (T_p), conclusion (T_c) and enthalpy of gelatinization (ΔH).

2.4.5 Color measurement

CIEL*a*b measurements of rice grains were measured (10 replicates) using a Color Quest XE (Hunter Lab, Reston, USA) in reflectance mode. Color parameters were expressed in terms of L^*, a^*, and b^* and white index (WI) was calculated using Formula (1).

$$W.I. = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

2.5. Bioactive compounds

2.5.1. γ-aminobutyric acid (GABA), ferulic acid, sinapic acid and p-coumaric were determined according to the methodology described by Oliveira et al. (2022). In order to determine ferulic acid, sinapic acid and *p*-coumaric acid, the sample extractions were performed according to Pérez-Jiménez et al. (2008). Chromatographic analysis was performed according to Nascimento et al. (2017) with quantification by external standardization with *trans*-ferulic acid (Ref.128708, Sigma Aldrich, St. Louis, USA), sinapic acid (Ref 530-59-6, Sigma Aldrich, St. Louis, USA) and *p*-coumaric acid (Ref 501-98-4,

Sigma Aldrich, St. Louis, USA) and identification through comparison of retention time and UV/VIS spectra in a Waters® Alliance model 2690/5 high performance liquid chromatographic system (Waters Corporation, Milford, USA), with a Waters® diode array detector model 2996 (quantification at 325 nm) and Empower® software (2002) (Waters Corporation, Milford, USA), with two Thermo® BDS HYPERSIL C 18 columns (Thermo Fisher Scientific, Waltham, USA) in series (50 × 4.6 mm, 2.4 μm; and 100 × 4.6 mm, 2.4 μm). The columns' temperature was at 30°C. The elution was performed in gradient mode with phosphoric acid 1.5 ml/L in water (Phase A) and acetonitrile (Phase B) with a flow of 1.2 ml/min. The injection volume was 15 μl and the running time was 30 min.

2.6. Cooking quality

The cooking time was performed according to the Ranghino test (Juliano & Bechtel, 1985) in three different cooking procedures (conventional pan, domestic microwave and electric rice cooker). For the conventional pan, 100 mL-deionized water was boiled (99 °C) in a 250 mL using a glass beaker and 5 g of rice were placed into it. For the microwave oven, a plastic pan (Plasvale®, 13.5 x 24.0 x 21.0 cm, Gaspar-SC, Brazil) containing 300 mL of pre-heated (99 °C) of deionized water were added to 15 g of rice, and then placed in the domestic microwave, level 1 (620 W) (Consul®, model CMA20BBBNA, Joinville-SC, Brazil). For an electric rice cooker (400 W) (Mondial® Bianca Rice, model NPE-05-5X, Sorocaba-SP, Brazil) the same proportion of water:rice (20:1) was applied. The cooking time started immediately after the rice came into contact with the boiling water. Ten grains of rice were removed and pressed between two clean glass plates to assess their degree of cooking. The final cooking time was recorded when at least 90% of the grains no longer had an opaque core or an uncooked center. Then, the rice was allowed to simmer for 2 min longer to ensure that the cores of all the grains were completely gelatinized and therefore, the optimal cooking time (t_c) was determined. Water uptake ratio was carried out according to the methodology described by Juliano & Bechtel (1985).

2.7. Carbohydrate characteristics

Total starch (TS), amylose (AM) and resistant starch (RS) contents were determined using a K-TSTA-100, K-AMYL and K-RSTAR standard kits, respectively (Megazyme International, Bray, Ireland).

2.8 Texture profile

Textural profile analysis (TPA) of the rice samples was performed according the methodology described by Paiva et al. (2015) using a texture analyzer (TA-XT2, Texture Technologies Corp., UK) with a 5 kg load cell using a two-cycle compression method. The texture analyzer was coupled to a computer that recorded the data via the XT.RA Dimension software program (v. 8, Texture Technologies Corp., USA). Each sample was prepared according to the optimal cooking time (t_c). The cooked rice was completely drained using a plastic sieve. A SMS P/25 probe was used to compress 2–3 grains, with pre-test and post-test speeds of 1 mm s^{-1} and a test speed of 5.0 mm s^{-1}.

2.9. Statistical analysis

Statistical analysis was performed with Tukey's test ($p < 0.05$). One-way ANOVA and radar chart were using XLSTAT software (Addinsoft, Paris, France). Correlogram for correlation analysis and their significant test was generated in R package "corrplot" in R-version 1.2.5042.

3. Results and discussion

3.1. Proximate composition

The proximate composition is presented in Table 1. CPR showed the highest value of moisture (10.88 %) and GBR the lowest value (6.88 %), which may be due to the drying conditions. A little decrease in percentage on ash (11.87%), protein (0.91 %) and lipids (27.72 %) was observed between NGBR and GBR samples. In fact, germination can contribute to a decrease in protein content may be due to the hydrolysis of protein by protease (Cornejo et al., 2015) and in lipid content by the amylose-lipid complex formation (Xu et al., 2012b). The carbohydrate content (3.97%) and energy value (0.43%) had increased a little after germination. During germination the activity of α- and β-amylase enzymes are involved in

the transformation of starch into malts and simple sugars (Pino et al., 2022). According dietary fiber, CPR showed the lowest value (3.98 %) and this was to be expected, since the fibers are concentrated in the external part of the grain, which is removed by polishing (Ma & Tan, 2020). Germination did not increase the content of dietary fiber but, the GBR rice presented 14.41% higher than CBR rice.

Table 1. Proximate composition of brown rice before and after germination.

Sample	Moisture	Ash	Protein	Lipid	Carbohydrate	Dietary Fiber	Energy value
NGBR	8.25 ± 0.67^{bc}	1.60 ± 0.09^{a}	6.61	3.39 ± 0.23^{a}	73.37 ± 0.16^{c}	6.79	350.43
GBR	6.88 ± 0.22^{c}	1.41 ± 0.07^{ab}	6.55	2.45 ± 0.21^{b}	76.39 ± 0.85^{b}	6.52	351.92
CBR	9.10 ± 0.65^{ab}	1.16 ± 0.03^{b}	7.13	2.14 ± 0.03^{b}	74.86 ± 0.52^{c}	5.58	347.31
CPR	10.88 ± 0.35^{a}	0.24 ± 0.00^{c}	6.55	0.31 ± 0.02^{c}	78.04 ± 0.26^{a}	3.98	341.15

Note. Mean ± SD. Different letters in the same column mean significant differences among samples ($p < 0.05$).

3.2. Physical properties

3.2.1. Falling number

The FN method used in the global wheat industry for detecting starch degradation by the α-amylase activity (Perten, 1964) was used to indirectly measure the increase in α-amylase in germinated rice (Table 2). FN values were ranged between 366 s (GBR) to > 400 s (CBR and CPR), showing statistical difference ($p < 0.05$). Commercial samples showed higher FN values, indicating minimal enzyme activity contrary to NGBR and GBR that present lower FN values, i.e. germination leads to α-amylase activation even in short germination time (< 24 h). α-amylase activity and starch degradation during germination is complex and depends on several variables e.g cultivar and germination time, corroborating with our previous findings (Oliveira et al., 2022). α-amylase is synthesized *de novo* during seed germination in the presence of endogenous GA (gibberellic acid) and the peak occurs after 48 h (Damaris et al., 2019).

3.2.2. Pasting and gelatinization properties

Pasting curves (RVA) are show in Figure 1. Pasting curves of NGBR and GBR showed a similar profile, however GBR showed a peak viscosity (PV) 41% higher than

NGBR and the final viscosity (FV) 6.59 % higher than NGBR. Our previous study (Oliveira et al., 2023) carried out under short germination conditions, also presented no reduction in paste viscosity of GBR. It was noteworthy that the impact of germination on pasting viscosity of rice depends on several factors as (i) time, (ii) cultivar, (iii) temperature, (iv) amylose/amylopectin ratio and (v) lipids and protein content (Li et al., 2020; Oliveira et al., 2022). The initial pasting temperature (PT) ranges between 68.5 (NGBR) to 79.0 °C (CPR). In relation to commercial samples, CPR showed the highest values of peak viscosity (PV) (2592 cP), minimum viscosity (MV) (960 cP), breakdown (BDV) (1632 cP), final viscosity (FV) (5254 cP) and setback (SBV) (4294 cP).

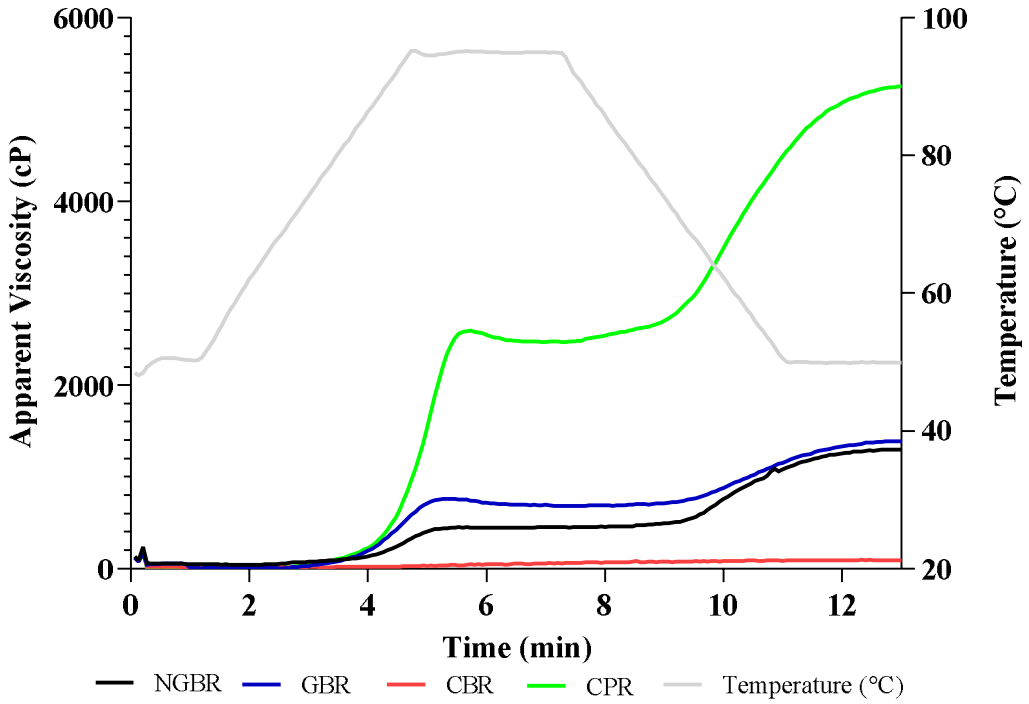


Figure 1. Rapid visco-analyzer curves of non-germinated, germinated and commercial samples.

The values obtained by DSC are presented in Table 2. There was no statistical difference ($p < 0.05$) between NGBR and GBR for all parameters (T_o, T_p, T_c and enthalpy). The short process time was not able to change the thermal properties of the material. The

CPR sample showed the highest value of T_o (61.42 °C), T_c (79.83 °C) and enthalpy (9.42 J/g), which was to be expected, since this sample does not have the pericarp that was removed by polishing and starch content is increased. Our results of gelatinization properties corroborate to those found in FN and in the pasting properties by RVA, and are in agreement with Oliveira et al. (2022) but contrary to the results reported by Pal et al. (2023) that founded lower gelatinization temperatures and enthalpy of gelatinization in GBR samples after 24 h at 28±1°C of soaking and 48 h at 28±1°C of germination.

3.2.3 Color measurement

The color measurement are presented in Table 2. The lightness (L^*) ranged from 60.79 (CBR) to 76.20 (CPR) and the W.I. ranged 55.26 (CBR) to 71.69 (CPR). No statistical difference ($p < 0.05$) was observed between NGBR and GBR samples in all parameters. The short germination time (< 24 h) was not enough to cause significant changes in the color of the grains. The changes in color parameters could be attributed to the enzymatic hydrolysis that took place during germination (Chinma et al., 2015), as Maillard reaction between the reducing sugars and proteins (Chung et al., 2012).

Table 2. Physical characteristics of brown rice before and after germination.

Sample	Falling Number (s)	DSC				Color characteristics			
		T_o	T_p	T_c	ΔH (J/g)	*L**	*a**	*b**	W.I.
NGBR	369.00±27.87^{b}	58.63±0.31^{a}	65.66±0.39^{a}	78.45±1.06^{a}	8.66±0.71^{a}	66.13±0.05^{d}	3.77±0.00^{b}	18.35±0.00^{b}	62.29±0.00^{b}
GBR	366.00±2.12^{c}	59.88±0.83^{a}	65.68±0.26^{a}	79.00±0.12^{a}	8.13±0.34^{a}	68.10±0.11^{b}	3.52±0.06^{b}	18.40±0.18^{b}	63.00±0.19^{b}
CBR	> 400±0^{a}	43.55±2.38^{b}	67.32±0.03^{a}	45.24±0.90^{a}	0.78±0.35^{b}	60.97±0.26^{c}	5.65±0.07^{a}	21.11±0.10^{a}	55.26±0.14^{c}
CPR	> 400±0^{a}	61.42±1.07^{a}	56.75±0.80^{b}	79.83±0.00^{a}	9.42±0.64^{a}	76.20±0.18^{a}	-0.35±0.50^{c}	15.30±0.21^{c}	71.69±0.11^{a}

T_0= Temperature onset, T_p = Peak temperature, T_c= Conclusion temperature, ΔH= Enthalpy. Values are (mean ± standard deviation). Data with the same letters in the same column do not differ significantly by Tukey test ($p < 0.05$).

3.2.3 Crystalline structures

The x-ray diffraction patterns of NGBR, GBR, CBR and CPR are shown in Figure 2. CBR showed a reduction of peak intensity, as expected considering that this commercial sample is parboiled. The peaks at 15°, 17° and around 23° demonstrate a typical A-type polymorphic form, and the peak around 23° represents the amylopectin molecules, the complex formed between starch and the endosperm cell wall (Pal et al., 2023). He et al. (2022) observed a reduction in relative crystallinity (RC) (32.18 to 28.59 %) after 12 hours after germination process, and greater reduction 48 hours after the process (32.18 to 12.08 %). The reduction in the relative crystallinity during germination may be due to the increase in α-amylase activity, resulting in the disruption of double-helical structures. In addition, Pal et al. (2023) also noted a reduction in RC in five cultivars of *Indica* rice after germination, attributing this reduction to the partial degradation of starch during germination by amylolytic enzymes.

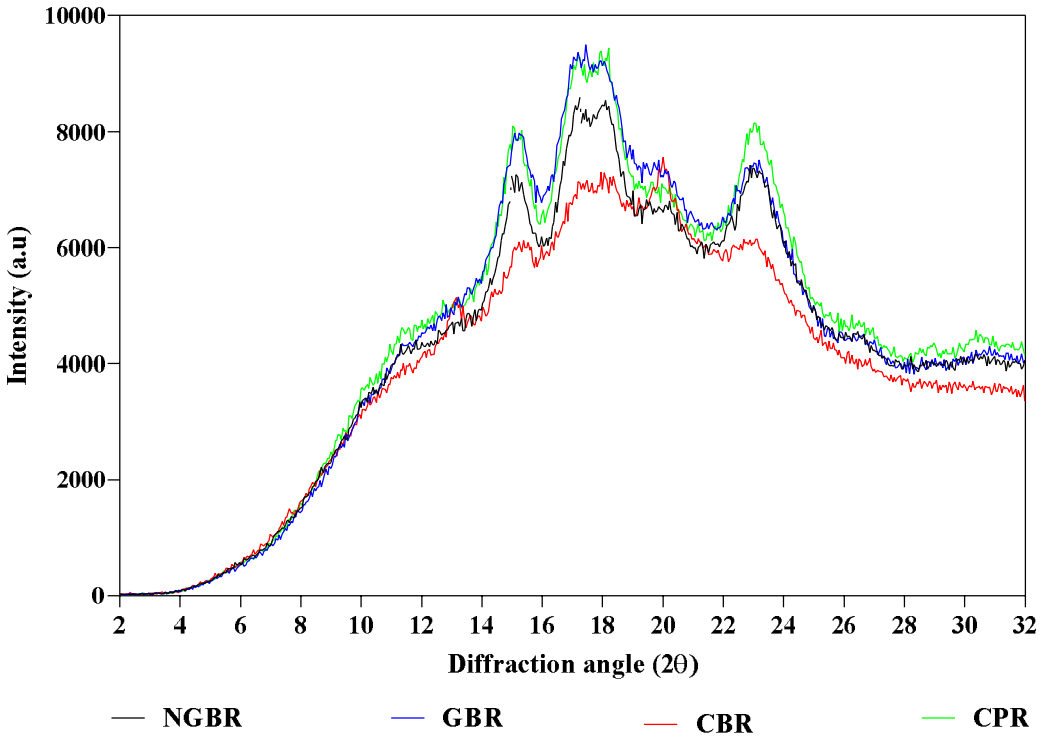


Figure 2. X-ray diffraction spectra of non-germinated, germinated and commercial samples.

3.3. Bioactive compounds

The results of ferulic, sinapic, *p*-coumaric acid and GABA are presented in Figure 3. CBR showed the highest value of ferulic, sinapic and *p*-coumaric acid and CPR the lowest values. High levels of phenolic acids are detected in rice bran and husk (90 %) contrary to other parts of the grain (Ding et al., 2018). During polishing, this layer is removed, causing a decrease in phenolic acids in the free and bound fractions (Liu et al., 2015).

There was no statistical difference ($p < 0.05$) between NGBR and GBR in ferulic acid, but germination caused an increase (54 %) in sinapic acid and a great reduction (87 %) in *p*-coumaric acid. Despite the soluble phenolic acids leaching out during soaking, these compounds could increase in the final stages of germination in a longer process (96 h). Germination induces the activity of two enzymes that are associated with the synthesis of phenolic compounds in the GBR: (i) phenylalanine ammonia-lyase (PAL) that reaches its peak 48 hours after germination and (ii) cell wall peroxidase (CW-PRX) that became activated in the early stage (Cho & Lim, 2018). Phenolic acids are common in rice and have the antioxidant and anti-inflammatory activity demonstrated in several studies (Butsat & Siriamornpun, 2010; Nignpense et al., 2022; Perez-ternero et al., 2017). In addition to these benefits, the literature has highlighted the prebiotic effects of phenolic compounds and the modulation of the gut microbiota with beneficial action on multiple disorders, including obesity, diabetes, cardiometabolic syndrome and neurodegenerative diseases (Kasprzak-drozd et al., 2021; Yen et al., 2020).

Regarding GABA contents, CPR showed the lowest value than GBR, as expected. Short germination time caused an increase of 91 % (12.85 to 160 mg GABA/100g). These findings are in agreement with our previous study, Oliveira et al. (2022) that also observed an increase of GABA in *Indica* rice species (*BRS Formoso* and *BRS Guaporé*) despite the short germination time. GABA has innumerous benefits for human health, including neuroprotection, anti-insomnia, anti-depression, anti-hypertensive, anti-diabetes, and anti-inflammatory (Hou et al., 2023).

In case of CBR, the parboiling process demonstrates an important strategy in favoring the diffusion of ferulic, sinapic and *p*-coumaric acid (14. 5 µg/g, 1.8 µg/g and 23.5

µg/g) from the pericarp to the endosperm. Parboiling process increases the phenolic acid content due to the instability of cell-wall structure caused by heat treatment and consequently, release of these acids and the derivatives from the wall cells to the starchy endosperm (Thammapat et al., 2016).

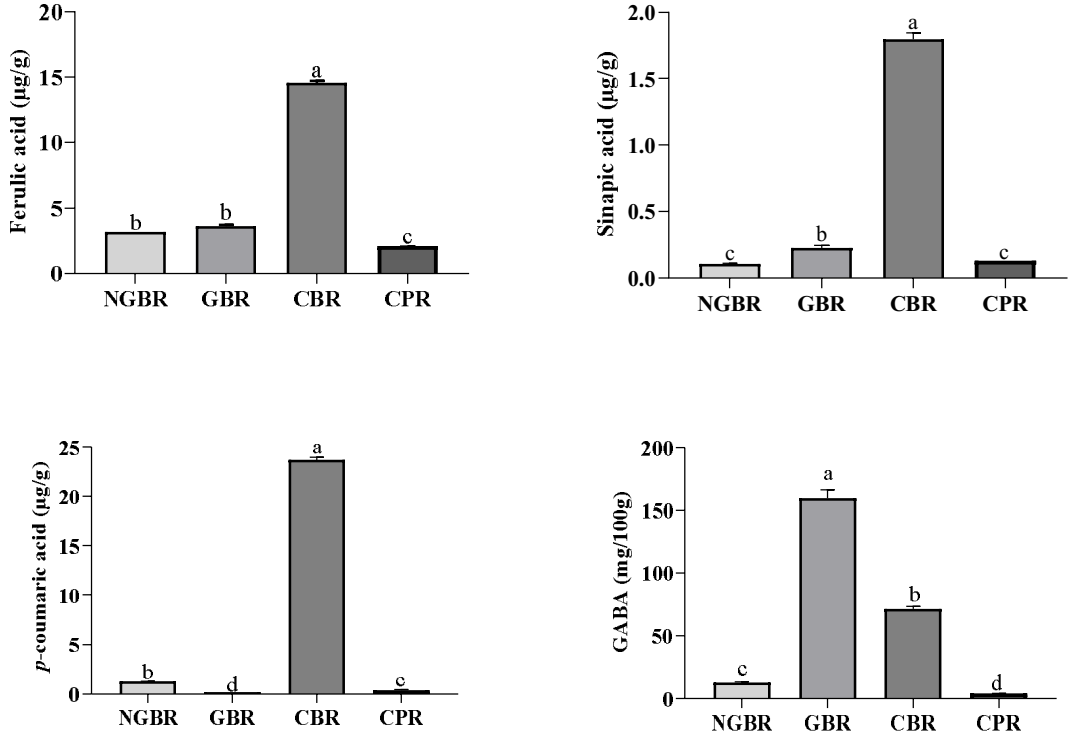


Figure 3. Bioactive compounds in rice samples.

3.4. Cooking quality and carbohydrate evaluation

The cooking time and water uptake ratio are presented in Table 3. The results of total starch (TS), amylose (AM) and resistant starch (RS) are shown in Figure 4, Figure 5 and Figure 6. In general, cooking methods determine the cooking time necessary to reach a complete cooking point of rice and samples showed statistical differences among them ($p < 0.05$). Regarding commercial samples, CBR showed the highest value for conventional pan (37 min) in contrast to electric rice cookers (24 min). CPR showed the lowest cooking time unrelated to method used (12 to 18 min). Respecting GBR, short germination was effective

in reduction of cooking time (30 %) in the case of conventional pan, which is the most traditional method used.

With respect to water uptake ratio, CPR (168.42-206.85 %) obtained the highest value probably due to the high starch contents (79.27-83.79 %) (Figure 4A) in opposition to GBR obtained by microwave method (101.85 %). Short germination induced an increase of 40 % in water uptake ratio in electric rice cookers that can be related to an increase of amylose content (14.74 to 15.70 %) (Figure 4). Changes observed in cooking quality of GBR are probably due to hydrolysis of high molecular weight polymers by hydrolytic enzymes activated during germination (Sirisoontaralak et al., 2015). Du et al. (2019) observed reduction (26 to 21 min) in cooking time and water absorption in germinated-parboiled rice compared to polished rice (330 to 197 %), attributing this phenomenon to the partial starch gelatinization. In addition, drying step after parboiling process could also contribute to reducing the water absorption due to the moisture sorption hysteresis.

Table 3. Cooking time and water uptake ratio of rices.

Sample	Cooking time (min)			Water uptake ratio (%)		
	Conventional pan	Microwave	Electric rice cookers	Conventional Pan	Microwave	Electric rice cookers
NGBR	31 ± 0.15^{b}	23 ± 2.15^{b}	23 ± 0.12^{a}	153.55 ± 1.39^{c}	109.29 ± 6.73^{bc}	76.82 ± 9.32^{c}
GBR	22 ± 1.14^{c}	23 ± 0.01^{b}	24 ± 0.00^{a}	143.12 ± 3.81^{d}	101.85 ± 10.06^{c}	129.80 ± 0.35^{b}
CBR	37 ± 0.00^{a}	25 ± 0.32^{a}	20 ± 0.04^{b}	186.13 ± 1.42^{b}	137.19 ± 2.86^{b}	114.07 ± 0.33^{b}
CPR	18 ± 0.00^{cd}	12 ± 0.00^{c}	16 ± 0.01^{c}	206.85 ± 0.93^{a}	168.42 ± 8.62^{a}	202.05 ± 7.55^{a}

The literature did not report changes in TS, AM and RS under different storage conditions. In this study, TS ranged from 57.72 (GBR microwave) to 85.22 % (NGBR microwave) immediately after cooking (t=0). At cooling temperature (4°C, t=24 h), TS ranged from 71.86 (NGBR) to 91.38 % (CPR) both for electric rice cookers and under freezing temperature, (-4 °C, t=30 days), TS varied between 63.45 (GBR) to 84.95 % (CPR) both for conventional pan.

Concerning AM, germination leads to a decrease in contents regardless of the cooking method used. In relation to commercial samples, a distinct reduction was observed in microwave-cooked rice after t= 24 h, especially in case of CBR (22.60 to 12.22 %). However, CBR storage under freezing conducted to an increase of AM (48 %). CPR demonstrated reductions of AM contents regardless of cooking preparation and storage.

In this study, the RS ranged from 1.72 to 2.98 % (*BRS Catiana*) and 0.38 to 2.50 % (commercial samples), and these values are in agreement with Kim et al. (2019) and Reed et al. (2013) that reported values ranging between 0.70-2.63 %. Interestingly, RS content was only detected in rice cooked by conventional pan and microwave (t= 0 and t=24 h). In the case of electric rice cookers that are the most used preparation method in Asia, RS content was only observed after 30 days under freezing, in which a significant increase (100 %) was observed and demonstrated a time-dependent phenomenon. When starch is gelatinized during cooking, its original crystalline structure is converted to an amorphous structure and subsequently retrograded. Refrigeration can facilitate starch retrogradation resulting in low digestibility of cooked and processed starchy foods. The amorphous structure becomes rearranged and ordered during a prolonged storage period (Singh et al., 2010). Many factors affect the RS content, such as degree of polishing, cooking method and the *Indica* genotype displayed the greatest value due to of its largest amylose content (Reed et al., 2013). Germination and parboiling processes increased the RS content due to the starch retrogradation and the cooling during storage (Du et al., 2019).

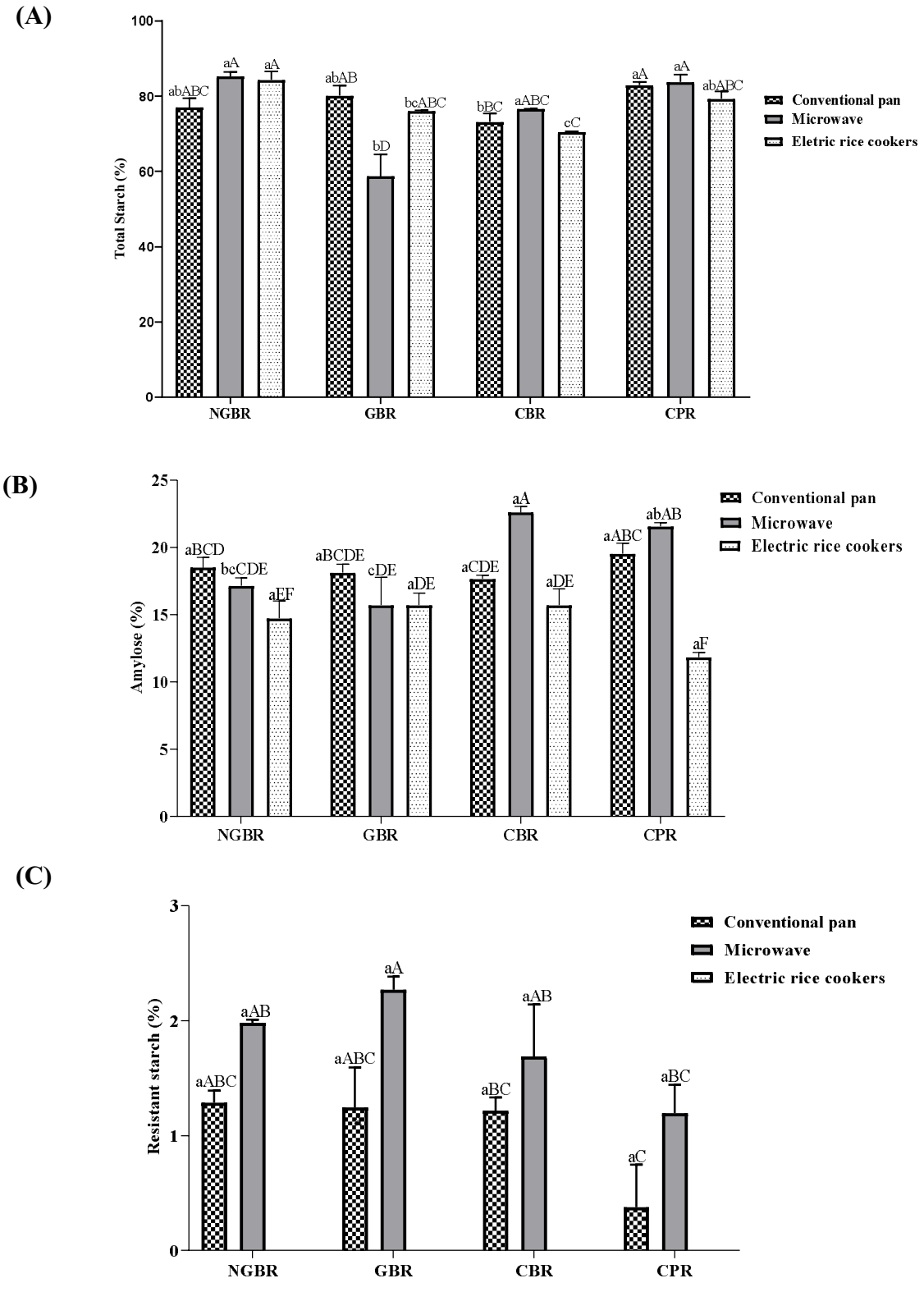


Figure 4. Total starch (A), amylose (B) and resistant starch (C) contents of rices after cooking (t=0 h).

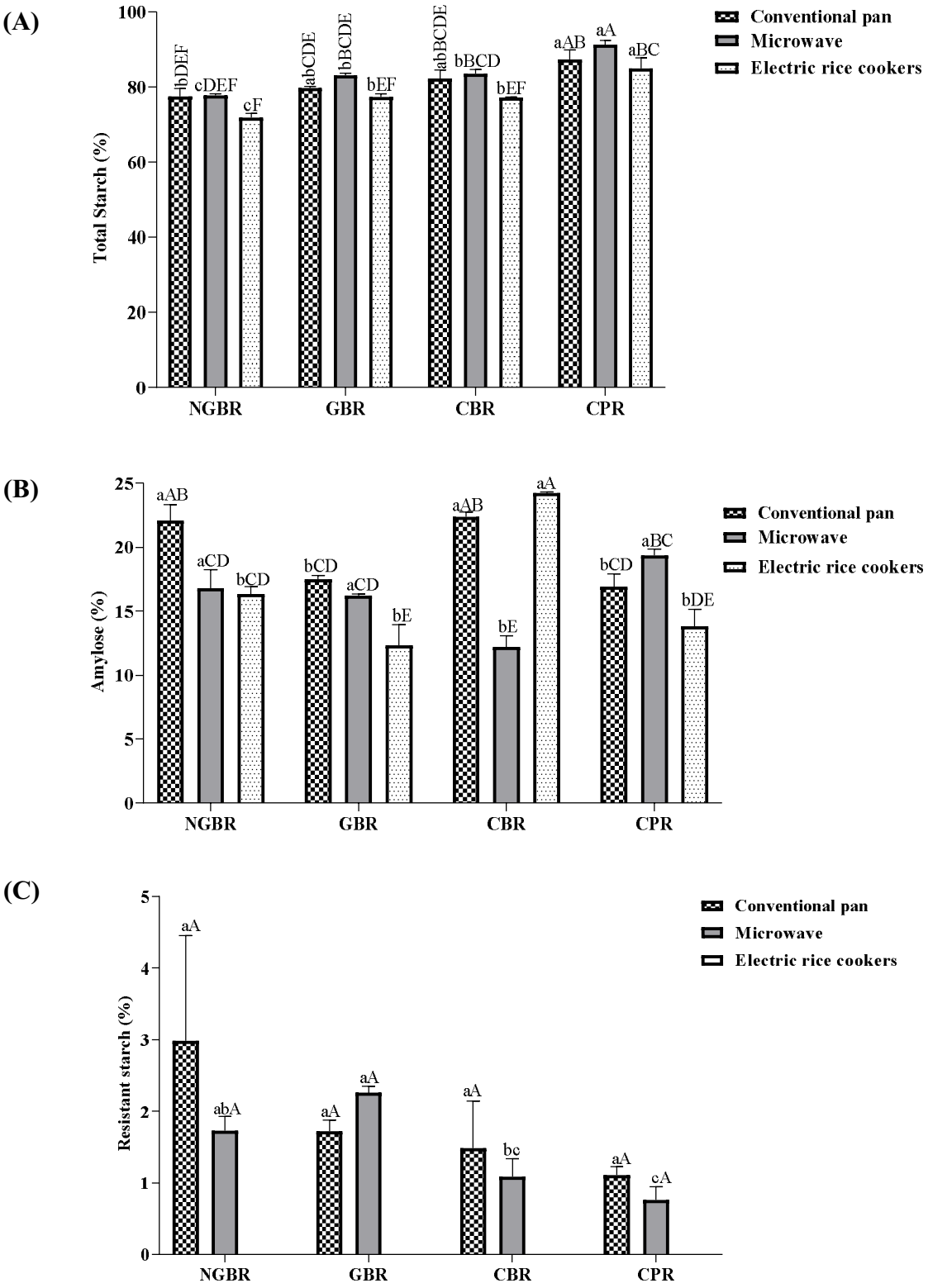


Figure 5. Total starch (A), amylose (B) and resistant starch (C) contents of rices at 24 h.

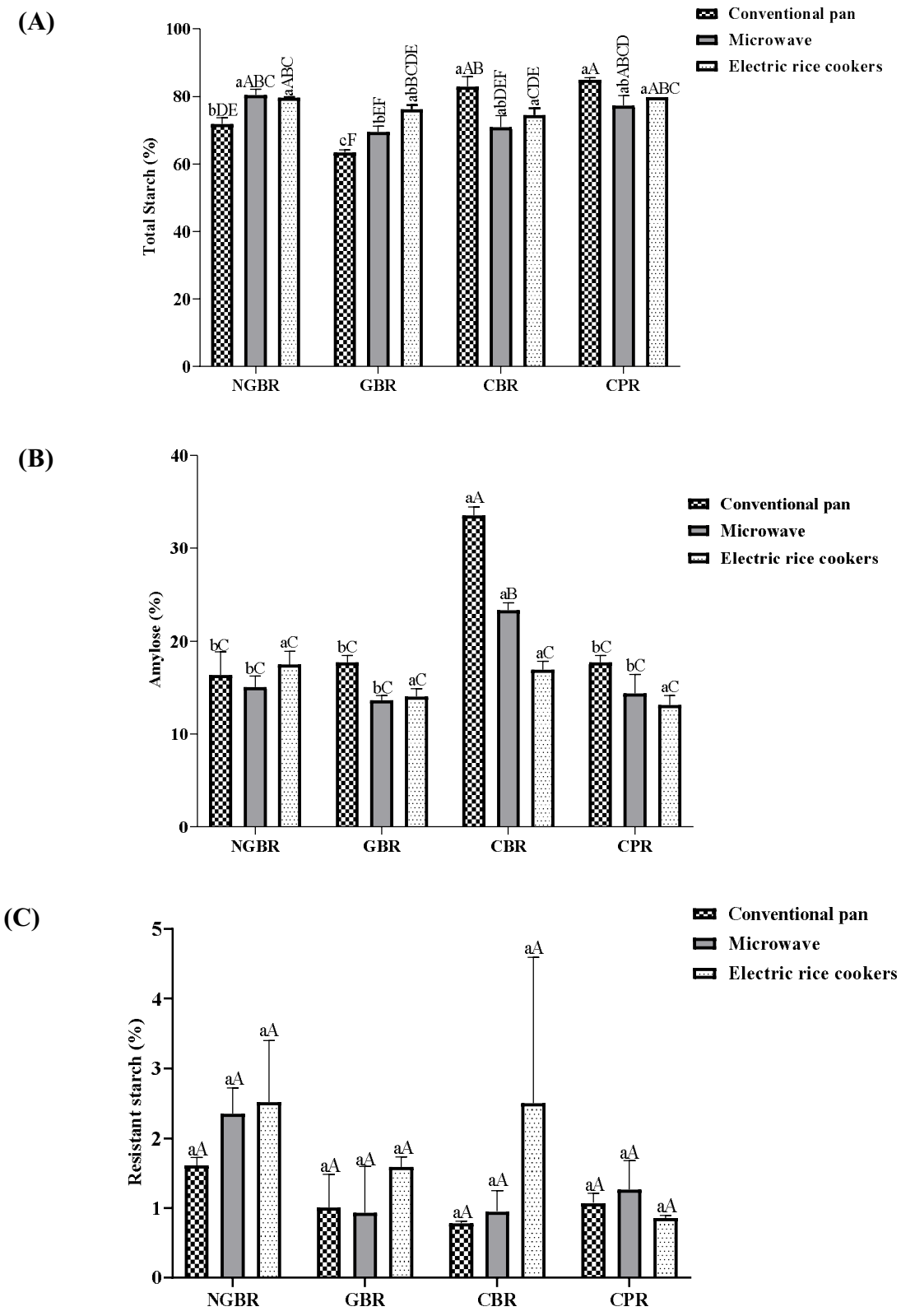


Figure 6. Total starch (A), amylose (B) and resistant starch (C) contents of rices at 30 days.

3.5 Texture profile

TPA profiles are presented in Figure 7. After cooking (t= 0 h) germination induced in GBR prepared by an electric rice cooker, the highest values of hardness (19.7 N) and chewiness (24.6 N). This behavior was also observed by CBR (commercial sample) cooked by electric rice cooker, that present a hardness of 21.8 N and a chewiness of 19.3 N (Figure 5 A).

Cooling (t= 24 h) induces the increase of hardness (17.5-32.3 N) and chewiness (7.4- 17.4 N) on CBR obtained by all preparation methods. However, germination leads to a reduction of 10 % in the hardness of GBR prepared by electric rice cookers, which consists of desirable characteristics in terms of sensorial properties. This behavior was also observed in terms of chewiness, in which germination conduct a reduction of 14 % compared to CBR sample cooked by electric rice cooker (Figure 5 B). This finding is corroborated by the highest AM content (24.2 %) observed by electric rice cookers after cooling (Figure 4) and higher correlation (r= 0.44) between AM and hardness ($p < 0.05$) (Figure 6).

Freezing (t= 30 days) caused a decrease in hardness (12.7-19.2 N) and chewiness (5.3-17.1 N) in comparison with commercial and non-germinated materials (Figure 5 C) unconcerned about preparation method. According to GBR, freezing was more effective in reducing hardness (64 %) than cooling (t=24h) in which, electric rice cooker caused the highest values of commercial samples, i.e. CBR (66.4 N) and CPR (25.5 N). A positive correlation between AM with hardness (r= 0.44), gumminess (r= 0.30) and chewiness (r= 0.14) was found (Figure 8).

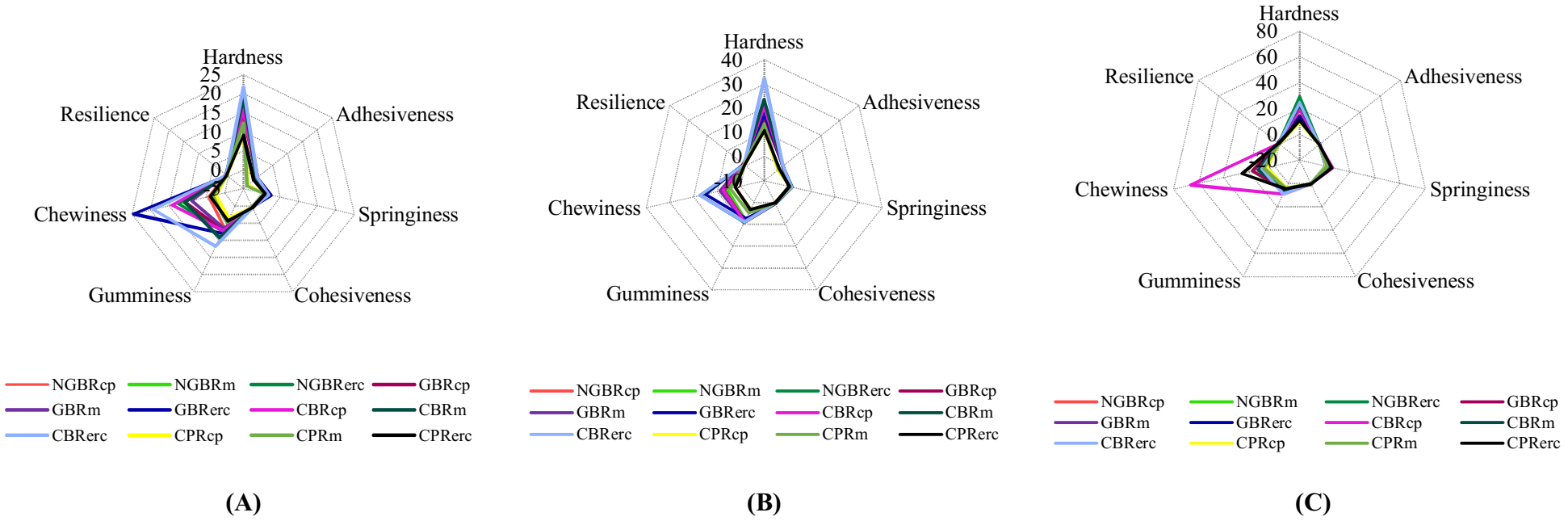


Figure 7. TPA profile of rices at t=0 h (A); t= 24 h (B) and t= 30 days (C). Where: cp= conventional pan; m= microwave and erc= electric rice cooker.

Figure 8. Pearson correlogram of carbohydrate characteristics and texture properties.

4. Conclusion

In this study, the effect of the germination process on proximate composition and physical properties of rice grains associated with the effect of cooking methods and storage time were evaluated. Germination was not able to cause an increase in dietary fiber content, neither lead to reduced the α-amylase activation in a short time (< 24 h). However, GBR demonstrate PV 41% higher than NGBR and FV 6.59 % higher than NGBR. Regarding thermal properties (T_o, T_p, T_c and enthalpy), there was no statistical difference ($p < 0.05$) between NGBR and GBR. In addition, germination caused an increase (54 %) of sinapic acid and GABA (103 %). Short germination induces changes in carbohydrate characteristic, specialty to AM content that influence the cooking quality. RS content was observed after 30 days under freezing, only in electric rice cooker showing a significant increase (100 %)

demonstrating a time-dependent phenomenon. Short germination was effective in reduction of cooking time (30 %) in the case of conventional pan, which is the most traditional method used. Texture profile showed that germination caused in GBR prepared by electric rice cooker, the highest values of hardness (19.7 N) and chewiness (24.6 N) after cooking (t= 0 h). In contrast, freezing of GBR obtained by an electric rice cooker caused a decrease in hardness (53 %) and chewiness (11.5 %) in comparison with non-germinated material that is an attractive characteristic considering BR consumption. These results sheds lights on understanding the effect of germination and storage in different types of rice, which can assist in recommending preparation methods to consumers.

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Chapter VII

The metagenomic approach reveals different microbial profiles in high and low-amylose rice after germination and polishing processes

Maria Eugenia Araujo Silva Oliveira[1], Bruno Gerfi Bertozzi[3], Priscila Zaczuk Bassinello[2], José Manoel Colombari Filho[2], Liliana de Oliveira Rocha[3], Carlos Wanderlei Piler Carvalho[4], Otniel Freitas-Silva[1,4], Dirce Yorika Kabuki[3], Cristina Yoshie Takeiti[1,4]

[1] Food and Nutrition Graduate Program (PPGAN), Federal University of the State of Rio de Janeiro, UNIRIO, 22290-240, Rio de Janeiro, RJ-Brazil

[2] Embrapa Arroz e Feijão, Rodovia GO-462, Km 12, Zona Rural, 75375-000 - Santo Antônio de Goiás, GO-Brasil

[3]Department of Food Science and Nutrition (DECAN), State University of Campinas, UNICAMP, Campinas, SP-Brazil

[4] Embrapa Agroindústria de Alimentos, Avenida das Américas, 29501, 23020-470 - Rio de Janeiro, RJ – Brasil

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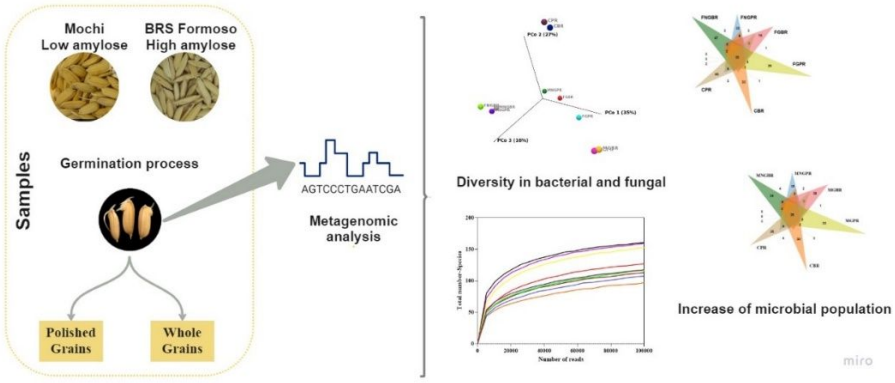


Highlights

- There was difference in microbial profile of *Indica* and *Japonica* ecotypes after germination.
- The germination of *Indica* ecotype showed the highest population of Bacteriodota.
- Rice polishing reduced firmicutes phyllo in *Japonica* ecotype.
- Combination of germination and polishing leads to Mucoromycota phyllo appearing with prevalence on *Japonica* type.

ABSTRACT

Germination is a sustainable process used to improve the nutritional quality of rice. However, microbiome profile of rice after this process is unknown and neglected. This study aimed to evaluate the microbiota composition of low and high-amylose rice after germination and polishing using metagenomics techniques. Alpha diversity was observed for bacterial microbiome, showing the highest Chao index for CBR (220.80) and the lowest in FNGPR (70.45). In case of fungi, CBR showed the highest value of Chao (32.65) and the lowest in MNGPR (11.36). Beta diversity suggested that the germination process had a similar effect on bacterial abundances presented in FGBR, FGPR, MGBR, and MGPR. Furthermore, (Principal Coordinate Analysis - PCoA) indicated that polishing treatment did not affect the microbiome to distinguish the studied population from commercial rice samples. The microbial profile of Mochi and BRS Formoso rice samples including bacteria and fungi counts indicated the prevalence of Cyanobacteria, Proteobacteria, and Ascomycota phylum. From Proteobacteria, Chryseobacterium, *Clostridium* sensu strictu 1 and *Clostridium* sensu strictu 5 represented the most frequent genera observed. Regarding fungi identification from Ascomycota, *Alternaria* and *Aspergillus* were more prevalent genera identified and from Mucoromycota, *Rhizopus*. In conclusion, germination treatment on Motí and BRS Formoso rices influenced both the bacterial and fungal microbiome communities.

Keywords: Germination, Metagenomics, Alpha-diversity

1. Introduction

Rice (*Oryza sativa* L.) is the most cultivated cereal in the world (FAOSTAT, 2021), and an important source of carbohydrates, fibers, vitamins, minerals, and bioactive compounds (Sen et al., 2020). According to the latest projections by the OECD-FAO Agricultural Outlook, global rice production (The majority of the projected production increase (52 Mt, i.e. 89.7%) is expected to occur in Asia where India (+20 Mt), China (+6 Mt), Vietnam (+4.5 Mt) and Thailand (+2.5Mt) (OECD-FAO, 2021a). *Indica* and *Japonica* are the two major types of rice traded on the global market. The species can be distinguished summarily by the shape and amylose content. *Indica* has longer grains and more amylose content than *Japonica* ones (Oka, 1988).

Brown rice (BR) contains all parts of rice grains (endosperm, embryo, and bran layers) different from the polished rice (PR) that lost these parts after the milling process (Juliano, 1985). From a nutritional perspective, BR is superior to PR, but some characteristics e.g. the taste, the sensory perception, and the shorter shelf life difficult its consumption. New technologies such as germination are needed as strategies to help market and improve the sensory quality of BR to increase its consumption (Mir et al., 2020).

Germination is an economic process used to improve the nutritional and sensory quality of rice. During this process, the enzymatic activity increases causing changes in the grain and the increment of a lot of substances including γ-aminobutyric acid (GABA), phenolic compounds, flavonoids, and γ-oryzanol (Oliveira et al., 2022). Previous studies has demonstrated the health benefits of consuming sprouted rice on starch digestibility, total antioxidant activities (Xia et al., 2017), and blood pressure (Nishimura et al., 2014).

Consumers are looking for products made with natural ingredients that help maintain and support a healthy heart, immune, digestive, and cognitive health (Mintel, 2023). In this scenario, germination can be used to improve the sensory characteristics of BR and the nutritional value of PR, as polishing after germination does not cause a significant decrease in bioactive compounds compared to non-germinated rice. This consisting an advantage since it does not change the consumption habits of PR, which is preferred by most consumers (Oliveira et al., 2022).

Cereal contamination can occur in different forms: (i) in the field, (ii) during harvest, and (iii) during storage (Scholtz et al., 2021). Germination can raise microbial growth due to the conditions under which the germinated brown rice (GBR) is produced as time, temperature, humidity, pH, and available nutrients after degradation of macromolecules (Z. Lu et al., 2010). In addition to these factors inherent in the process, whole grains usually have a higher content of microorganisms as they are in the surface layer (Doran & Bricgs, 1993), and the species, which *Japonica* rice (glutinous) demonstrate more water uptake during soaking stage which allowed for an increase in microorganisms (Gong et al., 2019).

Identifying the main microorganisms present in rice after germination and polishing can help control and solve problems related to the process. Metagenomics analysis provides a lot of information about the microbial composition of the food matrix and explores its diversity and functionality (Nam et al., 2023). Furthermore, there are no reported studies investigating the diversity of microorganisms after germination and polishing process of two different ecotypes of rice. Thus, this work aimed to investigate and characterize the microbial profile of *Indica* and *Japonica* rice after the germination and polishing process using metagenomics.

2. Material and Methods

2.1. Samples

Two rice (*Oryza sativa* L.) genotypes were selected from Active Germplasm Bank of Embrapa Rice and Beans (Santo Antônio de Goiás- GO, Brazil) belonging to two ecotypes: BRS Formoso (*O. sativa* subsp. indica) (F) and Mochi (M) (*O. sativa* subsp. japonica). The materials were chosen according to the apparent amylose content (Table S1) determined by the method ISO 664 (ISO, 2007). All genotypes were multiplied in the 2018/2019 harvest using a flood-irrigated system in the Embrapa Rice and Beans experimental field (6°29′8″S, 49°18′32″W). After harvest, the rice grains were naturally dried and storage at least for 4 months until germination.

2.2. Germination process

Germination was performed according to the methodology described by Zhang et al. (2014) with some modifications. The seeds (500 g) of paddy rice were soaked in deionized water (1 L) at pH 5.6 by adding L- glutamic acid (L- Glu) at 1.0 g/L (Sigma Aldrich, Ref. RES5063G- A701X, St. Louis, USA) and gibberellic acid (GA3) (Sigma Aldrich, Ref. G7645- 5G, St. Louis, USA) at 0.25 mg/L for 24 h in a fan oven (Fabbe-Primar, São Paulo-SP, Brazil) at 30°C. After this step, the grains were drained and allowed to germinate in a bread fermentation cabinet (National Mfg. Co., Lincoln, USA) at a controlled temperature of 35°C and relative humidity of 95% for 24 h. The germinated paddy rice grains were dried in a circulated air oven (Macanuda, Joinville-SC, Brazil) at 50°C overnight, then husk and pericarp (10%) were removed with the help of a rice polisher machine Suzuki (Santa Cruz do Rio Pardo- SP, Brazil) for 2 min and then ground in an lab hammer mill 3100 (Perten Instruments AB, Huddinge, Sweden) fit with a 0.8 mm sieve aperture obtaining a flour that was stored at room temperature until further analyses. Thus, the samples were denominated as: FNGBR- BRS Formoso non-germinated brown rice; FNGPR: BRS Formoso non-germinated polished rice; FGBR: BRS Formoso germinated brown rice; FGPR: BRS Formoso germinate polished rice; MNGBR: Mochi non-germinated brown rice; MNGPR: Mochi non-germinated polished rice; MGBR: Mochi germinated brown rice; MGPR: Mochi germinated polished rice; CBR: Commercial brown rice and CPR: Commercial polished rice.

2.3. Extraction of DNA and sequencing preparation.

All DNA samples were extracted through DNeasy PowerSoil Pro Kits from QIAGEN® (Qiagen, Hilden, Germany), following the manufacturing procedure. DNA quality control and library validation were made through High Sensitivity D1000 (Agilent, Santa Clara, California, United States), and samples were prepared according to 16S Metagenomic Sequencing Library and Fungal Metagenomic Sequencing Library according to the guidelines for 16S and ITS genes (Illumina®, Albany, New York, USA). The resulting data were 251 bp reads for which gene in 10 libraries for 16S and ITS, the beginning of data processing and removal of adapters was made using QIIME2 2022.2 pipeline, associated with quality control DADA2 software was used as read filtering.

2.4 Bioinformatic processing and statistical analysis

Filtered reads were inserted into CLC Genomics Workbench version 22.02, (Qiagen, Hilden, Germany) for taxonomic profiling and posterior abundance diversity inference, CLC Microbial Genomics Module was used for metagenomics processing data. For the taxonomic profiling the read mapping consisted of the adjusted read count abundances according to the QMI-PTDB Genus taxonomic profiling index (v.2.0) database for 16S gene. ITS gene database was constructed by downloading separately in NCBI, containing approximately 33.866 sequences, and inserted in CLC Genomics Workbench, subsequently converted into index database. Abundance results were merged and generated alpha and beta diversity, the results of the respective diversities were exploited through the total number of species, Chao 1 bias-corrected, Shannon entropy, and Bray-Curtis, respectively. A one-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) was performed analysis to verify the difference between the effect of germination and polish in rice samples using the XLSTAT version 2023.2.0 (Lumivero, Denver, CO, USA) and Principal Coordinates Analyses (PCoA) was performed by (CLC Genomics Workbench).

3. Results and discussion

3.1. Metagenomic sequencing and processing

The sequences uploaded for analysis on the CLC Genomics Workbench are shown in Table S1. The 16S inserted data contained MGPR (811,878,108) with the most nucleotide value and CPR (463,107,258) at the lowest counting. ITS raw data indicated CPR (1,036,266,965) with more nucleotide count and MNGPR (53,832,115) with least number of nucleotides. After evaluation bacteria presented the highest value of nucleotides still counted in MGPR (485,816,788) and CPR remained with the lowest number of nucleotides (277,076,337). Fungal nucleotide count maintained CPR (620,772,919) and MNGPR (32,229,827) in the same position.

3.2 Alpha diversity

The rarefaction curves for bacterial (Figure 1A) and fungi (Figure 1B) measurements were all saturated, indicating that gene sequences possibly reflected microbial profile after germination and polish process. 16S analyses indicated that the bacteria rate

demonstrated the highest diversity, both in MGBR (160.33) and MGPR (159.31), followed by FGPR (152.41) and FGBR (126.92). Fungi diversity, exploited through the ITS gene, did not shown saturation on the rarefaction curve (Figure 1B). MNGPR stopped the number of reads at approximately 60,000. Alpha diversity from CBR (213.74) indicated more richness of species, followed by CPR (105.48).

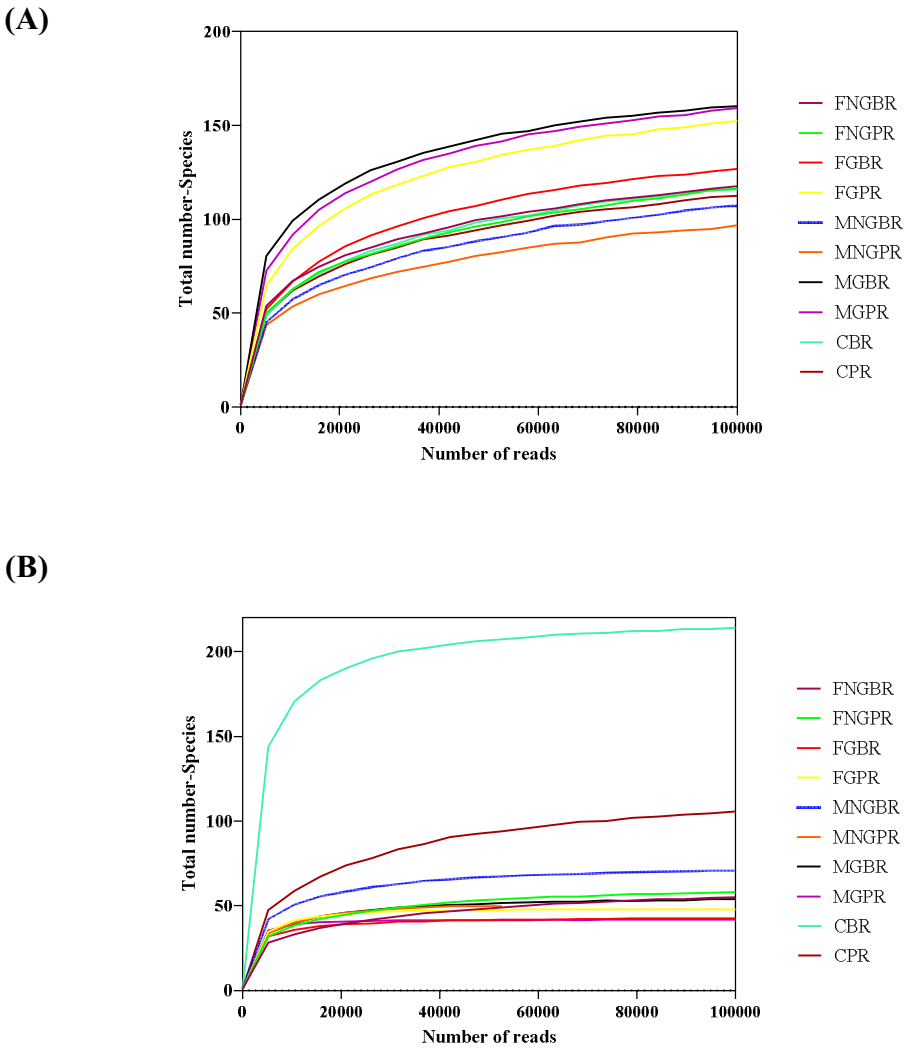


Figure 1. Rarefaction curves of (A) bacterial and (B) fungal sequences of DNA from rice before and after germination and polishing.

The results demonstrated that the germination process tends to raise bacterial diversity, probably due to high humidity and amylolytic enzymes (α-amylase; β-amylase) released in the substrate during germination process (Liao et al., 2013; Zhang et al., 2014).

Although the literature shows that polishing decreases the count of microorganisms in rice (Mir et al., 2020; Oh et al., 2010), in our work, polishing did not decrease the microbiome modulation of both *japonica* and *indica* rice.

Fungi are susceptible to environmental changes and their fitness process is closely related to these conditions. When coexisting bacteria, the competition between these groups is mainly related to nitrogen fixation and how they are going to react to rice processing after germination and polishing (Shuyan Li et al., 2022). Our results demonstrated that after the germination and polishing process, the bacterial growth could be acting as a potential competitor for fungal development diminishing their diversity in both processes. Germination can influence the microbiome of the samples, creating an environment that could modulate the species, and bacterial growth would be faster accordingly to their physiological and reproduction characteristics (Andreo-Jimenez et al., 2019; W. Wang et al., 2016).

Veen diagrams were based on Operational Taxonomic Unit (OTU) abundance and used to shown the unique and shared OTUs between non-germinated x germinated and brown x polished samples. BRS Formoso shared 22 and 23 species and Mochi shared 25 and 21 species for bacterial and fungal respectively (Figure 2). For bacterial, the number of unique OTUs decreased after germination (47 to 15) and polishing (32 to 26) for BRS Formoso and Mochi (38 to 32) but had a little increase (34 to 38) for Mochi after germination and CPR, had the highest OTU abundance values. Concerning the fungi, BRS Formoso samples shared 23 and Mochi 21 OTUs. The number of unique OTUs decreases for non-germinated samples but increases slightly after germination and polishing (Figure 4 C, Figure 4 D).

Alpha diversity analyses were assessed through Chao and Simpson indexes. The diversity (Simpson index) and richness (Chao index) for bacterial and fungal are shown in Table S4 and Table S5, respectively. The bacterial and fungal Simpson diversity index showed that all the samples were diverse and decreased after polishing both for the germinated and non-germinated samples. Maximum diversity was shown by CBR (0.94) and lowest by FNGPR (0.65) for bacteria and by MGPR (0.31) for fungi, respectively.

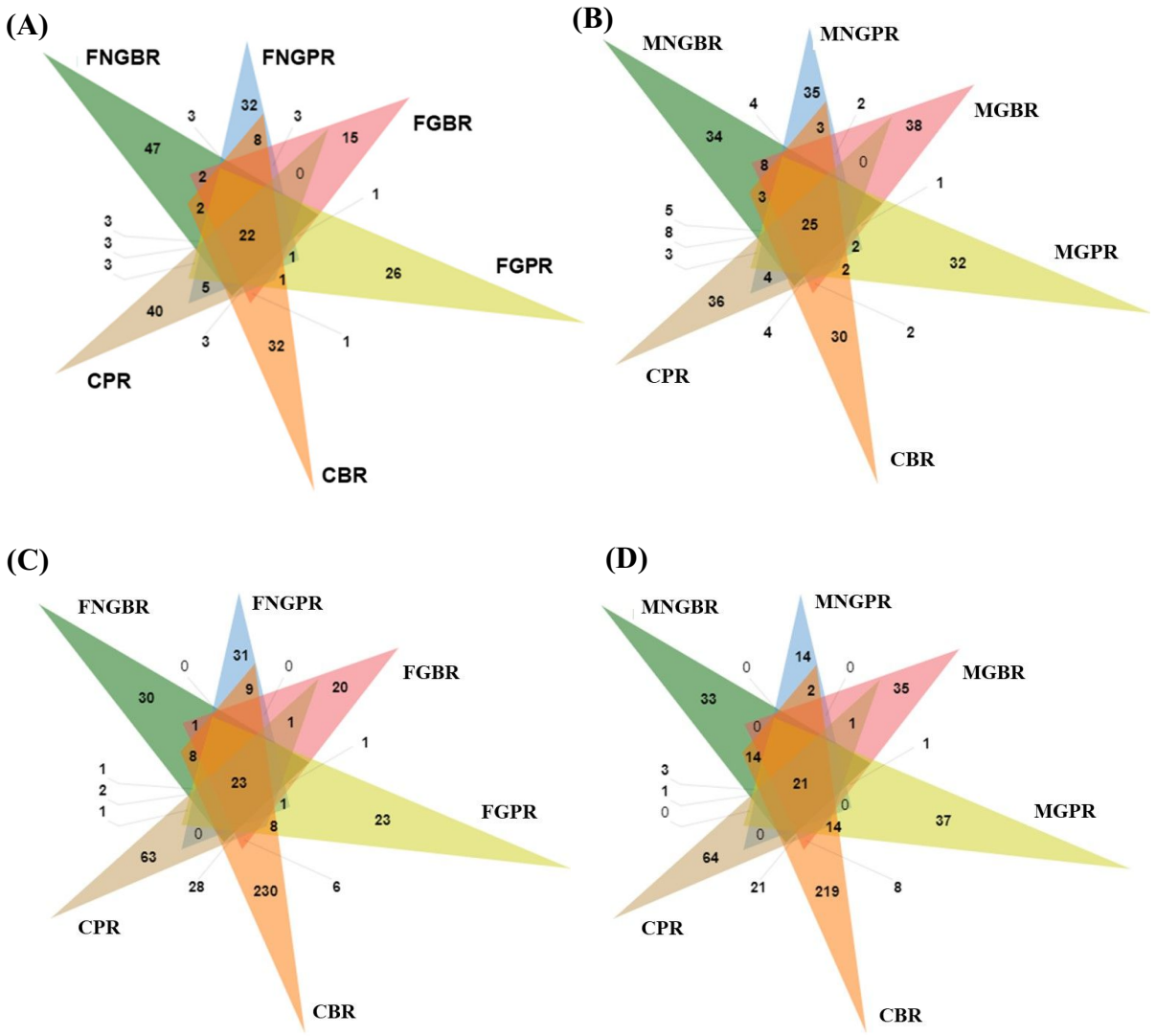


Figure 2. Veen diagram for numbers of shared of bacterial (A, B) and fungal (C, D) operational taxonomic units (OTUs) in samples of rice before and after germination and polishing showing the number of shared and unique OTUs in those samples.

Regarding bacteria, the highest Chao index was found in CBR (220.80) and the lowest in FNGPR (70.45). For fungi, CBR showed the highest value of Chao (32.65) and the lowest in MNGPR (11.36). Thus, the Chao index was low in the polished samples, which suggests that this process decreases the species richness, regardless the cultivar. Treated reads from ITS (Table S4) and 16S (Table S5) libraries indicated 99% similarity with the aligned

references abundance and effective reads. All samples demonstrated a little decrease after germination and polishing for the two parameters, but no statistical difference was observed ($p < 0.05$). The decrease in the Chao and Simpson indexes suggest that germination led to an increase and dominance of groups of bacteria and fungi during the process (Yang et al., 2023). Polishing does not reduce the diversity and richness in samples, regardless of the cultivar.

3.2 Beta diversity

Principal coordinates analyses (PCoA) shown data similarities and discrepancies in the abundance of the bacterial and fungal populations between the samples after the polishing and germination process. The pattern of bacterial abundances was presented in FGBR, FGPR, MGBR, and MGPR suggesting that the germination process had a similar effect through all germinated rice samples (Figure 3A). In contrast, FNGBR, FNGPR, MNGBR, MNGBR, CBR, and CPR were closely related indicating that the polishing treatment did not affect the microbiome to distinguish the population from the commercial samples (Figure 3A). Fungal PCoA shows a more diversified pattern of sample grouping, FNGBR and FNGPR, MGBR, and MGBR, CPR, and CBR, whereas MNGPR remained isolated from the other samples and MNGBR, FGBR, and FGPR coupled together. According to OTU clustering, MGBR and MGPR were the only samples that presented a distinctive percentage of fungi genus after germination for the Mochi genotype, presenting *Rhizopus* as the most clustered sequence among the OTUs, which explains the pair separation in the PCoA.

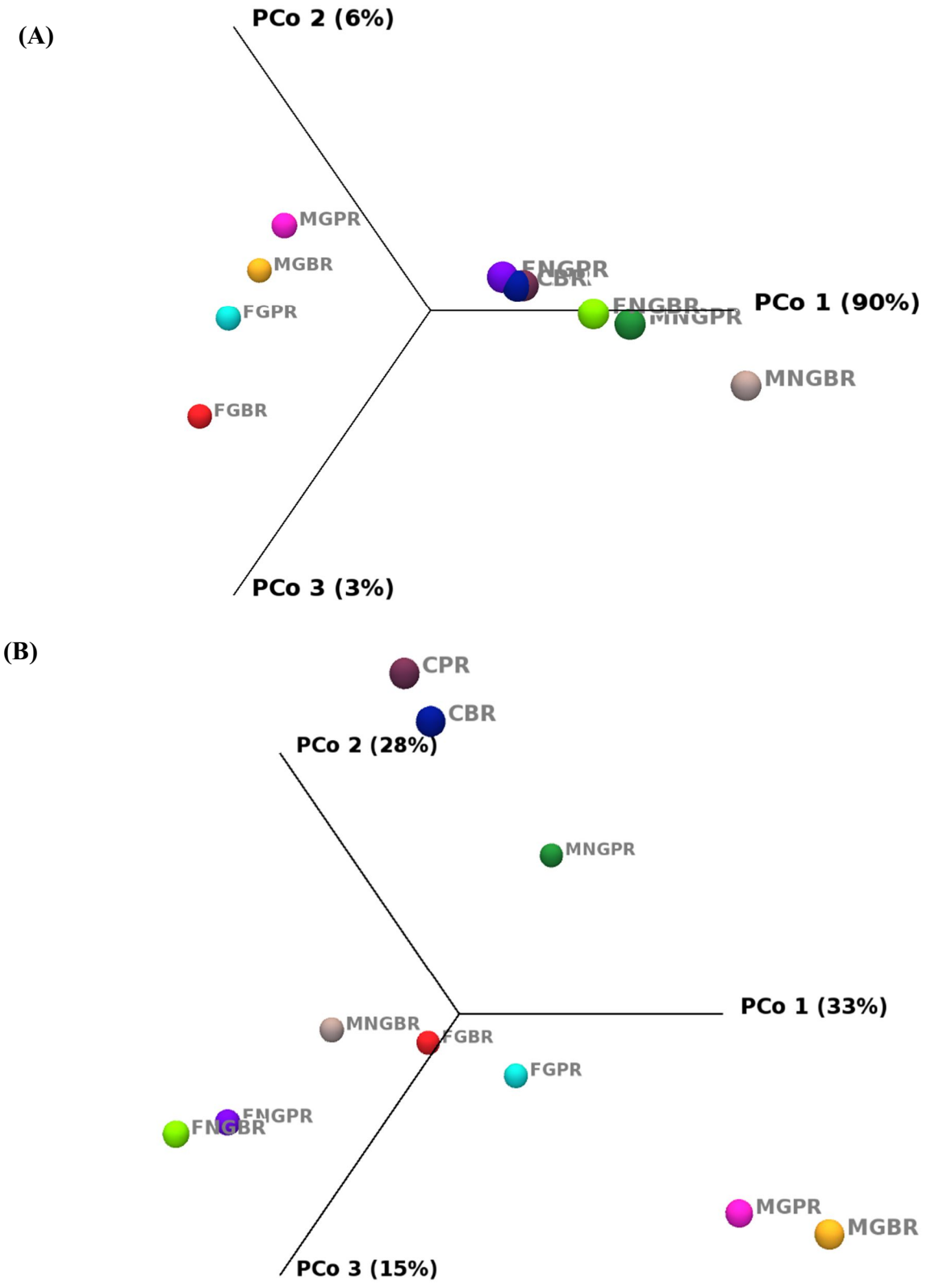


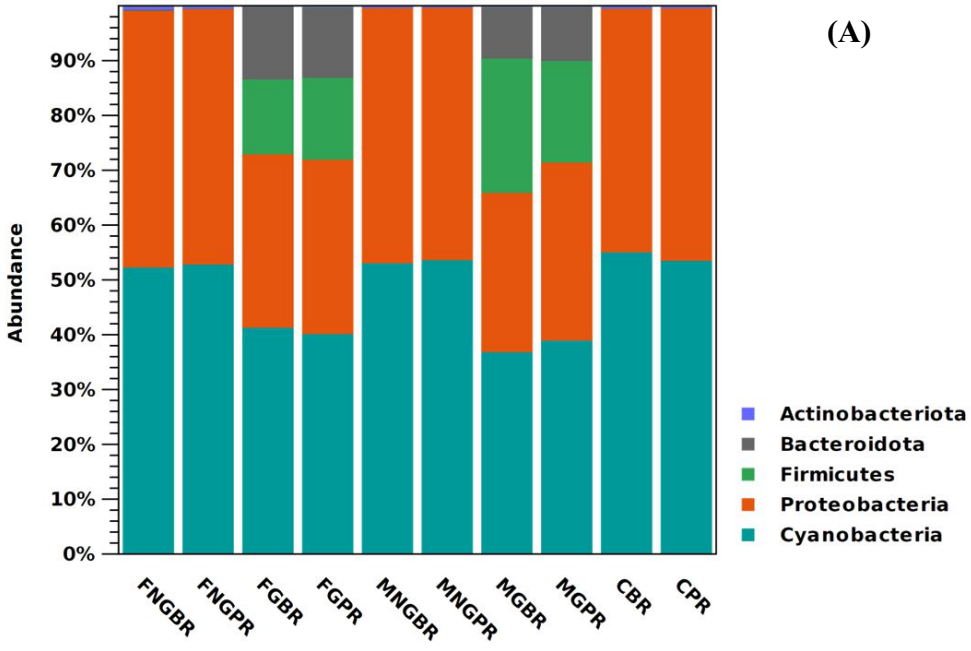
Figure 3. PCoA plots (A) bacterial and (B) fungal diversity based on Bray–Curtis distances.

Overall, the diversity analyses demonstrated that the communities of bacteria and fungi were directly influenced by the germination process, although for bacteria communities the effects of the process were more distinctive in the results since their growth in the environment was more competitive than fungi species, and their results had better resolution in data analysis. It has been reported that the germination process is more impactful than polishing for the microbiome in rice samples (Yang et al., 2023; Zhang et al., 2014).

3.3. Taxonomic profile of the metagenome

3.3.1. Phylum level distribution

The microbial profile of 10 Mochi and 10 BRS Formoso rice samples including bacteria and fungi counts indicated the prevalence of Cyanobacteria, Proteobacteria, and Ascomycota phylum. Bacteria population prevalence n the samples indicated the samples were followed by Firmicutes, Bacteriodota, Actinobacteriodota, and Deinococcota (Figure 4A), genus inference the samples could not classify the two most abundant genera in the samples, although in the FGBR, FGPR, MGBR, and MGPR showed a significant percentage of *Chryseobacterium* (FGBR-13%; FGPR-12%; MGBR-9.2%; MGPR-9.6%), *Clostridium senso strictu* 1 (FGBR 5.4%; FGPR-4.5%; MGBR-18%; MGPR-13%), *Clostridium sensu strictu* 5 (FGBR-8.1%; FGPR-10%; MGBR-5.7%; MGPR-5.2%) (Figure 4B).



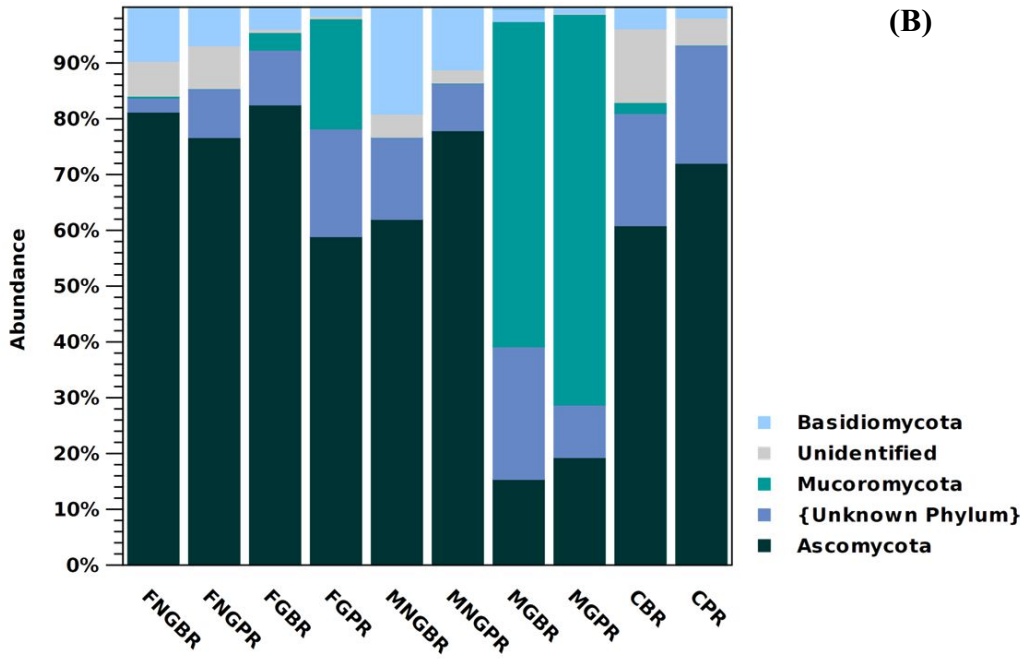


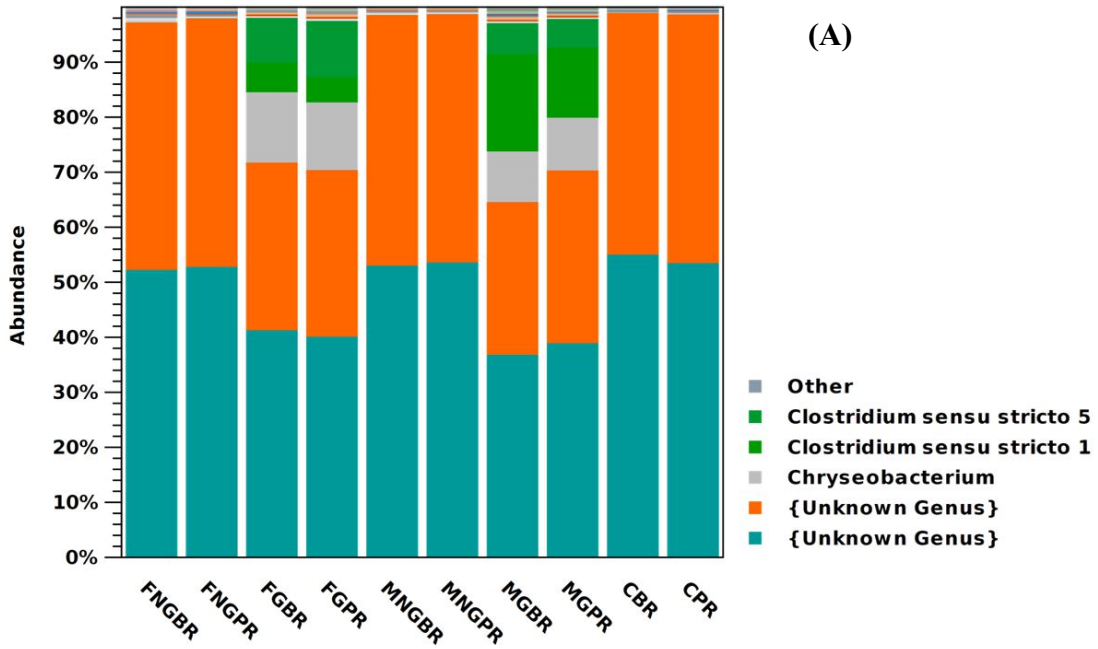
Figure 4. Taxonomic profiles of dominant bacterial (A) and fungal (B) communities at the phylum level.

In prevalence bacterial composition, Cyanobacteria excelled the other phylum possibly due to nitrogen concentration in the soil during fertilization. Studies have proven the relationship between biochemical aspects and the beneficial relation of fixing nitrogen by cyanobionts, providing an enhancement of rice seed germination, root, and growth (Bidyarani et al., 2015; Das et al., 2015) and Proteobacteria is related to metagenomic approach as most predominant bacteria groups in rice plants and agricultural environmental studies (Jang et al., 2020; Ojha et al., 2017). *Chryseobacterium* can be associated with several ecological niches, including agricultural soil, desert soil and plants, therefore is a very ubiquitous organism. Although when related to human hosts can cause pneumonia, meningitis, pyomyositis, and skin diseases. Recently, species from *Chryseobacterium* demonstrated a potential nematocidal activity, besides the antimicrobial function against *Ralstonia, Fusarium, Rhizoctonia,* and *Phytophthora.* Furthermore, *Chryseobacterium endophyticum* was used for biocontrol of rice blast and induced the expression of rice defense genes indicating a potential evolutionary relation between these species (Kumar et al., 2021). *Clostridium* is commonly associated with paddy rice soil, even at a lower frequency than

other reported species, although it also contribute to ecological maintenance as an organic source (Dhondge et al., 2022; Horino et al., 2015).

3.3.2. Genus level distribution

Fungal composition was subdivided mostly in Ascomycota phylum for all rice, although in MGBR and MGPR the prevalent phylum was Mucoromycota (=Zygomycota) (Figure 5A). The general classification in the samples exploited showed a big diversity in both samples, in which *Rhizopus* was the most frequent in MGBR (58%) and MGPR (70%). In contrast, in other samples a large miscellaneous of species was inferred and *Alternaria* was counted in FNGBR (15%), FNGPR (19%), MNGBR (17%), MNGPR (8.7%), CBR (3.1%) and CPR (19%) and *Aspergillus* was scored in MGBR (12%) and CBR (14%) (Figure 5B). Fungi composition differed only for MGBR and MGPR associating the *Rhizopus* species from Mucoromycota, as in the other samples it was not possible to distinguish a pattern for species identification. Ascomycota was predominantly identified in the other samples which corroborates to other ITS studies, since there is a potential to be found in almost every ecological niche (Allwood et al., 2023).



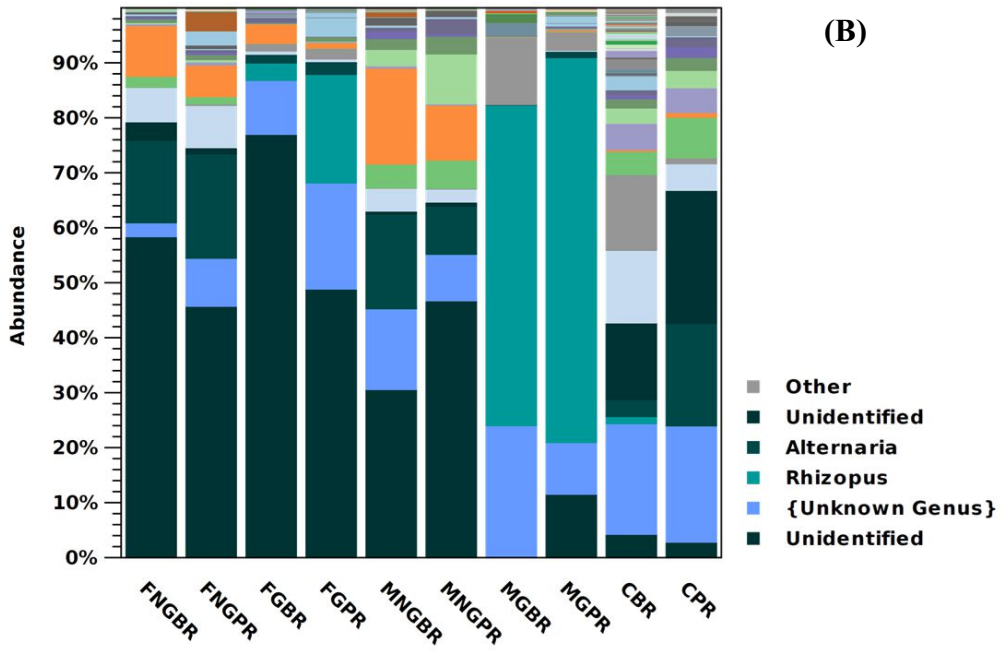


Figure 5. Taxonomic profiles of dominant bacterial (A) and fungal (B) communities at the genus level.

4. Conclusion

Germination treatment on Mochi and BRS Formoso rice influenced microbiome bacterial and fungal microbiome communities. Polishing was expected to reduce the bacterial and fungal abundance in the samples, although it was possible to notice that bran removal associated with the germination process raised the bacterial diversity of the samples. Fungal development in the samples was reduced in germinated and polished samples, affected not only by the treatment but also by the higher competition between the species favoring bacterial growth. Furthermore, it was possible to observe that germination could provide better conditions for bacterial species diminishing polishing effectiveness. Therefore, future research towards the use of adjuvant processes to germination and polishing, such as cold plasma or decontamination by UV-C post-treatment, could modulate or mitigate the presence of undesired microorganisms and reduce the diversity of species providing safe food products.

Credit authorship contribution statement

Maria Eugenia Araujo Silva Oliveira: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing; **Bruno Gerfi Bertozzi:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing; **Priscila Zaczuk Bassinello:** Project administration, Resources, Writing – review & editing; **José Manoel Colombari Filho:** Project administration, Resources. **Liliana de Oliveira Rocha:** Writing – review & editing, Supervision, Resources; **Carlos Wanderlei Piler Carvalho:** Conceptualization, Writing – review & editing, Supervision, Project administration, Resources; **Otniel Freitas-Silva:** Conceptualization, Writing – review & editing, Supervision, Project administration, Resources; **Dirce Yorika Kabuki:** Writing – review & editing, Supervision, **Cristina Yoshie Takeiti:** Conceptualization, Writing – review & editing, Supervision, Project administration, Resources.

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Main conclusion

Main conclusion

Although Asia is the largest producer and consumer worldwide, Latin America rises as an important producer driven mainly by plant-based market. Decreasing in *per capita* rice consumption and market stagnation associated with the demand for clean label products stimulate the use of germination process as a simple and natural strategy to deliver metabolites that impact technological characteristics thus promoting human health. Among metabolites, phenolic compounds play an important role against some chronic diseases, especially in the regulation of blood glucose level, and the interactions between phenolic compounds-starch that are neglected in the literature, and could be an effective alternative to delay digestibility. Short germination (< 24 h) associated with polishing affects differently bioactive compounds, amino acid profiles and pasting properties of rice genotypes containing different amylose levels. Short germination led to significant increase in free amino acids (FAA), especially in *Guaporé* (intermediate amylose content) that demonstrated an expressive increase in umami amino acids (Glu and Asp) as well as in *Mochi* (low amylose content) rice. In addition, *Guaporé* and *Mochi* presented high levels of proline, a precursor for the synthesis of 2-acetyl proline that provides popcorn aroma in *Jasmine* or *Thai* rice. *BRS Formoso* (high amylose content) lead to the highest FN value, suggesting that germination conditions were insufficient to activate the α-amylase. *BRS Catiana* (high amylose content) submitted on short germination time (< 24 h) did not cause enzyme activation or changes in physical properties. Metabolomics approaches reveal that short germination was effective to increase the phenolic compounds levels such as *trans*-ferulic, *p*-coumaric and caffeic acids in free and bound extracts. As expected, polishing caused a decrease in phenolic compounds, amino acids and GABA, but this reduction was more pronounced in non-germinated than in germinated rice, probably due to the diffusion of these components from the pericarp to the endosperm during the soaking step. Regarding germinated starch, soaking and germination steps promoted pits and pores on the granule surface. Both steps decreased protein content, total starch, melting enthalpy, and relative crystallinity. Differences in color characteristics and resistant starch comparing soaking and germinated starches were not found, which constitute an advantage in terms of functionality and applicability in formulations where starch properties are important. Gel strength of the GRS was 16.37 % higher than the SRS and 28.58 % higher than NGRS. Short germination

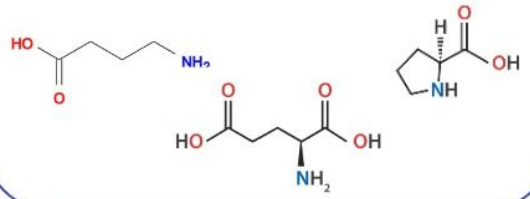
was effective in reduction of cooking time (31 to 21 min) using conventional pan that is the most traditional method used. Texture profile showed that germination caused in GBR prepared by electric rice cooker, the highest values of hardness (19.7 N) and chewiness (24.6 N) after cooking (t= 0 h). In contrast, freezing of GBR obtained by electric rice cooker caused a decrease in hardness (53 %) and chewiness (11.5 %) in comparison with non-germinated material that is an attractive characteristic considering BR consumption. Regarding metagenomics aspect there was a difference in bacterial and fungal profile of *Indica* and *Japonica* ecotypes after germination. In this way, the germination of *Indica* ecotype (*BRS Formoso*) showed the highest population of Bacteriodota, whereas rice polishing reduced Firmicutes phyllo in *Japonica* ecotype. Concerning fungal profile, the combination of germination and polishing leads to Mucoromycota phyllo appearence, with prevalence on *Japonica* type (*Mochi*). Despite the global food prices, our findings can help the food industry which in turn is constantly pursuing diversification of product niches that combine higher quality, nutritious properties, sensory experience and convenience foods. In fact, it is a competitive advantage to have genotypes capable of developing products with differentiated characteristics. Future works are necessary to explore possible applications of these rice cultivars in different GABA rice-based products, by monitoring the food safety aspects.

SHORT GERMINATION

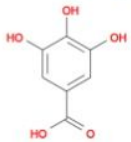
Short time (< 24 h): reduces costs

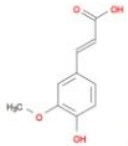
Germination caused little changes in the physical properties of starch.

Increase of GABA and *umami* amino acids



Increase in bioactive compounds even after polishing





Cooking techniques: changes in texture and carbohydrate chemistry under different preparation and storage

Diversity in bacterial and fungal population after combination of germination and polishing