Universidade de Aveiro
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Elisabete Cristina Fonseca Aguiar

Estudo de variedades de arroz- Informações químicas e nutricionais

Study of rice varieties – Chemical and nutritional facts

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Relatório de Estágio apresentado à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia – Ramo Biotecnologia Alimentar, realizado sob a orientação científica da Doutora Ivonne Delgadillo Giraldo, Professora Associada com Agregação do Departamento de Química da Universidade de Aveiro e do Dr. Diogo Barbosa Amorim de Lemos, Responsável pela Direção do Departamento de Qualidade da Empresa Novarroz – Produtos Alimentares, S.A.

Em memória da minha madrinha.

“A saudade é a luz viva que ilumina a Estrada do passado.”

Fernando Pessoa

.

o júri

presidente

Doutor Jorge Manuel Alexandre Saraiva
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Professora associada com agregação do Departamento de Química da Universidade de Aveiro

agradecimentos

Chega ao fim mais uma etapa.
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keywords

Rice, chalky area, whiteness, starch, amylose, resistant starch, glycemic index, viscosity, breakdown, retrogradation, germination, GABA

abstract

This traineeship was developed in partnership with Novarroz – Produtos Alimentares, S.A., a familiar company whose major activity is the processing and commercialization of rice and its by-products. Rice (*Oryza sativa*) is one of the most important cereal crops and it is consumed by 60% of the world's population, being one of the main sources of nutrients and energy. Thus, the knowledge of the composition of rice varieties is of total importance to Novarroz, Produtos Alimentares, S.A. Therefore, this work comprised two main goals.

The first goal consisted in the characterization of 23 milled rice samples from the new harvest, namely in terms of physical characteristics of the rice grains, pasting properties and chemical and nutritional composition.
Physical analyses of the rice grains enabled detecting higher length and length-to-width ratio values to *indica* rice varieties. Strong correlations were found between these parameters. *Japonica* rice samples showed higher chalky area % compared to *indica* varieties. Chalky area was strongly and positively correlated with total whiteness and kett.
Starch behaviour during cooking and cooling was assessed through the determination of pasting properties. In general, peak and breakdown viscosities were higher for *japonica* rice samples. These two parameters were found strongly and positively correlated. On the other hand, setback viscosities were higher for *indica* rice varieties and negatively and strongly correlated with peak and breakdown viscosities.
Total starch ranged from 74.79 to 84.45 %, in dry matter. The determination of amylose assigned the highest values to *indica* varieties. This parameter was strongly correlated with pasting properties.
Glycemic index (GI) of rice samples was determined. Samples in which starch hydrolysis was faster over time presented the higher GI values. *Indica6* presented the lowest GI (76.40 ± 1.06) being seen as a possible low GI rice variety destined to type 2 diabetic people. A negative correlation was found between glycemic index and amylose content corroborating some literature reports.

The second goal of this internship consisted in the germination of two brown rice samples: *japonica14 G0h* and *japonica15 G0h*. These samples were germinated in Novarroz's water (pH = 5.3), pH = 3.0 and pH = 4.0 for 24, 48 and 72 hours in order to assess the biochemical changes occurring during germination, mainly in terms of γ - aminobutyric acid (GABA) content, a non-protein amino acid that has been implicated in many health benefits.
A significant decrease was detected in total starch and amylose contents. As expected, starch hydrolysis led to an increase in reducing sugars content over time.
In general, the germination process contributed to the increase of the GI. However, *japonica15 G24_w*, *japonica15 G24_3.0* and *japonica15 G24_4.0* presented a significant decrease in GI compared to *japonica15 G0h*.
A relation between acid germination conditions and GABA content was effectively detected. Germination at pH = 3.0 led to a significant increase in GABA content in the rice grains over time, mainly in *japonica14* samples: each 24 hours, the GABA content increased significantly ranging from 9.27 (at 0 hours) to 43.63 mg/100 dry matter (at 72 hours), showing an increase of almost 5 times in relation to ungerminated *japonica14*, corroborating previous reports.

palavras-chave

Arroz, área gessada, brancura, amido, amilose, amido resistente, índice glicémico, viscosidade, rutura, retrogradação, germinação, GABA

resumo

Este estágio curricular foi desenvolvido em parceria com a Novarroz – Produtos Alimentares, S.A., uma empresa familiar cuja atividade principal é o processamento e comercialização de arroz e dos seus subprodutos. O arroz (*Oryza sativa*) é uma das mais importantes culturas de cereais e é consumido por 60% da população mundial, sendo uma das principais fontes de nutrientes e energia. Desta forma, o conhecimento da composição das variedades de arroz é de total importância para a Novarroz, Produtos Alimentares, S.A. Assim, este trabalho englobou dois objetivos principais.

O primeiro objetivo consistiu na caraterização de 23 amostras de arroz branqueado da nova colheita, nomeadamente em termos de caraterísticas físicas dos grãos de arroz, propriedades de gelificação, composição química e nutricional.
A determinação das caraterísticas físicas dos grãos de arroz permitiu observar maiores comprimentos e proporções comprimento/largura para as variedades de arroz *indica*. Foram obtidas correlações elevadas entre estes dois parâmetros. As amostras de arroz *japonica* mostraram maiores percentagens de área gessada comparando com as variedades *indica*. Assim, obteve-se uma correlação elevada e positiva entre a brancura total e o kett.
O comportamento do amido durante o cozinhamento e arrefecimento foi avaliado através da determinação das viscosidades. Em geral, as viscosidades do pico e rutura foram mais elevadas para as amostras de arroz *japonica*. Por outro lado, as viscosidades de retrocesso foram superiores para as variedades *indica* e negativa e fortemente correlacionadas com as viscosidades do pico e de rutura.
O conteúdo de amido total variou de 74.79 a 84.45%, em matéria seca. A determinação do conteúdo de amilose atribuiu os maiores valores às variedades *indica*. Este parâmetro estava fortemente correlacionado com as propriedades de gelificação.
O índice glicémico (IG) das amostras de arroz foi determinado. As amostras cuja hidrólise de amido foi mais rápida ao longo do tempo apresentaram valores de IG mais elevados. *Indica6* apresentou o menor IG (76.40 ± 1.06) sendo visto como uma variedade de arroz de baixo IG destinada a diabéticos do tipo 2. O IG e o conteúdo de amilose apresentaram uma correlação negativa, estando de acordo com a literatura.

O segundo objetivo deste estágio consistiu na germinação de duas amostras de arroz integral: *japonica14 G0h* e *japonica15 G0h*. Estas amostras foram germinadas em água da Novarroz (pH = 5.3), a pH = 3.0 e a pH = 4.0 durante 24, 48 e 72 horas, a fim de verificar as alterações bioquímicas que ocorreram durante a germinação, principalmente em termos do conteúdo de ácido gama-aminobutírico (GABA), um aminoácido não proteico que tem sido implicado em muitos benefícios para a saúde.
Os conteúdos de amido total e amilose diminuíram significativamente. Como esperado, a hidrólise de amido levou a um aumento do conteúdo de açucares redutores ao longo do tempo.
Em geral, o processo de germinação contribuiu para o aumento do IG. Contudo, as amostras germinadas *japonica15 G24_5.3*, *japonica15 G24_3.0* e *japonica15 G24_4.0* apresentaram uma diminuição significativa no IG comparando com a amostra *japonica15 G0h*.
Efectivamente foi encontrada uma relação entre o conteúdo de GABA e as condições acídicas da germinação. A germinação a pH = 3.0 levou a um aumento significativo de GABA nos grãos de arroz com o tempo, principalmente nas amostras *japonica14*: a cada 24 horas, o conteúdo de GABA aumentou significativamente, variando entre 9.27 (0 horas) e 43.63 mg/100 de matéria seca (72 horas), mostrando um aumento de quase 5 vezes em relação à amostra não-germinada *japonica14*, corroborando estudos anteriores.

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ABREVIATIONS

AACC	American Association of Cereal Chemists
ANOVA	Analysis of Variance
approx.	approximately
BHT	Butylated Hydroxytoluene
BRC	British Retail Consortium
cP	centiPoises
DNS	3,5 – Dinitrosalicylic Acid
e.g.	for example
EUREKA	European Research Coordination Agency
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GABA	γ - Aminobutyric Acid
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
GC-MS	Gas Chromatography – Mass Spectrometry
GI	Glycemic Index
GOD-POD	Glucose Oxidase-Peroxidase
HCl	Hydrochloride acid
IAPMEI	Institute for Support of Small and Medium Enterprises and Innovation
IFS	International Featured Standards
ISO	International Organization of Standardization
KCl	Potassium Chloride
KOH	Potassium Hydroxide
NaOH	Sodium Hydroxide
RVA	Rapid Visco Analyser
SGS	Société Générale de Surveillance S.A
SME	Small and Medium Enterprises
SD	Standard Deviation

1. OVERVIEW

This report was developed under the master's thesis in Food Biotechnology of University of Aveiro, in partnership with Novarroz – Produtos Alimentares, S.A. and is divided in six parts. The present point contextualizes this internship, presenting the organization and objectives of this work. Point 2 comprises a literature review based on nutritional and chemical composition of rice; also in point 2, germination of brown rice is presented as a mechanism to increase the content of γ - aminobutyric acid (GABA), compound that is reported as a good modulator of insulin response, being an alternative to type 2 diabetic people. Then, in point 3, Novarroz – Produtos Alimentares, S.A. and its mission and policies are presented, as well as the rice processing and the work carried out in the company. Point 4 lists and describes all samples and methodologies used to achieve all the results, presented and discussed in point 5. Finally, point 6 presents a brief conclusion.

The accomplishment of needs and requirements of consumers is one of the many purposes of the companies and the focus on research is one of the tools to achieve this goal. The internship with Novarroz – Produtos Alimentares, S.A. materializes itself in this context. The major activity of this company is the processing and commercialization of rice and its by-products [1]. Therefore, the deep knowledge about rice and understanding of its composition and features are important steps in the improvement of existing products and development of new products, answering the needs of consumers and extending the company to new markets, contributing to its economic growth. This internship aimed to provide professional experience in the business environment, absorbing the knowledge of organizational policies of the company and its mission, from the entry of raw materials to the finished products. Thus, a part of the internship took place in the company, namely in the laboratory of quality control, where the physical analysis of rice and its by-products is performed to assess its commercial price and quality in all processing stages; another part was developed in the university, in the laboratories, in a research context.

Thus, this work was divided in two distinct parts.

The first part was about the characterization of milled rice and two brown rice varieties from the new harvest in order to provide chemical and nutritional information about their composition to the company. This characterization comprised physical analysis of the rice grains and pasting properties, carried out in the laboratory of Novarroz, and chemical analyses, performed in the laboratories of the university. The chemical analyses included moisture determination, total starch, amylose, resistant starch and protein quantifications. Also, nutritional studies were performed to determine the rate of starch hydrolysis of rice varieties and their respective hydrolysis index in order to estimate the glycemic index [2]. This last parameter is of great importance to Novarroz since one of its main concerns has been the research for a low glycemic index rice variety able to respond to the needs of diabetic type 2 consumers.

The second part of this thesis consisted in the germination process of the two brown rice varieties characterized before. The germination of brown rice has been touted as a successful strategy to increase γ - aminobutyric acid (GABA) content[3]. GABA is a non-protein amino-acid that is known for its numerous benefits [4, 5]. The idea was to test different GABA increment strategies in order to study the possibility of developing a new product in Novarroz that presented health benefits. Recent studies suggested that GABA increase was more effective if germination occurred in acidic conditions [6]. Therefore, the two brown rice varieties were germinated in Novarroz's water, at pH = 5.3, pH = 3.0 and pH = 4.0, for 24, 48 and 72 hours. Then, the chemical analyses and nutritional studies performed to milled and brown varieties were also performed to the germinated rice varieties. Moreover, reducing sugars, phenolic compounds and GABA contents were also determined in order to evaluate the chemical changes occurring during germination.

2. LITERATURE REVIEW

2.1 Rice

Rice is one of the most important cereal crops of the world [7] and it is consumed, mainly, as a whole grain [8] for nearly 60 % of the world's population [9], having an important role in the supply of energy and nutrient needs [10]. In developing countries, rice accounts for 715 kcal/capita/day: 27% of dietary energy supply, 20% of dietary protein and 3% of dietary fat [11].

Rice belongs to the Gramineae family [12] and genus *Oryza*, which comprises 23 species but only *Oryza sativa* and *Oryza glauberrima* are cultivated [11, 12]. Currently, almost all existing rice varieties derived from *Oryza sativa* [11]. This species can be divided into three sub-species: *indica*, *japonica* and *javanica* [11]. The most common sub-species are *indica* and *japonica* [11, 13], wherein *indica* represents about 80 % of all cultivated rice [11].

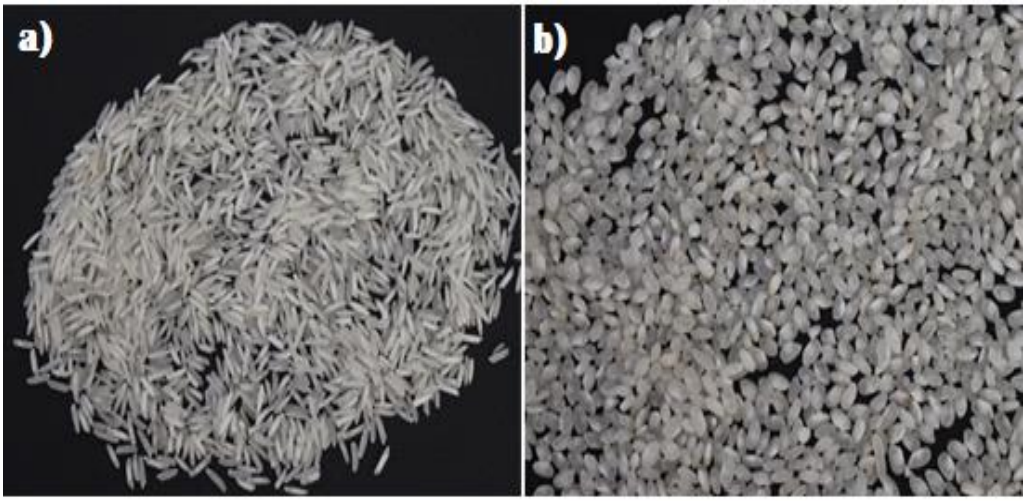

Figure 1 - The most common rice sub-species: *indica* **(a)** and *japonica* **(b)**.

Indica sub-species can be found in tropical [11, 14] and subtropical rice planting regions, with low latitudes or altitudes [14] because of its high tolerance to flooding, heat and strong light conditions [15]. *Japonica* sub-species are mostly cultivated in temperate regions with high latitudes [14], supporting cold and low light levels [15]. Normally, *japonica* grains are shorter than *indica* grains [13] (see Figure 1). *Indica* rice has long kernels with a length-to-width proportion of 4 to 5 (e.g basmati and jasmine) [6] and,

after cooking, presents a firm and non-glutinous texture due to the high content of amylose, characteristic of *indica* sub-species [11, 15]. *Japonica* rice has a length-to-width ratio of 2 to 3 [6] and its low content of amylose [11, 15] contributes to its sticky and glutinous texture [6, 11] after cooking, a desirable feature in Mediterranean and Asian cuisine (e.g. sushi) [6].

2.1.1 Grain structure

Rice grain is a heterogeneous and complex system of distinct constituents [16].

The rough rice (paddy) comprises an outer protective layer (hull or husk) and the fruit [17, 18]. The rice fruit is a caryopsis, wherein the single seed is fused with the pericarp, forming a seed-like grain; the grain is the seed-like grain with the husk. The husk comprises the lemma, palea, rachilla, sterile lemmas and awn [18], as shown in Figure 2, and represents about 20% of the rough rice weight [17, 19].

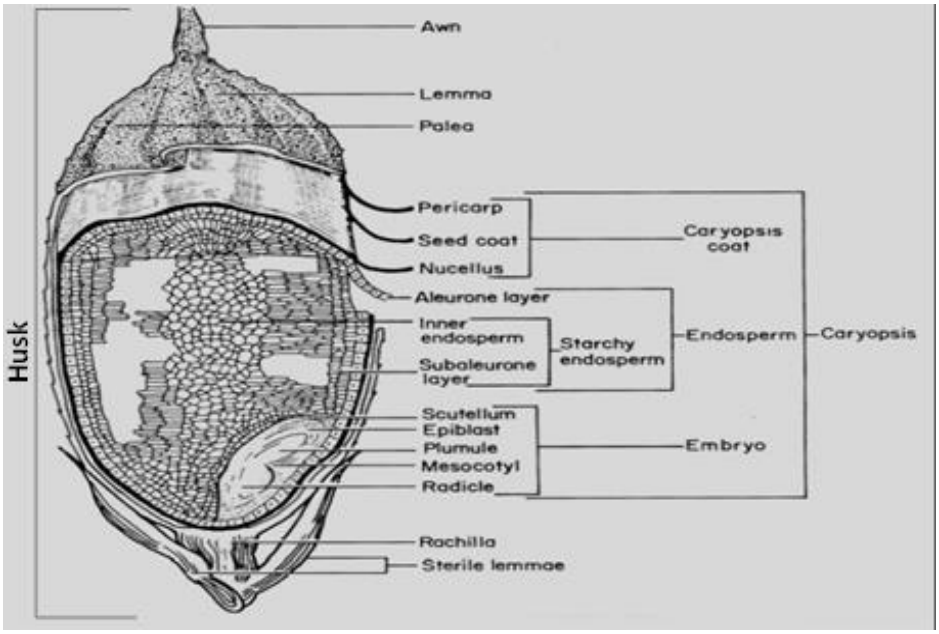

Figure 2 – Rice grain morphology (adapted from[17]).

The husked rice grain is composed by the caryopsis coat (pericarp, seed-coat and nucellus), the germ (or embryo) and endosperm [17]. It's called brown rice due to the brownish and fibrous pericarp [17, 18]. The embryo contains the embryonic leaves (plumule) and the embryonic primary root (radicle) [18]. Their cells are abundant in protein and lipid bodies [17]. The endosperm, which occupies the major space of the

grain, has an important role on the nutrition of the germinating embryo [18], being a source of starch, proteins and lipids [17]. The aleurone layer, rich in protein and lipid bodies [17], surrounds the endosperm [18]. The starchy endosperm (see Figure 2) is composed by the inner endosperm and subaleurone layer [17] and consists of a proteinaceous matrix containing starch granules [17, 18], sugars, fats, crude fibre and organic matter [18]. Frequently, chalky white spots appear in the starchy endosperm: white spots showing up in the middle of the ventral side are called white bellies; a white region expanding to the edge of the ventral side and toward the middle of the endosperm is called a white core [18].

During the milling and polishing processes, the husk is firstly removed from the grain and then the pericarp, embryo, aleurone layer and a small part of the starchy endosperm are removed as the bran, originating the white rice grain, the most consumed type all over the world [18, 20].

2.1.2 Composition

The detailed knowledge of the nutritional composition of rice is very important, considering it constitutes the world's principal source of food [21]. Previous studies have shown that some factors can affect the composition and the nutritional value of the rice grain, such as environmental influences, phenotypic variation, fertilizers, degree of milling and storage conditions [22-24].

The rice grain contains 75% of starch, 12% of water, 7-8% of proteins, 1.5-1.7 % of lipids and 2% of fibre. Furthermore, rice is also a good source of vitamins and minerals. The brown rice presents a bigger content of lipids, fibre and vitamins. The content of these compounds decreases significantly with milling [11, 25, 26].

2.1.2.1 Starch

Starch is the most abundant compound in the rice grain and it consists of different glucose polymers [27]. Chemically, starch can be divided in two types of glucan polymers: amylose (Figure 3a) and amylopectin (Figure 3b) [24, 27, 28]. Amylose is a long linear

chain of glucose units linked by α (1, 4) linkages, weakly branched (approx. 1 branch per 1000 residues) and smaller molecular weight (10^{5-6}), whereas amylopectin possesses the same linear structure but with a vast number of short branches with α (1, 6) linkages and higher molecular weight (10^{7-8}) [24, 27, 28].

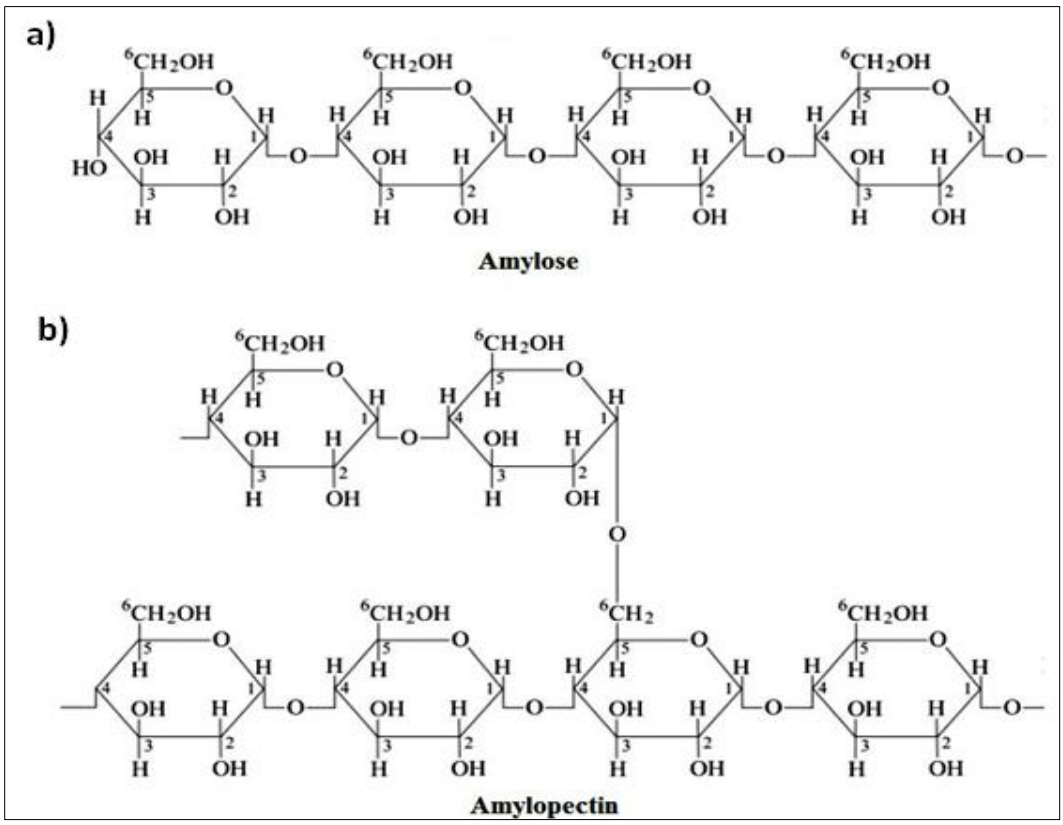

Figure 3 – Chemical structure of amylose **(a)** and amylopectin **(b)** (adapted from [29]).

Rice starch occurs in small particles called granules [20, 30, 31]. Rice starch granules are the smallest known to exist in cereal grains, with its size reported in the range of 2 to 7 μm [30]. These granules have ordered and disordered regions. Ordered regions consist of packaging double helices originated from short branches of amylopectin molecules [31]. Most of these double helices are arranged into semi-crystalline layers, forming a "semi-crystalline structure" [27, 31]. Disordered regions (or amorphous lamella) mainly incorporate internal amylopectin chains (long chains) and branch points. The position of amylose in relation to amylopectin and structure of the amorphous growth ring are still obscure. The organization of starch chains and the formation of crystalline lamellae are controlled primarily by the combination of enzyme

activity and environmental conditions during starch biosynthesis [32]. Starch properties are influenced by the granule size, granule distribution, amylose/amylopectin ratio and mineral content [30].

The amylose of rice starch ranges from 0 to 30% (w/w) [24] and is directly related to water absorption, volume expansion and good apartness of cooked rice grains, being inversely proportional to cohesiveness and glossiness [33]. Amylose content has been evaluated by the iodine-binding procedure, based on the capacity of iodine to form an inclusion complex with amylose. However, iodine can also binds to amylopectin molecules with a polymerization degree superior to 60%, causing overestimation of amylose content. On the other hand, phospholipids and free fatty acids have affinity to bind to amylose, competing with iodine, which causes an underestimation of amylose content. Due to this fact, amylose measured using this method is denominated as apparent amylose [24].

Starch can be hydrolysed by three enzymes: α-amylase, β-amylase and amyloglucosidase. The α-amylase is an endoamylase that is responsible for the cleavage of intern α-1,4 glycosidic bonds of amylose and amylopectin molecules, originating oligosaccharides with α-configuration. The β-amylase and amyloglucosidase are denominated as exoamylases: β-amylase hydrolyses units of maltosyl from the nonreducing ends of amylose and amylopectin, leading to the formation of β-maltose whereas amyloglucosidase cleaves both α-1,4 and α-1,6 glycosidic bonds at the branching point, releasing β-D-glucose units from starch polymer [34].

Starch rice properties are obtained after cooking the native starch granules in an excess of water to hydrate and disperse amylopectin and amylose [35], leading to gelatinization and pasting. Gelatinization comprises several changes in the starch granule, such as losing crystallinity, absorbing water, swelling and leaching of some components, like amylose. Thus, rheological properties occur during heating [35, 36]. The swelling behaviour of the rice is primarily the property of its amylopectin content, and amylose acts both as a diluent and as an inhibitor of swelling, especially in the presence of lipids [37]. Pasting covers the changes occurring after gelatinization, during further heating, involving the same alterations that characterize gelatinization, and eventually the

disruption of the granules with the application of shear forces [35]. It has been reported that waxy (very low amylose content) rice starches have a higher swelling volume, resulting in a higher viscosity than the other rice classes [36].

Cooked rice is known by the declination in texture and taste over time. These changes are caused by a process called retrogradation. Retrogradation is characterized by the reassociation of gelatinized starch molecules to form crystallites upon cooling, implicating fully reversible recrystallization in the case of amylopectin and partially irreversible recrystallization in the case of amylose. Thus, rice retrogradation is influenced by starch composition, granule architecture, lipids, physical processing technologies and retrogradation conditions during storage. Retrogradation is responsible for increasing the level of enzyme-resistant starch [38]. Resistant starch is characterized by a smaller and linear structure with a length of 20-25 glucose residues; Resistant starch molecules can link among themselves through hydrogen bonds [39]. This type of starch is known to escape the digestion in the small intestine by amylolitic enzymes, being only partially fermented by the gut microflora [9, 39]. Therefore, Resistant starch has physiologic effects that are like those of dietary fibre, affecting body weight and energy balance, lowering the intestine's pH and promoting the absorption of some minerals, like zinc, calcium and magnesium. The food and agriculture organization (FAO) reported resistant starch as a dietary fibre to be consumed for the prevention of type 2 diabetes mellitus [39].

2.1.2.2 Protein

Rice proteins are colourless, bland taste, hypoallergenic and hypocholesterolemic. Like most of the cereals, protein content is not particularly high, but the amino acid composition is more complete than the other cereals [40]. Rice protein is considered of high quality since it contains eight of ten essential amino acids [41], having as limiting amino acids lysine (only 3.8 to 4.0%) [42, 43] and threonine[43]. The analysis of protein rice profile shows that it is high in glutamic and aspartic acid [26].

Milled rice accumulates in its endosperm 3.8-8.8% albumin, 9.6-10.8% globulin, 2.6-3.3% prolamin and 66-78% glutelin [44]. In rice endosperm cells, seed storage

proteins are accumulated in two types of granules, known as protein bodies, located between the starch granules [45-47].

The major storage protein of rice is glutelin [40, 42, 44], stored in type II protein bodies as an alkaline (and/or acid)-soluble protein [40, 45, 46]. These protein bodies can be digested by the human digestive system [45]. Rice glutelin encompasses two major polypeptide subunits classified as α and β units, with apparent molecular weight of 30-39 and 19-25 kDa, respectively, and polymerizes by disulphide bonding and hydrophobic interactions to form very large macromolecular complexes [44, 47]. These higher-order structures may explain the lack of functional properties of rice glutelin [44].

Prolamin, present in lower quantity, is stored in type 1 protein bodies as an alcohol-soluble protein [45, 46]. These types of protein bodies cannot be digested by human digestive system [45]. Prolamin is constituted by three polypeptide units with apparent molecular weights of 10, 13 and 16 kDa. The 13 kDa polypeptide is readily solubilised in alcoholic solutions, while the 10 and 16 kDa polypeptides with a high level of sulphur containing amino acids require a reducing agent for solubilisation in alcoholic solutions [44, 47].

Albumin is soluble in water [41], existing in a wide range of molecular weight from 10 to 200 kDa [44]. Globulin is soluble in salts [41] and consists of four types of globulins with apparent molecular weights in a range of 16 to 200 kDa [47].

2.1.2.3 Lipids

Milled rice possesses low lipid content, however, lipid content has a great impact on cooking and quality properties of rice [48]. Rice lipids can be divided by structure into neutral lipids, glycolipids and phospholipids. In spite of these lipid types don't differ between i*ndica* and *japonica* rice, its distribution in the grain isn't uniform and endosperm cells contain a higher quantity of polar lipids [49]. Takano *et al.* (1989) determined the ratio of neutral lipids, glycolipids and phospholipids as 98.6:0.5:0.9. Furthermore, triacylglycerol, steryglycoside and phosphatidylcholine were determined as being the major lipid components in the above neutral lipids, glycolipids and

phospholipids, respectively [50]. Some reports list that an interaction between glycolipids and phospholipids has the effect of increasing pasting temperature in rice [48].

Lipids can be classified according to cellular distribution and its association. If lipids are associated to starch granules, they are called starch lipids; if they are distributed throughout the grain, they are known as non-starch lipids. Generally, starch lipids have about 0.5-1.0% of milled rice, being generally present in greater quantities than non-starch lipids; however this is not universal and there are differences between waxy and non-waxy varieties, due to the formation of amylose-lipid complexes [49].

The degradation of lipid compounds during storage has been reported as responsible for the rice deterioration [51, 52]. Free fatty acids content in the milled rice surface is used to assess the development of potential off-flavours and colours. These compounds are present because bran lipids, such as triacylglycerols, and lipase are deposited together on the rice grain during milling, resulting in free fatty acids formation on the rice surface, due to lipase's action [51]. Consequently, oxidation of free fatty acids and other lipids occurs: the lipids present in the outer areas of the grain suffer oxidation reactions, being that they are more exposed to the air than those in the inner areas, leading to off-flavours development [49, 51, 52].

2.1.2.4 Antioxidants

Rice has been reported as a source of antioxidant compounds with benefits for human health [53]. Previous studies reported phenolic compounds as the major hydrophilic antioxidants in rice while carotenoids, tocopherols and oryzanols as the main lipophilic antioxidant constituents [54].

The lipophilic antioxidants have been reported as protectors of cell membranes from lipid peroxidation [55].

Phenolic compounds are the most abundant and those reported as having the highest antioxidant activity in rice [53]. They appear in rice bran as soluble free, soluble conjugates and insoluble bound forms. Free forms are present inside the plant cell vacuoles, soluble esters or conjugates are esterified to sugars and other low molecular mass components and insoluble bound forms are covalently linked to cell wall structure

components, like cellulose, hemicellulose, lignin, pectin and rod-shaped structural proteins. About 74% of the total phenolic compounds present in rice are in the insoluble bound forms [56]. Phenolic compounds in rice are mainly represented by hydroxycynnamic (e.g. ferulic and p-coumaric acids) and hydroxybenzoic acids (e.g. sinapic, p-hydroxybenzoic and protocatechuic acids) [53]. Zhou *et al.* (2004) found higher levels of phenolic compounds in brown rice compared to milled rice. Their study also indicates that storage leads to a decrease in phenolic acid contents in brown and milled rice, being more accented at 37 °C than at 4 °C storage [57].

2.1.2.5 Vitamins and minerals

Rice is a good source of vitamins from complex B (riboflavin, thiamine, niacin) [11, 26, 43]. Thiamine (B1) is present in the scutellum (44%), pericarp and aleurone (35%), while riboflavin (B2) is uniformly present in the embryo [43]. Thiamine, riboflavin and niacin (B3) have been found in the ranges of 0.117-1.74 mg/100 g, 0.011-0.403 mg/100 g and 1.972-9.218 mg/100 g, respectively, for different varieties [11]. Vitamin E is reported as being present in rice bran, having health benefits [51].

Minerals like calcium, magnesium and phosphorus are present in rice along with some traces of iron, cooper, zinc and manganese [26].

2.1.2.6 Dietary fibre

Rice, namely brown rice, is rich in dietary fibre, which contributes to decrease the risk of intestinal disorders and fight constipation, especially insoluble fibre [26]. Dietary fibre comprises a wide range of carbohydrate-based non-digestible molecules, such as cellulose, β-glucans, hemicelluloses (arabinoxylans and arabinogalactans), pectins, gums and mucilages [58]. Dietary fibre is highest in the bran layer and lowest in milled rice. One cup (160 g) of cooked brown rice contains around 2.4 g of dietary fibre, which equates to 8% of an average man's daily fibre needs and 9.6% of an average woman's daily fibre needs [26]. Some reports indicate that cold water-insoluble dietary fibres prepared from milled rice or rice bran frequently exhibit inconsistent influences on the pasting viscosity or gel elasticity of starch dispersions [58].

Arabinoxylans and β-D-glucans are the major components of cereals, particularly of rice. They are known to alleviate disease symptoms, such as diabetes, atherosclerosis and colon cancer [59].

2.2 Glycemic index

The glycemic index (GI) is the indexing of the glycemic response of a fixed amount of available carbohydrate from a test food in relation to the same amount of available carbohydrate from a standard test food, normally white bread [60]. Thus, foods can be classified according to their effect on postprandial glycaemia [61-63]. Foods whose carbohydrates rapidly increase blood glucose are known as high GI foods, whereas those foods whose carbohydrates increase the blood glucose slowly are called low GI foods [61, 63]. They can be divided into low GI (GI≤ 55), medium GI (56≤GI≤69) and high GI (GI≥70) [61].

According to the aforementioned classification, white rice is generally known to have a relatively high GI when compared with other starchy foods. Rice GI ranges from 54 to 121 [64]. However, starch rice contributes to lower postprandial serum glucose and insulin responses compared to potato starch, for example, in healthy and diabetic people [65, 66].

Rice with low amylose content is easily digested by amylolytic enzymes present in the human organism, which contributes to the rapid increase of blood glucose whereas high amylose rice has been reported to exhibit lower GI [64, 65]. Goddard *et al.* (1984) tested the insulin and glucose responses to three ingested milled rices given to healthy people. The intermediate (20-25%) amylose rice has been reported as having a significantly lower serum glucose response at 30 minutes than waxy rice (0-2% amylose) and low amylose (10-20%) rice [67]. Thus, amylose content of foods plays an important role in controlling the starch digestion rate, being used to predict the blood parameters mentioned before [64]. In addition, lipids may also help delay starch digestion due to the formation of complexes with amylose [65, 67]. Therefore, rice with higher amylose content possesses more lipid-amylose complexes, which prevents the enzymes attack,

contributing to a decrease in the rate of carbohydrates utilization, resulting in lower insulin and glucose responses [67].

Apart from amylose, resistant starch can influence the decrease of insulin secretion and control postprandial blood glucose, preventing diabetes [9, 39, 64]. The degree of starch hydrolysis was found to be negatively correlated with resistant starch [64].

2.3 Brown rice vs milled rice

As already mentioned, brown rice is hulled directly from paddy rice and consists of a bran layer (6–7% of its total weight), embryo (2-3%) and endosperm (about 90%) [55]. White rice can be obtained by milling brown rice to remove germ and bran layers [19]. In fact, white rice is the most processed type due to consumer preference [21]. From an economic viewpoint, the quality of milled rice is of supreme importance [19, 21] since the grain size and shape, whiteness and cleanliness are strongly correlated with the transaction price of rice [21].

The rice processing generates four fractions: brown rice, hull, white rice and bran. Each of these fractions can vary in chemical content according to the variety of rice and the type of processing performed [26].

Brown rice is described as having more nutritional compounds, such as proteins, lipids, dietary fibre, vitamins and minerals compared to white rice [26, 55, 68]. These nutrients exist mainly in germ and bran layers of the rice. The complete milling and polishing that converts brown rice in white rice destroys about 67% of vitamin B3, 80% of vitamin B1, 90% of vitamin B6, half of the manganese, half of the phosphorus, 60 % of the iron, and a great part of the dietary fibre and essential fatty acids. These nutrients are present in rice bran, acquired as by-product of rice. Minerals are chiefly located in the bran of the rice grain. Therefore, rice can only contribute significantly to the iron supply if it is eaten as brown rice [26].

The studies developed by Ito *et al.* (2005) showed that brown rice possesses a low GI compared to white rice, being a good food to prevent the rapid increase of postprandial blood glucose concentration without increasing the insulin secretion [69].

This can be explained based on the highest dietary fibre content in brown rice: the fibre inhibits the α-amylase activity in the intestine, decreasing starch hydrolysis [69].

Despite all advantages, brown rice is less desirable due to its poor cooking and eating qualities; when cooked it presents a dark appearance and unpalatability with its hard texture and chewiness which is attributed to tough fibrous bran layer [55]. Some studies were done in order to reduce the cooking time of brown rice. Heat-cold treatment, pre-gelatinization [55] and germination [70] are some of the methods indicated to the lowering of cooking time of brown rice [55, 70]. Brown rice surface contains pigments; some studies showed that the colour of cooked rice decreases with increasing degree of milling [19].

2.4 Type 2 diabetes mellitus

Diabetes mellitus has been described as an epidemic of contemporary society and it is estimated that the number of people worldwide suffering this chronic problem will be about 300 million by 2025 [71].

Diabetes mellitus is a syndrome characterized by an absolute or relative deficiency of insulin caused by various mechanisms resulting in hyperglycaemia [72], leading to two major forms of diabetes: insulin-dependent diabetes mellitus (type 1) and non-insulin dependent diabetes mellitus (type 2) [73]. Both types of diabetes present the same pattern: a phase of latent susceptibility, followed by preclinical dysfunction that can be identified; if not, the disease identification appears with clinical symptoms and signs [72].

Type 1 diabetes mellitus is an autoimmune disease deriving from the selective destruction of insulin-producing beta cells in the pancreatic islets, requiring the insulin intake [74].

Type 2 diabetes mellitus is a major chronic disease with serious implications to the society and the economy [75]. This disease seems to result from the interaction between a genetic predisposition and behavioural and environmental risk factors, existing strong evidence that some modifiable risk factors as obesity and physical inactivity are the main determinants of the disease [73, 76]. The disease can be classified into two subtypes: non-obese diabetes and obese diabetes [72]. The high-risk groups should be submitted to

the impaired glucose tolerance screening. This analysis allows the evaluation of the degree of glucose intolerance. Subjects with impaired glucose tolerance a have greater risk of type 2 diabetes developments requiring a better diet care and health supervision [76]. Type 2 diabetic people should regulate their diet, preventing excessive energy intake. Carbohydrates with rapid absorption should be replaced by complex carbohydrates and dietary fibre, given the fact that the latter has been suggested has a protective factor against type 2 diabetes [72]. It is known that low-GI foods are able to produce low blood glucose and insulin responses in normal people, and improve glycemic control in patients with well-controlled type 2 diabetes [62], since the release of sugars is slower [60]. As already mentioned, rice has a relatively high glycemic index; however its glycemic index is better to the diet of type 2 diabetic people when compared with other foods [64, 66, 77].

2.5 Gamma - Aminobutyric acid (GABA)

GABA is a non-protein amino acid [78] and a signal molecule in pancreatic islets [5]. This compound is highly soluble in water and structurally it is a flexible molecule that can assume several conformations in solution, including a cyclic structure that is similar to proline and is zwitterionic at physiological pH values of 4.03 and 10.56 [79]. It is produced in plants, microorganisms and mammals by α-decarboxylation of L-glutamic acid that is catalysed by the glutamate decarboxylase in beta cells, which leads to a glutamate decrease [4, 5, 80].

GABA is metabolized through a reversible transamination catalysed by GABA transaminase, leading to the formation of succinic semi-aldehyde. This product is oxidized to succinate in an irreversible reaction catalysed by succinate aldehyde dehydrogenase. These reactions constitute a pathway known as GABA shunt, represented in

Figure 4 [81, 82].

Recent reports indicate that glutamate decarboxylase is activated by the increase in the cytosolic levels of H^+ and Ca^{2+} and that GABA accumulation is important in pH regulation [83]. Some reports show indications of GABA increase in various tissues in

response to mechanical stimulation, mechanical damage, cold shock, heat shock, hypoxia, cytosolic acidification, water stress and phytohormones [82].

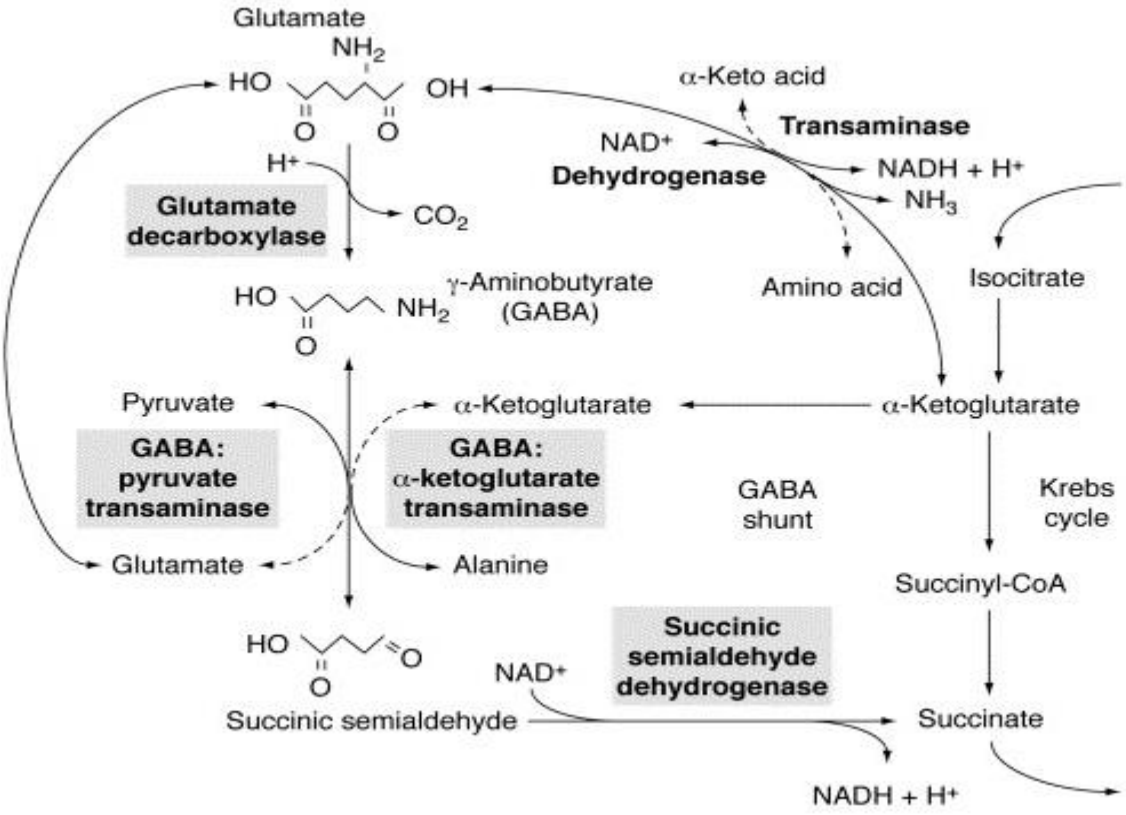

Figure 4 – The GABA shunt and its relationship to other metabolic pathways. Enzymes are indicated in bold; those specifically associated with GABA shunt are in bold and highlighted in grey (adapted from [81]).

GABA presents several pharmacological functions. The studies developed by Adeghate *et al.* (2002) showed that GABA can significantly increase insulin secretion from the pancreas of normal rats [4]. Another recent study suggests that interstitial GABA activates $GABA_a$ channels and $GABA_b$ receptors and effectively modulates hormone release in islets from type 2 diabetic and normoglycaemic subjects [5]. Hayakawa *et al.* (2004) proved that low-oral GABA administration has a hypotensive effect in spontaneously hypertensive rats, in other words, it contributes to a decrease in blood pressure [84].

Due to the aforementioned benefits, GABA-enriched foods are seen as functional foods and have become popular in reducing pain and anxiety, as well as overcoming

insomnia and chronic alcohol-related symptoms [6]. In natural forms, GABA can be found in rice germs, green tea and soybean, although GABA concentration in these natural products is not enough to promote any significant medicinal activity. Thus, recent studies have been focusing on increasing the GABA content in food [78].

Germinated rice has been described as a good functional food, possessing some health benefits, given by its high GABA content. Ito *et al.* (2005) describes pre-germinated rice as a better source of GABA when compared to brown and white rice. The study supports the conclusion that the intake of pre-germinated rice instead of white rice is effective for the control of postprandial blood glucose concentration without increasing the insulin secretion in healthy subjects [69]. Other studies have demonstrated that rice germ increased serum adiponectin levels in mice and that its active compound was GABA [85].

2.6 Germination

Germination is an effective and common process to improve the nutritional quality of cereals consumed around the world [86], allowing the development of new products with advantages and health benefits [86, 87]. Germinated brown rice has gained a special attention, especially in Asian countries [87], considering it has higher nutrient levels, sweetness and better digestion and absorption characteristics than non-germinated brown rice [6].

Germinated rice is achieved by soaking the whole grain of brown rice in water until its embryo begins to bud [87]. Germination process is affected by external factors such as germination time and absence or presence of light, both of which can aid or inhibit germination in relation to the reserve within the seed [86]. This process leads to the degradation of some seed reserves that are used for respiration and synthesis of new cell constituents for the developing embryo [86]. These changes cause alterations in the biochemical, nutritional and sensory characteristics of the cereal [86-88].

During germination, hydrolytic enzymes (e.g. amylases and proteases) are activated in order to decompose large molecules (e.g. starch, non-starch polysaccharides and protein) into small molecular substances. Thus, an increase of reducing sugars,

peptides and amino acids is observed with germination [87]. The most significant change occurs in starch, however, the degree of starch degradation depends on several germination conditions such as temperature, humidity, culturing media, soaking and germination time [87, 89].

Beyond these nutritional changes, biochemical activities occurring in germination can also lead to the formation and/or increase of bioactive compounds, with medical benefits [87, 89]. Some of these are antioxidants, such as ascorbic acid, tocopherols, tocotrienols and phenolic compounds, enhancing antioxidant activity [87]. Ohtsubo *et al.* (2004) detected a higher content of ferulic acid (an increase of 126%) in germinated rice when compared to brown rice [3]. Another study reports a global increase in the phenolic content with rice germination [89].

Kayahara *et al.* (2000) showed that the volume of nutrients in germinated brown rice relative to milled rice is nearly 4 times that of dietary fibre, vitamin E, niacin and lysine, and about 3 times as much for vitamin B1 and B6, and magnesium [90].

As noted above, germinated brown rice is known for its high GABA content. The study conducted by Kayahara *et al.* (2000) detected an increase of GABA content of 10 times more in germinated rice compared to milled rice [90]. The study carried out by Ohtsubo *et al.* (2004) allowed determining the GABA content (per 100 g of dry weight) in polished rice, brown rice and germinated brown rice. Brown rice was soaked in 120 L of water at a controlled temperature of 30 °C. GABA content after 72 hours was 40 times higher compared to polished rice and 11.5 times higher in relation to non-germinated brown rice [3]. Karladee *et al.* (2012) also studied the effect of germination in GABA content at different conditions for 21 varieties of brown rice concluding that GABA increases with germination [88]. The work developed by Thitima *et al.* (2012) deduced that the germination time influences GABA content, water absorption as well as the texture of cooked rice when compared to non-germinated brown rice [79]. A recent study performed by Zhang *et al.* (2014) had as goal to determine the effects of pH of soaking water on GABA production in two rice cultivars (*indica* and *japonica*). There were three pH treatments (pH 5.6, 7.0 and 8.4).The pH value was adjusted by citric acid and

sodium citrate. GABA content was highest at pH 5.6 which suggests that acidic conditions have a major significant effect on GABA content [6].

Iman *et al.* (2014) studied the effects of white rice, brown rice and germinated rice in the dietary management of cardiovascular diseases. Diet-induced hypercholesterolaemic rats were fed with the three types of rice, in comparison with normal, high-fat diet and Simvastatin rats. Germinated brown rice reduced weight gain and improved lipid parameters, suggesting that this type of rice can ameliorate cardiovascular disease risk by modulation of lipid metabolism and oxidative stress [91].

3. THE INTERNSHIP

3.1 The company: Novarroz, Produtos Alimentares, S.A.

Novarroz – Produtos Alimentares, S.A. is a family company founded in 1979 and it is located in Adães (Oliveira de Azeméis), being represented by the logo of Figure 5. The main activities of this enterprise are the husking, whitening, packaging and selling of rice and rice by-products (such as broken rice, bran and hull) [1].

Figure 5 - Novarroz logo[1]**.**

The company processes different rice varieties from Portugal and around the world and has an operating capability of 24 hours per day employing 63 collaborators. Novarroz possesses storage silos with 30 000 and 800 metric tons of capacity to paddy and milled rice, respectively, and a vertical mill with a processing capacity of 15 tons per hour. The final products are packed in polypropylene packing complex, vacuum bags, and raffia bag card [1].

The Novarroz mission is to place on the market quality products that are healthy, diversified and safe, following the food safety standards, contributing to the total satisfaction of customers and consumers. To meet these goals, the company works with the Integrated Management System, extending its corporative policy for the entire organization based on the following strategies:

- **The focus on the customers and markets** – trying to anticipate the needs of its customers and consumers;
- **Quality assurance and food safety** – guaranteeing the elaboration of quality products, fulfilling all legal requirements of food safety and customers;

- **Continuous improvement** – achieving a sustainable and credible business development, applicable to all sectors and processes, based on strict quality criteria;
- **Health protection and promotion of safety at work** - working to protect the health of workers, promote all the conditions to ensure safety at work and maintain an adequate level of social protection;
- **Respect for the environment** – preventing pollution, minimizing the atmospheric emissions and emitted noise, energetic costs reduction and correct separation of wastes;
- **Reduction of energy consumption and costs** – improving the energetic performance and efficiency in the rationalization of consumption and related costs, encouraging the purchase of products, services and energy efficient processes;
- **Ethics and legality** - full compliance with ethical and transparent code and legislation applicable to the company [1].

Novarroz possesses BRC Food and IFS certifications by SGS, demonstrating its commitment with quality and satisfaction of its customers. Recently (2015), Novarroz acquired the certification of ISO 50001, on energy management. The company also has the status of SME Leader by IAPMEI, it is part of the project "Portugal Sou Eu" and its manufacturing process is attested by EUREKA program [1].

3.2 Rice: legal and commercial definitions

In Portugal, rice is commercialized according to a set of characteristics regulated by Decreto-Lei n. º 62/2000 that specifies the different classifications of rice, taking into account several factors. Rice can be classified according to its physical state, grain length and treatment it has undergone [92].

As to physical state, rice can be paddy, hulled, semi-milled and milled. Paddy rice is obtained after threshing, still maintaining the hull, whereas hulled rice is the rice without the hull, also known as brown rice. Semi-milled rice is obtained after removing the husk,

part of the germ and outer layers of pericarp, while milled rice (or white rice) is achieved after hulling and total removal of germ and all layers of pericarp.

In relation to length, the rice grains can be round, medium and long. Round rice grains have a length that doesn't exceed 5.2 mm and a length/width ratio of less than 2. Medium rice grains present a length that is more than 5.2 mm and less than or equal to 6 mm and its length/width ratio is less than 3. Long rice grains have a length superior to 6 mm, but in relation to length/width ratio they can be divided in two: the rice grains with a length/width ratio between 2 and 3 and the rice grains which length/width ratio is equal or superior to 3.

According to the treatment applied, rice can be parboiled, pre-cooked, glazed or oil-polished. Parboiled rice is characterized by the complete gelatinization of its starch content due to a vaporization process; first, rice (paddy rice or hulled) is immersed in water, then vaporized and, finally, dried. Pre-cooked rice is obtained after applying a physical treatment, allowing the reduction of cooking time. Glazed rice is the white rice wrapped in a film of glucose and talc, proper to human consumption. Finally, oil-polished rice is obtained after milled rice being surrounded by a layer of comestible oil, in accordance to food regulations [92].

There are other commercial designations to rice, according to the grain size and culinary purposes. Thus, the different rice varieties can be grouped in [93]:

- *Agulha* – Rice grains are long and thin, belonging to *indica* sub-specie. Therefore, its amylose content can vary from intermediate to high; so after cooked and cooled, the rice grains present a firmer texture. This rice cooks easily and presents a low capacity to absorb the cooking water.
- *Carolino* – This rice belongs to *japonica* sub-specie and the grains are long and rounded. The *carolino* varieties present lower amylose content in relation to *agulha* varieties, which contributes to its sticky texture after cooking. Furthermore, this rice absorbs the cooking water easily and can retain all the flavours added.
- *Risotto* – This type of rice grain absorbs the water easily, forming a creamy pasta after cooking, being frequently used in Italian dishes.

- *Basmati* – Aromatic rice with long grains, usually used in Indian dishes and considered the rice with better quality.
- *Jasmine* – This rice is similar to basmati; however, after cooking, presents a stickier texture, being extremely used in Chinese cuisine.
- *Brown rice* – This designation is related to any type of husked rice that wasn't submitted to milling, being rich in fibre, vitamins and minerals; its high fibre content is responsible for the increase in cooking time.
- Wild rice – Instead of belonging to the *Oryza* genus, it is considered a grass seed from the *Zizania* genus, an aquatic and wild herb from North America. The grains are long, slender and black; presenting a high content of vitamins from complex B. Wild rice is frequently used in salads, mixed with *basmati* rice.

3.3 The industrial processing of rice

Rice needs to be submitted to several technological operations and analyses to reach the consumers under appropriate conditions. This point focuses on the industrial processing of rice conducted by Novarroz.

The paddy rice must be conveniently dried by the producer, before being taken to the factory. The rice moisture must be around 13%, with a maximum of 14%, considered a safe value for cereals, according to the Decreto-Lei n.º 63/2000 [94]. All vehicles arriving at the factory loaded with paddy rice (Figure 6a) are immediately weighted and a representative sample is collected from different points of the cargo. After that, the samples are subjected to several analyses in the laboratory, to assess each cargo relative to control, security and food quality. If all analyses are in accordance with guidelines fixed by the company, the paddy rice is taken out of the vehicle and stored in appropriate silos (Figure 6b), properly cleaned and disinfected. These silos possess adequate ventilation and monitoring systems of temperature and moisture, and if necessary, mechanisms of grain protection against pest attacks, based upon treatments legally accepted and authorized.

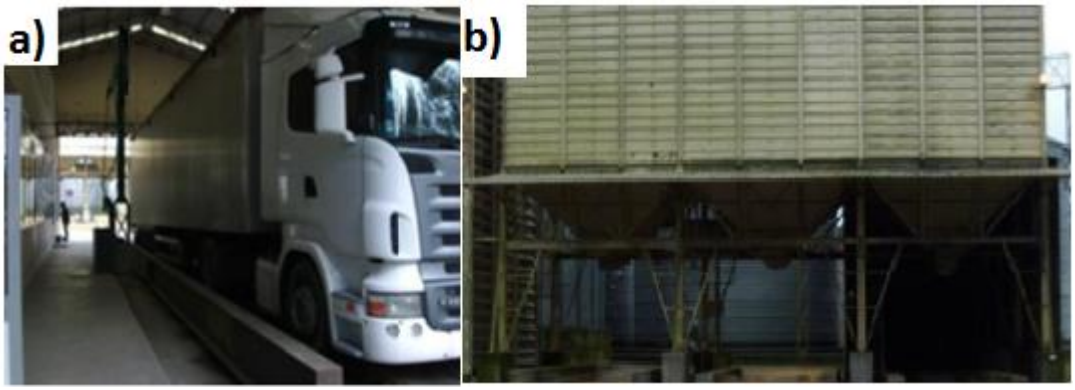

Figure 6 - Weighing of paddy rice cargo **(a)**. Silos for paddy rice storage **(b)**.

To assess the rice quality, different procedures are implemented in the laboratory, such as moisture analyses, checking of pest parasites (e.g. weevil) and other cereals (e.g. corn). Here, the collected rice samples suffer all industrial transformation processes in a small scale: hulling, milling and separation of good rice grains from broken rice grains, using the equipment showed in Figure 7a, b and c, respectively. The resulting rice is used to verify its physical characteristics, such as length, width, length-to-width ratio, chalky area percentage, whiteness degree (kett), among others.

Figure 7 - Novarroz laboratory: hull removing **(a)**, milling **(b)** and separation of the whole rice grains and broken rice grains **(c)**.

The analyses pertaining to the presence of genetic modified organisms, pesticides and others are done by an external certified laboratory.

The industrial processing comprises the transformation of paddy rice grains into grains adequate to human consumption, according to the commercial type, taking into account the current legislation.

The first step of the industrial processing is to remove all impurities from rice, like stones, straw and sticks, using cleaner machinery and de-stoners. Then, rice is routed to the machinery responsible for the hull removing, which, by the action of the pressure of rotating cylinders, facilitates the husk removal by friction, releasing the brown rice. When the rice leaves the machine, it passes by a control system responsible for separating the rice that has not been properly hulled, forwarding it again to the beginning of the process. The husk, obtained as a by-product, is stored to be sold to the local industries. After being dehulled, the rice is milled in the mill, having its germ and pericarp removed from the grains according to the desirable milling degree. The result, at the end of this process, is white rice along with two by-products: the rice bran and broken rice. The rice bran is taken out of the production line and stored in adequate silos, being sold afterwards as an ingredient of animal feed or food products, due to its high nutritional value.

Then, milled rice is submitted to a polishing process with the polisher showed in Figure 8. This is done with water, but only the enough quantity to the desirable effect, to prevent it from being absorbed by the rice grains. Beyond that, this step is important to the temperature lowering, elevated by the milling process. After polishing, the grains are routed to graders and shifters, where occurs the separation of whole grains and broken grains. The broken grains are stocked in proper silos to be sold subsequently. Lastly, the whole grains pass through the selectors, which monitor the physical quality of the grains, removing those exhibiting visual defects (e.g. red or striated grain, green, fissured and chalky). The perfect grains, as they are called, follow to a weighing screen which ascertains the product's weight at the end of the process. The final product is then stored in specific silos for subsequent packaging.

Throughout the process, there are magnets in strategic points to remove any metallic objects present in rice, ensuring the product quality maintains the high standards food safety.

Figure 8 - Rice polisher.

Rice is packed in the form of desired consumption units, variable weight, with or without vacuum, each containing the respective lot identification. The packaging is crucial to protect rice from microorganism's attacks, moisture penetration and other threats to its quality. At the end of the process, the packages pass by a metal detector (Figure 9a), to guarantee the absence of foreign metallic objects.

Figure 9 - Metal detector (after packaging) **(a)** and storage of packed rice **(b)**.

The rice packages are stored at controlled temperature and moisture conditions (as shown in Figure 9b), preventing the microbial development, until being dispatched.

3.4 Work developed in the company

Part of the internship was developed in the laboratory of the company and the methodologies and works done are described below:

- **Company policies and Portuguese legislation** – Knowledge of company policies and mission as well as the Portuguese legislation relative to rice;
- **Rice samples** – Preparation of rice samples: dehulling, milling and separation of good rice grains from broken ones;
- **Rice analyses** – Physical characterization of rice grain varieties relative to length, width, length-to-width ratio, chalky area, whiteness degree (kett) and other parameters;
- **Equipment calibration** – calibration of greenhouse and thermal balances;
- **Grain analyses** – Recognition of chalky grains, deformed, damaged, striated and broken;
- **Chlorine in the water** – Determination of chlorine content in the water in several points of the company;
- **Packaging and labelling** – Knowledge of rules and Food and Drug Administration (FDA) guidelines relative to packaging and labelling in order to update the packages of some products, with respect to allergens and nutrition facts;
- **Product sheets** – Elaboration of product sheets containing relevant information to customers in different languages;
- **Cooking tests** – Some rice varieties were cooked in order to define the cooking time.
- **Complaints** – Attend to customer and consumer complaints and performing the adequate tests to assess the causes for the complaint;
- **Pasting Properties –** Determination of the pasting properties of rice varieties with the Rapid Visco Analyser (RVA).

4. MATERIAL AND METHODS

4.1 Rice samples

Rice samples from different geographic locations were provided by Novarroz – Produtos Alimentares, S.A. and properly encoded according to their sub-species (*indica* or *japonica*), since their real name and provenance are confidential information of the company. Paddy rice samples were processed in the laboratory of quality control of Novarroz to obtain milled and brown rice for different analyses.

A total of 23 milled rice varieties were studied, namely 8 *indica* and 15 *japonica* sub-species, as listed in Table 1. These samples were analysed in terms of physical characteristics (grain dimensions, total and crystalline whiteness, chalky area and kett), pasting properties and chemical composition such as moisture, starch (total, resistant and amylose), protein and glycemic index.

Table 1 - Milled rice samples provided by Novarroz, Produtos Alimentares, S.A.

Sample	Rice Type	Crop
indica1	Agulha (aromatic)	2014/2015
indica2	Agulha (aromatic)	2014/2015
indica3	Agulha	2014/2015
indica4	Agulha	2014/2015
indica5	Agulha	2014/2015
indica6	Agulha	2014/2015
indica7	Agulha	2014/2015
indica8	Agulha	2014/2015
japonica1	Carolino	2014/2015
japonica2	Carolino	2014/2015
japonica3	Carolino	2014/2015
japonica4	Carolino	2014/2015
japonica5	Carolino	2014/2015
japonica6	Carolino	2014/2015
japonica7	Carolino	2014/2015
japonica8	Carolino	2014/2015
japonica9	Medium	2014/2015
japonica10	Medium	2014/2015
japonica11	Medium	2014/2015
japonica12	Medium	2014/2015
japonica13	Medium	2014/2015
japonica14	Risotto	2014/2015
japonica15	Round	2014/2015

Brown rice samples corresponding to milled varieties *japonica14* and *japonica15* were provided by the company. All the parameters used to evaluate the chemical composition of milled rice were analysed in brown rice samples with addition of reducing sugars, soluble and insoluble phenolic compounds and GABA measurement. Furthermore, these two brown rice varieties were germinated in different pH conditions and time. Thus, these samples were codified according to Table 2.

Table 2 - Brown rice samples provided by Novarroz, Produtos Alimentares, S.A.

Sample	Rice Type	Crop
japonica14 G0h	Brown risotto	2014/2015
japonica15 G0h	Brown round	2014/2015

Some commercial rice samples sold by other companies were acquired, analysed and compared with rice varieties under this study. Three of these samples are indicated as good rice for diabetics by supplier since they possess low GI. GABA1, GABA2 and GABA3, purchased from another supplier, have been advertised as a good source of GABA. Table 3 lists all commercial samples acquired and studied.

Table 3 - Commercial rice samples from external companies for comparison.

Sample	Rice Type	Characteristics
indica9	Aromatic parboiled	Low GI
indica10	Aromatic parboiled	Low GI
indica11	Aromatic	Low GI
GABA1	Aromatic germinated, wild and red	GABA enriched
GABA2	Germinated round	GABA enriched
GABA3	Germinated round	GABA enriched

4.2 Determination of physical characteristics of rice grains

Analysis of physical characteristics of rice grains comprises the determination of length, width, length-to-width ratio, total and crystalline whiteness, chalky area % and Kett.

All physical analyses, excluding Kett, were conducted using an S21 Rice Statistic Analyser (Agromay Soluciones Técnicas, S.L.) in the laboratory of quality control of Novarroz. This apparatus is an inspector of rice grains that, through image processing and

subsequent statistical analysis, allows the systemic identification of defects and characterization of varieties. It consists of two basic elements: the physical structure of the analyser and the software associated to a computer (Figure 10). The body of the analyser is a pump casing with a vertical dispenser, a ramp fitted with vibration, that separates the grains of rice on the surface of the ramp, and a high-speed camera that captures images of individual grains of rice that run on the surface of the ramp. About 60-70 g of rice grains were deposited into the vertical dispenser container, the button was pressed to capture images, the vibrator system was connected and the mechanism that allows to pass grains to the ramp was opened. The camera started to take pictures using a high speed continuous shooting at moving grain on the ramp until the entire sample was completed. Hereafter, the program selected the best image of each grain discarding those images with defective frame or fuzzy, getting a number of correct photographs of grains representative of the sample and presented the results [95].

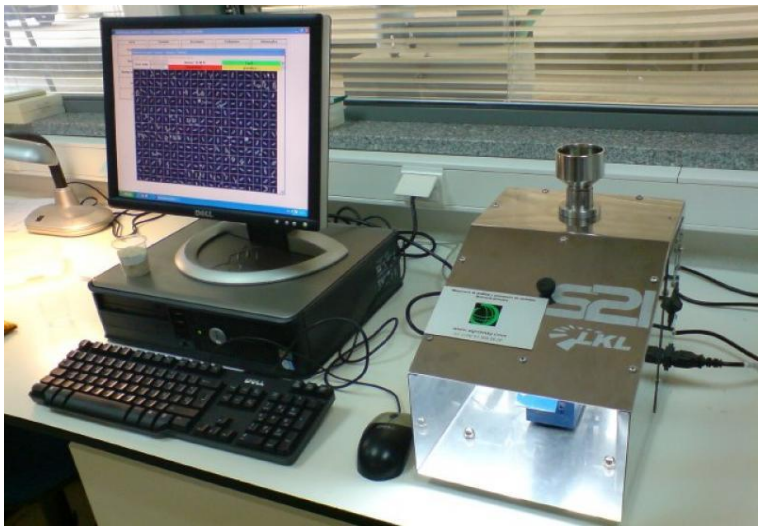

Figure 10 - S21 Rice Statistic Analyser in the laboratory of quality control of Novarroz.

Kett determination was performed using the *Kett Electric Laboratory, model C-300-3* equipment (commonly called kett), also in the laboratory of quality control of Novarroz. Kett is another parameter used to measure rice whiteness being an indicator of the level of milling as well as the quality of the rice. Therefore, Kett value correlates positively with whiteness and its determination is based on the light reflectance principle and obtained in a scale of 0 to 100. Before the analysis, the kett equipment was zeroed

with its optical standard. Then, the sample container was filled with rice, closed and inserted into kett [96], as shown in Figure 11.

Figure 11 - *Kett Electric Laboratory, model C-300-3* equipment in the laboratory of quality control of Novarroz.

4.3 Determination of the pasting properties of rice with the Rapid Visco Analyser (RVA)

The determination of pasting properties of milled rice varieties was performed through Rapid Visco Analyser (RVA), *model TecMaster*, located in the laboratory of quality control of the company (see Figure 12). This equipment is a rotational viscometer that is able to continuously record the viscosity of a sample under controlled temperature conditions. RVA is combined with the Thermocline for Windows (TCW) software program, being able to heat and cool samples through more than 100 temperature ramps [97]. These determinations were carried out according to the American Association of Cereals Chemistry (AACC) International Method 61-02.01 [98]. The parameters obtained (peak viscosity, pasting temperature, breakdown, setback and final viscosity) are indicators of gelatinization and paste viscosity characteristics of milled rice flour, being predictors of rice cooking and processing properties. This methodology is based on the simultaneously heating and stirring of an aqueous suspension of ground rice disposed in a canister placed

in the equipment, followed by temperature lowering. A precisely controlled heat-hold-cold temperature cycle is used to cause viscosity changes in the mixture [98].

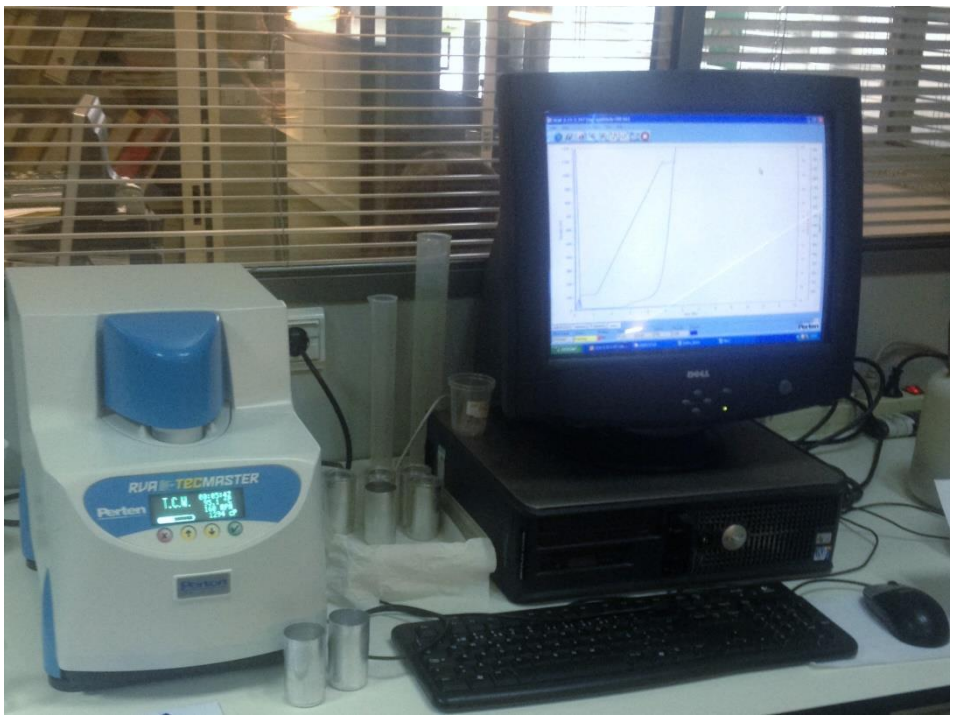

Figure 12 - Rapid Visco Analyser (RVA), *model TecMaster,* located in the laboratory of quality control of Novarroz.

The instrument and associated computer were switched on and control software was opened. Firstly, a test was run, without any canister in the equipment, to warm RVA. Then, the apparatus was zeroed. These two steps need to be done every morning before sample tests. After this, the analysis of samples was performed. The test profile for rice was selected, consisting in the time/temperature cycle (according to AACC) represented in Table 4. The name of the sample was entered in the file used to record viscosity data of the test and test run option was selected. In the next window of RVA software it was necessary to enter the moisture value of the sample, since the correct sample and water weight are calculated by the program based on the moisture content of the sample [98]. Therefore, the sample was ground, and one part of the rice flour was used for determining moisture content throughout AACC International Method 44-15.02 [99]. The other part of rice flour was passed throughout a 0.5 mm sieve and weighted into the test canister. Distilled water was also weighing into a new test canister. The rice flour was transferred into water surface in the canister; a paddle was placed into this canister and

the mixture vigorously jogged through the sample up and down 10 times, until all the flour was dispersed in the water. The paddle was placed into the canister and this assembly was firmly inserted into the paddle coupling. The test was initiated by depressing the motor tower of RVA [98].

Table 4 - Time/temperature cycle used in the test profile for rice, according to AACC[98].

	Temperature (°C)	Time (min:sec)
	50.0 (Idle temperature)	
1rst	50.0	1:00
2nd	95.0	4:45
3rd	95.0	7:15
4th	50.0	11:06
End of test		12:30

At first, the temperature remained at 50.0 °C for 1 minute; then, heating did begin with an increase in rate temperature of 14°C per minute begun in an increase rate temperature of 14°C per minute, until reaching 95.0 °C; During 3 minutes and 45 seconds the temperature remained at 95 °C; lastly, the temperature begun to drop, taking about 3 minutes and 51 seconds to reach 50.0 °C, remaining so until the end of the test. The instrument dispersed the mixture by rotating the paddle at 960 rpm during 10 seconds; and then the viscosity was measured using a constant paddle rotation speed of 160 rpm. The test terminated automatically and the content of the canister was discarded [98]. At the end of the test, a typical RVA curve was obtained, as exemplified in Figure 13.

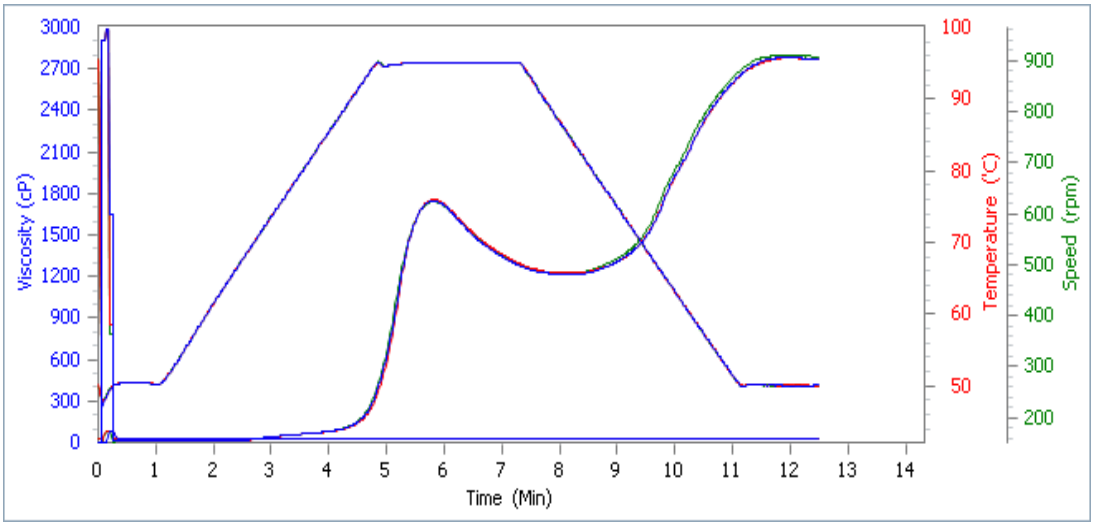

Figure 13 – Typical complete RVA curve.

4.4 Determination of moisture content

The determination of moisture content was performed through a freeze dryer VirTis, *model benchtop K*.

Empty sample containers of 5 mL were weighed and the values were registered. Whole rice grains were grounded and passed through a 1 mm sieve. Then, about 1 gram of the rice flour obtained was measured in the sample containers previously weighted and the values were also recorded. Before the freezing process, the containers' lids were removed and stored. The containers were sealed with parafilm, which was then punched with a needle. Then, the samples were frozen and dried for 3 to 4 days.

After drying, the sample containers were weighted (with lids) and kept in a desiccator for use in the protein and GABA analysis.

Moisture calculation was based on weight loss according to the following formula:

$$\%\ \mathbf{Moisture} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}}$$

4.5 Determination of protein content

The protein content was determined by elemental analysis using a *TruSpec 630-200-200 CNHS* Analyser in the microanalysis laboratory of the university.

The Analyser is responsible for the dynamic combustion of the sample. About 3 mg of lyophilised rice samples (point 4.4) were weighted in tin capsules and introduced in the combustion reactor through an automatic sampler with a certain amount of oxygen. The samples were combusted in a furnace operating at 1075 °C and with an afterburner at 850 °C to generate N_2, CO_2, H_2O and SO_2 gases. These gases were transported by a helium stream through the internal copper surface of the reactor, separated by gas chromatography and quantified with thermal conductivity [100]. The nitrogen amount was multiplied by factor 5.95 in order to determinate protein content [3]. The results were obtained in dry matter, in total percentage.

4.6 Determinations of starch content

4.6.1 Solutions

HCl-KCl buffer 25 mM at pH = 1.5 – Hydrochloride acid (HCl) and potassium chloride (KCl) solutions were prepared separately with a final concentration of 0.25 mM. The buffer was prepared adding HCl solution to KCl solution until a final pH = 1.5. The buffer solution was completed with distilled water according to the final volume.

Tris-maleate buffer 0.6 M at pH = 6.9 – Maleic acid and tris(hydroxymethyl)aminomethane (Trisma) were weighted according to a final concentration of 0.6 M, being mixed and dissolved with distilled water. The pH of the solution was adjusted to 6.9 with a sodium hydroxide (NaOH) 5 M solution. Finally, the buffer solution was completed with distilled water up to the required volume.

Sodium-acetate buffer 0.4 M at pH = 4.75 – Sodium acetate was measured to a final concentration of 0.4 M and dissolved with distilled water. Then, the solution was adjusted with an acetic acid (CH_3COOH) 1 M solution and completed with distilled water.

Glucose oxidase-peroxidase (GOD-POD) reagent – About 30 mL of GOD-POD reagent buffer was diluted in 1 L of distilled water. GOD-POD enzymes were dissolved in approximately 20 mL of GOD-POD buffer prepared before and quantitatively transferred to the bottle containing the remainder of GOD-POD buffer. The bottle was covered with aluminium foil to protect the enclosed reagent from light. The GOD-POD reagent was distributed by 10 containers covered with aluminium foil. The container in use was stored in the refrigerator at -4 ° C and the other nine were frozen at -20 °C.

Iodine (I_2) solution – To prepare this solution, 2000 ± 5 mg of potassium iodide (KI) were weighted and dissolved with enough distilled water to form a saturated solution. Then, 200 ± 1 mg of I_2 crystals were weighted and added to the previous solution, being quickly dissolved. This solution was transferred to a 100 mL flask covered with aluminium foil in order to protect it from light. The solution was completed with distilled water and stored in the dark until being used.

4.6.2 Total starch content

The determination of total starch content was accomplished according to Soares [101] (2014).

Five to seven rice grains were ground and passed through a 0.5 mm sieve. Then, about 25-35 mg of ground rice sample were weighted into a test tube and 2 mL of potassium hydroxide (KOH) 2 M were added. This mixture was placed under agitation for 72 hours at room temperature.

After 72 hours, the pH mixture was adjusted with HCl 2 M to 6.9 and 3 mL of tris-maleate buffer 0.4 M at pH = 6.9 were added in order to stabilize pH at 6.9, since this is the optimum pH of activity of α-amylase (**Sigma-Aldrich** – A3176). α-Amylase enzyme solution was prepared with a concentration of 40 mg/mL in tris-maleate buffer 0.6 M at pH = 6.9 and 1 mL was added. The starch hydrolysis proceeded for 48 hours at room temperature.

Three aliquots of 100 µL were collected from the previous solution and transferred to microcentrifuge tubes. Then, 1 mL of sodium acetate buffer 0.4 M, pH = 4.75 and 50 µL of amyloglucosidase (**Sigma-Aldrich** – A10115), prepared with a concentration of 6mg/mL in sodium acetate buffer, were added. The mixture was carefully shaken by inversion of the microcentrifuge tubes and placed in a water bath at 60 °C, leaving it to react overnight. The next day the samples were taken from the bath and centrifuged at 10 000 rpm for 1 minute. Glucose content was quantified using the enzymatic kit glucose oxidase-peroxidase (GOD-POD) (**nzytech**, AK00161), using a Thermo Scientific *Multiskan Go* UV/Visible microplate spectrophotometer to measure the absorbance at 510 nm. Glucose content was determined using the Beer-Lambert law and converted into starch through the conversion factor 0.9. Total starch content was presented in dry matter, according to the moisture content determined (see point 4.4).

4.6.3 Resistant starch content

The extraction and quantification of resistant starch was also performed according to Soares [101] (2014) at the university's laboratories.

Seven to ten rice grains were ground and passed through a 1.0 mm sieve. About 100 mg of ground rice sample were weighted into a test tube and 2 mL of HCl-KCl buffer 25 mM at pH = 1.5 were added. This mixture was placed in a water bath at 40 °C. Pepsin (**Riedel-de Haën** – 20895) solution was prepared in HCl-KCl buffer 25 mM at pH = 1.5, with a concentration of 0.1g/mL. Then, 200 µL of this enzyme were added to the mixture to hydrolyse proteins, reacting at 40 °C for 1 hour, under constant stirring. The tubes were then, taken from the bath to cooled until room temperature. The pH was adjusted with sodium hydroxide (NaOH) 3 M to 6.9 and 3 mL of tris-maleate 0.6 M at pH = 6.9 were added under stirring. α-Amylase solution was prepared as in point 4.6.2 and 1 mL was added to the tubes at room temperature leaving it to react for 72 hours under agitation.

After 72 hours, a HCl 6 M solution was used to adjust the pH to 4.75 and 2.8 mL of sodium acetate buffer 0.4 M at pH = 4.75 were added. The tubes were transferred to a water bath at 60 °C and 400 µL of amyloglucosidase (prepared as in point 4.6.2) were added, to react overnight.

The next day, tubes were centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded. Then, the residue was washed with an ethanol 50% solution to extract all the sugars digested by the hydrolysis process, being the supernatant also discarded. This step was performed three times ensuring the complete extraction of sugars to avoid the quantification of resistant starch by excess. Then, the residue obtained reacted with 1.2 mL of KOH 2 M solution for 30 minutes at room temperature, under stirring, in order to solubilize the starch that was not digested by the enzymes. The pH solution was adjusted with HCl 2 M to 4.75.

Three aliquots of 300 µL were collected from the tubes and transferred to microcentrifuge tubes. After, 700 µL of sodium acetate buffer 0.4 M at pH = 4.75 and 50 µL of amyloglucosidase (prepared as before) were added. The microcentrifuge tubes were closed, carefully shaken and placed in a water bath at 60 °C in order to promote the hydrolysis overnight. The next day, the samples were centrifuged at 10 000 rpm for 1 minute. The quantification of glucose and starch was carried out as described in point 4.6.2, being the resistant starch content also presented in dry matter basis.

4.6.4 Amylose content

The determination of amylose content was carried out according to the ISO 6647 [102] with slight modifications. The rice samples were totally ground and passed through a 0.180 mm sieve. After, 100±0.5 mg of the resultant flour was weighted in test tubes. Then, 1 mL of ethanol solution at 95% and 9 mL of NaOH 1 M were added into the tubes. The samples were placed in the orbital shaker, model *Agitorb 200 ICP*, at 180 rpm and room temperature for 2 days.

After 2 days, the tubes were covered with parafilm, shaken and placed in a boiling bath for 10 minutes. Then, the tubes were taken from the bath and left to cooldown at room temperature. The samples were transferred to 100 mL flasks and completed to volume with distilled water.

Three aliquots of 500 µL were collected from each flask and transferred to test tubes. After this, 5.00 mL of distilled water, 100 µL of acetic acid 1 M, 200 µL of iodine solution (see point 4.6.1) and 4.20 mL of distilled water were added, completing a final volume of 10 mL. The tubes were shaken in a vortex mixer. The absorbance was obtained using a Jenway UV/Visible spectrophotometer, *model 6405*, at 620 nm.

The same procedures were applied to rice samples with known amylose content in order to get the standard curve. Thereunto, five standard samples with 0.00, 12.10, 14.10, 14.25 and 22.80 % of amylose were used, being the analysis also done in triplicate. The blank tube was prepared in the same conditions of the sample and standard tubes but using 500 µL of NaOH 0.09 M instead of sample.

4.7 Starch hydrolysis to determinate glycemic index

4.7.1 Solutions

HCl-KCl buffer 50 mM at pH = 1.5 – Hydrochloride acid (HCl) and potassium chloride (KCl) solutions were prepared separately with a final concentration of 0.50 mM. The buffer was prepared adding HCl solution to KCl solution until a final pH = 1.5. The buffer solution was completed with distilled water according to the final volume.

HCl-KCl buffer 25 mM at pH = 1.5 – Prepared as in 4.6.1.

Tris-maleate buffer 0.6 M at pH = 6.9 – Prepared as in 4.6.1.

Sodium-acetate buffer 0.4 M at pH = 4.75 – Prepared as in 4.6.1

Glucose oxidase-peroxidase (GOD-POD) reagent – Prepared as in 4.6.1.

4.7.2 Method

The rate of starch digestion of rice at different times was measured to estimate glycemic index based on the procedure developed by Goñi *et al.* (1997) [2] with some alterations.

About 50 mg of rice grains were weighted in capped tubes and 5.00 mL of distilled water were added. The samples were boiled at 100 °C for 30 minutes. After 30 minutes, all the content of the tubes was transferred to 50 mL plastic samplers and 5 mL of HCl-KCl buffer 50 mM at pH = 1.5 were added. The mixture was ground and homogenised for 2 minutes using an Ultra-Turrax homogenizer, *model T25, Ika Verke*, at velocity 5 (21500 rpm). The final solution was transferred to 30 mL erlenmeyer flasks being placed in a water bath at 40 °C. Thereafter, 200 µL of pepsin enzyme (prepared as in 4.6.3) were added to the erlenmeyer flasks and left to react in the water bath for 60 minutes.

One hour later, the erlenmeyer flasks were withdrawn from the bath and allowed to cool to room temperature. After, the flasks were put in a stirring plate, the solution's pH was adjusted next to 6.9 with 100 µL of NaOH 3 M solution and stabilized at 6.9 adding 14.9 mL of tris-maleate buffer 0.6 M at pH = 6.9. Then, 5 mL of α-amylase (2.6 U prepared in tris-maleate buffer 0.6 M at pH = 6.9) were added to each sample. Three aliquots of 300 µL were taken from each erlenmeyer flask every 30 minutes from 0 to 3 hours and transferred to microcentrifuge tubes. The tubes were placed at 100 °C for 5 minutes to inactivate the enzyme. Then, 1 mL of sodium acetate buffer 0.4 M at pH = 4.75 was added to each aliquot, and, 25 µL of amyloglucosidase (4.2 U prepared in sodium acetate buffer 0.4 M at pH = 4.75) were added. The microcentrifuge tubes were closed,

carefully shaken and placed in a water bath at 60 °C overnight to promote the hydrolysis of digested starch into glucose by amyloglucosidase.

Finally, the samples were centrifuged at 10 000 rpm for 1 minute. The quantification of glucose and starch was performed as described in point 4.6.2. The rate of starch digestion was expressed as the percentage of total starch hydrolysed at different times (30, 60, 90, 120, 150 and 180 minutes), represented by hydrolysis curves. The area under hydrolysis curves (AUC) was calculated by numerical integration using the composed Simpson rule. The hydrolysis index (HI), expressed as a percentage, was obtained as the relation between the AUC for a sample and the AUC for a reference food, namely white bread, treated under the same conditions of the sample. The glycemic index (GI) was estimated according to the Goñi model, represented by the following equation:

$$\mathbf{GI} = 39.71 + (0.549 * HI)$$

4.8 Germination process

The germination process was based on the procedures of Zhang *et al.* (2014) [6] with several modifications. The brown rice samples were germinated under three different pH conditions (3, 4, and 5.3) for 24, 48 and 72 hours to evaluate the effects on the chemical composition such as starch, GABA, phenolic compounds, among others. For this, Novarroz provided some samples of water from the factory. In spite of the content of chlorine in the water being evaluated in the laboratory of quality control of the company, other parameters are controlled through chemical and microbiological analysis carried out by an external laboratory. The pH is one of the parameters analysed and was important to perform the germination process, described in Figure 14.

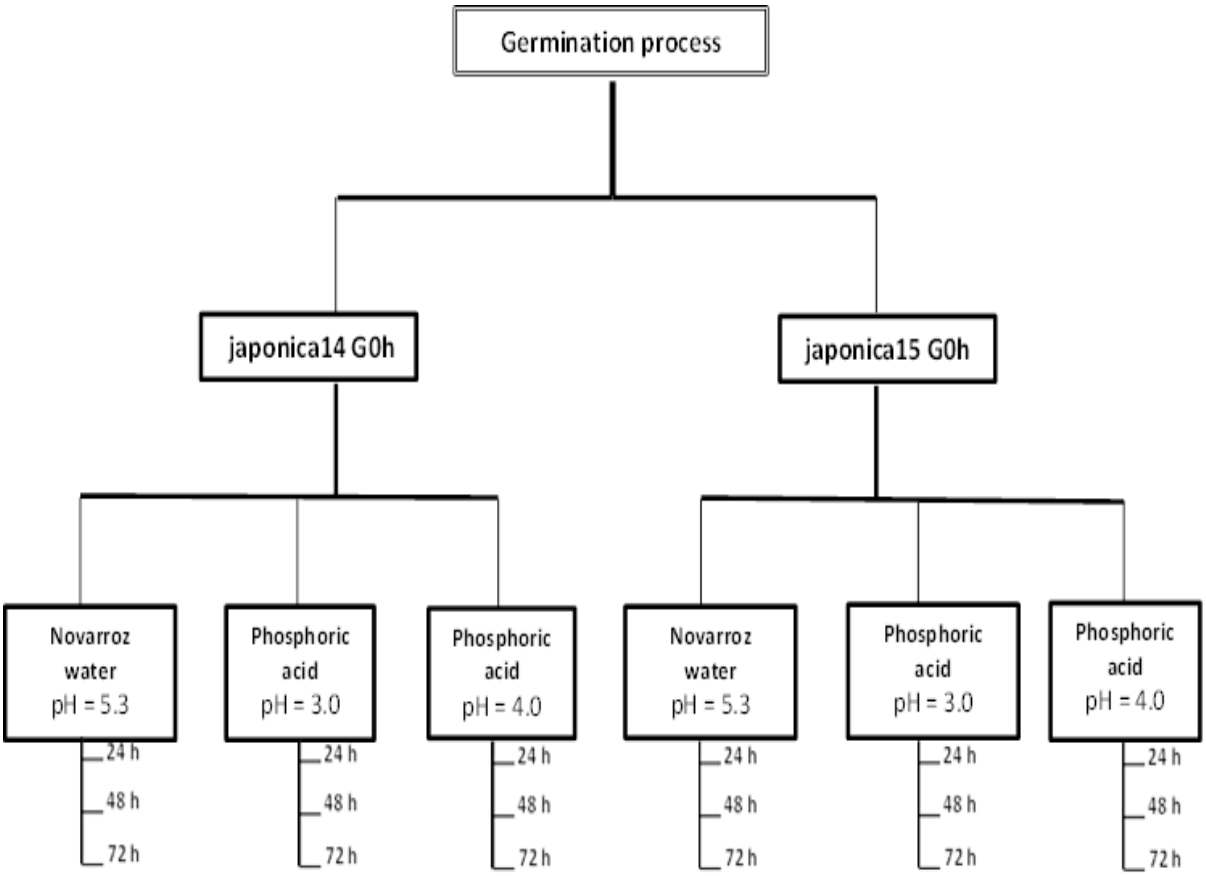

Figure 14 – Germination process of two brown rice varieties at different pH conditions for 24, 48 and 72 hours.

Novarroz's water was used as the germination medium and solvent of phosphoric acid solutions, being its pH near to 5.3. The phosphoric acid solutions were prepared according to a final pH of 3.0 and 4.0 respectively, using the water of the company as solvent. Unlike distilled water, the Novarroz's water wasn't a pure solution, containing minerals and other components that interfere with phosphoric acid salts. Therefore, the phosphoric acid mass was adjusted in order to acquire the intended pH: for example, to obtain the final pH = 3.0 and 4.0.

The rice grains of two brown cultivars (*japonica14 G0h* and *japonica15 G0h*) were carefully selected, thus excluding the broken and damaged grains. Then, about 10 g of rice grains were weighted in Petri dishes for each pH and germination time, giving a total of 9 Petri dishes per rice variety, according to Figure 14. The seeds were surface-sterilized through dipping in sodium hypochlorite 0.1% solution for 30 minutes, and then washed with Novarroz's water in abundance.

The grains were covered with Novarroz's water at pH = 5.3, phosphoric acid solution at pH = 3.0 and phosphoric acid solution at pH = 4.0, respectively, and germinated for 24, 48 and 72 hours (as shown in Figure 14) at room temperature. The samples were put in a location with enough sunlight to promote germination.

Every 24 hours, that is, at 24, 48 and 72 hours, rice samples were taken and placed to dry in a laboratory oven with air circulation at 45 °C until they lost the most of the absorbed water in order to stop the germination. The germinated rice samples were stored in a desiccator until being analysed.

4.9 Determination of reducing sugars content

4.9.1 Solutions

3,5-dinitrosalicylic acid (DNS) – 1 g of DNS was dissolved in about 50 mL of distilled water. To this solution about 30 g of sodium potassium tartarate were added. This mixture was placed in a water bath not exceeding 100 °C to facilitate the dissolution. Then, 20 mL of NaOH 2M solution was added. The final solution was transferred to a dilution flask of 100 mL and completed with distilled water. DNS solution was stored in an amber coloured bottle.

4.9.2 Method

The extraction of reducing sugars was carried out based on Ohtsubo *et al.* (2005) [3] methodology with alterations. The quantification was performed according to Miller [103] (1959) procedures with slight modifications.

The brown and germinated rice grains were ground and passed through a 1.0 mm sieve. Then, 1.0 g of rice flour obtained was weighted to test tubes and 1.6 mL of ethanol 80% was added. The mixture was placed under stirring for 1 hour in order to extract all reducing sugars. After 1 hour, the samples were centrifuged at 3000 rpm for 10 minutes and supernatant was collected. Then, three aliquots of 200 µL of supernatant were transferred to test tubes and 200 µL of DNS solution were added to each tube. The

mixtures were shaken and put in a water bath at 100 °C for 5 minutes. After 5 minutes, the tubes were placed in a cold bath and 1.6 mL of distilled water was added. The tubes were shaken in a vortex mixer. The absorbance was measured in a *Biotek Eon* microplate spectrophotometer UV/Visible at 540 nm and the content of reducing sugars was calculated through a standard curve of solutions of glucose in ethanol 80% with concentrations between 0.0 and 1.00 mg/mL, treated as the rice samples.

4.10 Determination of phenolic compounds content

4.10.1 Soluble phenolic compounds content

The extraction of soluble phenolic compounds was performed according to Lin *et al.* (2011) [104] with modifications. The quantification was achieved based on the procedures developed by Iqbal *et al.* (2005) [105] with alterations.

The brown and germinated rice seeds were ground and passed through a 1.0 mm sieve. 1.0 g of ground and sieved rice was weighted and 1.6 mL of ethanol 80% solution was added. The mixture was placed under stirring for one hour. Then, the samples were centrifuged at 3000 rpm for 10 minutes. After that, the supernatant was collected. Three aliquots of 50 µL of supernatant were transferred to test tubes and 200 µL of distilled water and 50 µL of Folin-Ciocalteau reagent (**VWR** – 31360.264) were added. The samples were shaken and allowed to react for 5 minutes. Hereafter, 1.0 mL of sodium carbonate 10% and 400 µL of distilled water were added. The tubes were stirred and placed in a dark location to react for 90 minutes. After 90 minutes, the absorbance was read through a *Biotek Eon* microplate spectrophotometer UV/Visible at 760 nm. The quantification of phenolic compounds was accomplished through a standard curve of solutions of gallic acid in ethanol 80% with concentrations between 0.00 and 300.00 mg/L.

4.10.2 Insoluble phenolic compounds content

The insoluble phenolic compounds were also extracted according to Lin *et al.* (2011) [104] with modifications and quantified based on the procedures developed by Iqbal *et al.* (2005) [105] with alterations.

The brown and germinated rice grains were ground and passed through a 1 mm sieve. Then, 0.200 g of sieved rice were weighted to a test tube and 1.6 mL of ethanol 80% was added. The mixture was agitated and left to react under stirring for one hour. One hour later, the samples were centrifuged at 3000 rpm for 10 minutes and the supernatant was discarded. 320 µL of KOH 1 M were added to the residue and left to react overnight.

On the next day, the mixture was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected. After, the supernatant was diluted: 50 µL of supernatant were transferred to a test tube and 450 µL of distilled water were added. From the diluted solution were collected three aliquots of 50 µL to test tubes and added 200 µL of distilled water and 50 µL of Folin-Ciocalteau reagent. The mixture was left to react for 5 minutes. Then, 1mL of sodium carbonate 10% and 400 µL of distilled water were added. The solution was shaken and let to react in the dark for 90 minutes. Finally, the absorbance was read in the same way as in point 4.10.1 and the quantification of phenolic compounds was performed using a standard curve of solutions of gallic acid in KOH 0.1 M with concentrations between 0.00 and 750 mg/mL.

4.11 Determination of GABA content

4.11.1 Solutions

Isobutanol – HCl was prepared with isobutanol previously dried with calcium hydride, distilled and stored in a bottle with 4Å molecular sieves. For each mL of dry dried isobutanol 270 µL of acetyl chloride were added.

Butylated hydroxytoluene (BHT) solution was prepared in a concentration of 0.2 mg/mL of BHT in ethyl acetate.

4.11.2 Method

GABA was extracted from brown and germinated rice samples according to Jannoey *et al.* 2010 [106] procedures with modifications. The amino acids separation was performed through gas chromatography and detection by mass spectrometry (GC-MS) analysis based on the procedures carried out by Coimbra *et al.* (2011) [107], being the amino acids derivatization accomplished through the methodology developed by MacKenzie *et al.* (1974) [108].

About 0.250 g of freeze-dried rice powder (see point 4.4) were weighted to microcentrifuge tubes and 800 µL of ethanol 70% were added. The mixture was vigorously shaken for 1 minute at room temperature and then centrifuged at 7.2 g at 4 ° C for 20 minutes. The supernatant was collected in a test tube. The same volume of 70% ethanol solution was added to the pellet as described above and the extraction was repeated. Then, 250 µL of the internal standard solution (ornithine 10 µmol/mL in ethanol 70%) were added to the collected supernatant (1.6 mL). The mixture was shaken, filtered through a 0.45 µm Millipore filter and dried under vacuum using a centrifugal evaporator, yielding a dry residue.

Derivatization is an important step of sample preparation in GC or GC-MS applications since it allows converting non-volatile compounds to volatile derivatives which can easily migrate to the gaseous phase by a heating process. GABA, ornithine and the other amino acids present in the samples were derivatized to their N(O,S)-heptafluorobutyryl isobutyl esters, according to Figure 15.

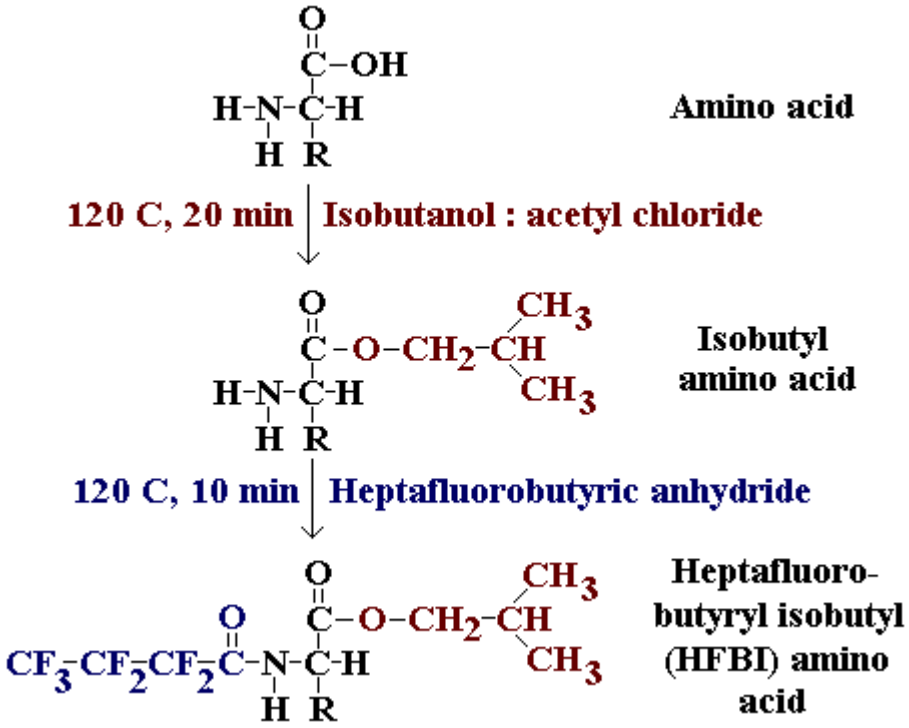

Figure 15 - Process of amino acids derivatization (adapted from [109])**.**

The dry residue obtained before was dissolved in 200 µL of isobutanol - HCL (prepared as explained in point 4.11.1). The mixture was heated to 120 ° C for 10 minutes and, after shaking in a vortex mixer, was heated for 30 minutes more. Then, the solution was cooling down to ambient temperature and the excess of reagent was evaporated under vacuum through the centrifugal evaporator. Hereafter, 200 µL of BHT solution (see point 4.11.1) were added, shaken and the solvent was removed under vacuum in the centrifugal evaporator. After that, 100 µL of heptafluorobutyric anhydride were added, mixed and the solution was heated during 10 minutes at 150 °C. After 10 minutes, the samples were left to cool at room temperature and the excess of solvent was removed under vacuum. The residue obtained was dissolved in 150 µL of ethyl acetate and analysed through GC-MS or frozen at -20 °C until subsequent analysis.

The GC-MS analysis was performed using the Shimadzu single quadrupole GCMS-QP2010 Ultra gas chromatograph-mass spectrometer. A DB1–MS (30 m length, 0.25 mm inner diameter and 0.10 µm thickness) (J & W Scientific) was used for separation. The GC oven temperature program was as follows: 1 minute hold at 70 °C, increase to 170 °C at 2.0 °C/ minute and then to 250 °C (5 minutes hold) at 16 °C/minute. The total time of GC analysis was 61 minutes. Helium was used as the carrier gas at a flow rate of 1.86 mL/minute. 3 µL of each sample were injected in split mode. The initial injector temperature was 70 °C. The septum purge flow rate was 3.0 mL/minute. The transfer line and ion source temperature were 300 and 250 °C, respectively. Ion source fragmentation was performed with an electron impact energy of 70 eV. The compounds were detected using scan mode. Mass spectra were recorded in the mass range 50-700 *m/z*.

Quantification was based on the internal standard method using ornithine and the calibration curve for GABA was built in the concentration range 0.0-0.5 mg/mL.

4.12 Statistical analysis

The physical characteristics of the rice grains were measured just once, since the values given are means of the parameters of more than one hundred grains calculated by the equipment. Moisture content was also acquired once. The other determinations done were reported as the means ± standard deviations (SD), in dry matter basis. GABA and protein analysis were performed in duplicate while the remaining parameters were determined in triplicate.

One way-analysis of variance (ANOVA) by Tuckey's test ($p < 0.05$) was applied to check the relevant differences between samples, using the GraphPad Prism 6 software statistical program. Pearson correlation analysis was also conducted in order to study the relation between all the parameters studied using Microsoft Office Excel 2010.

The detailed results and correlations can be consulted in Annexes A, B, C, D, E and F.

5. RESULTS AND DISCUSSION

5.1 Milled, brown and commercial rice samples

5.1.1 Physical characteristics of milled rice grains

Rice quality is of great importance since it affects the commercial and nutritional value of the grains and is assessed according to defined criteria. The relevance of these criteria depends on the particular use of each customer or consumer. The grain appearance is one of the most important quality parameters. This parameter is mainly evaluated through the grain shape, namely the grain length, grain width and the length-to-width ratio, and the chalkiness of the endosperm [110]. Therefore, the analysis of these characteristics is extremely important to Novarroz in order to respond to the needs of its customers.

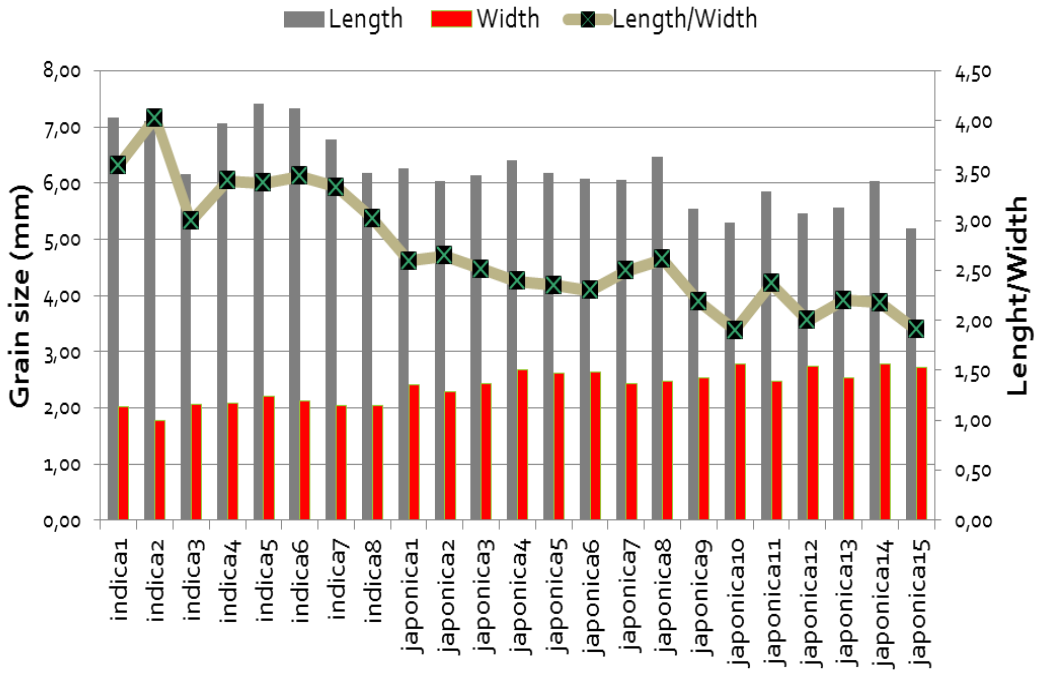

Figure 16 – Grain size, in mm, namely length (grey bars) and width (red bars) of the grains, and the ratio value between these parameters (brown line).

The analysis of Figure 16 allows us to verify that the grains of *indica* varieties are longer and narrower compared to the grains of *japonica* sub-species, corroborating previous reports [6, 13]. All *indica* varieties studied presented a grain length superior to

6.0 mm and a length-to-width ratio equal or superior to 3, therefore being classified as long rice kernels, as expressed in the Portuguese Decreto-Lei n.º 62/2000 [92]. These sub-species are commercially designated by *agulha* varieties. Other group of long rice grains comprises those varieties whose length of the grains is also superior to 6.0 mm but the length-to-width proportion is between 2 and 3. The first eight *japonica* sub-species (1, 2, 3, 4, 5, 6, 7 and 8), presented in Figure 16, are examples of this type of classification, being commonly named *carolino* varieties by producers and consumers. J*aponica9*, 10, 11, 12, 13 and 14 belong to the group of medium rice grains since its length mean is less than 6.0 mm and superior to 5.2 mm and the length-to-width ratio is inferior to 3. *Japonica14* is the variety with the larger rice grains, a desirable characteristic to the confection of risotto dishes, being commercially designated as *risotto*. Finally, *japonica15* is designated as a variety of round grains because its length doesn't exceed 5.2 mm and its length-to-width ratio is less than 2, being the variety with the smallest grains.

The chart of Figure 16 illustrates interrelations between the acquired size parameters. In fact, strong correlations were found between length-to-width ratio and both average length and width of all samples studied, as shown in Figure 17. Grain length was positively correlated with length-to-width ratio (Figure 17a)), exhibiting a Person's correlation coefficient (*r*) of 0.90, indicating that the selection of rice grains with greater length would contribute to a positive response in length-to-width ratio. On the other hand, average width was negatively correlated with length-to-width ratio (*r* = -0.95), as it is possible to see in Figure 17b). These results suggest a trend to an inverse relationship

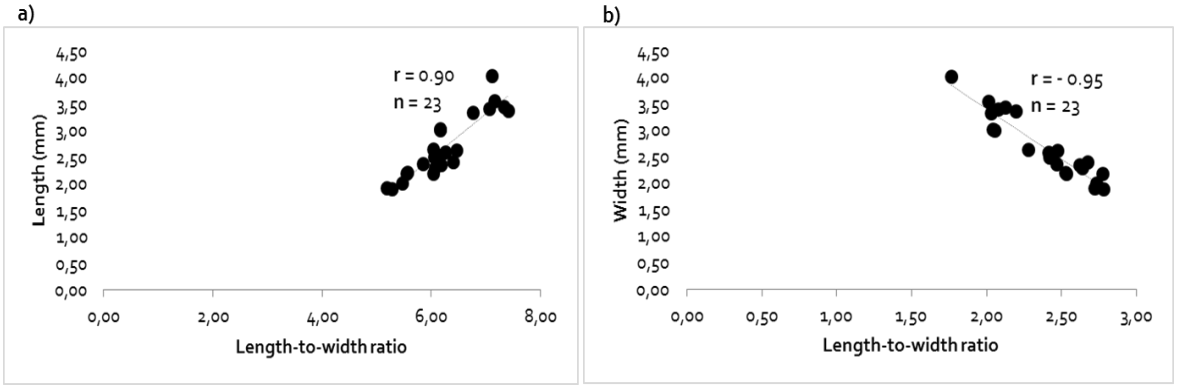

Figure 17 – Positive correlation between length (mm) and length-to-width ratio **(a)** and negative correlation between width (mm) and length-to-width ratio **(b)** having taken the 23 samples into account.

between length and width, which is confirmed by Figure 18. The studies carried out by Koutroubas *et al.* (2004) [110] also found similar relations between size parameters, corroborating the results obtained.

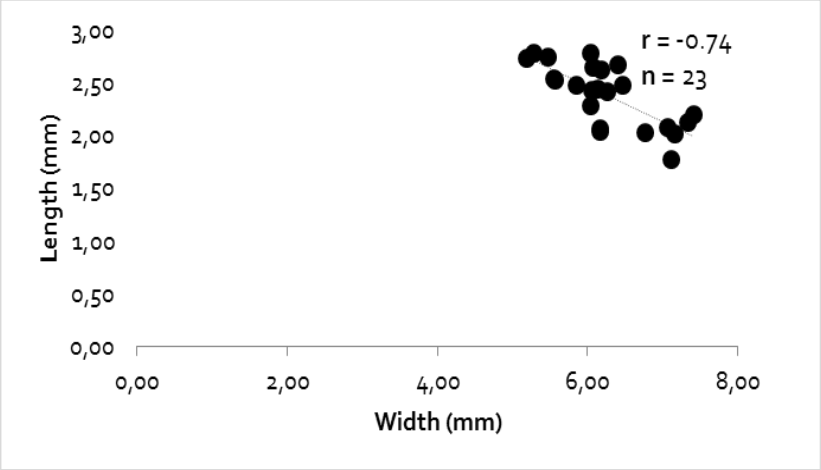

Figure 18 – Negative correlation between length (mm) and width (mm) of 23 rice samples.

Chalkiness is also an important criterion to assess the quality of rice in most of the world markets, having a great influence in its final commercial value [111]. Kim *et al.* (2000) [112] evidenced that a proportion of chalky area superior to 15% decrease the eating quality of rice. The chalky endosperm consists of loosely packed, round and large compound starch granules while the vitreous endosperm includes polyhedral and well organized small single starch granules [113]. The formation of chalky areas in the grains is associated with high temperature stress during grain maturation putting global warming as a causer of a global problem for rice agriculture in the future [111]. Thus, the evaluation of total and crystalline whiteness and chalky area % of the rice grains is an important analysis to the company, since it allows establishing the commercial value of rice varieties in the market.

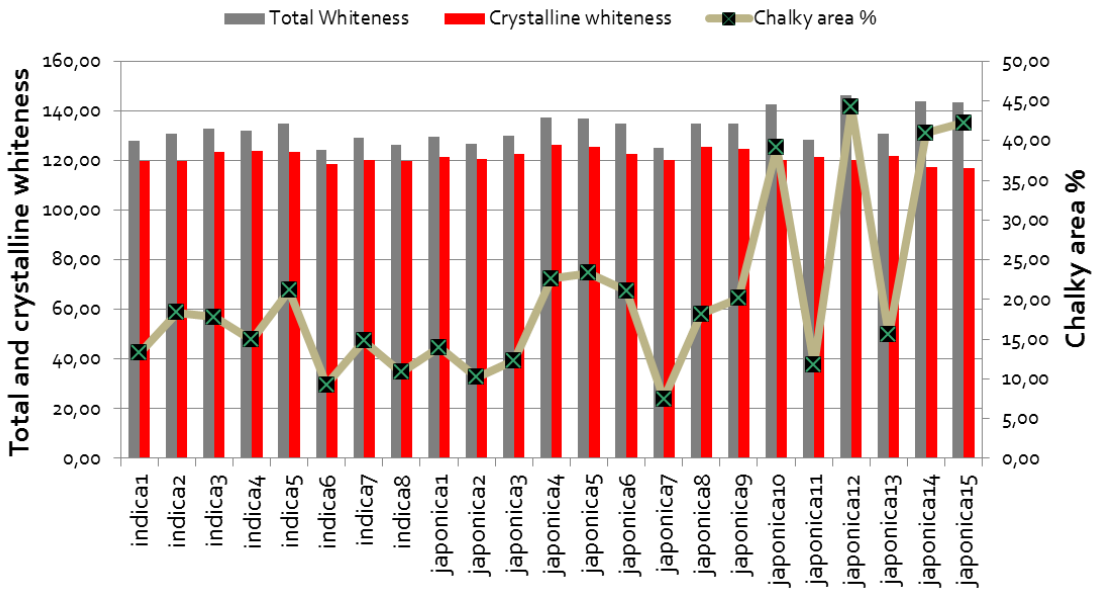

Figure 19 – Grain chalkiness, namely total (grey bars) and crystalline (red bars) whiteness of the grains' endosperm, represented in the left axis, and chalky area % associated, represented in the right axis (brown line).

As shown in Figure 19, the values of total whiteness ranged from 124.37 to 146.42, assigned to *indica6* and *japonica12*, respectively. Relative to vitreous whiteness, the minimum value belonged to *japonica15* and *japonica4* exhibited the maximum value. The higher values of chalky area % were attributed, mainly, to *japonica* sub-species, being 44.21 % (*japonica12*) the maximum value. In general, *indica* sub-species presented lower values of chalky area % compared to *japonica* sub-species. However, the minimum value belonged to *japonica7* (7.50 %), a *carolino* rice. Therefore, *japonica7* possesses a better starch organization compared to *japonica12*, according to the information mentioned before.

Kett value is also a measure of rice grains whiteness achieved after the milling process. As already explained, milling process consists in removing the bran layer of the rice grain, until it achieves the white colour, typical of the most consumed rice kernels. Thus, the degree of milling will be connected with whiteness and translucency level, being an important quality factor to the rice industry. Figure 20 represents the results relative to kett values for all 23 milled samples.

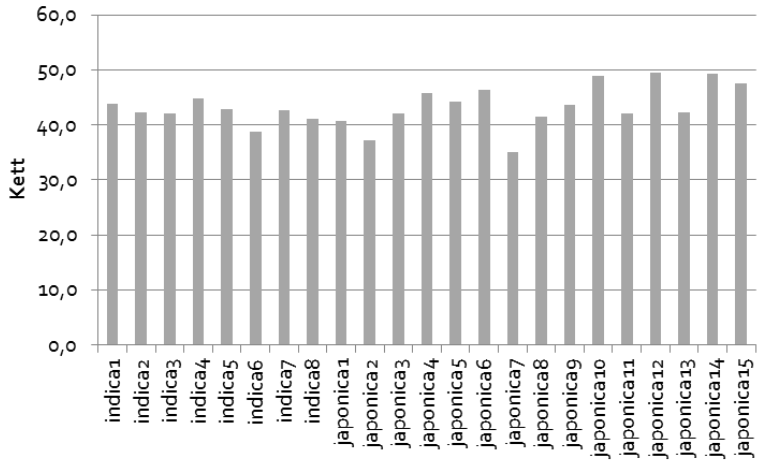

Figure 20 – Kett values for all 23 milled rice samples.

Japonica7 possessed the lower kett value (35.0) while *japonica12* presented the highest value (49.4). These results seemed to suggest a relation with chalky area % results. Also, in general, the highest kett values belong to *japonica* varieties, suggesting a higher degree of milling applied by the company.

Actually, robust correlations were found between some whiteness criteria, as attested by Figure 21. The linear regression lines traced between total whiteness and both chalky area % (Figure 21a)) and kett value (Figure 21b)) show positive and strong correlations (r = 0.97 and r = 0.88, respectively). Chalky area % and kett values were also compared, being strongly correlated (r = 0.86) in the positive direction (Figure 22). These results and correlations also were found by Soares (2014) [101].

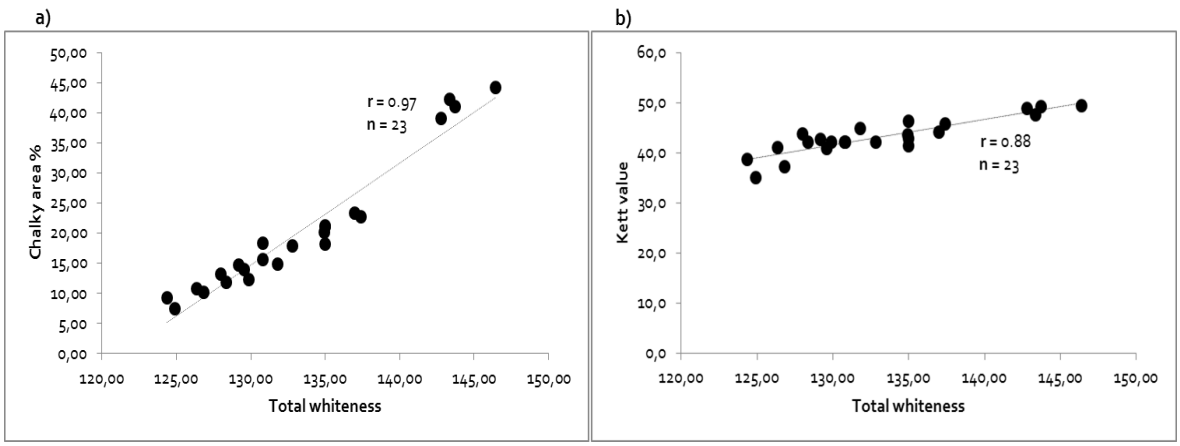

Figure 21 – Positive correlations between both chalky area % **(a)** and kett value **(b)** and total whiteness for all 23 milled samples.

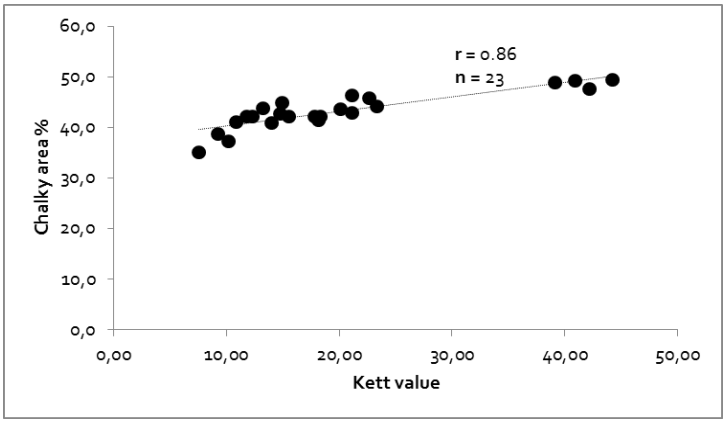

Figure 22 – Positive correlation between chalky area % and kett value for all 23 rice samples.

The analysis of physical parameters of rice grains is a useful tool used by Novarroz, Produtos Alimentares S.A. since it allows to quickly evaluate the quality of rice varieties.

The presentation and interpretation of the remaining parameters was based on the same approach used in this point. However, from now on, the correlation results of milled rice samples will be presented and discussed in point 5.1.8 in order to study all the parameters together. The correlations related to physical analysis were presented here to exemplify the approach used to present and discuss the results of this thesis.

5.1.2 Pasting properties of milled rice samples

The eating and cooking quality of rice is also indicated as one of the most important traits affecting consumer acceptability. The acquisition and analysis of pasting properties through RVA is one of the well-established physicochemical methods to assess and predict the rice cooking and eating quality [114]. Pasting of starch occurs after gelatinization involving granular swelling, exudation of molecular components from the granule and eventually the disruption of the granules [115].

From RVA tests some parameters used to describe pasting process are obtained. These parameters are illustrated in Figure 23. The RVA curve of Figure 23 illustrates pasting process occurring in *indica7*. The analysis of represented parameters allows the interpretation of the modifications that are happening in the starch granules.

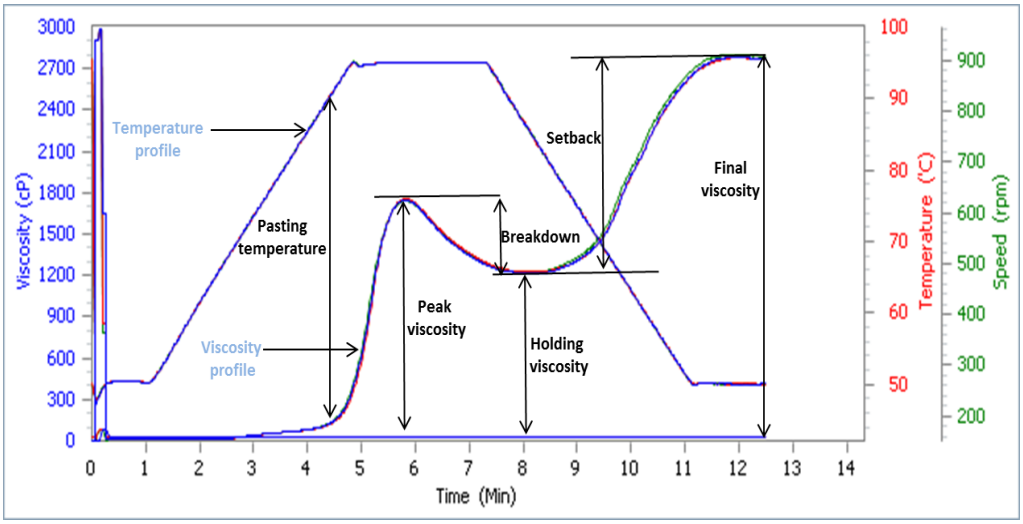

Figure 23 – Complete RVA curve from *indica7*, in triplicate, showing the main parameters used to describe the pasting process.

During hydration, the water binds to starch granules and other components, such as proteins. If temperatures are less than 50 °C, the swelling of the starch grains occurs but it is minimal and reversible. When the mixture is heated, the starch grains begin to swell, occurring gelatinization process with loss of orderly structure leading to irreversible swelling of starch granules [97]. In the course of the process, the granules absorb and bind water more as they swell, reducing the available water and causing collisions between granules, thus beginning the pasting phenomenon, which is visualized through the sudden increase in viscosity during the heating phase of the test (Figure 23). The temperature at which the mentioned phenomenon starts is called pasting temperature (Figure 23) and, in practice, gives an indication of the minimum required temperature to cook a sample. If nothing else was happening, the viscosity would continue to increase until all starch granules reach a maximum size. Then, when the rate of swelling is equal to the rate of breakdown of the granules, the maximum viscosity is achieved, being illustrated by peak viscosity in Figure 23. Thus, peak viscosity provides an indication about the starch capacity to bind water molecules, being correlated with the final quality of the rice [97]. From this heating stage results three parameters: pasting temperature, peak viscosity and time of the peak.

Figure 24 represents all average pasting temperatures resulting from all 23 milled rice samples analysis, acquired through RVA tests. For each sample, three RVA tests were performed and the values of all parameters were presented as mean ± standard deviation. The letters result from statistical analysis, through ANOVA following a Tuckey's test, with 95 % of significance level. Samples with different letters are significantly different.

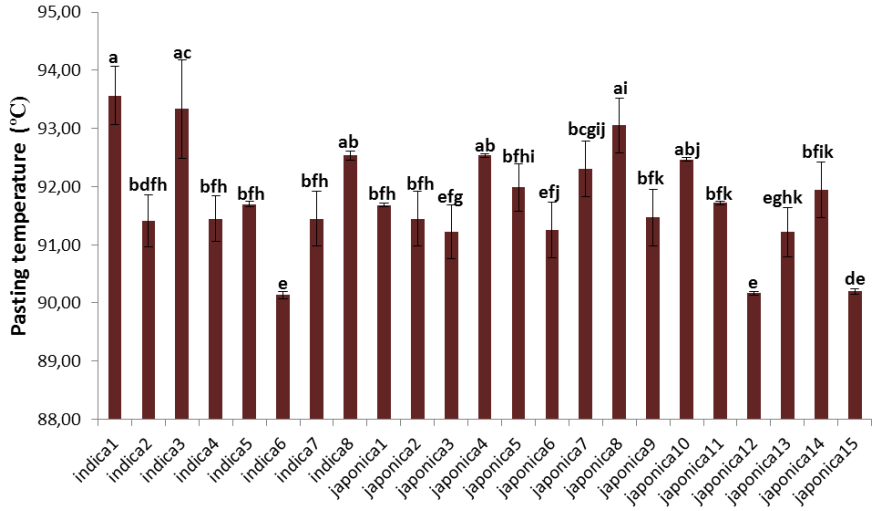

Figure 24 – Average pasting temperature (°C) for each milled rice sample, acquired through RVA tests.

According to Figure 24, *indica1* and *indica3* possess the highest values of pasting temperatures, being similar to *indica8*, *japonica4*, *japonica8* and *japonica10*. Thus, for these samples, pasting process requires a higher temperature to begin. *Indica6*, *japonica12* and *japonica15* presented the lowest pasting temperature values. Lin *et al.* (2010) [116] suggested that higher pasting temperatures may be associated with higher amylose contents: the bonding force between amylose molecules is strong due to hydrogen bonds so pasting of starch with high amylose content is more difficult than that of starch with low amylose content, requiring higher temperatures. However, this parameter doesn't allow us to distinguish the samples since, in general, the values are very close, that being confirmed by statistical analysis. Therefore, this criterion can't be analysed individually. By the way, a specific value obtained for one of the parameters doesn't mean the same thing for different samples. It's necessary to take all factors and parameters into consideration when comparing samples [97].

Another important factor is the peak viscosity, represented in Figure 25. Peak viscosity ranged from 887.33 to 2155.67 centiPoises (cP). In general, the values of peak viscosity were lower in *indica* varieties. *Indica3* and *indica8* presented the lowest values while *japonica6*, 12 and 15 presented the highest values. Rice varieties with lower amylose content have been associated with higher peak viscosity values [116]. As mentioned before, *indica* varieties present higher amylose contents (also determined in this work, in point 5.1.5) compared to *japonica* which may explain the results of peak viscosity.

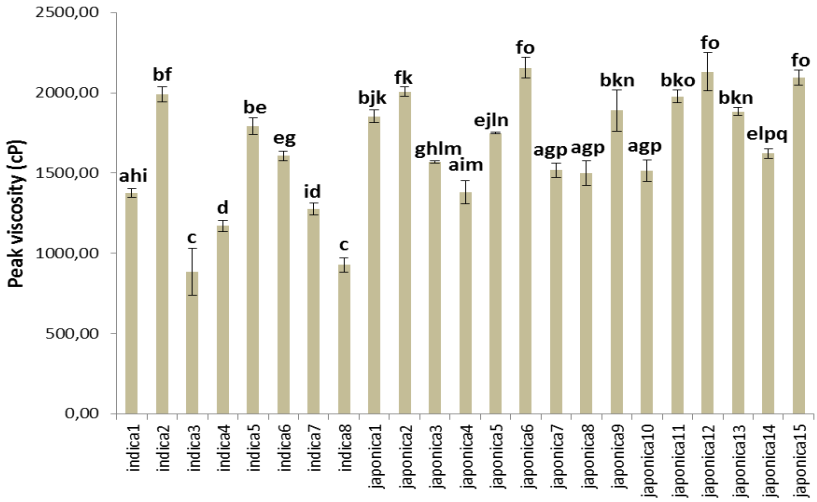

Figure 25 - Peak viscosity (centiPoises) cP for each milled rice sample, acquired through RVA tests.

Figure 26 highlights the differences in RVA pasting curves of *indica* and *japonica* varieties. Figure 26a) represents the pasting curve belonging to *indica3* and Figure 26b)

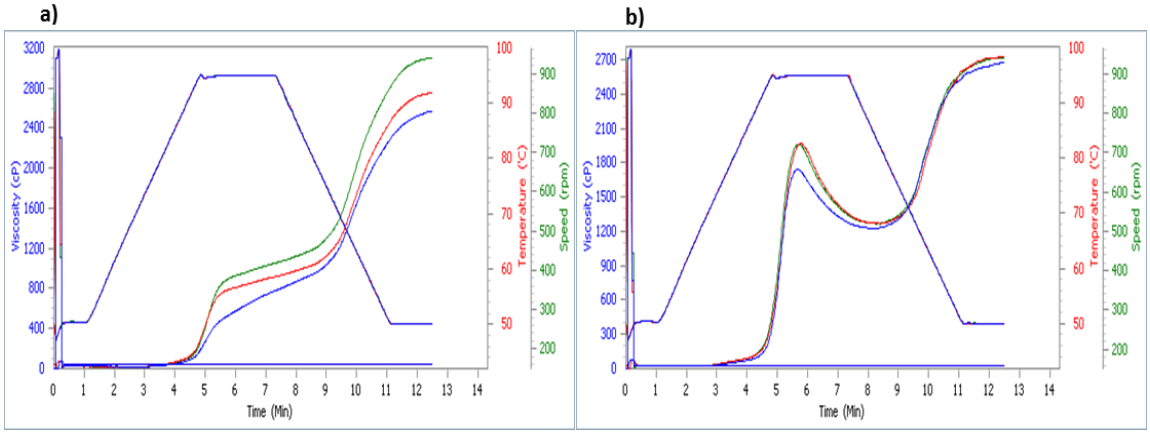

Figure 26 – RVA pasting curves of *indica3* **(a)** and *japonica9* **(b)**, in triplicate.

illustrates the RVA curve of *japonica9*, attesting the significant differences observed in Figure 25.

Time of the peak is the time, in minutes, corresponding to the maximum viscosity achieved. Figure 27 focuses the time, in minutes, corresponding to the maximum viscosity acquired in the heating phase, for all samples. For *japonica* sub-species the maximum viscosity was achieved at around 7 minutes and, curiously, *indica2* and *indica7* also showed the same tendency, while the remaining *indica* varieties taken less one minute to reach their maximum viscosity. However, this pasting property isn't so relevant since it isn't very selective, given the results of statistical analysis.

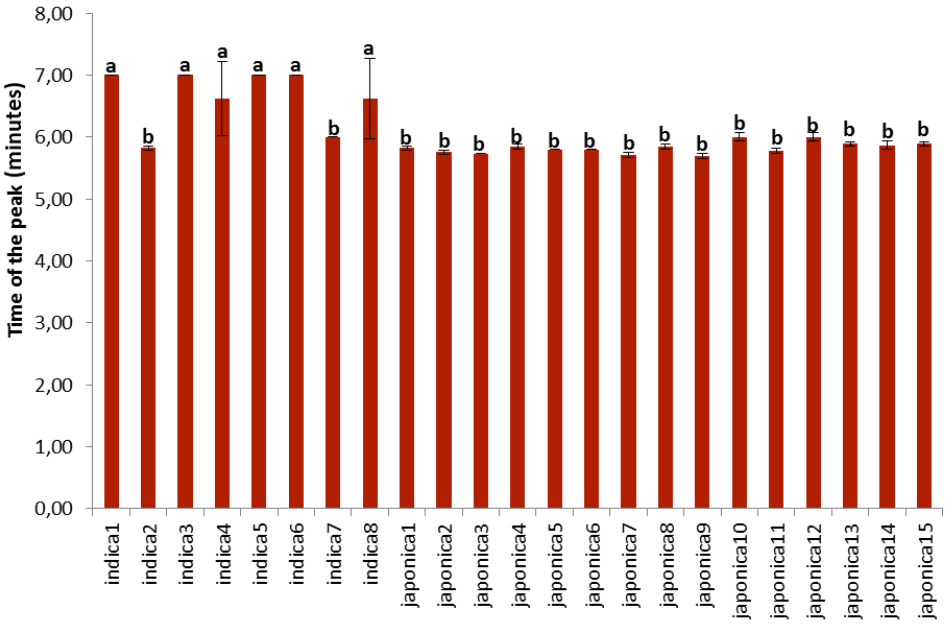

Figure 27 – Time of the peak (minutes) for each milled rice sample, acquired through RVA tests.

After maximum viscosity is reached, the disruption of the granules is observed as a reduction (or breakdown) in viscosity. In general, this breakdown starts to occur after the maximum temperature is achieved, continuing until the end of the holding stage [97]. However, in some cases, breakdown may start to occur before maximum temperature is reached. The rate of decrease is initially rapid, but after a short time the curve starts to flatten out. With almost all starches, about 4 minutes is usually enough for a constant rate of decrease to be achieved. At the end of the holding stage, the holding viscosity is

achieved, that being the minimum viscosity of the formed paste. The breakdown is another pasting parameter that expresses the starch's stability at high temperatures, pointing the rate of the disruption of starch granules. It results from the difference between peak viscosity and holding viscosity [97]. Figure 28 expresses the results of holding viscosity values for all 23 milled rice samples.

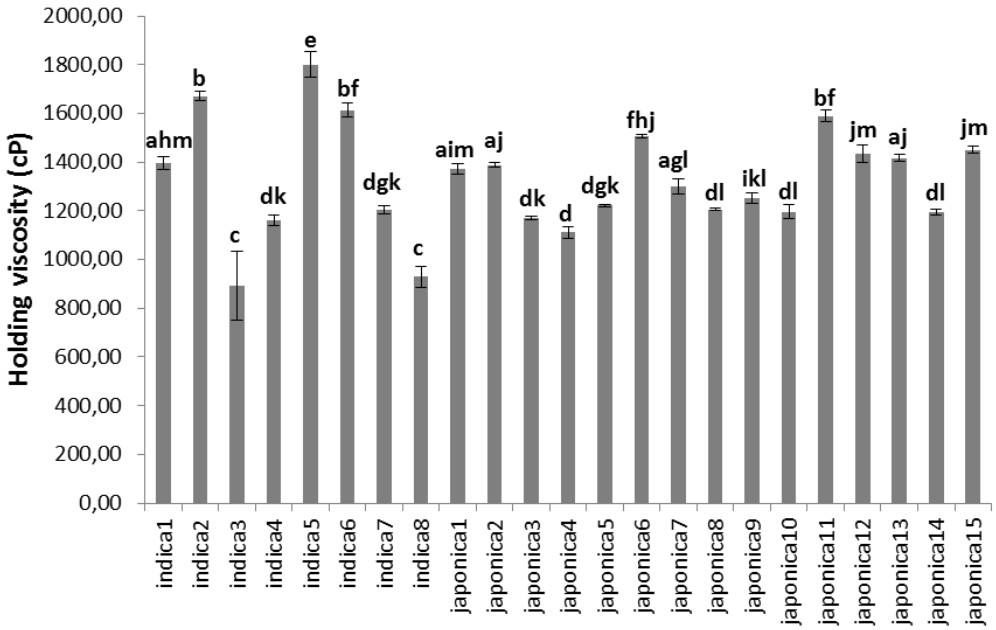

Figure 28 – Holding viscosity (cP) at the end of holding stage for each milled rice sample, acquired through RVA tests.

Holding viscosities ranged from 893.67 to 1800 cP. The lower values were achieved for *indica3* and *indica8*, being statistically similar. The higher values were found in *indica2*, *indica5*, *indica6* and *japonica11*. Thus, it's possible to suggest that lower values of holding viscosity might be correlated with higher levels of granule disruption. However, this suggestion needs to be done with prudence, since the breakdown is also calculated taking peak viscosity in account.

Figure 29 presents the results related to breakdown, calculated through the difference between peak viscosity and holding viscosity for all 23 milled samples, with the respective statistical analysis. Breakdown values of *indica* rice starch were lower than those of *japonica* rice starch. Indeed, the breakdown values of all *indica* samples (except *indica2* and *indica7*) were significantly different compared to all *japonica* samples. The

analysis of the results allows the visualization of a marked profile for *indica* and *japonica* rice samples since, in general, the breakdown values of *indica* are extremely low compared to *japonica* rice. The negative values of breakdown for *indica* samples displayed the tendency for peak viscosity being lower than holding viscosity, being this tendency the main responsible to define the format of RVA curve and identify the rice sub-specie (*indica* or *japonica*) under studies. However, as it is possible to see in Figure 29, some *indica* varieties can possess breakdown values similar to some *japonica* samples. Thus, as was already mentioned, it's important to conjugate all the other parameters to establish a definitive and conclusive analysis.

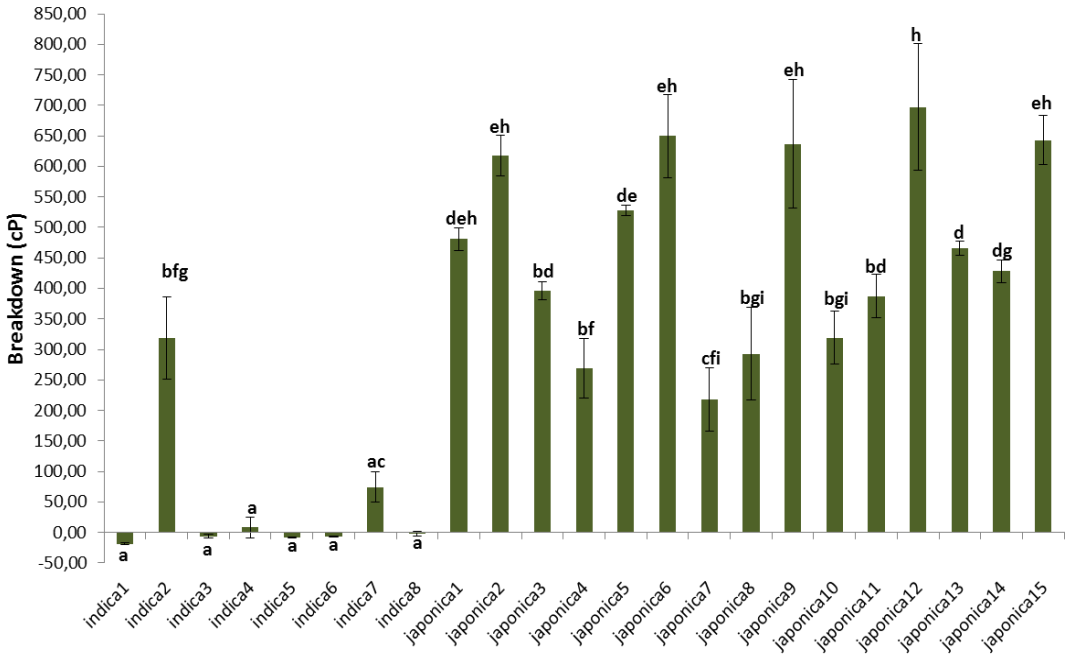

Figure 29 – Breakdown viscosity (cP) for each milled rice samples, acquired through the difference between peak viscosity and holding viscosity.

After the holding stage, temperature begins to decrease leading to an increase in viscosity. As the temperature falls, the glucan chains from starch entangle and reorganize with each other creating a new starch network (retrogradation process) [97]. This phenomenon leads to the formation of a gel which has a great contribution to the increase in viscosity as the temperature drops. In waxy starches, the reorganization of starch is limited by the chains being relatively short and by the branch points preventing associations of the type that can occur with the longer unbranched amylose molecules. In starches with higher amylose contents, the degree of reorganization is higher, leading to

a greater increase in viscosity in cooling state [97]. At the end of the test, final viscosity will be achieved and setback can be calculated through the difference between final viscosity and holding viscosity, being associated to the re-association of starch molecules during cooling, or, in other words, the retrogradation process. Setback has been correlated with the final texture of the rice [97]. The final viscosities for all samples in study are exposed in Figure 30, in cP, with the respective statistical analysis.

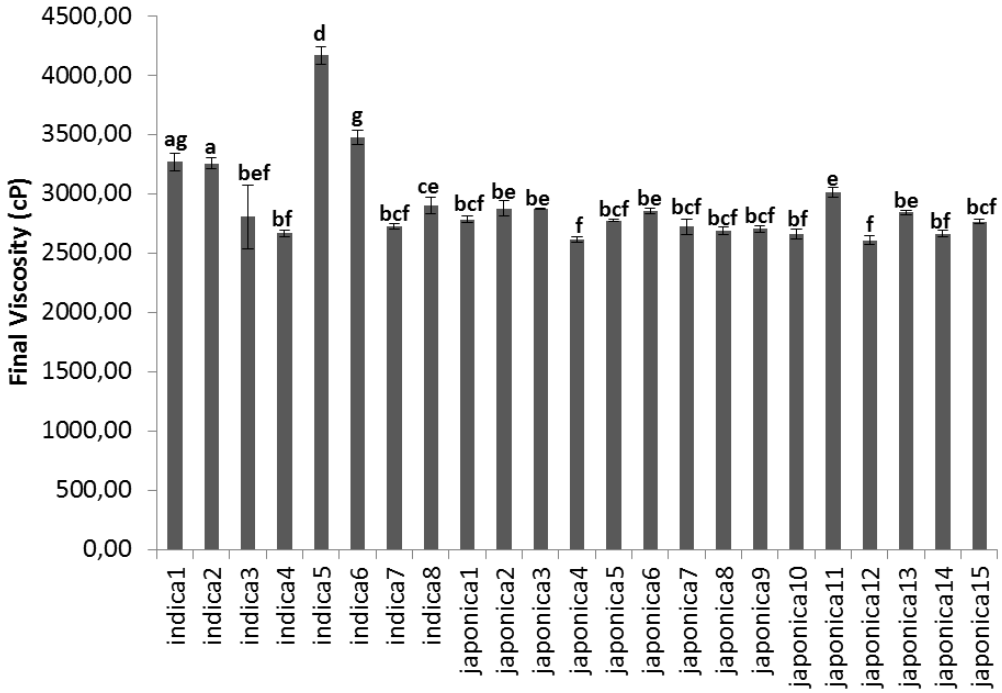

Figure 30 – Final viscosity (cP) of all 23 milled rice samples, acquired through RVA tests.

Final viscosity values ranged from 2608.0 and 4169.00 cP. *Indica5* and *indica6*, clearly, presented the highest values of final viscosity, followed by *indica1* and *indica2*. These results corroborate the literature reports since the highest values are associated to samples with higher amylose content. However, four *indica* varieties don't follow this tendency, revealing a similar behaviour to *japonica* samples. In spite of final viscosity being the pasting parameter commonly used to assess the quality of a particular sample since it reveals the capacity of that sample to form a gel or a viscous pasta after cooked and cooled [97], it can't be used alone to analyse the quality of rice, as mentioned above. Setback is also important to complement the analysis of the behaviour of the sample's starch after the cooling stage of the test. Thus, setback values, obtained from the

difference between final viscosity and holding viscosity for each sample, are represented in Figure 31.

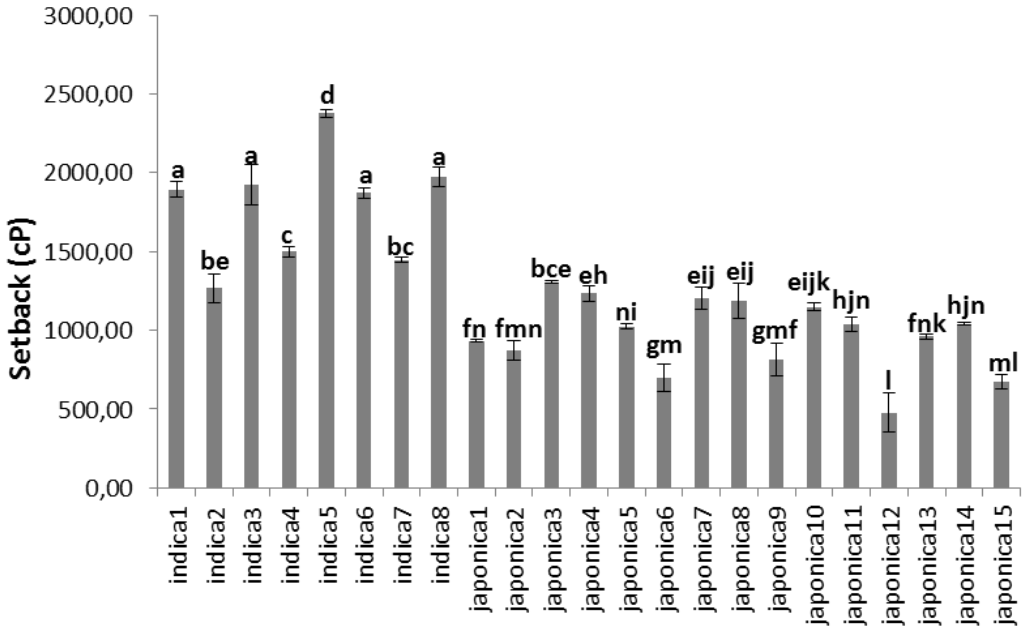

Figure 31 – Setback viscosity (cP) for each milled rice samples, calculated through the difference between final viscosity and holding viscosity.

A quick analysis of the histogram of Figure 31 allows the conclusion that the highest values of setback belong to *indica* samples. Only *indica2* and *indica7* present a similar behaviour to some *japonica* samples but, in general, all *indica* samples show higher values, being significantly different from the majority of *japonica* samples. Higher values of setback are, clearly, correlated with final viscosity values. Thus, samples with highest values of final viscosity will show higher setback values. In point 5.1.8 the correlations between all analysed parameters are presented, opening up the possibility to verify the correlations between all pasting properties and other characterization parameters analysed for milled rice samples. From the analysis of Figure 31 it is possible to suggest that *indica* samples suffered a greater degree of re-organization of starch molecules.

The results found for pasting properties were consistent with the studies developed by Lin *et al.* (2010) [116]. These researchers also discovered lowest values of peak viscosity and breakdown viscosity in *indica* rice starches compared to *japonica* rice starches. The tendencies observed in this study for setback and final viscosity in *indica*

and *japonica* samples were also found in Lin's work [116]. Another study carried out by Zheng *et al.* (2012) [113] showed the same trends of pasting parameters found in this work.

The correlations of physical, pasting and chemical parameters between all milled samples are discussed in point 5.1.8.

5.1.2 Moisture content

This point comprises the results related to moisture content of milled, brown and commercial rice samples. Moisture % is of great importance to present the results of all chemical and nutritional parameters in a dry matter basis. Furthermore, moisture content has been described as an important influence on rice quality during storage. Under practical storage conditions, moisture is the most responsible factor in controlling the deterioration rate [117]. According to Decreto-Lei n.º 63/2000 [94], the moisture content of rice cannot exceed 14%. Figure 32 represents moisture % of milled, brown and commercial rice samples.

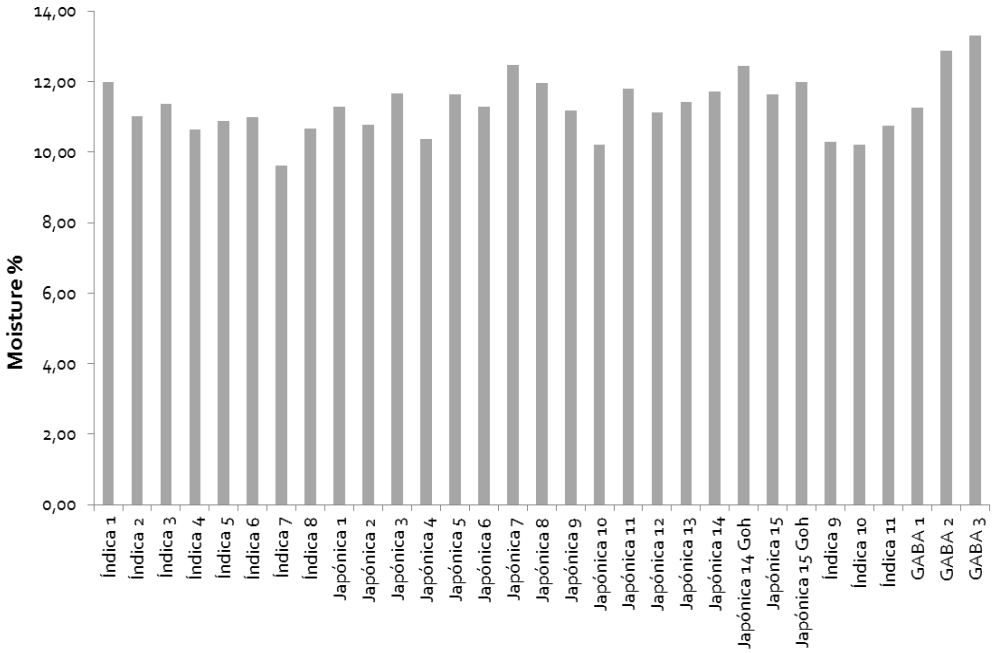

Figure 32 – Moisture content (%) of milled, brown and commercial rice samples.

The moisture content of all rice samples was in accordance with the Portuguese legislation, ranged from 9.61 to 13.31 %. *GABA2* and *GABA3*, two commercial samples, presented the highest values. *Indica7* showed the lowest value. The samples commercialized by Novarroz which showed the highest moisture contents were *Japonica7* and *japonica14 G0h* (brown rice).

5.1.3 Total starch content

Starch is the most abundant constituent of rice. Total starch of a rice grain encompasses a digestible portion, represented by amylose and amylopectin polymers, and a small non-digestible portion (resistant starch) [9]. This point focuses on the results concerning total starch percentage in the grain of milled, brown and commercial rice samples. Figure 33 comprises these results, in dry matter, with respective statistical analysis.

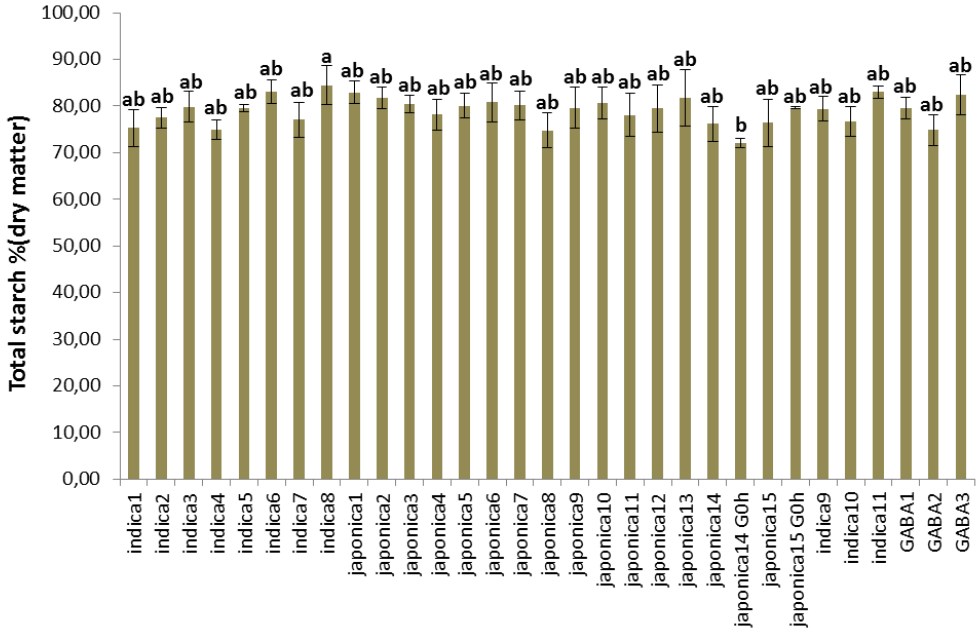

Figure 33 – Total starch % in the grain, in dry matter, of milled, brown and commercial rice samples and respective statistical analysis.

The content of total starch ranged from 72.04 to 84.32 %, in dry matter. *Indica8* was the rice variety with the greater starch content. On the other hand, *japonica14 G0h* presented the lowest value of starch content, being significantly different of *indica8*,

according to Figure 33. Except *japonica14 G0h* and *indica8*, all other rice varieties didn't present significant differences between them, being statistically similar. Even starch content of commercial rice samples (*indica9*, *indica10*, *indica11*, *GABA1*, *GABA2* and *GABA3*) was similar to that of company samples. In fact, the results were consistent with literature reports [2, 10, 118], also presented in a dry matter basis. The relevant differences are related to the proportion between different starch types (amylose, amylopectin and resistant starch) which confer distinct properties between rice varieties [8]. Thus, it is normal the non-existence of many significant differences relatively to total starch content between samples since, in general, its content is similar for all non-germinated rice samples.

5.1.4 Resistant starch content

Resistant starch, known to escape hydrolysis in the small intestine, has been implicated as having a great impact on the rate of starch digestion, improving glycemic and insulinemic responses [9]. Some investigators suggested that proteins and lipids may be the main responsible for the resistant starch type I (physically inaccessible starch) formation since they interact by binding to amylose and/or amylopectin polymers [119]. The content of resistant starch in rice grains was determined for all milled, brown and commercial samples and is illustrated in Figure 34. The results are presented in dry mater, through a histogram with the respective statistical analysis ($p < 0.05$).

The average content of resistant starch, per rice grain, ranged from 0.44 to 7.18 %. The lowest values belonged to *indica9*, *indica10* and *GABA1* (0.67, 0.66 and 0.44 %, respectively). According to the supplier's indications on the package, *indica9* and *indica10* rice grains were previously parboiled. Parboiling basically involves soaking of paddy rice to sufficiently moisten the starchy endosperm followed by boiling it with heated steam under or without added pressure to gelatinize the starch and then drying it. Generally, this process is applied in order to decrease kernel damage during milling proceedings. Parboiling has been associated to with the increase of rice starch digestibility, reducing significantly resistant starch content [120]. Thus, results for *indica9* and *indica10* are

explained. Also *GABA1*, another commercial sample, presented a very low content of resistant starch, probably due to the fact of being a mixture of different types of rice grains in different proportions (germinated, wild and red rice grains). The analysis of *GABA1* was performed considering a homogeneous amount of all types of rice grains. However, the differences of resistant starch content in the different rice grains have contributed to lowering the average resistant starch content in *GABA1*. No reports were found relative to specific resistant starch content in wild and red rice grains.

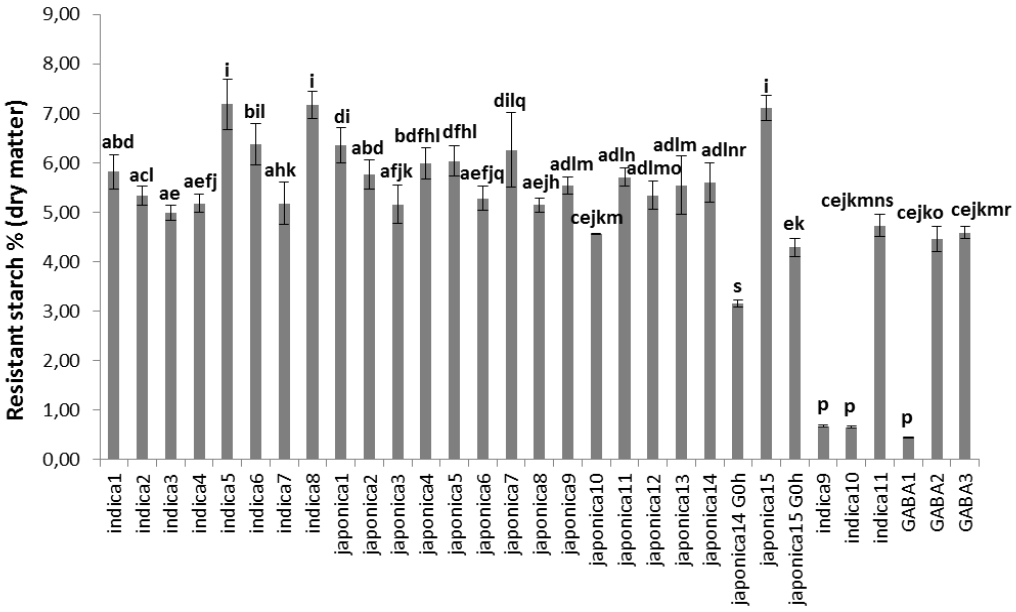

Figure 34 – Resistant starch content (%) in the grain, in dry matter basis, for milled, brown and commercial samples with the respective statistical analysis ($p < 0.05$).

Indica5 and *indica8* presented the highest values of resistant starch content of the *indica* varieties (7.18 and 7.17 %), being statistically similar. *Indica6* revealed a resistant starch content of 6.37 % per rice grain, showing statistical similarities with *indica5* and *indica8*. The remaining *indica* sub-species exhibited an amount of resistant starch under 6 %, with no statistical differences between them. *Japonica15* presented the highest value of *japonica* samples, being statistical analogous to *indica5* and *indica8*. Also resistant starch contents of *japonica1* and *japonica7* presented the same trends of *japonica15*. Brown rice samples presented lower values of resistant starch content, compared to the correspondent milled samples.

In general, the results of resistant starch content are in accordance with literature reports [9, 121]. Furthermore, the calculations to assess what amount of total starch (determined in point 5.1.4) corresponded to resistant starch were done. These results are presented in Figure 35 with the respective statistical analysis. A rapid analysis allows concluding that there aren't any relevant differences in graph of Figure 35 comparing to Figure 34, keeping the trends and differences between samples, even with slight differences in total starch content.

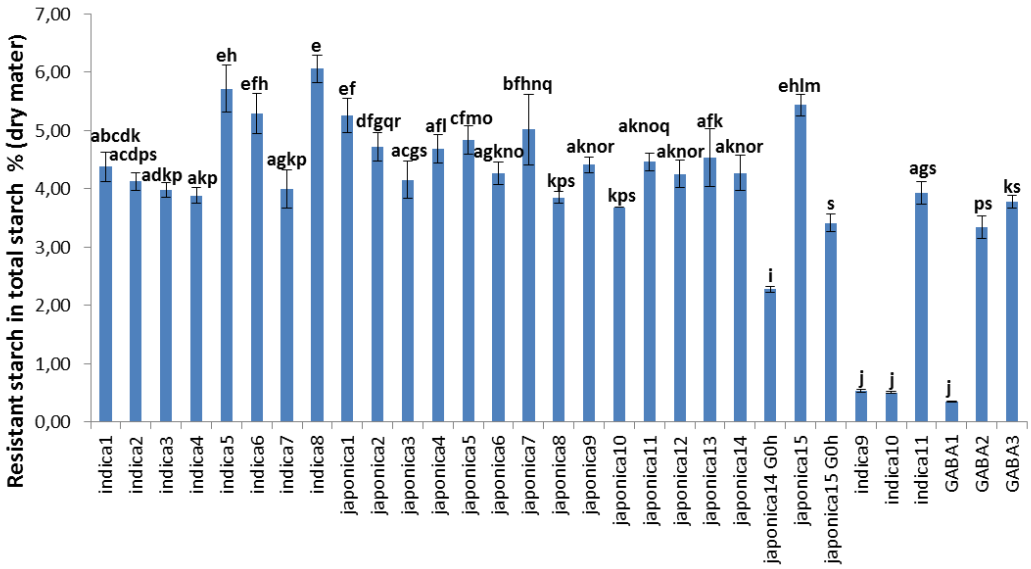

Figure 35 – Resistant starch in total starch (%), in dry matter, with the respective statistical analysis ($p < 0.05$).

5.1.5 Amylose content

Cooked rice texture and rice starch functional properties are related with each other being primarily impacted by amylose content [24]. Thus, the determination of amylose content, complemented with pasting properties analysis, is of great importance to Novarroz, Produtos Alimentares S.A. since it allows to predict the final texture of cooked rice, directing different varieties according to customer's purposes. Previous reports found great variations in amylose/amylopectin ratio in rice grains allowing their classification as waxy (1 – 2% amylose), very low amylose content (2 – 12%), low amylose content (12 -20%), intermediate amylose content (20 – 25%) and high amylose content (25 -33%) [122]. Frei *et al.* (2003) [123] indicates that starchy food with higher amylose

contents is associated with lower blood glucose levels compared to those with lower levels of this polymer. Figure 36 represents the results related to amylose content, per rice grain, obtained through iodine procedure, in dry matter for milled, brown and commercial rice samples and the respective statistical analysis ($p < 0.05$).

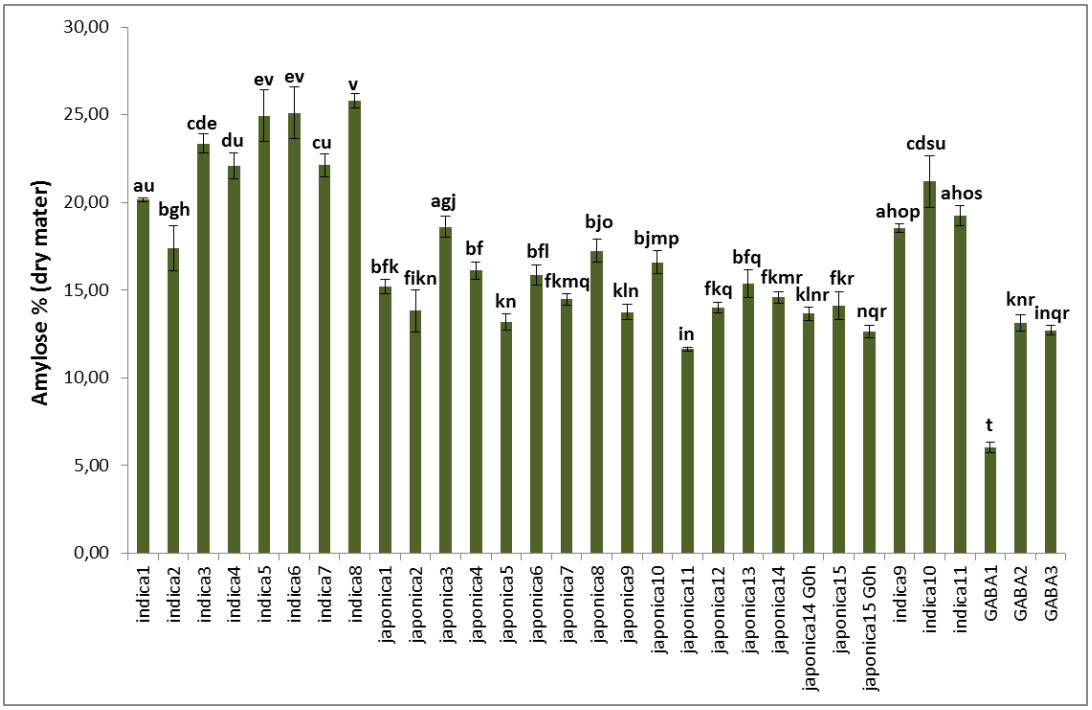

Figure 36 – Amylose content (%), per rice grain, in dry matter, with respective statistical analysis ($p < 0.05$).

The analysis of Figure 36 allows, immediately, the association of the greater amylose contents to indica samples, as expected. These associations are clearly supported by statistical analysis which indicates significant differences between *japonica* and *indica* samples. The values of amylose content varied from 6.04 (*GABA1*) and 25.79 % (*indica8*). *Indica5*, *indica6* and *indica8* have the higher amylose contents, being statistically similar between them. *Indica2* was the *indica* sub-specie that presented the lowest amylose amount. The amylose content of *japonica* rice samples ranged from 11.64 (*japonica11*) to 18.61% (*japonica3*). *Japonica14 G0h* and *japonica15 G0h* showed an amylose content similar to that of their milled grains (*japonica14* and *japonica15*, respectively). The commercial samples *indica9*, *indica10* and *indica11* presented similarities with indica rice samples of Novarroz. On the other hand, *GABA2* and *GABA3* presented lower values of amylose content, being statistically similar to *japonica* varieties

and completely different of *indica* samples. *GABA1* exhibited an amylose content of 6.04%. Naturally, red and wild rice grains possess lower amylose contents. The germinated rice grains also possess lower amylose amounts due to the germination process. Thus, it is possible to explain the amylose content of *GABA1*.

All samples studied were classified having its amylose content into account, according to the previously established criteria [122]. This classification is shown in Table 5 for milled, brown and commercial samples.

Table 5 – Classification of rice amylose content of milled, brown and commercial analysed samples.

Classification	Samples
Waxy (1 - 2% amylose)	None
Very low amylose content (2 - 12%)	*japonica11* *GABA1*
Low amylose content (12 - 20%)	*indica2* *Indica9* *indica12* All *japonica* varieties (excluding *japonica11*) *GABA2* *GABA3*
Intermediate amylose content (20 - 25%)	*indica1* *indica3* *indica4* *indica5* *indica7* *indica10*
High amylose content (25 - 33%)	*indica6* *indica8*

The determination of amylose content allowed concluding that the main *indica* samples possess relatively high amylose content compared to that of *japonica* varieties, corroborating previous reports [11, 15]. In spite of being *indica* sub-species, *indica2*, *indica9* and *indica12* were classified as samples with very low amylose content, showing trends of behaviour similar to that of the *japonica* varieties. It should be remembered

that *indica2* also exhibited some similar tendencies to japonica samples with respect to the pasting properties that could be correlated with their amylose content. This and other relations between analysed parameters are discussed in point 5.1.8 related to correlations analysis.

As for resistant starch, the content of amylose in total starch was also calculated, presenting, as expected, lower values compared to amylose content achieved per rice grain. However, the same tendencies and differences were verified, showed in Figure 37, through histogram and statistical analysis ($p < 0.05$).

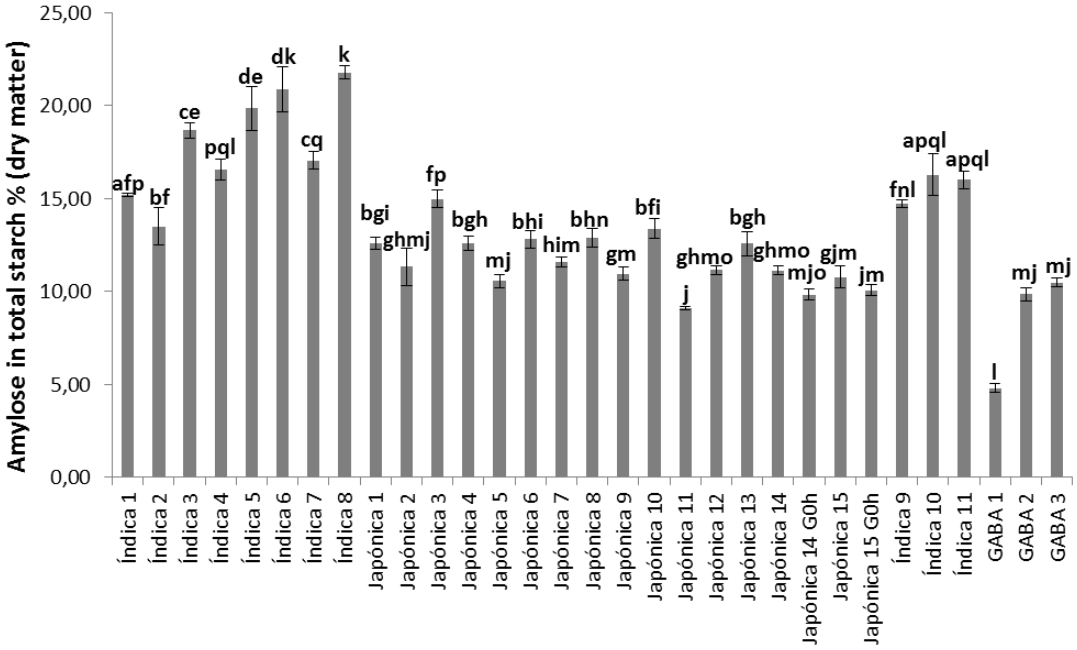

Figure 37 – Amylose in total starch (%), in dry matter, with the respective statistical analysis ($p < 0.05$).

5.1.6 Glycemic index

As mentioned in point 2.2, rice, namely white rice, is known for its relatively high glycemic index, being associated with a significantly increased risk of type 2 diabetes mellitus development [64]. One of the main concerns of Novarroz has been the demand for rice varieties possessing low glycemic index values in order to respond to the needs of type 2 diabetic consumers since low glycemic foods are reported to be able to produce

low glucose and insulin responses, reducing the need of medication and diabetic complications contributing to a better life quality of the patients [62].

Starch content, namely amylose, has been implicated as one of the main factors influencing the rate of starch hydrolysis. High amylose rice was reported exhibiting lower glycemic index values than low amylose varieties [64]. Thus, in vitro glycemic studies of rice varieties were performed in order to achieve the rate of starch digestion, through the hydrolysis index, to estimate glycemic index values. The hydrolysis curves of all milled, brown, commercial samples and white bread (reference) were constructed and starch hydrolysis index of total starch (determined in 5.1.3) was determined. Figure 38 represents the rate of total starch digestion of white bread, used as reference. The hydrolysis index obtained for white bread was 100 corroborating Goñi *et al.* (1997) [2] results.

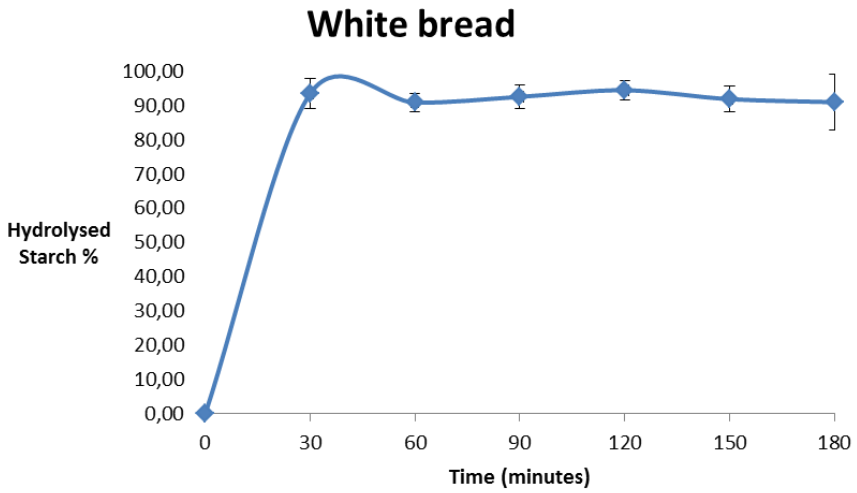

Figure 38 - Hydrolysis curve relative to total starch rate digestion of white bread, the reference used.

The hydrolysis curves for rice samples were also traced and hydrolysis indexes obtained through the calculation of the area under the curve (AUC). Figure 39 shows the hydrolysis curves of *indica6*, the Novarroz rice sample with the lowest hydrolysis index (66.83), and *japonica8*, the Novarroz sample with the highest hydrolysis index (92.98). The analysis of Figure 39 allows verifying that total starch hydrolysis of *indica6* is slower over time compared to *japonica8*. Therefore, samples whose starch hydrolysis is higher would present higher values of glycemic index since these two parameters are correlated

through Goñi's model (GI = 39.71 + (0.549*HI)) [2]. The accurate inspection of hydrolysis curves and hydrolysis indexes of white bread (Figure 38) and rice samples (Figure 39) allows corroborating the reports that indicate white bread as a higher GI food comparatively to rice. White bread presented a glycemic index of 100 as in Goñi's studies [2].

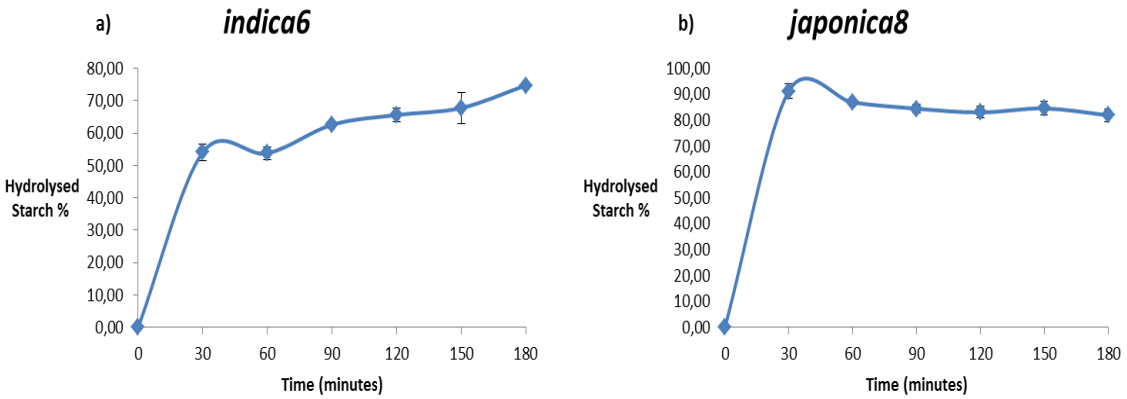

Figure 39 – Hydrolysis curves relative to total starch rate digestion of *indica6* **(a)** and *japonica8* **(b)**, two rice samples of Novarroz.

The glycemic indexes of milled, brown and commercial rice samples ranged from 70.92 to 90.75 as exhibited by a histogram with respective statistical analysis in Figure 40.

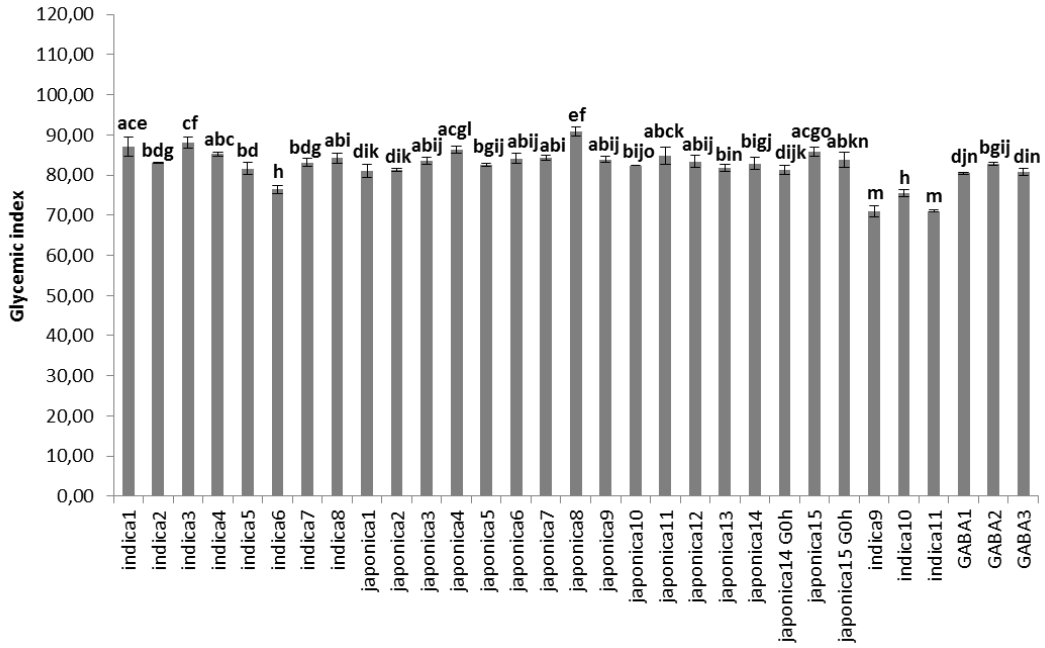

Figure 40 – Glycemic index of milled, brown and commercial rice samples with the respective statistical analysis ($p < 0.05$).

The lowest GI values corresponded to *indica9*, *indica10* and *indica11*. As mentioned in point 4.1, these samples are commercial samples indicated as good rice for diabetic people since they possess low GI values, according to the supplier. In fact, these samples presented the lowest values, however, the GI values obtained for these samples (70.92, 75.46 and 71.05 for *indica9*, *indica10* and *indica11*, respectively) are slightly different from those indicated by the supplier. The information printed in the package of these products indicates a GI value of 55 for these three samples. This doesn't mean that the information given by the supplier is false. These results, probably, are connected to the procedures used to achieve the GI values, namely in relation to the reference used. A recent study carried out by Kale *et al.* 2015 [124] used two different references to estimate glycemic index. The starch hydrolysis was also based on Goñi *et al.* procedures using white bread and glucose as references. When the reference was the white bread, the GI values ranged from 77.59 to 83.44 being in accordance with the results obtained in this thesis. However, when the reference was glucose, the GI values ranged from 54.31 to 58.41. The information about the procedures used to estimate glycemic index of the commercial samples by the supplier weren't provided and so it wasn't possible to confirm the hypothesis presented. Yet, the GI values obtained in this thesis were used to compare commercial with Novarroz samples, considering *indica9*, *indica10* and *indica11* as low GI rice in order to assess what sample of Novarroz could be commercialized as rice for diabetics. This was evaluated using statistical analysis through ANOVA, followed by a Tuckey's test with $p < 0.05$. According to Figure 40, *indica6* presented statistical similarities with *indica10*, one of the low GI samples. *Indica6* presented the lower GI value (76.40) and significant differences compared to all other samples of Novarroz. The analysis of amylose content, in point 5.1.5, revealed that *indica6* possesses the second higher value which can be related with the GI value obtained. Besides amylose content, other factors related to this parameter can influence GI values, namely its arrangement in starch granules and physical availability in the rice grains. The analysis of correlations, in point 5.1.8, allows to determinate which parameters are more or less correlated with GI values in milled rice samples.

On the other hand, all other samples of Novarroz presented higher values compared to low GI commercial samples. *Japonica8* presented the highest GI value and was statistically similar to *indica1* and *indica3* meaning the digestion starch of these samples is faster over time. This finding can also open interesting commercial perspectives to Novarroz because, if on the one hand, diabetic people should consume low GI foods, on the other hand, there are consumer groups needing to consume foods with high GI carbohydrates (for example, competition athletes). So, rice varieties with high GI could be advantageous in this context, being the main energy source on the development of high GI rice products.

In point 2.3 it was mentioned that previous studies carried out by Ito *et al.* (2005) [69] revealed that brown rice varieties presented lower GI values compared to their corresponded milled samples. *Japonica14 G0h* (81.26) and *japonica15 G0h* (83.82) presented, effectively, lower GI values compared to *japonica14* (82.83) and *japonica15* (85.86). However, as shown in Figure 40, these differences weren't statistically significant.

These GI studies are extremely important to the food industry since consumers are getting more informed and concerned with health and specific diet orientations. The companies need to be more and more informed being able to respond to all needs. The knowledge about specific GI value of each rice variety allows the opening of new market options reaching a wider range of consumers.

5.1.7 Protein content

The number of people affected with celiac disease (an autoimmune disorder caused by an allergenic reaction to gliadin and glutenin, commonly called as gluten protein, present in wheat, rye and barley proteins) comprises about 1% of the world's population [125]. Therefore, rice is indicated as one of the most appropriated cereal grains for celiac people and production of gluten-free products due to its protein benefits. Thus, the determination of protein content is of extreme importance to Novarroz. The histogram of Figure 41 illustrates the results related to protein content, in dry matter, per rice grain, and the respective statistical analysis with a p-value less than 0.05.

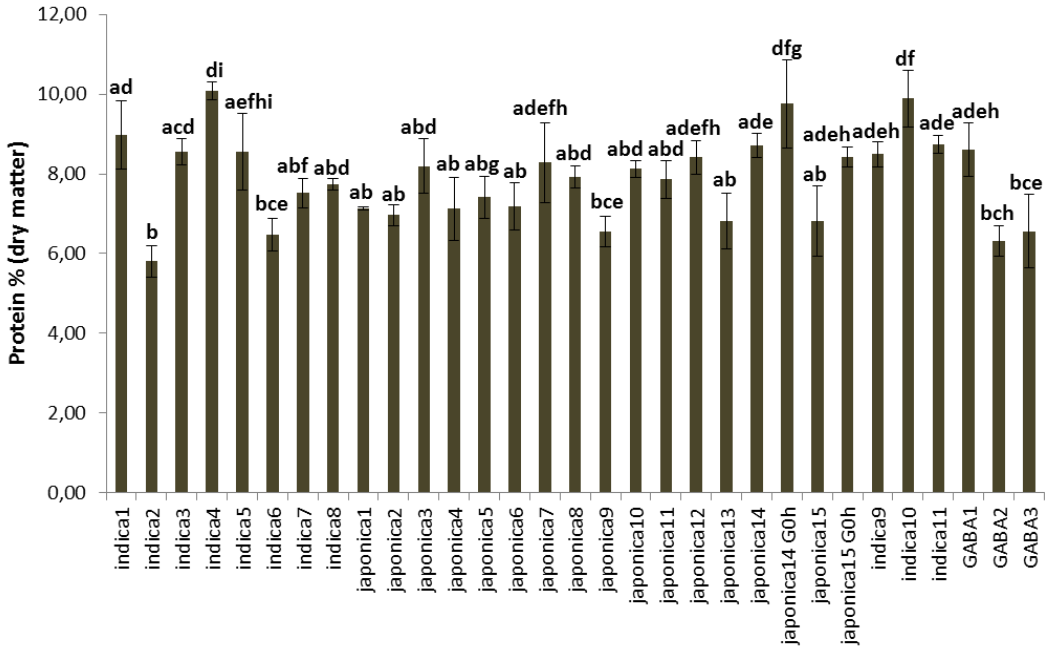

Figure 41 – Protein content (%), in dry matter, of all milled, brown and commercial samples with the respective statistical analysis ($p < 0.05$).

Protein content ranged from 5.81 to 10.08 %, *indica2* possessing the lowest value and *indica4* the highest. *Indica4* was statistically similar to *indica1*, *indica3*, *indica5*, *indica8*, *japonica3*, *japonica7*, *japonica8*, *japonica10*, *japonica11*, *japonica12*, *japonica14*, *japonica14 G0h*, *japonica15 G0h*, *indica9*, *indica10*, *indica11* and *GABA1*. These results are consistent with previous reports [26, 47].

As mentioned in point 2.3, brown rice is known for its higher nutritional value compared to white rice and protein content is also implicated. Literature reports indicate that brown rice has a slightly higher content of protein since it still possesses the extern aleurone layers [26, 40]. In fact, *japonica14 G0h* (9.75%) and *japonica15 G0h* (8.42%) presented higher values of protein content compared to the corresponded milled samples (8.72 % for *japonica14* and 6.81% for *japonica15*). However, as shown in Figure 41 by the letters in the histogram, these differences weren't significant.

5.1.8 Correlations

This point discusses the most relevant Pearson's correlations found between all parameters determined for milled rice samples. Correlations were classified, according to their coefficients (r) as: very weak ($0 < r < 0.19$), weak ($0.2 < r < 0.39$), moderate ($0.40 < r < 0.59$), strong ($0.60 < r < 0.79$) strong and very strong ($0.80 < r < 1.0$). Three correlation tables were achieved and all detailed and complete data can be consulted in Annex D. Table a), in Annex D, represents all the calculated correlations when considered just milled *indica* rice samples; table b) is referent to all correlations only with *japonica* milled samples and table c) shows all correlations related to all milled rice samples.

The analysis of correlation coefficients of all milled rice samples (Annex D – table c) allowed detecting some important correlations. This discussion focuses the correlations of table c, in Annex D. When relevant and necessary, the other two tables are mentioned.

As mentioned in point 5.1.1, length and width parameters were strongly negatively correlated ($r = -0.74$, $p < 0.05$). Thus, is natural, the strong negative correlation observed between length-to-width ratio and width ($r = -0.95$, $p < 0.05$). In terms of whiteness, chalky area % was strongly positively correlated with kett ($r = 0.86$, $p < 0.05$) and total whiteness ($r = 0.88$, $p < 0.05$).

Pasting parameters were found closed correlated one each other. Final viscosity was strongly correlated with holding viscosity and setback ($r = -0.67$ and $r = -0.66$, respectively, $p < 0.05$). Breakdown values shown a moderate correlation with final viscosity ($r = -0.44$, $p < 0.05$). Peak viscosity and breakdown were strongly negatively correlated with setback ($r = -0.68$ and $r = -0.93$, respectively) while breakdown and holding viscosities were found strongly positive correlated with peak viscosity ($r = 0.80$ and $r = 0.74$, respectively). These results confirmed prior studies about pasting properties of rice [114, 116].

Amylose content was strongly negatively correlated with breakdown ($r = -0.85$, $p < 0.05$) and peak viscosity ($r = -0.64$, $p < 0.05$). These correlations were consistent with a previous study developed by Lin *et al.* (2010) [116]. Final viscosity and setback were found positively correlated with amylose content ($r = 0.51$ and $r = 0.88$, respectively) corroborating some literature reports [114, 116]. Amylose was found negatively (r = -

0.68, $p < 0.05$) correlated with width (mm). On the other hand, length and length-to-width ratio were found positively correlated with amylose content ($r = 0.66$ and $r = 0.70$, respectively). A very weak negative correlation ($r = - 0.08$, $p < 0.05$) was found between amylose content and glycemic index. However, when considered only the correlations of *indica* milled rice samples, a higher negative correlation was found between amylose and glycemic index ($r= - 0.38$, $p < 0.05$). These findings are coherent with some articles correlating higher amylose contents with low GI [10, 64].

Glycemic index presented a weak negative correlation with peak viscosity ($r = -0.33$, $p < 0.05$) and final viscosity ($r = -0.35$, $p < 0.05$) and a moderate negative correlation with holding viscosity ($r = -0.44$, $p < 0.05$). As expected, a strong negative correlation was achieved between glycemic index and total starch ($r = -0.62$, $p < 0.05$) since lower total starch contents lead to higher hydrolysis indexes. Resistant starch content was discovered negative weakly correlated with glycemic index ($r = -0.23$, $p < 0.05$) being in accordance with literature [64].

5.2 Brown and germinated rice samples

5.2.1 Germination

The pH of Novarroz's water was measured before the beginning of the germination process, being about 5.3. The analysis report carried out by the external laboratory responsible for Novarroz's water inspection was provided by the quality department of Novarroz. In that report pH value was about 6.1. This variation is normal since pH measurements were done in different days, using different equipment and in different temperature conditions. However, to perform the germination process and prepare acidic solutions using Novarroz's water as solvent, the pH value obtained in the beginning day of germination (pH = 5.3) was considered. Therefore, the germination process was carried out at pH = 5.3 (Novarroz's water) and pH = 3.0 and 4.0 (phosphoric acid solutions using Novarroz's water as solvent), at ambient temperature.

As mentioned in point 4.8, *japonica14 G0* (Figure 42a)) and *japonica15 G0* (Figure 42b) were the chosen samples to germinate. The main macroscopic differences between these two rice varieties reside in the grains' dimensions, as it is possible to see. Both ungerminated brown rice samples present a typical brown colour caused by the presence of pigments on the surface of the rice grains [19].

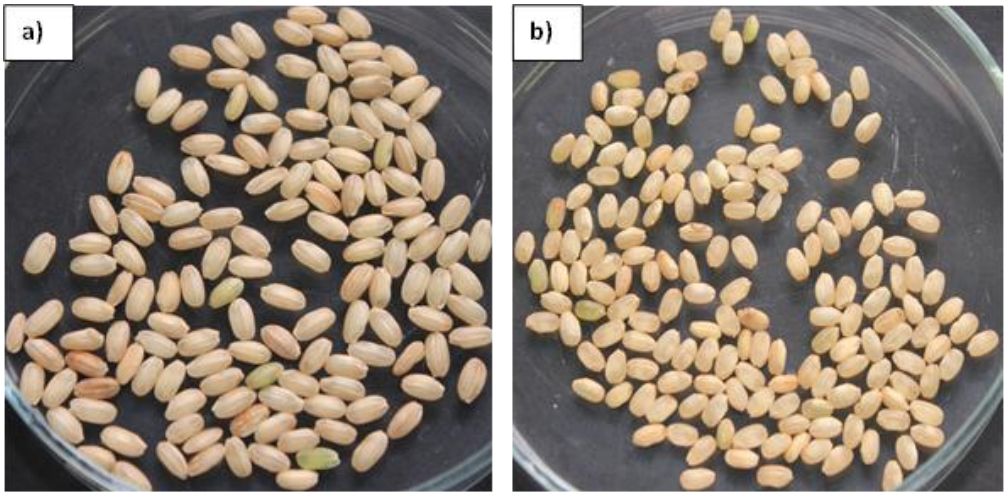

Figure 42 – Ungerminated brown rice samples: *japonica14 G0h* **(a)** and *japonica15 G0h* **(b)**.

Brown rice samples were soaked in the three aforementioned pH conditions for 24, 48 and 72 hours. Thus, different germinated brown rice samples were obtained, as shown in Table 6. The designation of each germinated sample was attributed according to the stoppage time of germination and pH condition.

Table 6 – Designation of each germinated brown rice samples according to pH and time conditions of germination.

Germinated brown rice sample	pH condition
japonica14 G24h_5.3	**pH = 5.3**
japonica14 G48h_5.3	
japonica14 G72h_5.3	
japonica14 G24h_3.0	**pH = 3.0**
japonica14 G48h_3.0	
japonica14 G72h_3.0	
japonica14 G24h_4.0	**pH = 4.0**
japonica14 G48h_4.0	
japonica14 G72h_4.0	
japonica15 G24h_5.3	**pH = 5.3**
japonica15 G48h_5.3	
japonica15 G72h_5.3	
japonica15 G24h_3.0	**pH = 3.0**
japonica15 G48h_3.0	
japonica15 G72h_3.0	
japonica15 G24h_4.0	**pH = 4.0**
japonica15 G48h_4.0	
japonica15 G72h_4.0	

The germination process continued and physical modifications of the rice grains were recorded. After 24 hours, the rice grains exhibited few alterations. A slight budding in rice samples was observed for all pH conditions.

The changes in the rice grains began to be significant after 48 hours of germination, mainly in *japonica14 G48h*, that presented more significant differences compared of *japonica15 G48h*. A visual inspection allowed to verify that brown rice samples germinated at pH = 3.0, at 48 hours, presented a greater rootlets development, as showed in Figure 43b) and Figure 43e). On the other hand, the samples germinated at

pH = 5.3 showed the lowest radicle development at 48 hours, as illustrated in Figure 43a) and Figure 43b).

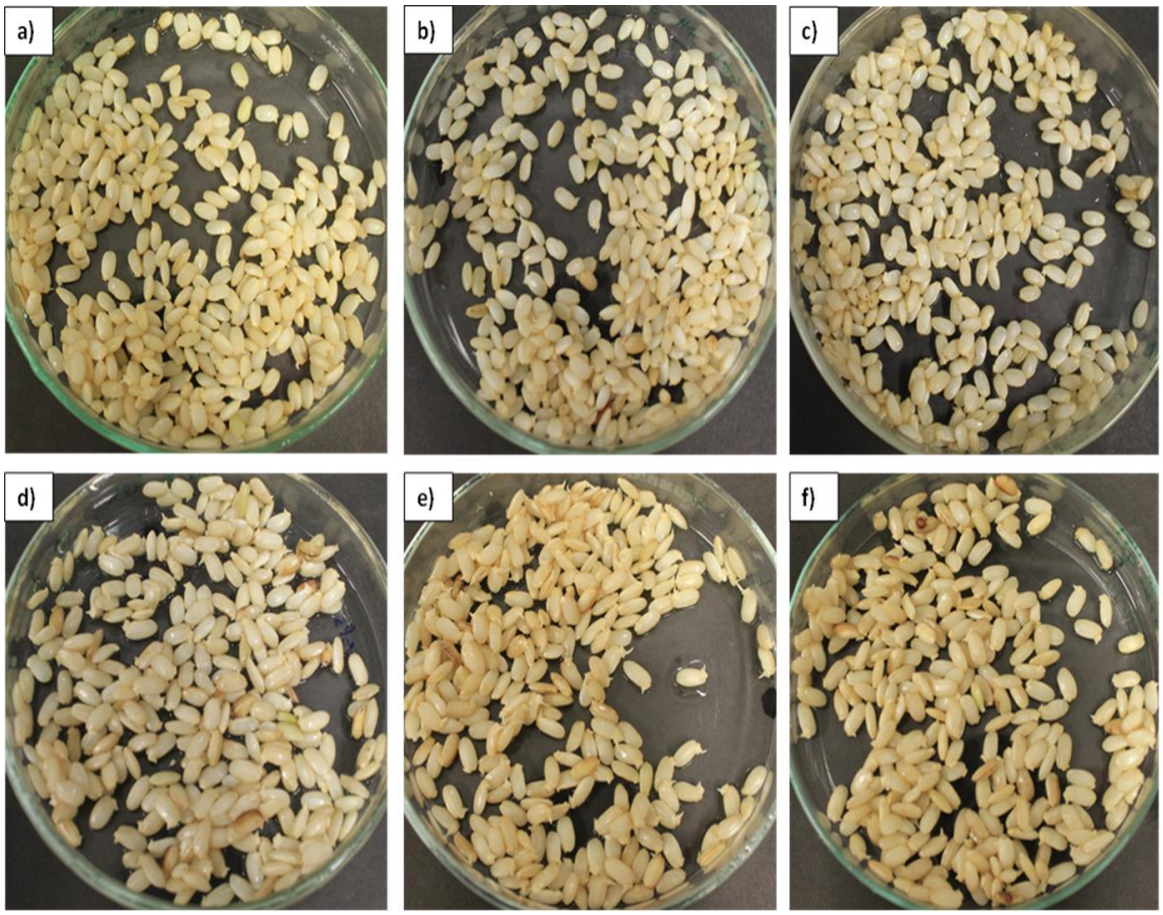

Figure 43 – Germinated brown rice samples at 48 hours, immediately before drying. **(a)** *japonica15 G48h_5.3*; **(b)** *japonica15 G48h_3.0*; **(c)** *japonica15 G48h_4.0*; **(d)** *japonica14 G48h_5.3*; **(e)** *japonica14 G48h_3.0*; **(f)** *japonica14 G48h_4.0*.

With 48 hours of germination, the rice grains of all germinated samples presented a less brownish colour being more clean and white. The beginning of the formation of fissures was also registered.

At 72 hours, germinated brown rice samples presented very significant differences, especially both brown rice samples germinated at pH = 3.0. Many grains of the mentioned samples presented very large radicles compared to the remaining germinated samples. These observations were sharper in *japonica14 G72h_3.0* (Figure 44e)) than in *japonica15 G72h_3.0* (Figure 44b)). The samples germinated at pH = 5.3 showed smaller rootlets, especially *japonica15 G72_w*. The loss of brownish colour was more pronounced at the end of the germination process for all samples.

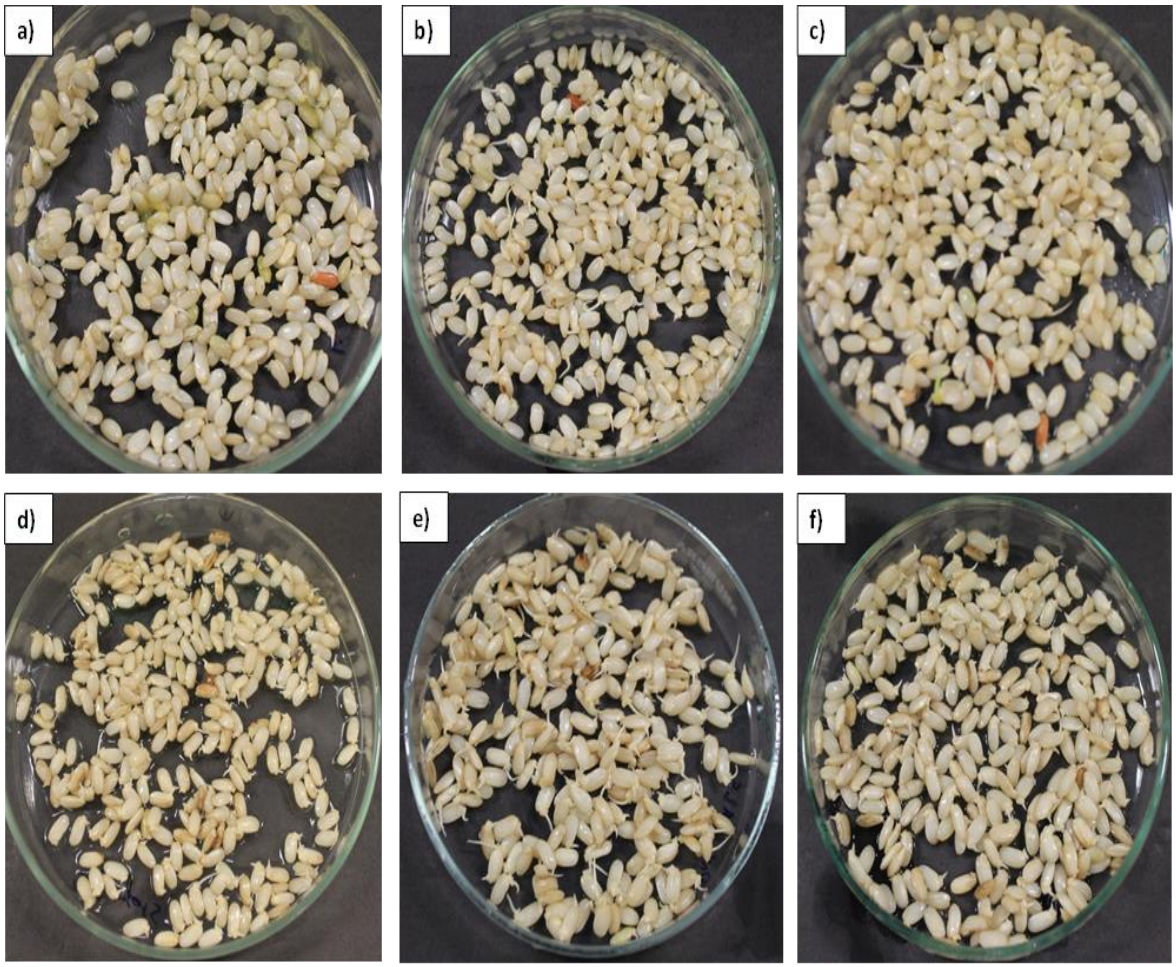

Figure 44 - Germinated brown rice samples at 72 hours, immediately before drying. **(a)** *japonica15 G72_5.3*; **(b)** *japonica15 G72h_3.0*; **(c)** *japonica15 G472h_4.0*; **(d)** *japonica14 G72h_5.3*; **(e)** *japonica14 G72h_3.0*; **(f)** *japonica14 G72h_4.0*.

The germination process was stopped by drying the samples at 45 ºC overnight. The next morning, the Petri plates with the samples were taken from the laboratory oven and placed into a desiccator until cooldown. Then, all sets (Petri plate + germinated rice sample) were weighted in order to verify their weight and compared to the initial weight (about 10 grams). Figure 45 shows the germinated brown rice samples at 72 hours, after drying, for all pH conditions. Germinated brown rice samples were stored into a desiccator until used and analysed.

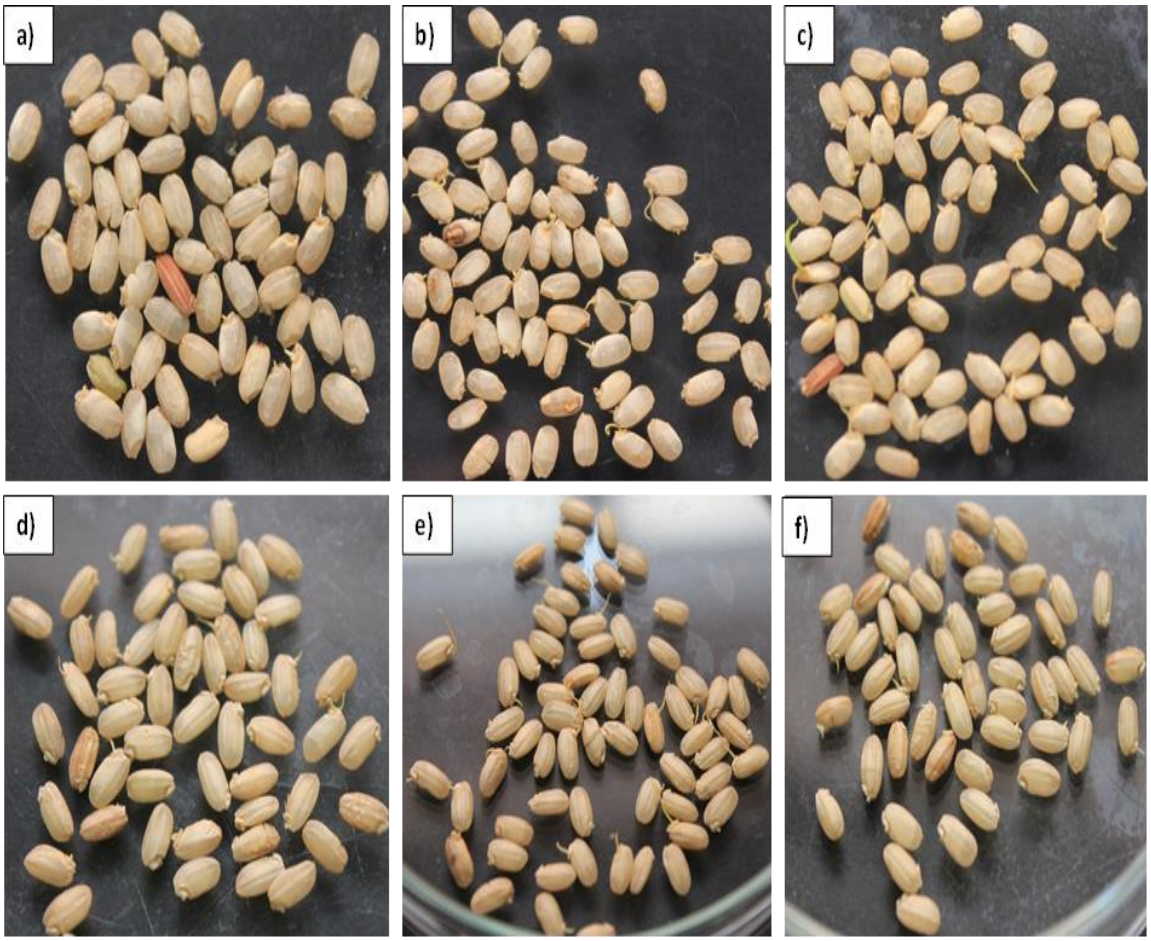

Figure 45 - Germinated brown rice samples at 72 hours, after drying. **(a)** japonica15 G72_5.3; **(b)** japonica15 G72h_3.0; **(c)** japonica15 G472h_4.0; **(d)** japonica14 G72h_5.3; **(e)** japonica14 G72h_3.0; **(f)** japonica14 G72h_4.0.

5.2.2 Moisture content

The determination of moisture content of germinated brown rice samples was carried out as described in point 4.4. The results of moisture % of ungerminated and germinated brown rice samples are presented in Figure 46.

The highest moisture contents corresponded to the ungerminated brown rice samples *japonica14 G0h* and *japonica15 G0h* (12.44 and 11.99 %, respectively), determined in point 5.1.2, along with milled and commercial samples.

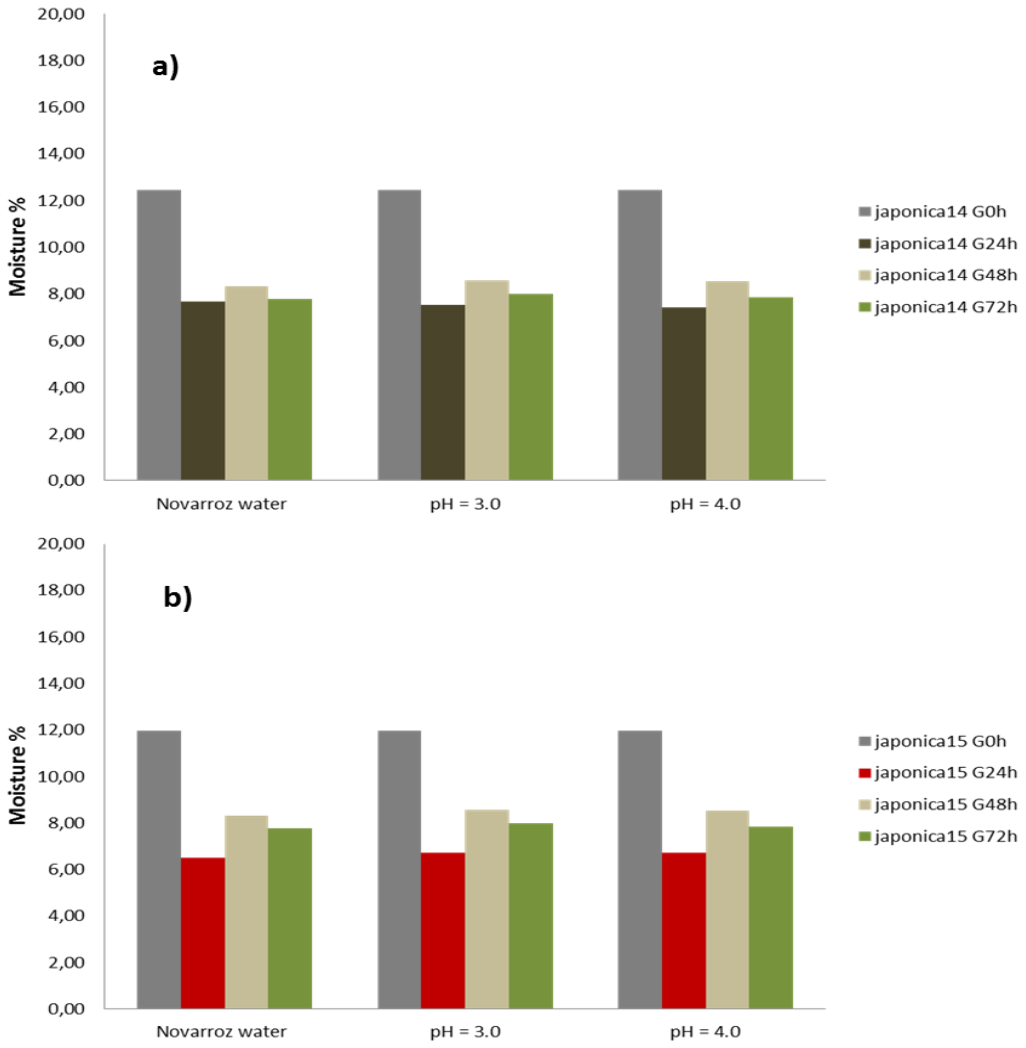

Figure 46 – Moisture content (%), per rice grain, for ungerminated and germinated brown rice samples: **(a)** *japonica14* and **(b)** *japonica15*.

Germinated samples of *japonica14* exhibited moisture values ranging from 7.42 to 8.59 % while moisture contents of germinated samples of *japonica15* varied from 6.53 to 7.78%. Curiously, a pattern in the variation of moisture content of germinated samples was observed. At 24 hours, all germinated samples presented a decrease in moisture content followed by a slight increase at 48 hours. At the end of 72 hours, all samples displayed a small decrease. This profile may be attested by the histogram of Figure 46.

5.2.3 Total starch content

As mentioned above, the germination process leads to drastic changes in the chemical composition of the rice grains. These changes result from the biochemical activity occurring inside the grain in order to produce essential compounds and energy for the formation of the radicle. This is achieved through hydrolytic enzymes that are activated to cleave larger compounds into smaller and simpler molecules. Starch is one of the molecules firstly affected by α-amylase, amyloglucosidase and other enzymes. From the starch hydrolysis results lower polymers and simple sugars. Thus, during the germination process, the total starch content decreases while simple reducing sugars increase inside the rice grains [87, 126].

Total starch content was assessed in the germinated rice samples as mentioned in point 4.6.2 but with a slight initial modification. Firstly, simple reducing sugars (quantified in point 5.2.8) resulting from starch hydrolysis were extracted preventing the quantification of total starch per excess. Therefore, after the rice samples being ground and weighted into test tubes, 2 mL of ethanol 80% solution were added. This mixture was placed under stirring for one hour with the purpose of extracting the reducing sugars. After one hour, the samples were centrifuged at 3000 rpm for 10 minutes, the supernatant obtained was discarded and 2 mL of KOH 2 M were added to the residue. The procedure continued as explained in point 4.6.2.

The results related to total starch content for ungerminated and germinated brown rice samples, in dry matter, per rice grain, are illustrated in Figure 47 by a histogram.

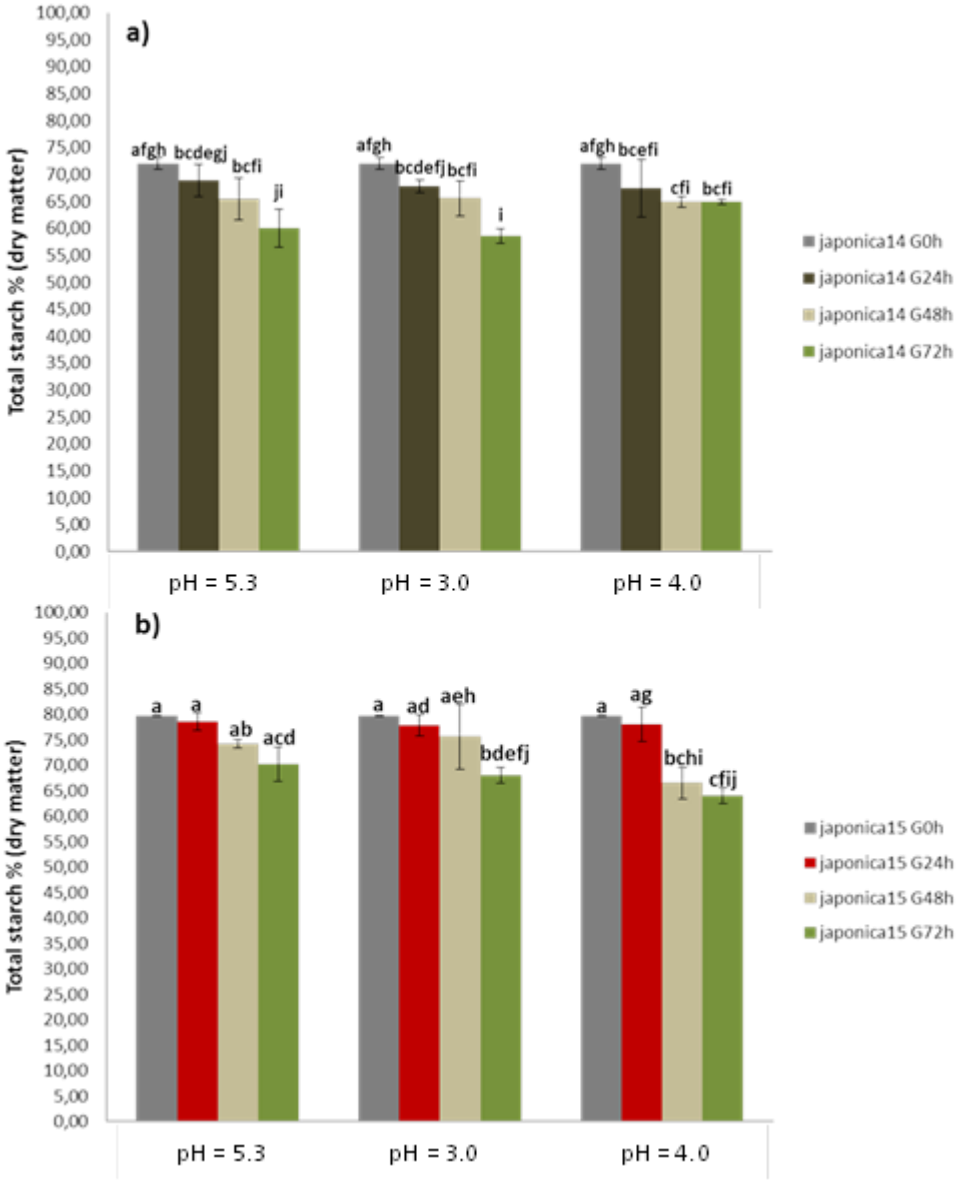

Figure 47 – Total starch content (%), in dry matter, per rice grain, for ungerminated and germinated brown rice samples: **(a)** *japonica14* and **(b)** *japonica15*, with statistical analysis ($p < 0.05$).

In general, a decrease in total starch content was observed for all germinated samples compared to the ungerminated brown rice samples.

When germinated at pH = 5.3, the total starch content of *japonica14* decreased from 72.04 (*japonica14 G0h*) to 60.01 % (*japonica14 G72h*). At 24 and 48 hours of germination, the variation of total starch content wasn't significantly different from the correspondent ungerminated rice sample (G0h). However, at 72 hours, the decrease in total starch was significant when compared to japonica14 G0h. These results weren't very

different from those samples germinated at pH = 3.0 and 4.0. At pH = 3.0, total starch content was lower at 72 hours (58.61 %) being statically similar to japonica14 G48h (65.60 %). The germination carried out with a pH = 4.0 led to a decrease in total starch amount. However, this depletion wasn't significant.

The germination of *japonica15*, at pH = 5.3, led to an insignificant decrease in total starch %. Although, when the pH of the germination medium was 3.0, the total starch amount per rice grain presented a significant decrease from 79.65 % (*japonica15 G0h*) to 68.04 % (*japonica15 G72h*). This tendency was similar to *japonica15* samples resultant from the germination carried out at pH = 4.0. In this case, a significant decrease in total starch content was already noted at 48 hours, comparatively to *japonica15 G0h* (79.65 % to 66.46 %).

Japonica14 presented a higher starch decrease (about 12 %), when germinated at pH = 5.3, comparatively to *japonica15* that showed a starch decrease of less than 9 %, after 72 hours. The same trend also happened when the brown rice samples were germinated in a medium with pH = 3.0. However, when germinated in pH = 4.0, *japonica15* exhibited a higher depletion of total starch (about 15 %), after 72 hours, compared to *japonica14* (less than 8 %).

In general, the largest differences were observed at 72 hours in all situations which suggests that germination time is, probably, the main factor affecting starch hydrolysis.

Wu *et al.* (2013) [127] also germinated three brown rice varieties using distilled water as the germination medium. The total starch content was also found decreased over time. Another recent study performed by Chinma *et al.* (2015) [126] also reported a same depletion in total starch content with time associated to the enzymatic hydrolysis of starch to simple sugars. No reports were found relating starch hydrolysis and pH conditions of germination. However, it's plausible to consider that starch hydrolysis not only occurred due to enzymatic hydrolysis but also due to acidic conditions of the germination medium (pH = 3.0 and 4.0).

5.2.4 Amylose content

Amylose content was achieved as explained in point 4.6.4. The results related to amylose content are shown in Figure 48, in dry matter, per rice grain, with statistical analysis ($p < 0.05$).

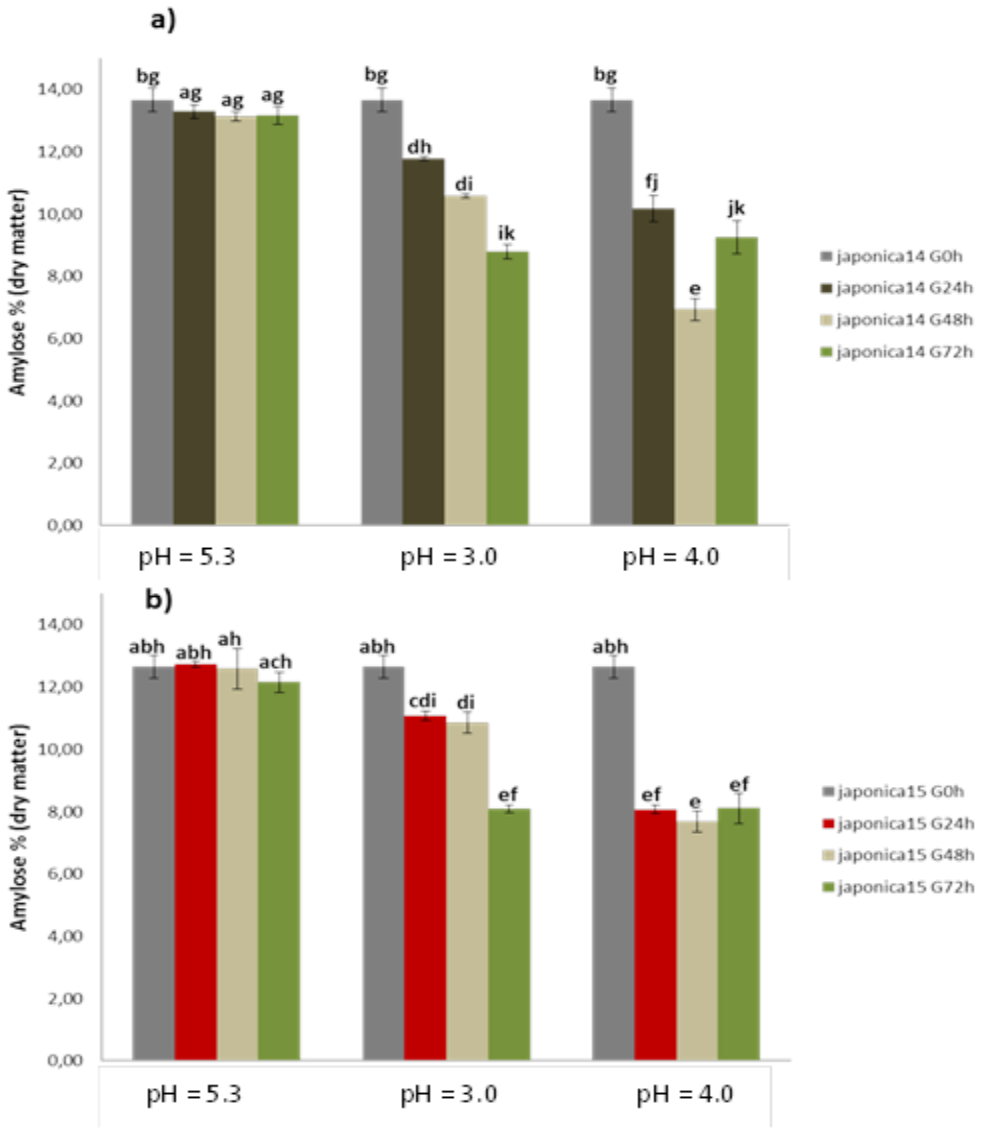

Figure 48 – Amylose content (%), in dry matter, per rice grain, for ungerminated and germinated brown rice samples: **(a)** *japonica14* and **(b)** *japonica15*, with statistical analysis ($p < 0.05$).

The germination at pH = 5.3 led to a negligible decrease in amylose content for both *japonica* varieties, ranging from 13.66 to 13.15 % and 12.63 to 12.14 % to

japonica14 and *japonica15*, respectively. The germination in more acidic conditions caused a greater fluctuation in amylose contents.

The amylose contents of *japonica14* presented a higher range compared to those of *japonica15* when germinated in lower pH conditions. The amylose amount of the rice grains of *japonica14* decreased almost 5%, after 72 hours when germination happened in a medium with pH = 3.0, presenting significant differences from ungerminated *japonica14* brown rice each 24 hours. On the other hand, *japonica15* only presented significant differences in amylose content, comparing to ungerminated *japonica15,* after 72 hours of germination. These results may suggest that the decrease in total starch % (described in the last point) is firstly related to the hydrolysis of amylose molecules in the rice grains. At pH = 4.0, amylose content of *japonica14* decreased significantly after 24 and 48 hours of germination. Curiously, at 72 hours, a significant increase was observed in the amylose content of *japonica14*. This increase may be caused by amylopectin debranching leading to the formation of linear starch polymers (amylose). For *japonica15*, at 24 hours, a relevant depletion of amylose content was observed compared to ungerminated brown rice *japonica15*. After 24 hours, no more relevant variations were found.

The results found are consistent with those found by Wu *et al.* (2013) [127]. This study reported a significant decrease in amylose content during germination, showing a similar trend to the total starch content.

The amylose % in total starch (determined in point 5.2.3) was also calculated for germinated samples. The graph bars of Figure 49 illustrates the same trends observed in Figure 48 but with more significant differences, as expected.

The analysis of Figure 49 allows verifying pronounced differences in amylose content for samples whose germination medium was at pH = 5.3. This is normal since the variation of total starch over time was taken into account for the calculation. Also more significant differences were found for *japonica14_3.0* rice as well as for *japonica14_4.0* germinated samples. However, *japonica15_3.0* and *japonica15_4.0* showed the same trends observed in the histogram of Figure 48.

With these significant changes in total starch and amylose content, and taking into account all the information cited and analysed, it makes sense to think that starch

digestibility was affected and, consequently, the glycemic index (determined in point 5.2.6). Pearson's correlations were performed to assess what relations are found between chemical parameters of germinated brown rice samples being shown in Annex F and discussed in point 5.2.12.

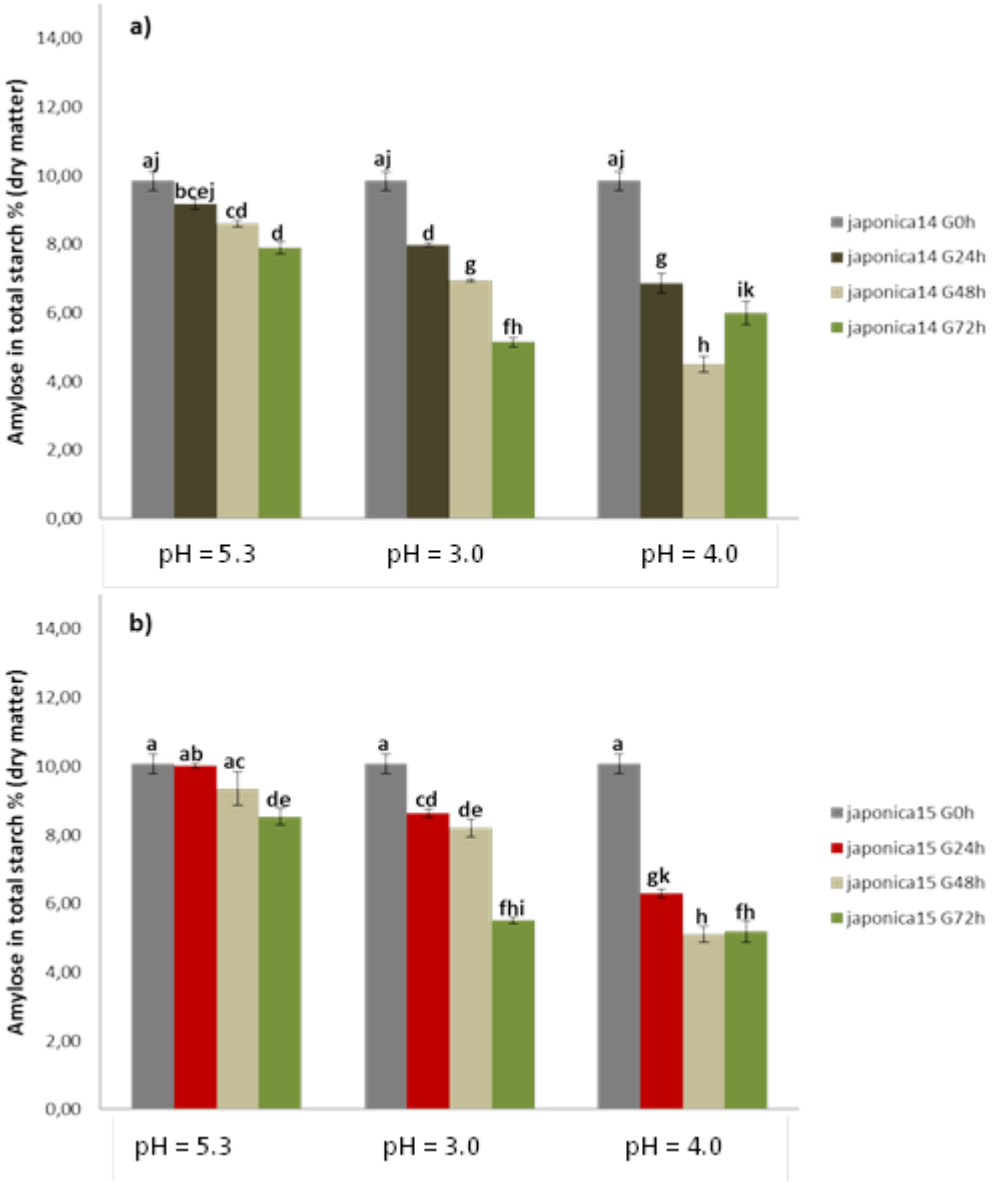

Figure 49 - Amylose in total starch %, in dry matter, for ungerminated and germinated brown rice samples: **(a)** *japonica14* and **(b)** *japonica15*, with statistical analysis ($p < 0.05$).

5.2.5 Resistant starch content

The determination of resistant starch content during germination was also assessed as explained in point 4.6.3. The results were expressed in a histogram form, illustrated by Figure 50.

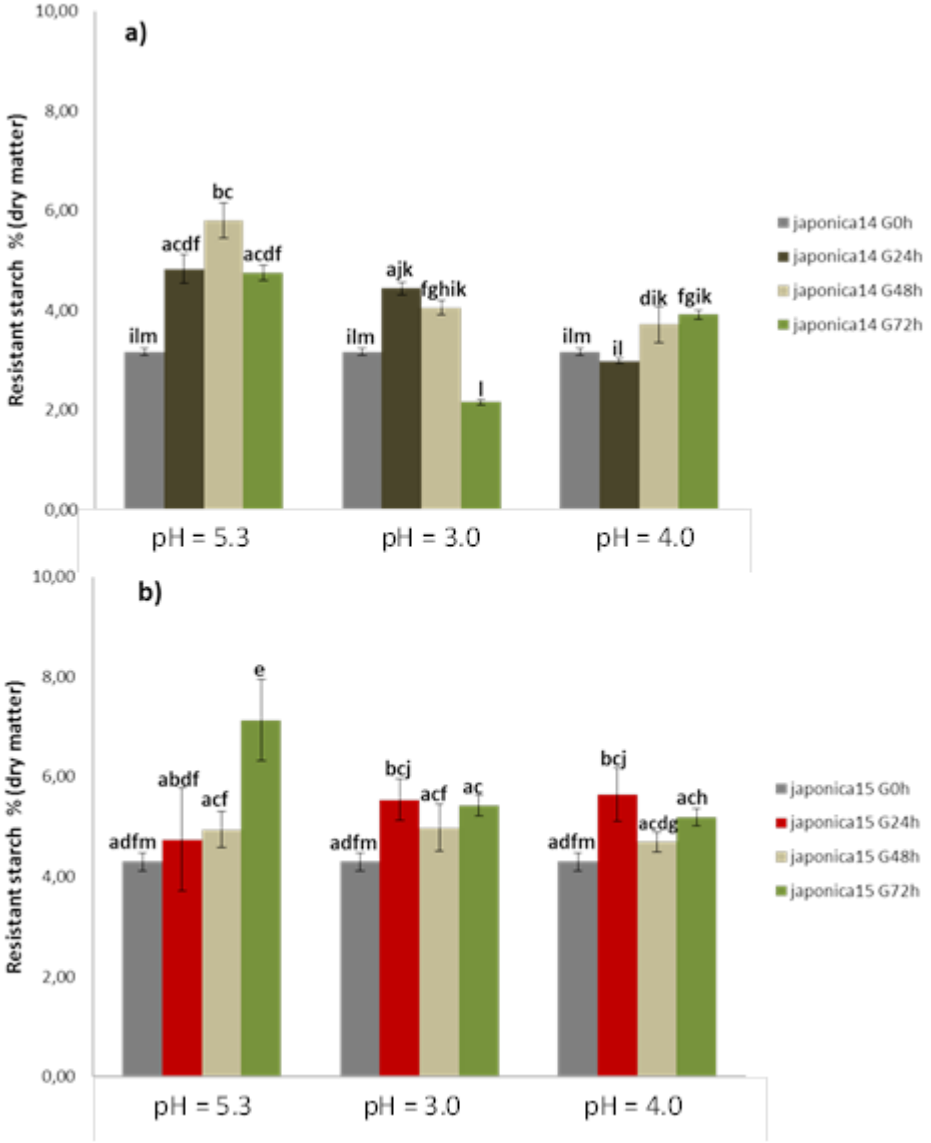

Figure 50 - Resistant starch content (%), in dry matter, per rice grain, for ungerminated and germinated brown rice samples: **(a)** *japonica*14 and **(b)** *japonica15*, with statistical analysis ($p < 0.05$).

A general increase in resistant starch content in relation to the ungerminated rice samples was noted. Only *japonica14*, germinated at pH = 3.0, at 72 hours, presented a lower resistant starch content compared to the initial ungerminated rice sample being

the only germinated sample in accordance with literature reports [128]. No reports were found to justify the remaining alterations. However, this increase in resistant starch may happen if first the hydrolysis of the more accessible starch occurred, as probably happened.

5.2.6 Glycemic index

As mentioned above, starch digestibility is affected with germination being of great importance to evaluate that effect in glycemic index values. In fact, some authors reported an increase in starch digestibility after the germination process [128, 129]. The rate of starch hydrolysis was determined and glycemic index was estimated for germinated brown rice samples as explained in point 4.7. Figure 51 represents glycemic index values of all germinated samples along with statistical analysis.

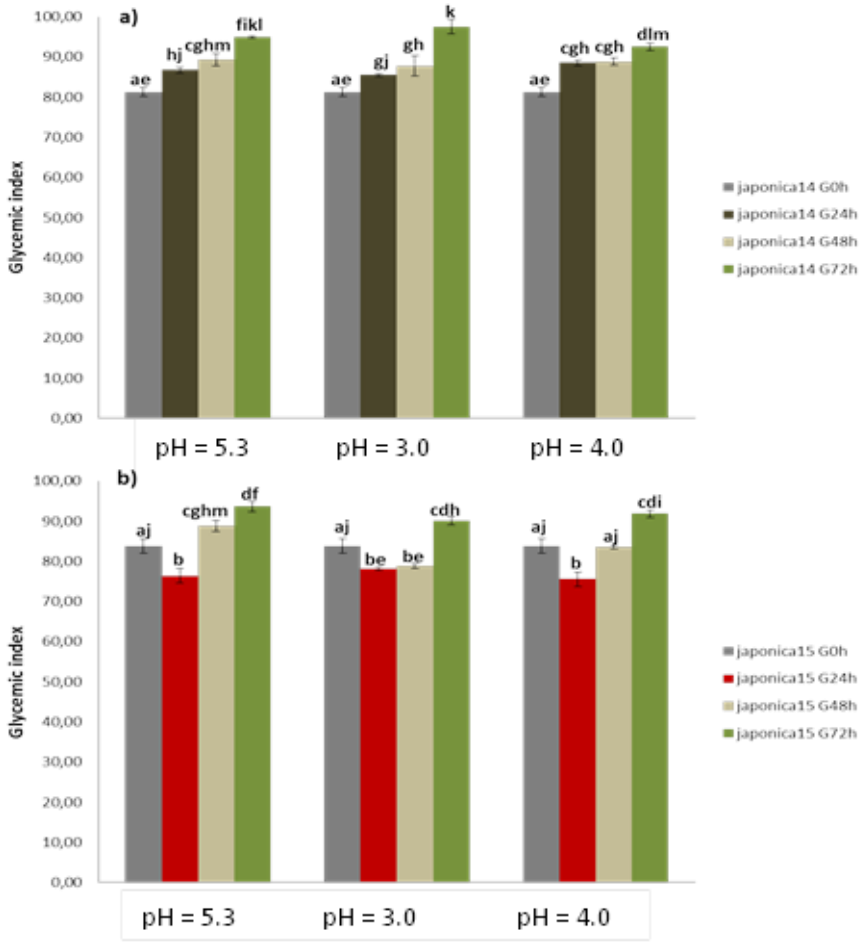

Figure 51 – Glycemic index values for ungerminated and germinated rice brown samples with respective statistical analysis ($p < 0.05$).

In general, significant variances were found between ungerminated and germinated samples.

The germination at pH = 5.3 increased significantly the glycemic index of *japonica14* (81.26 to 86.81) after 24 hours. After 48 hours, no relevant differences were found comparatively to *japonica14_3.0 G24h*. However, at 72 hours of germination, glycemic index was maximal with a significant value of 94.96. The same trends were detected in *japonica14_3.0* and *japonica14_4.0* rice samples, being the maximum values also reached at 72 hours (97.57 and 92.56, respectively). These results indicate high starch hydrolysis indexes caused by the increase of starch digestibility, corroborating recent literature reports [129].

Japonica15 revealed a curious behaviour relatively to glycemic index variation. After 24 hours, for all different pH conditions, a significant decrease was observed (76.40, 78.15 and 75.54 for *japonica15_5.3*, *japonica15_3.0* and *japonica15_4.0*, respectively). After 48 hours, a significant increase was detected for *japonica15_5.3* and *japonica15_4.0*. For all situations, the maximum value was also achieved at 72 hours.

In general, the germination process doesn´t seem very favourable to the development of low glycemic index rice samples. However, *japonica15 G24* rice samples presented lower glycemic index values compared to the initial ungerminated rice sample. This result could be interesting since these glycemic index values are closed to those of commercial low glycemic index samples (see point 5.1.6).

5.2.7 Protein content

The variation in protein content during germination process was achieved through the methodology described in point 4.5. The results for this parameter are illustrated by the histograms of Figure 52.

The analysis of Figure 52 enables, immediately, to verify that no relevant changes occurred in protein content during germination.

Germinated *japonica14* samples demonstrated a slight increase over time compared to ungerminated brown rice. On the other hand, *japonica15* exhibited a slight

reduction of protein content, for all germination conditions, when compared to the ungerminated sample. However, as mentioned before, these changes weren't significant.

These results were consistent with previous germination studies [86, 126]. The determination of protein content was performed through the quantification of total nitrogen. Probably, free amino acids were also quantified along with protein content which led to insignificant changes during germination.

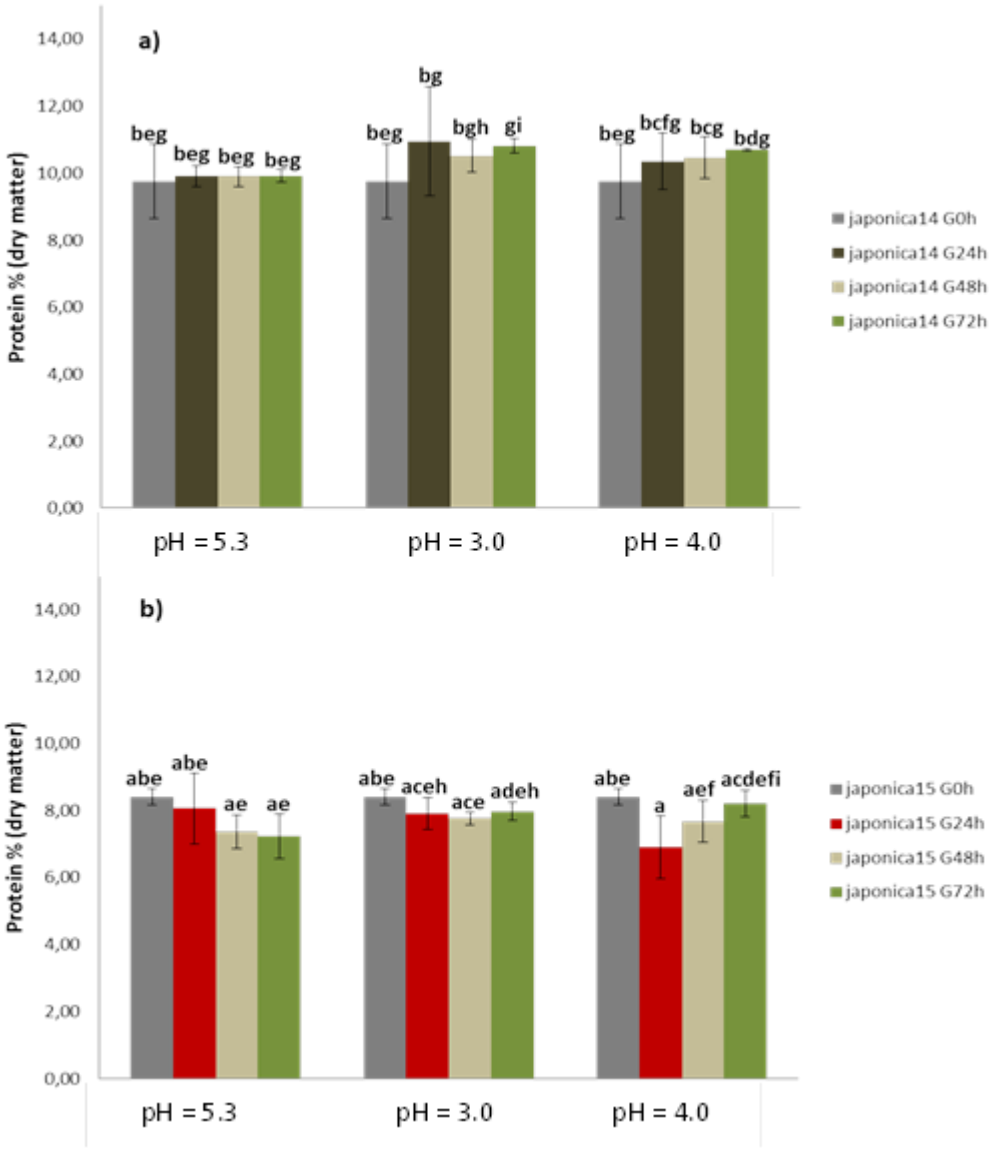

Figure 52 – Protein content (%), in dry matter, for ungerminated and germinated *japonica14* **(a)** and *japonica15* **(b)** brown rice samples, for all pH conditions, and respective statistical analysis ($p < 0.05$).

5.2.8 Reducing sugars content

As mentioned before, the germination process activates the enzymatic hydrolysis of starch leading to a decrease in its content and an increase in reducing sugars (glucose, fructose, maltose…) content. The reducing sugars content was determined through DNS method (described in point 4.9) and the results are presented in mg/100 g of dry rice sample. The histograms of Figure 53 show these results with the respective statistical analysis.

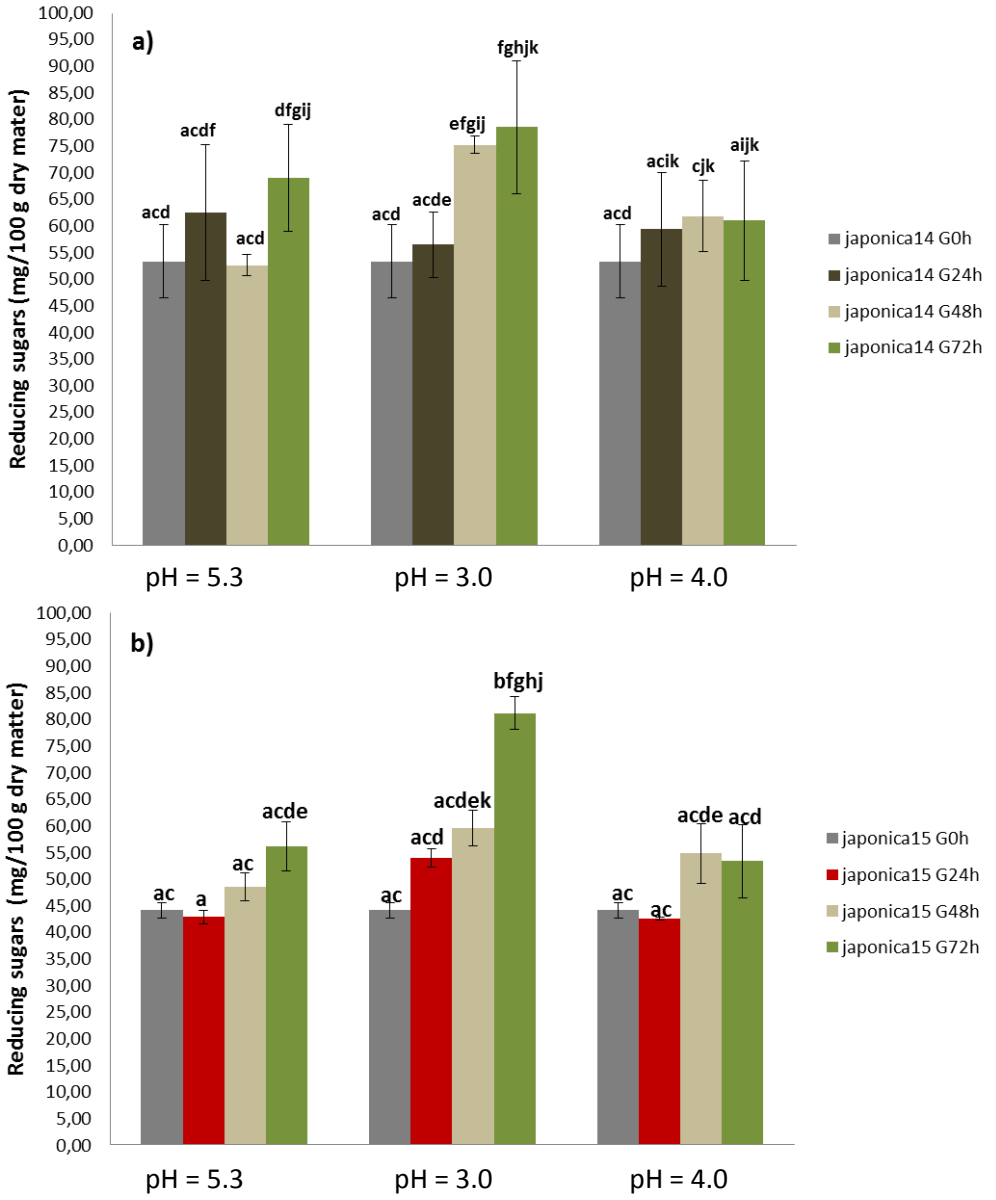

Figure 53 – Reducing sugars content (mg/100 g of dry matter) for ungerminated and germinated brown rice samples of *japonica14* **(a)** and *japonica15* **(b)** with statistical analysis ($p < 0.05$).

In fact, the inspection of Figure 53 shows a general increase in reducing sugars content for all germinated samples. This increment is more pronounced for that samples germinated in pH = 3.0, especially at the end of 72 hours, being significantly different compared to the respective ungerminated rice sample. The remaining germinated samples showed lower and no relevant variations comparatively to ungerminated brown rice samples.

Kim *et al.* (2012) [86] germinated brown rice samples in water at 15 °C, for 72 hours and also found a significant increase in reducing sugars content over time. The recent studies performed by Chinma *et al.* (2015) [126] led to the same conclusions. However, no reports were found relating the variation of reducing sugars content with pH conditions.

5.2.9 Soluble phenolic compounds content

Soluble phenolic compounds were quantified using the Folin-Ciocalteu colorimetric method (described in point 4.10.1). Gallic acid was used as the standard and the phenolic content is expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample dry weight. The results related to soluble phenolic compounds are presented in Figure 54 with the respective statistical analysis ($p < 0.05$).

All *japonica14* germinated rice samples presented significant variations comparatively to the correspondent ungerminated brown rice sample. A great decrease in soluble phenolic content was noted immediately at 24 hours of germination. After 24 hours, no more relevant changes were verified until 72 hours (Figure 54a)). These tendencies were also verified for *japonica15* rice samples, as shown in Figure 54b).

In general, *japonica14* rice samples presented a higher content of soluble phenolic values compared to the *japonica15* rice samples.

Some studies detected a slight increase in the soluble phenolic content of germinated rice samples compared to ungerminated rice samples. Some authors have associated this increase to the biosynthesis of phenolic compounds caused by enzyme hydrolysis during germination [126]. However, this wasn't verified in this thesis.

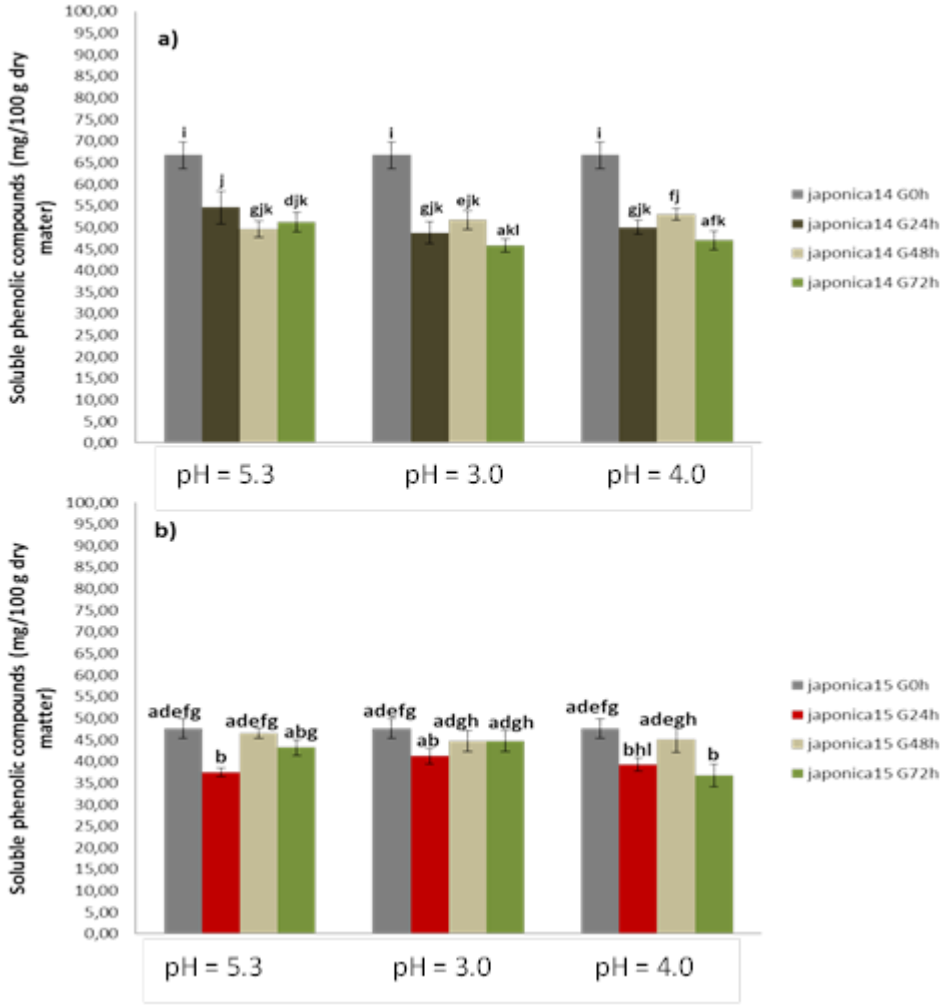

Figure 54 – Soluble phenolic content (mg/100 g of dry matter) for ungerminated and germinated brown rice samples of *japonica14* **(a)** and *japonica15* **(b)** with statistical analysis ($p < 0.05$).

Soares [101] (2014) found the same trends when evaluating the content of soluble phenolic compounds of rice samples germinated in distilled water (pH = 6.0). Soares associated the decrease of phenolic compounds content to the production of energy. However, this hypothesis doesn't make sense. If rice grains needed more energy sources, after the starch depletion, protein would be used primarily, whose content is higher, as seen in point 5.2.7. Phenolic compounds are known for their antioxidant activity [130]. In stress conditions, the rice grain needs to use its antioxidant capabilities in order to respond to medium fluctuations. Thus, in acidic conditions, the rice grain responds to the oxidative stress using its antioxidant system immediately at the beginning of the germination process. Initially, the rate of formation of phenolic compounds will be lower

compared to the rate of utilization. Therefore, a decrease is observed, in general, at 24 hours, comparatively to the ungerminated brown rice samples. Then, there is an attempt to replenish the normal values of phenolic contents with time. However, this process will be slow given the acidic conditions of the medium. This is a possible explanation for these results.

5.2.10 Insoluble phenolic compounds content

Insoluble phenolic compounds content was also assessed through Folin-Ciocalteu method and their results are also presented in mg/100 g of dry sample, in Figure 55, with the respective statistical analysis ($p < 0.05$).

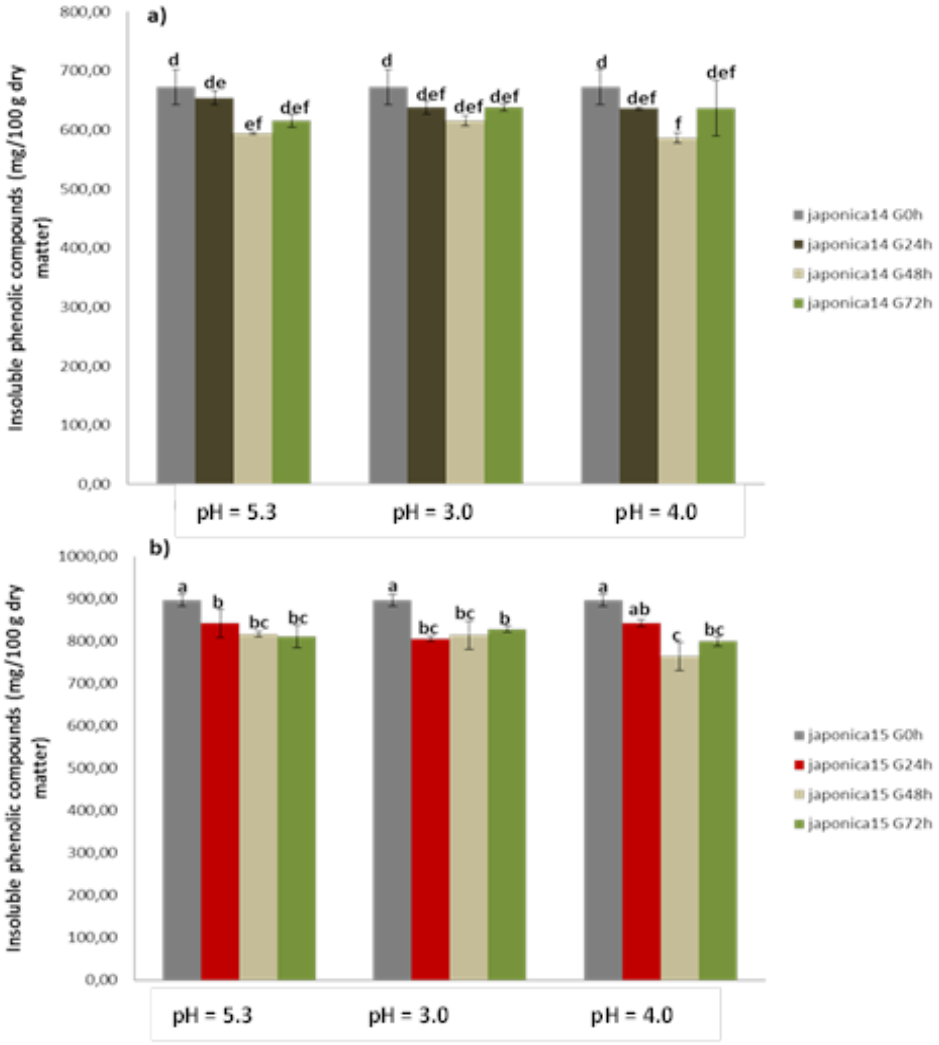

Figure 55 – Insoluble phenolic content (mg/100 g of dry matter) for ungerminated and germinated brown rice samples of *japonica14* **(a)** and *japonica15* **(b)** with statistical analysis ($p < 0.05$).

Insoluble phenolic content was clearly higher compared to soluble phenolic content. In fact, according to literature reports, about 74 % of the total phenolic compounds present in rice are of the insoluble form [56].

Insoluble phenolic content followed the same trends found for the content of soluble phenolic. A general decrease in phenolic compounds was noted. Soares [101] (2014) also reported these trends. The use of the antioxidant system of the rice grain can be used again to explain these variations.

5.2.11 GABA content

The germination at different pH values was performed, mainly, in order to evaluate the effects in GABA production. As mentioned before, GABA has been reported for its many health benefits and it exists, naturally, in the germen of brown rice grains. However, the amount presented isn't sufficient to exercise a significant effect in health. Thus, many studies have been developed in order to increase the content of this free amino acid in brown rice grains and other cereal grains. Germination is the main strategy used. As listed in point 2.5, the germination in acidic environments has been appointed as a more favourable strategy to obtain higher amounts of GABA. The objective was verifying that and analyse that tendency.

Therefore, GABA was extracted, derivatized, identified and quantified by GC-MS, as explained in point 4.11. However, some problems were found before achieving the final results. The initial internal standard used was norleucine, as mentioned in the procedures for amino acids analysis developed by Coimbra *et al.* (2011) [107]. Nevertheless, norleucine was found to have the same retention time of GABA (between 16.50 and 17.00 minutes) compromising all the quantification process since a good separation of two compounds wasn't possible.

The resolution of this problem focused in the choice of another internal standard. The compound used as internal standard was ornithine since is an amino acid, is absent in the rice grains and doesn't interfere with the rice samples. An ornithine solution was prepared in ethanol 80% (solvent used to extract GABA from rice), dried, conveniently derivatized and 2 µL were injected to determine its retention time. Figure 56 represents a

chromatogram obtained for a rice sample where it is possible identify the peaks of GABA and ornithine and their retention times. As it is possible to see, GABA and ornithine are perfectly separated.

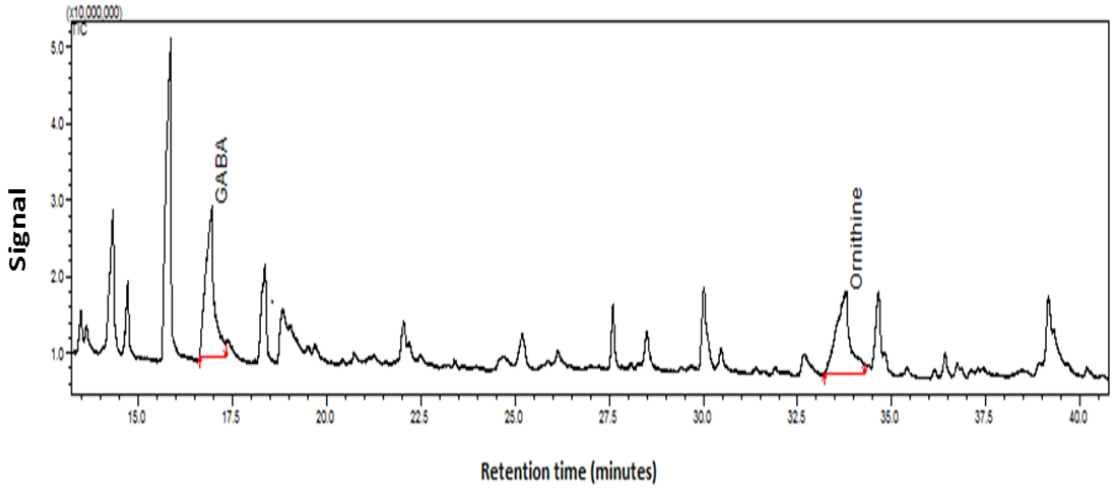

Figure 56 – Chromatogram relative to retention times of GABA and ornithine (internal standard).

The inspection of mass spectrum of derivatized GABA allowed identifying, unequivocally, a GABA peak in the chromatogram. In addition to GABA, GC-MS analysis enabled the identification of other compounds in the rice samples, already present in the database.

Figure 57 represents the chromatogram of *japonica14 G72_3.0* rice sample where it is possible to identify other amino acids such as leucine, isoleucine, proline and phenylalanine and a fatty acid (myristic acid). The chromatograms of ungerminated and germinated brown rice samples were obtained. Then, GABA and ornithine peaks were integrated in order to determine their areas to quantify GABA content. GABA quantification was performed in duplicate and the results are presented in mg/ 100g of dry matter.

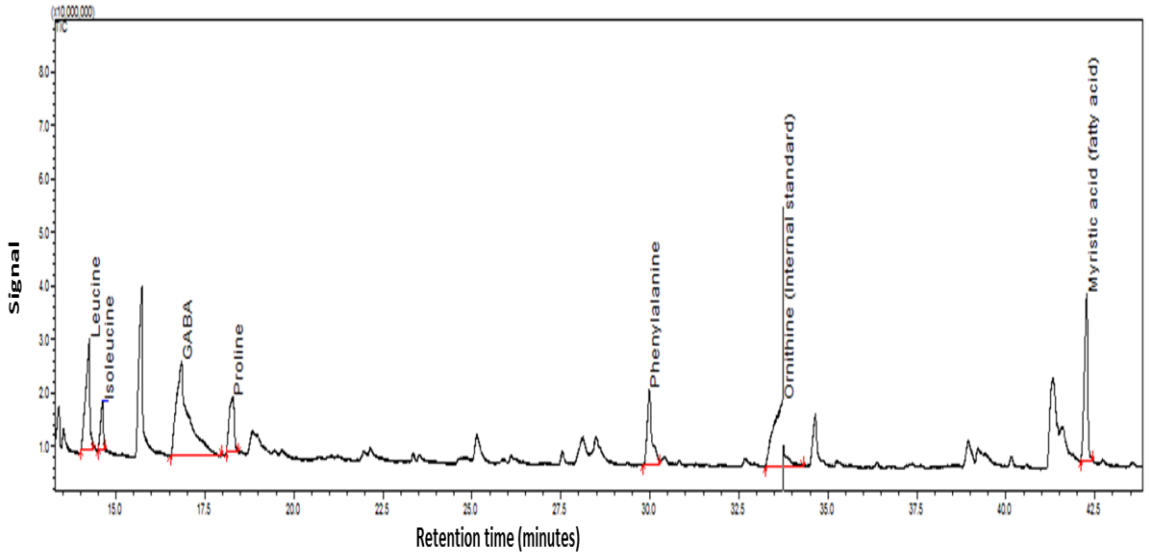

Figure 57 – Chromatogram of *japonica14 G72h_3.0*.

The histograms of Figure 58 exhibit GABA content variation for all pH conditions and the respective statistical analysis.

Figure 58 enables confirming that the germination process, effectively, has a significant influence in GABA production. Furthermore, the pH conditions of germination medium seem to have a particular and relevant effect, too.

The initial ungerminated *japonica14* brown rice shows low GABA content (9.27 mg/ 100 dry matter). The germination at (pH = 5.3) induced a significant increase in GABA at 24 and 48 hours (20.24 and 23.62 mg/ 100 dry matter, respectively), compared to the initial ungerminated sample. However, a slight decrease was observed at the end of 72 hours. The germinated *japonica14* samples germinated at pH = 3.0 presented higher and significant GABA contents. Each 24 hours, the GABA content increased significantly ranging from 9.27 (at 0 hours) to 43.63 mg/ 100 dry matter (at 72 hours), showing an increase of almost 5 times in relation to ungerminated *japonica14*. For pH = 4.0, a significant increase was observed at the first 24 hours. Yet, a decrease was observed at 48 and 72 hours. Thus, the highest GABA contents of germinated *japonica14* samples were observed when the germination was performed at pH = 3.0. At pH = 4.0, the highest increase in GABA amount was observed at 24 hours (3 times higher compared to the

initial japonica14 G0h). Even germinated at pH = 5.3 (slightly acidic), the maximum increase of GABA obtained was of 2.5 times (*japonica14 G48*), compared to the initial ungerminated sample. These results are consistent with some literature reports [6, 88]. The increase in H^+ concentrations has been reported as a stimulator factor of glutamate decarboxylase activity (responsible for glutamate decarboxylation giving GABA) [131]. This report corroborates the results found showing pH influences the production of GABA during germination.

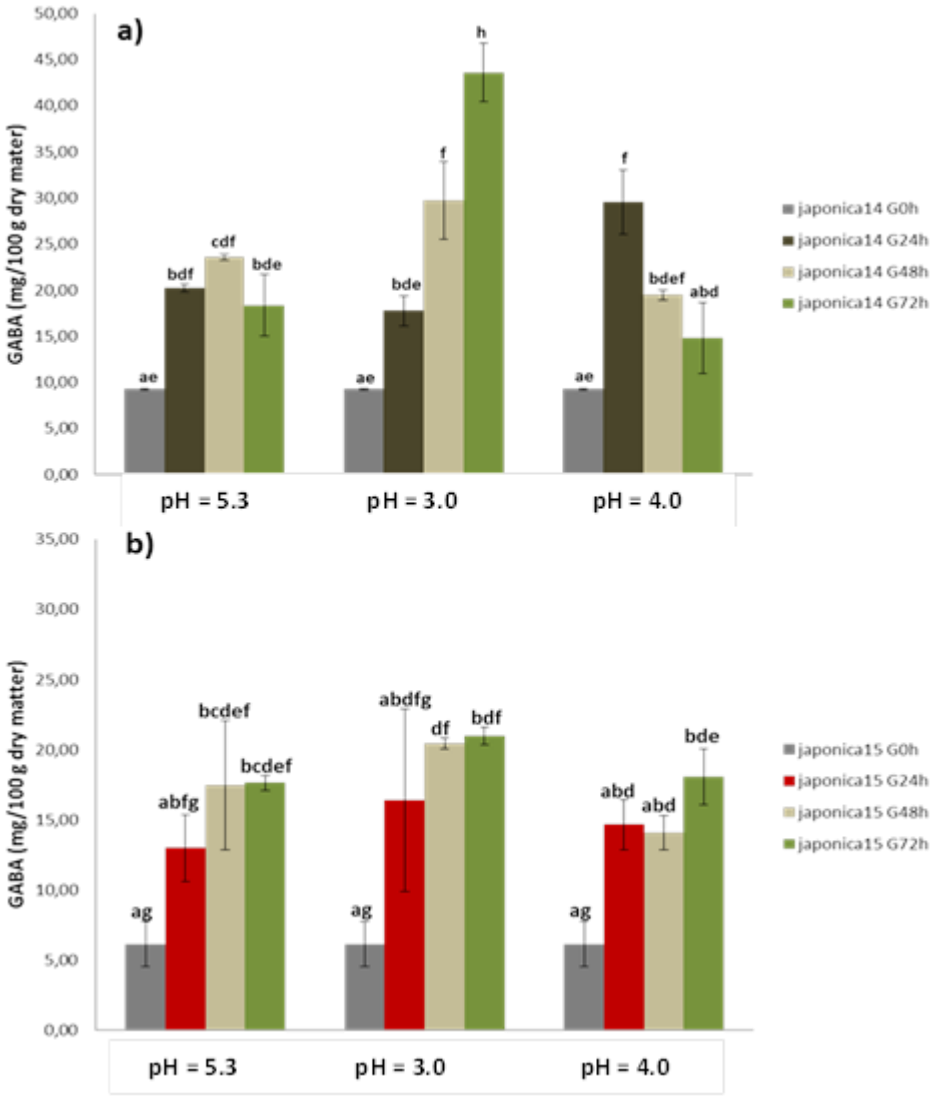

Figure 58 – GABA content (mg/ 100 mg dry matter) of ungerminated and germinated brown samples with statistical analysis ($p < 0.05$).

An increase in GABA content was also observed in germinated *japonica15* rice samples, however, this increase wasn't so pronounced and significant compared to that

occurring in *japonica14*. The germination at pH = 5.3 water allowed developing a germinated sample whose highest GABA content was achieved at 72 hours (17.61 mg/ 100 g dry matter), showing an increase of approximately 3 times compared to the initial brown rice. The highest GABA contents obtained to germinated *japonica15* at pH = 3.0 and 4.0 were, respectively, 3.5 and 3.0 times higher compared to ungerminated *japonica15*.

GABA1, *GABA2* and *GABA3* are three commercial samples described as being enriched in GABA content. The GABA contents of these samples were also assessed and are represented in Figure 59.

a)

Sample	GABA content (mg/100 g dry matter)
GABA1	$16,51 \pm 0,07^{bd}$
GABA2	$2,48 \pm 0,01^{g}$
GABA3	$16,59 \pm 0,10^{bd}$

b)

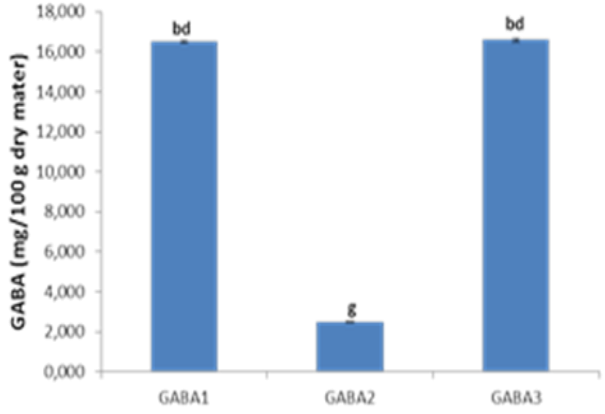

Figure 59 – GABA content in commercial rice samples *GABA1*, *GABA2* and *GABA3*, in mg/ 100 g dry matter and respective statistical analysis.

GABA2 is statistically similar to ungerminated *japonica15*. On the other hand, *GABA1* and *GABA3* presented statistical similarities with some germinated rice samples. *Japonica14 G48_3.0*, *japonica14 G72_3.0* and *japonica14 G24_4.0* GABA contents were significantly higher comparatively to the commercial samples.

5.2.12 Correlations

This section lists some of the most relevant Pearson's correlations found between all parameters determined for all ungerminated and germinated brown rice samples. The strength of correlations was classified as in point 5.1.8. Also, three correlation tables were made and all detailed and complete data can be consulted in Annex F. Table **a)**, in Annex F, represents all the calculated correlations when all ungerminated and germinated brown rice samples were considered; table **b)** focuses on all correlations taking only ungerminated and germinated *japonica14* samples into account and table **c)** shows all correlations related to ungerminated and germinated *japonica15* samples. This discussion focuses on the correlations of table **a)**, in Annex F, considering all ungerminated and germinated brown rice samples.

Weak correlations were found between total starch content and amylose and resistant starch ($r = 0.29$ and $r = 0.35$, respectively), associated to the variations observed. Strong negative correlations were noted between total starch and glycemic index and total starch and reducing sugars ($r = -0.83$ and $r = -0.70$, respectively). Germination leads to an increase in starch digestibility decreasing total starch content and increasing reducing sugars content. Consequently, starch hydrolysis is faster enhancing glycemic index. Also strong negative correlations were found between total starch and protein ($r = -0.64$, $p < 0.05$) and total starch and GABA content ($r = -0.61$, $p < 0.05$).

Resistant starch presented a strong negative correlation with protein content ($r = -0.68$, $p < 0.05$).

Glycemic index presented a strong positive correlation with reducing sugars content and moderate correlations with protein ($r = 0.46$, $p < 0.05$), insoluble phenolic compounds ($r = -0.48$, $p < 0.05$) and GABA content ($r = 0.52$, $p < 0.05$).

Protein content presented a negative very strong correlation with insoluble phenolic compounds ($r = -0.90$, $p < 0.05$) and moderate positive correlations with reducing sugars ($r = 0.48$, $p < 0.05$), soluble phenolic compounds ($r = 0.58$, $p < 0.05$) and GABA content ($r = 0.46$, $p < 0.05$).

Reducing sugars content was found strongly positively correlated with GABA content ($r = 0.69$, $p < 0.05$) and moderately correlated with insoluble phenolic compounds ($r = -0.48$, $p < 0.05$)

Soluble and insoluble phenolic compounds were found negatively strongly correlated ($r = -0.61$, $p < 0.05$), showing that an increase in soluble phenolic content led to a decrease in insoluble phenolic compounds.

6. CONCLUSIONS

The two main goals of this thesis were fulfilled.

The physicochemical characterization of 23 milled rice samples provided by Novarroz, Produtos Alimentares S.A. was carried out.

Physical analysis of the rice grains enabled attributing the higher length values and length-to-width ratios to *indica* rice varieties. The strong correlations found between size parameters suggested an inverse relationship between length and width. In general, the chalky area % of *japonica* rice samples was higher compared to *indica* varieties being positive strongly correlated with total whiteness ($r = 0.97$, $p < 0.05$) and kett value ($r = 0.86$, $p < 0.05$).

The determination of pasting properties allowed studying the starch behaviour during cooking and cooldown. *Japonica* rice samples were found with higher values of peak and breakdown viscosities. These two parameters were strongly positively correlated. Setback values were higher for *indica* varieties being strongly negative correlated with peak and breakdown viscosities.

Total starch content was determined in dry matter. Its content ranged from 74.79 to 84.45 % *(japonica8* and *indica8*, respectively) being consistent to literature reports.

The determination of amylose content assigned the highest values to *indica* varieties. This starch parameter was positively strongly correlated with setback viscosity ($r = 0.88$, $p < 0.05$) and negatively strongly connected with breakdown and peak viscosities ($r = -0.85$ and $r = - 0.67$, respectively).

Hydrolysis indexes were determined to estimate glycemic indexes of rice samples. Samples whose starch hydrolysis was faster over time presented the higher glycemic index values. The sample of Novarroz with lower glycemic index was indica6 (76.40 ± 1.06) that was statistically similar to a commercial low glycemic index rice sample (*indica10*). *Indica6* was seen as possible choice as a low GI rice variety destined to type 2 diabetic people. On the other hand, *japonica8* presented the highest GI value (90.75 ± 1.11), opening new perspectives to the development of high GI products directed to other type of consumers. A very weak negative correlation was found between glycemic index

and amylose content ($r = -0.08$, $p < 0.05$) corroborating some literature reports. Resistant starch was also found negative weakly correlated with glycemic index ($r = -0.23$, $p < 0.05$).

Protein content ranged from 5.81 to 10.08 % (*indica2* and *indica4*, respectively), in dry matter.

This characterization allows providing important information about new rice varieties composition to Novarroz, Produtos Alimentares S.A.

The second part of this thesis consisted in the germination of *japonica14 G0h* and *japonica15 G0h* in Novarroz's water (pH = 5.3), pH = 3.0 and pH = 4.0 for 24, 48 and 72 hours.

Germination process led to several changes in rice samples composition over time.

A significant depletion in total starch content was observed over time. No relevant differences were found comparing different pH conditions.

As expected, the decrease in amylose content helped to corroborate the depletion in total starch since amylose is the first starch polymer affected by enzymatic hydrolysis. This decrease was more pronounced in samples germinated in more acidic conditions.

A general increase in the resistant starch content was detected over germination. This parameter was found negatively strongly correlated with protein content ($r = - 0.68$, $p < 0.05$).

The germination process contributed to the increase of the glycemic index of almost all germinated samples. However, *japonica15 G24_5.3*, *japonica15 G24_3.0* and *japonica G24_4.0* presented a decrease in glycemic index values compared to *japonica15 G0h*. Glycemic index was found negatively strongly correlated with total starch content ($r = - 0.83$, $p < 0.05$), confirming that total starch content effectively influences glycemic index. Also a negative weak correlation was detected between glycemic index and amylose content ($- 0.11$, $p < 0.05$).

A clear increase in reducing sugars content was noted. This increase was higher at pH = 3.0 for both *japonica* germinated rice varieties. Total starch content and reducing sugars were negatively strongly correlated. Reducing sugars were positively strongly correlated with glycemic index ($r = 0.61$, $p < 0.05$).

Soluble and insoluble phenolic content decreased with germination, probably associated to the activation of the rice grains' antioxidant system caused by acidic conditions of the germination medium.

A relation between acid germination conditions and GABA content was effectively detected, corroborating previous reports. Germination at pH = 3.0 led to a higher and significant GABA content in rice grains over time, mainly in *japonica14* samples. Each 24 hours, the GABA content increased significantly ranging from 9.27 (at 0 hours) to 43.63 mg/ 100 dry matter (at 72 hours), showing an increase of almost 5 times in relation to ungerminated japonica14. Thus, *japonica14 G72_3.0* was the germinated sample with higher and significant GABA content. GABA content was strongly negatively correlated with total starch content ($r = -0.61$, $p < 0.05$) and strongly positively correlated ($r = 0.69$, $p < 0.05$) with reducing sugars content.

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8. ANNEXES

ANNEX A – Physical characteristics of milled rice samples.

ANNEX B – Pasting properties of milled rice samples.

ANNEX C1 – Chemical and nutritional characterization of milled, brown and commercial rice samples, in dry matter.

ANNEX C2 – Chemical and nutritional characterization of milled, brown and commercial rice samples, in dry mater.

ANNEX D – Correlations values for milled rice samples with all parameters analysed.

a) Milled indica samples

b) Milled japonica samples

c) All milled samples

ANNEX E1 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

ANNEX E2 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

ANNEX E3 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

ANNEX E4 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

ANNEX F – Correlations values for brown and germinated rice samples with all parameters analysed.

a) All ungerminated and germinated samples

b) Ungerminated and germinated *japonica14* samples

c) Ungerminated and germinated *japonica15* samples

ANNEX A – Physical characteristics of milled rice samples.

Sample	Type	Length (mm)	Width (mm)	L/W ratio	Total whiteness	Crystalline whiteness	Chalky area (%)	Kett
indica1	Agulha (aromatic)	7,108	1,766	4,025	130,79	119,94	18,34	42,2
indica2	Agulha (aromatic)	7,169	2,016	3,556	127,99	119,87	13,24	43,8
indica3	Agulha	6,16	2,057	2,995	132,82	123,57	17,82	42,1
indica4	Agulha	7,061	2,080	3,395	131,82	123,77	14,91	44,9
indica5	Agulha	7,414	2,198	3,373	135,00	123,61	21,15	42,9
indica6	Agulha	7,33	2,128	3,445	124,37	118,65	9,26	38,7
indica7	Agulha	6,771	2,031	3,334	129,19	120,39	14,77	42,7
indica8	Agulha	6,173	2,043	3,022	126,35	119,63	10,82	41,0
japonica1	Carolino	6,261	2,414	2,594	129,58	121,41	13,97	40,8
japonica2	Carolino	6,037	2,281	2,647	126,82	120,50	10,18	37,2
japonica3	Carolino	6,129	2,439	2,513	129,88	122,74	12,30	42,1
japonica4	Carolino	6,404	2,674	2,395	137,42	126,18	22,65	45,7
japonica5	Carolino	6,178	2,624	2,354	136,98	125,47	23,30	44,2
japonica6	Carolino	6,08	2,643	2,300	134,99	122,65	21,09	46,4
japonica7	Carolino	6,056	2,424	2,498	124,89	120,13	7,50	35,0
japonica8	Carolino	6,467	2,476	2,612	134,97	125,56	18,16	41,5
japonica9	Medium	5,548	2,536	2,188	134,94	124,79	20,13	43,6
japonica10	Medium	5,29	2,782	1,902	142,79	119,99	39,11	48,9
japonica11	Medium	5,855	2,469	2,371	128,35	121,48	11,80	42,1
japonica12	Medium	5,468	2,738	1,997	146,42	120,35	44,21	49,4
japonica13	Medium	5,561	2,526	2,202	130,83	121,69	15,56	42,2
japonica14	Risotto	6,039	2,775	2,176	143,72	117,27	40,95	49,2
japonica15	Round	5,196	2,725	1,907	143,37	117,05	42,22	47,5

ANNEX B – Pasting properties of milled rice samples.

Sample	Type	Peak viscosity (cP)	Time peak (minutes)	Pasting temperature(° C)	Holding viscosity (cP)	Breakdown (cP)	Setback(cP)	Final viscosity(cP)
indica1	Agulha (aromatic)	1376,00 ± 28,69^{ahi}	7,00 ± 0,00^{a}	93,57 ± 0,51^{a}	1395,00 ± 26,96^{ahm}	-19,00 ± 1,73^{a}	1894,33 ± 48,95^{a}	3270,33 ± 72,15^{ag}
indica2	Agulha (aromatic)	1990,33 ± 47,65^{bf}	5,82 ±0,04^{b}	91,42 ± 0,45^{bdfh}	1672,00 ± 19,70^{b}	318,33 ± 67,34^{bfg}	1267,00 ± 91,54^{be}	3257,33 ± 44,46^{a}
indica3	Agulha	887,33 ± 146,19^{c}	7,00 ± 0,00^{a}	93,33 ± 0,85^{ac}	893,67 ± 142,68^{c}	-6,33 ± 3,51^{a}	1920,67 ± 128,36^{a}	2808,00 ± 267,18^{bef}
indica4	Agulha	1170,00 ± 36,86^{d}	6,62 ± 0,60^{a}	91,45 ± 0,39^{bfh}	1161,67 ± 19,86^{dk}	8,33 ± 17,10^{a}	1498,33 ± 29,74^{c}	2668,33 ± 27,15^{bf}
indica5	Agulha	1791,67 ± 53,61^{be}	7,00 ± 0,00^{a}	91,70 ± 0,05^{bfh}	1800,00 ± 53,36^{e}	-8,33 ± 1,53^{a}	2377,33 ± 25,32^{d}	4169,00 ± 77,00^{d}
indica6	Agulha	1606,67 ± 29,26^{eg}	7,00 ± 0,00^{a}	90,13 ± 0,06^{e}	1613,00 ± 29,14^{bf}	-6,33 ± 0,58^{a}	1872,00 ± 32,14^{a}	3478,67 ± 60,34^{g}
indica7	Agulha	1278,00 ± 37,03^{id}	6,00 ± 0,00^{b}	91,45 ± 0,48^{bfh}	1203,67 ± 15,70^{dgk}	74,33 ± 24,58^{ac}	1447,67 ± 17,67b^{c}	2725,67 ± 20,03^{bcf}
indica8	Agulha	927,33 ± 44,99^{c}	6,62 ± 0,65^{a}	92,53 ± 0,08^{ab}	929,33 ± 43,62^{c}	-2,00 ± 3,61^{a}	1975,67 ± 63,81^{a}	2903,00 ± 73,37^{ce}
japonica1	Carolino	1853,00 ± 38,94^{bjk}	5,82 ± 0,04^{b}	91,68 ± 0,03^{bfh}	1371,67 ± 20,53^{aim}	481,33 ± 18,58^{deh}	933,67 ± 7,64^{fn}	2786,67 ± 31,37^{bcf}
japonica2	Carolino	2006,00 ± 30,41^{fk}	5,76 ± 0,04^{b}	91,45 ± 0,48^{bfh}	1388,67 ± 7,57^{aj}	617,33 ± 33,50^{eh}	872,67 ± 59,50^{fmn}	2878,67 ± 62,85^{be}
japonica3	Carolino	1569,00 ± 7,55g^{hlm}	5,73 ± 0,00^{b}	91,22 ± 0,46^{efg}	1172,67 ± 7,09^{dk}	396,33 ± 14,64^{bd}	1306,00 ± 8,19^{bce}	2875,00 ± 1,00^{be}
japonica4	Carolino	1381,33 ± 73,12^{aim}	5,84 ± 0,04^{b}	92,53 ± 0,03^{ab}	1112,33 ± 23,76^{d}	269,00 ± 49,39^{bf}	1233,00 ± 47,63^{eh}	2614,33 ± 25,58^{f}
japonica5	Carolino	1749,33 ± 6,51^{ejln}	5,80 ± 0,00^{b}	91,98 ± 0,40^{bfhi}	1221,67 ± 4,93^{dgk}	527,67 ± 8,39^{de}	1025,00 ± 17,09^{ni}	2774,33 ± 10,69^{bcf}
japonica6	Carolino	2155,67 ± 62,69^{fo}	5,80 ± 0,00^{b}	91,25 ± 0,48^{efj}	1505,67 ± 8,62^{fhj}	650,00 ± 68,02^{eh}	700,00 ± 86,24^{gm}	2855,67 ± 23,86^{be}
japonica7	Carolino	1518,67 ± 44,29^{agp}	5,71 ± 0,04^{b}	92,30 ± 0,48^{bcgij}	1300,67 ± 32,59^{agl}	218,00 ± 51,64^{cfi}	1205,33 ± 69,59^{eij}	2724,00 ± 64,86^{bcf}
japonica8	Carolino	1499,00 ± 78,58^{agp}	5,84 ± 0,04^{b}	93,05 ± 0,48^{ai}	1206,67 ± 3,79^{dl}	292,33 ± 75,96^{bgi}	1188,67 ± 110,19^{eij}	2687,67 ± 31,90^{bcf}
japonica9	Medium	1889,67 ± 127,91^{bkn}	5,69 ± 0,04^{b}	91,47 ± 0,49^{bfk}	1252,67 ± 23,09^{ikl}	637,00 ± 104,82^{eh}	814,33 ± 100,17^{gmf}	2704,00 ± 27,78^{bcf}
japonica10	Medium	1514,33 ± 65,59^{agq}	6,00 ± 0,07^{b}	92,47 ± 0,03^{abj}	1195,33 ± 28,92^{dl}	319,00 ± 43,09^{bgi}	1149,33 ± 27,43^{eijk}	2663,67 ± 40,41^{bf}
japonica11	Medium	1976,67 ± 38,68^{bko}	5,78 ± 0,04^{b}	91,72 ± 0,03^{bfk}	1589,33 ± 24,91^{bf}	387,33 ± 35,92^{bd}	1037,00 ± 46,78^{hjn}	3013,67 ± 44,07^{e}
japonica12	Medium	2131,00 ± 121,01^{fo}	6,00 ± 0,07^{b}	90,17 ± 0,03^{e}	1434,00 ± 36,51^{jm}	697,00 ± 103,81^{h}	477,00 ± 125,04^{l}	2608,00 ± 38,22^{f}
japonica13	Medium	1883,00 ± 26,06^{bkn}	5,89 ± 0,04^{b}	91,22 ± 0,43^{eghk}	1417,67 ± 14,98^{aj}	465,33 ± 11,68^{d}	960,33 ± 14,15^{fnk}	2843,33 ± 16,77^{be}
japonica14	Risotto	1622,33 ± 29,87^{elpq}	5,87 ± 0,07^{b}	91,95 ± 0,48^{bfik}	1194,00 ± 13,11^{dl}	428,33 ± 18,82^{dg}	1041,00 ± 10,58^{hjn}	2663,33 ± 26,54^{bf}
japonica15	Round	2093,33 ± 47,35^{fo}	5,89 ± 0,04^{b}	90,20 ± 0,05^{de}	1450,33 ± 15,50^{jm}	643,00 ± 40,26^{eh}	674,33 ± 44,23^{ml}	2767,67 ± 21,78^{bcf}

ANNEX C1 – Chemical and nutritional characterization of milled, brown and commercial rice samples, in dry matter.

Sample	Type	Moisture (%)	Total starch (%)	Glycemic index	Protein(%)
indica1	Agulha (aromatic)	12,00	75,26 ± 4,02^{ab}	87,04 ± 2,33^{ace}	8,97 ± 0,86^{ad}
indica2	Agulha (aromatic)	11,01	77,48 ± 2,22^{ab}	83,05 ± 0,06^{bdg}	5,81 ± 0,40^{b}
indica3	Agulha	11,37	79,81 ± 3,34^{ab}	88,09 ± 1,46^{cf}	8,55 ± 0,32^{acd}
indica4	Agulha	10,64	74,92 ± 2,09^{ab}	85,16 ± 0,62^{abc}	10,08 ± 0,22^{di}
indica5	Agulha	10,89	79,59 ± 0,77^{ab}	81,61 ± 1,59^{bd}	8,56 ± 0,95^{aefhi}
indica6	Agulha	10,99	83,05 ± 2,53^{ab}	76,40 ± 1,06^{h}	6,47 ± 0,42^{bce}
indica7	Agulha	9,61	77,08 ± 3,76^{ab}	83,05 ± 1,01^{bdg}	7,52 ± 0,37^{abf}
indica8	Agulha	10,67	84,45 ± 4,2^{a}	84,18 ± 1,31^{abi}	7,74 ± 0,14^{abd}
japonica1	Carolino	11,30	82,92 ± 2,48^{ab}	81,12 ± 1,63^{dik}	7,13 ± 0,04^{ab}
japonica2	Carolino	10,77	81,82 ± 2,28^{ab}	81,30 ± 0,45^{dik}	6,96 ± 0,27^{ab}
japonica3	Carolino	11,65	80,49 ± 1,91^{ab}	83,44 ± 0,90^{abij}	8,19 ± 0,69^{ade}
japonica4	Carolino	10,36	78,14 ± 3,36^{ab}	86,23 ± 0,85^{acgl}	7,13 ± 0,79^{ab}
japonica5	Carolino	11,63	80,07 ± 2,57^{ab}	82,52 ± 0,41^{bgij}	7,41 ± 0,53^{abf}
japonica6	Carolino	11,28	80,76 ± 4,11^{ab}	84,08 ± 1,26^{abij}	7,18 ± 0,60^{ab}
japonica7	Carolino	12,46	80,12 ± 3,09^{ab}	84,26 ± 0,67^{abi}	8,29 ± 1,00^{ade}
japonica8	Carolino	11,97	74,79 ± 3,74^{ab}	90,75 ± 1,11^{ef}	7,93 ± 0,28^{abd}
japonica9	Medium	11,18	79,65 ± 4,46^{ab}	83,86 ± 0,70^{abij}	6,56 ± 0,38^{bce}
japonica10	Medium	10,21	80,64 ± 3,47^{ab}	82,30 ± 0,00^{bijo}	8,13 ± 0,21^{abdf}
japonica11	Medium	11,79	78,11 ± 4,62^{ab}	84,86 ± 2,11^{abck}	7,86 ± 0,48^{abd}
japonica12	Medium	11,12	79,53 ± 5,06^{ab}	83,37 ± 1,48^{abij}	8,41 ± 0,42^{ade}
japonica13	Medium	11,41	81,67 ± 6,07^{ab}	81,65 ± 0,87^{bin}	6,81 ± 0,69^{ab}
japonica14	Risotto	11,73	76,16 ± 3,79^{ab}	82,83 ± 1,51^{bigj}	8,72 ± 0,30^{ade}
japonica14 G0h	Brown Risotto	12,44	72,04 ± 1,04^{b}	81,26 ± 1,10^{dijk}	9,75 ± 1,10^{df}
japonica15	Round	11,64	76,42 ± 5,04^{ab}	85,86 ± 1,13^{acgo}	6,81 ± 0,88^{ab}
japonica15 G0h	Brown Round	11,99	79,65 ± 0,12^{ab}	83,82 ± 1,87^{abkn}	8,42 ± 0,24^{ade}
indica9	Aromatic Agulha	10,28	79,43 ± 2,59^{ab}	70,92 ± 1,37^{m}	8,50 ± 0,32^{ade}
indica10	Aromatic Agulha	10,21	76,68 ± 3,28^{ab}	75,46 ± 0,81^{h}	9,89 ± 0,71^{d}
indica11	Aromatic Agulha	10,74	83,03 ± 1,34^{ab}	71,05 ± 0,23^{m}	8,74 ± 0,21^{ade}
GABA1	Basmati, red, wild	11,25	79,51 ± 2,34^{ab}	80,38 ± 0,30^{djn}	8,62 ± 0,67^{ade}
GABA2	Round	12,88	74,89 ± 3,28^{ab}	82,79 ± 0,36^{bgij}	6,30 ± 0,38^{bch}
GABA3	Round	13,31	82,32 ± 4,28^{ab}	80,72 ± 0,94^{din}	6,56 ± 0,92^{bce}

ANNEX C2 –Chemical and nutritional characterization of milled, brown and commercial rice samples, in dry matter.

Sample	Type	Amylose (%) (grain)	Amylose (%) (Total starch)	Resistant starch (%) (grain)	Resistant starch (%) (Total starch)
indica1	Agulha (aromatic)	20,17 ± 0,12^{au}	15,18 ± 0,09^{afp}	5,81 ± 0,34^{abd}	4,38 ± 0,25^{abcdk}
indica2	Agulha (aromatic)	17,40 ± 1,29^{bgh}	13,48 ± 1,00^{bf}	5,33 ± 0,20^{acl}	4,13 ± 0,15^{acdps}
indica3	Agulha	23,37 ± 0,55^{cde}	18,65 ± 0,44^{ce}	4,98 ± 0,16^{ae}	3,98 ± 0,12^{adkp}
indica4	Agulha	22,10 ± 0,74^{du}	16,56 ± 0,56^{pql}	5,18 ± 0,18^{aefj}	3,88 ± 0,14^{akp}
indica5	Agulha	24,95 ± 1,49^{ev}	19,86 ± 1,19^{de}	7,18 ± 0,51^{i}	5,72 ± 0,40^{eh}
indica6	Agulha	25,11 ± 1,47^{ev}	20,85 ± 1,22^{dk}	6,37 ± 0,42^{bil}	5,29 ± 0,35^{efh}
indica7	Agulha	22,12 ± 0,64^{cu}	17,05 ± 0,49^{cq}	5,18 ± 0,42^{ahk}	3,99 ± 0,33^{agkp}
indica8	Agulha	25,79 ± 0,40^{v}	21,78 ± 0,34^{k}	7,17 ± 0,28^{i}	6,06 ± 0,24^{e}
japonica1	Carolino	15,20 ± 0,40^{bfk}	12,60 ± 0,33^{bgi}	6,35 ± 0,36^{di}	5,26 ± 0,29^{ef}
japonica2	Carolino	13,84 ± 1,21^{fikn}	11,32 ± 0,99^{ghmj}	5,76 ± 0,30^{abd}	4,71 ± 0,25^{dfgqr}
japonica3	Carolino	18,61 ± 0,61^{agj}	14,98 ± 0,49^{fp}	5,16 ± 0,39^{afjk}	4,15 ± 0,31^{acgs}
japonica4	Carolino	16,13 ± 0,50^{bf}	12,60 ± 0,39^{bhg}	5,99 ± 0,31^{bdfhl}	4,68 ± 0,24^{afl}
japonica5	Carolino	13,18 ± 0,45^{kn}	10,55 ± 0,36^{mj}	6,04 ± 0,30^{dfhl}	4,83 ± 0,24^{cfmo}
japonica6	Carolino	15,87 ± 0,59^{bfl}	12,82 ± 0,47^{bhi}	5,28 ± 0,25^{aefjq}	4,26 ± 0,20^{agkno}
japonica7	Carolino	14,48 ± 0,33^{fkmq}	11,60 ± 0,27^{him}	6,26 ± 0,75^{dilq}	5,01 ± 0,60^{bfhnq}
japonica8	Carolino	17,25 ± 0,66^{bjo}	12,90 ± 0,50^{bhn}	5,14 ± 0,14^{aejh}	3,85 ± 0,10^{kps}
japonica9	Medium	13,76 ± 0,44^{kln}	10,96 ± 0,35^{gm}	5,53 ± 0,17^{adlm}	4,41 ± 0,14^{aknor}
japonica10	Medium	16,59 ± 0,66^{bjmp}	13,38 ± 0,53^{bfi}	4,56 ± 0,00^{cejkm}	3,68 ± 0,00^{kps}
japonica11	Medium	11,64 ± 0,10^{in}	9,09 ± 0,08^{j}	5,71 ± 0,19^{adln}	4,46 ± 0,14^{aknoq}
japonica12	Medium	14,02 ± 0,29^{fkq}	11,15 ± 0,23^{ghmo}	5,34 ± 0,29^{adlmo}	4,25 ± 0,23^{aknor}
japonica13	Medium	15,38 ± 0,78^{bfq}	12,56 ± 0,64^{bgh}	5,55 ± 0,60^{adlm}	4,53 ± 0,49^{afk}
japonica14	Risotto	14,60 ± 0,32^{fkmr}	11,12 ± 0,25^{ghmo}	5,60 ± 0,40^{adlnr}	4,27 ± 0,31^{aknor}
japonica14 G0h	Brown Risotto	13,66 ± 0,38^{klnr}	9,84 ± 0,27^{mjo}	3,16 ± 0,07^{s}	2,27 ± 0,05^{i}
japonica15	Round	14,11 ± 0,80^{fkr}	10,78 ± 0,61^{gjm}	7,11 ± 0,25^{i}	5,43 ± 0,19^{ehlm}
japonica15 G0h	Brown Round	12,63 ± 0,36^{nqr}	10,06 ± 0,29^{jm}	4,29 ± 0,18^{ek}	3,41 ± 0,15^{s}
indica9	Aromatic Agulha	18,54 ± 0,26^{ahop}	14,73 ± 0,21^{fnl}	0,67 ± 0,02^{p}	0,54 ± 0,02^{j}
indica10	Aromatic Agulha	21,21 ± 1,48^{cdsu}	16,27 ± 1,13^{apql}	0,66 ± 0,02^{p}	0,50 ± 0,02j
indica11	Aromatic Agulha	19,26 ± 0,56^{ahos}	15,99 ± 0,47^{apql}	4,73 ± 0,23^{cejkmns}	3,93 ± 0,19^{ags}
GABA1	Basmati, red, wild	6,04 ± 0,30^{t}	4,81 ± 0,24^{l}	0,44 ± 0,01^{p}	0,35 ± 0,01^{j}
GABA2	Round	13,15 ± 0,48^{knr}	9,85 ± 0,36^{mj}	4,46 ± 0,26^{cejko}	3,34 ± 0,20^{ps}
GABA3	Round	12,72 ± 0,28^{inqr}	10,47 ± 0,23^{mj}	4,59 ± 0,12^{cejkmr}	3,78 ± 0,10^{ks}

ANNEX D – Correlations values for milled rice samples with all parameters analysed.

a) Milled indica samples

VARIABLE	L	W	L/W	TW	CW	CA	K	PV	TP	PT	HV	BD	SB	FV	M	TS	RS	RST	AM	AMT	GI	PR
L	**1.00**																					
W	0,11	**1.00**																				
L/W	0,69	-0,65	**1.00**																			
TW	0,06	0,08	0,01	**1.00**																		
CW	-0,08	0,37	-0,32	0,89	**1.00**																	
CA	0,14	-0,11	0,21	0,95	0,70	**1.00**																
K	0,09	-0,08	0,13	0,63	0,61	0,50	**1.00**															
PV	0,82	-0,29	0,85	0,13	-0,22	0,35	-0,10	**1.00**														
TP	0,06	0,75	-0,51	0,00	0,26	-0,15	-0,13	-0,28	**1.00**													
PT	-0,53	-0,08	-0,35	0,24	0,24	0,19	0,43	-0,52	0,29	**1.00**												
HV	0,90	-0,03	0,73	0,11	-0,17	0,29	-0,12	0,96	-0,04	-0,52	**1.00**											
BD	0,13	-0,89	0,76	0,11	-0,25	0,33	0,01	0,58	-0,84	-0,26	0,34	**1.00**										
SB	0,02	0,75	-0,52	0,15	0,24	0,09	-0,19	-0,11	0,82	0,27	0,10	-0,70	**1.00**									
FV	0,66	0,31	0,30	0,20	0,00	0,34	-0,22	0,71	0,36	-0,22	0,83	-0,04	0,62	**1.00**								
M	0,13	-0,07	0,15	0,00	0,00	0,02	0,04	0,07	0,59	0,53	0,14	-0,16	0,35	0,31	**1.00**							
TS	-0,36	0,28	-0,46	-0,44	-0,35	-0,39	-0,85	-0,16	0,26	-0,20	-0,11	-0,22	0,47	0,20	-0,08	**1.00**						
RS	0,14	0,47	-0,22	-0,20	-0,20	-0,17	-0,39	0,15	0,39	-0,11	0,29	-0,35	0,74	0,64	0,06	0,66	**1.00**					
RST	0,03	0,45	-0,29	-0,28	-0,25	-0,24	-0,53	0,08	0,38	-0,14	0,20	-0,34	0,71	0,57	0,02	0,78	0,98	**1.00**				
AM	-0,24	0,84	-0,79	-0,17	0,12	-0,31	-0,45	-0,42	0,60	-0,13	-0,24	-0,75	0,72	0,18	-0,20	0,72	0,64	0,70	**1.00**			
AMT	-0,29	0,72	-0,74	-0,26	-0,02	-0,36	-0,59	-0,37	0,54	-0,15	-0,22	-0,65	0,69	0,19	-0,17	0,85	0,69	0,77	0,98	**1.00**		
GI	-0,54	-0,23	-0,26	0,42	0,45	0,31	0,68	-0,55	0,03	0,89	-0,62	-0,08	-0,08	-0,49	0,31	-0,47	-0,44	-0,47	-0,33	-0,39	**1.00**	
PR	-0,08	0,52	-0,45	0,42	0,70	0,15	0,72	-0,52	0,49	0,47	-0,39	-0,65	0,30	-0,20	0,17	-0,44	-0,09	-0,18	0,19	0,02	0,58	**1.00**

Strength of Pearson's correlation

0 < r < 0.19 – very weak

0.2 < r < 0.39 – weak

0.40 < r < 0.59 – moderate

0.60 < r < 0.79 – strong

0.80 < r < 1.0 – very strong

Length (mm) – L
Width (mm) – L
Length/Width ratio – L/W
Total whiteness – TW
Crystalline whiteness – CW
Chalky area (%) – CA
Kett – K
Peak viscosity (cP) – PV
Time of the peak (minutes) – TP
Pasting temperature (°C) – PT
Holding viscosity (cP) – HV
Breakdown (cP) – BD
Setback (cP) - SB
Final viscosity (cP) - FV
Moisture (%) - M
Total starch (%) - TS
Resistant starch (%) - RS
Resistant starch in total starch (%) - RST
Amylose (%) - AM
Amylose starch in total starch (%) - AMT
Glycemic index - GI
Protein (%) - PR

b) Milled japonica samples

VARIABLE	L	W	L/W	TW	CT	CA	K	PV	TP	PT	HV	BD	SB	FV	M	TS	RS	RST	AM	AMT	GI	PR
L	**1.00**																					
W	-0,44	**1.00**																				
L/W	0,86	-0,84	**1.00**																			
TW	-0,45	0,92	-0,79	**1.00**																		
CW	0,55	-0,24	0,45	-0,25	**1.00**																	
CA	-0,56	0,90	-0,84	0,98	-0,45	**1.00**																
K	-0,43	0,93	-0,79	0,93	-0,20	0,90	**1.00**															
PV	-0,45	-0,02	-0,27	0,08	-0,32	0,15	0,14	**1.00**														
TP	-0,45	0,70	-0,65	0,75	-0,34	0,77	0,70	0,07	**1.00**													
PT	0,60	-0,15	0,45	-0,25	0,46	-0,34	-0,28	-0,81	-0,17	**1.00**												
HV	-0,35	-0,17	-0,12	-0,19	-0,37	-0,09	-0,08	0,85	0,03	-0,58	**1.00**											
BD	-0,43	0,12	-0,33	0,30	-0,19	0,32	0,31	0,88	0,08	-0,80	0,50	**1.00**										
SB	0,53	-0,26	0,47	-0,40	0,33	-0,45	-0,37	-0,90	-0,30	0,78	-0,64	-0,90	**1.00**									
FV	0,06	-0,58	0,36	-0,65	-0,05	-0,59	-0,44	0,41	-0,46	-0,21	0,61	0,13	0,03	**1.00**								
M	0,22	-0,30	0,29	-0,33	-0,14	-0,30	-0,41	0,01	-0,43	0,00	0,19	-0,16	0,12	0,27	**1.00**							
TS	-0,07	-0,38	0,18	-0,45	0,04	-0,40	-0,33	0,24	-0,15	-0,20	0,20	0,22	-0,11	0,31	-0,32	**1.00**						
RS	0,00	-0,12	0,06	-0,12	-0,25	-0,05	-0,23	0,23	-0,24	-0,30	0,25	0,16	-0,21	0,09	0,33	-0,09	**1.00**					
RST	0,01	-0,23	0,13	-0,25	-0,22	-0,17	-0,33	0,28	-0,28	-0,33	0,29	0,21	-0,23	0,17	0,25	0,19	0,96	**1.00**				
AM	0,27	0,03	0,16	0,03	0,22	-0,04	0,06	-0,53	0,13	0,27	-0,55	-0,37	0,46	-0,25	-0,16	0,01	-0,45	-0,44	**1.00**			
AMT	0,24	-0,06	0,19	-0,07	0,21	-0,13	-0,01	-0,45	0,09	0,20	-0,48	-0,30	0,42	-0,16	-0,24	0,25	-0,45	-0,38	0,97	**1.00**		
GI	0,27	0,07	0,12	0,13	0,35	0,02	0,03	-0,29	-0,02	0,34	-0,16	-0,33	0,21	-0,23	0,29	-0,80	0,00	-0,21	0,24	0,02	**1.00**	
PR	0,12	0,24	-0,05	0,22	-0,30	0,26	0,17	-0,39	0,27	0,22	-0,24	-0,43	0,31	-0,25	0,29	-0,30	-0,40	-0,47	0,18	0,10	0,10	**1.00**

Strength of Pearson's correlation

0 < r < 0.19 – very weak

0.2 < r < 0.39 – weak

0.40 < r < 0.59 – moderate

0.60 < r < 0.79 – strong

0.80 < r < 1.0 – very strong

Length (mm) – L
Width (mm) – L
Length/Width ratio – L/W
Total whiteness – TW
Crystalline whiteness – CW
Chalky area (%) – CA
Kett – K
Peak viscosity (cP) – PV
Time of the peak (minutes) – TP
Breakdown (cP) – BD
Setback (cP) - SB
Final viscosity (cP) - FV
Moisture (%) - M
Total starch (%) - TS
Resistant starch (%) - RS
Resistant starch in total starch (%) - RST
Amylose (%) - AM
Amylose starch in total starch (%) - AMT

c) All milled samples

VARIABLE	L	W	L/W	TW	CW	CA	K	PV	TP	PT	HV	BD	SB	FV	M	TS	RS	RST	AM	AMT	GI	PR
L	**1.00**																					
W	-0,74	**1.00**																				
L/W	0,90	-0,95	**1.00**																			
TW	-0,49	0,69	-0,59	**1.00**																		
CW	0,13	0,06	-0,02	0,00	**1.00**																	
CA	-0,50	0,64	-0,55	0,97	-0,25	**1.00**																
K	-0,33	0,52	-0,41	0,88	-0,06	0,86	**1.00**															
PV	-0,33	0,44	-0,38	0,29	-0,15	0,31	0,16	**1.00**														
TP	0,59	-0,57	0,61	-0,24	-0,08	-0,19	-0,08	-0,54	**1.00**													
PT	0,17	-0,20	0,17	-0,16	0,34	-0,24	-0,13	-0,63	0,23	**1.00**												
HV	0,27	-0,07	0,20	-0,06	-0,23	0,00	-0,07	0,74	0,00	-0,51	**1.00**											
BD	-0,72	0,70	-0,73	0,47	-0,01	0,45	0,31	0,80	-0,79	-0,46	0,18	**1.00**										
SB	0,72	-0,69	0,72	-0,45	0,06	-0,44	-0,32	-0,68	0,84	0,42	-0,07	-0,93	**1.00**									
FV	0,64	-0,48	0,59	-0,31	-0,08	-0,27	-0,26	0,11	0,59	-0,07	0,67	-0,44	0,66	**1.00**								
M	-0,16	0,21	-0,22	-0,06	-0,04	-0,07	-0,21	0,22	-0,14	0,16	0,13	0,21	-0,17	0,00	**1.00**							
TS	-0,20	0,01	-0,14	-0,33	-0,08	-0,28	-0,38	0,07	0,02	-0,21	-0,01	0,10	0,04	0,13	-0,16	**1.00**						
RS	0,15	-0,08	0,09	-0,17	-0,23	-0,10	-0,26	0,08	0,25	-0,17	0,27	-0,13	0,28	0,46	0,14	0,29	**1.00**					
RST	0,10	-0,08	0,06	-0,25	-0,23	-0,18	-0,33	0,07	0,24	-0,20	0,23	-0,11	0,28	0,45	0,08	0,51	0,97	**1.00**				
AM	0,66	-0,68	0,70	-0,37	-0,02	-0,34	-0,20	-0,67	0,84	0,17	-0,14	-0,85	0,88	0,51	-0,39	0,12	0,20	0,22	**1.00**			
AMT	0,61	-0,66	0,66	-0,40	-0,05	-0,36	-0,24	-0,64	0,82	0,13	-0,14	-0,81	0,86	0,51	-0,39	0,27	0,25	0,30	0,99	**1.00**		
GI	-0,12	0,03	-0,08	0,19	0,37	0,08	0,17	-0,33	-0,04	0,60	-0,44	-0,08	-0,01	-0,35	0,30	-0,62	-0,23	-0,36	-0,08	-0,17	**1.00**	
PR	0,16	-0,02	0,06	0,13	0,09	0,09	0,22	-0,49	0,40	0,38	-0,33	-0,42	0,33	-0,06	0,13	-0,39	-0,18	-0,25	0,26	0,19	0,36	**1.00**

Strength of Pearson's correlation

0 < r < 0.19 – very weak

0.2 < r < 0.39 – weak

0.40 < r < 0.59 – moderate

0.60 < r < 0.79 – strong

0.80 < r < 1.0 – very strong

Length (mm) – L
Width (mm) – L
Length/Width ratio – L/W
Total whiteness – TW
Crystalline whiteness – CW
Chalky area (%) – CA
Kett – K
Peak viscosity (cP) – PV
Time of the peak (minutes) – TP
Pasting temperature (°C) – PT
Holding viscosity (cP) – HV
Breakdown (cP) – BD
Setback (cP) - SB
Final viscosity (cP) - FV
Moisture (%) - M
Total starch (%) - TS
Resistant starch (%) - RS
Resistant starch in total starch (%) - RST
Amylose (%) - AM
Amylose starch in total starch (%) - AMT
Glycemic index - GI
Protein (%) - PR

ANNEX E1 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

Sample	Type	Moisture (%)	Total starch(%)	Amylose (%) (grain)	Amylose (%) (in total starch)
japonica14 G0h	Brown Risotto	12,44	$72,04 \pm 1,04^{afgh}$	$13,66 \pm 0,38^{bg}$	$9,84 \pm 0,27^{aj}$
japonica14 G24h_w	Germinated Brown Risotto	7,67	$68,96 \pm 3,03^{bcdegj}$	$13,29 \pm 0,21^{ag}$	$9,16 \pm 0,15^{bcej}$
japonica14 G48h_w	Germinated Brown Risotto	8,33	$65,49 \pm 3,95^{bcfi}$	$13,13 \pm 0,14^{ag}$	$8,60 \pm 0,09^{cd}$
japonica14 G72h_w	Germinated Brown Risotto	7,77	$60,01 \pm 3,49^{ji}$	$13,15 \pm 0,28^{ag}$	$7,89 \pm 0,17^{d}$
japonica14 G24h_3.0	Germinated Brown Risotto	7,54	$67,88 \pm 1,16^{bcdefj}$	$11,77 \pm 0,07^{dh}$	$7,99 \pm 0,05^{d}$
japonica14 G48h_3.0	Germinated Brown Risotto	8,59	$65,60 \pm 3,31^{bcfi}$	$10,58 \pm 0,07^{di}$	$6,94 \pm 0,05^{g}$
japonica14 G72h_3.0	Germinated Brown Risotto	8,01	$58,61 \pm 1,31^{i}$	$8,79 \pm 0,23^{fj}$	$5,15 \pm 0,14^{fh}$
japonica14 G24h_4.0	Germinated Brown Risotto	7,42	$67,42 \pm 5,31^{bcefi}$	$10,17 \pm 0,42^{ik}$	$6,85 \pm 0,28^{g}$
japonica 14 G48h_4.0	Germinated Brown Risotto	8,53	$64,95 \pm 1,00^{cfi}$	$6,93 \pm 0,36^{e}$	$4,50 \pm 0,23^{h}$
japonica 14 G72h_4.0	Germinated Brown Risotto	7,85	$64,93 \pm 0,52^{bcfi}$	$9,24 \pm 0,53^{ik}$	$6,00 \pm 0,35^{ik}$
japonica15 G0h	Brown Round	11,99	$79,65 \pm 0,12^{a}$	$12,63 \pm 0,36^{abh}$	$10,06 \pm 0,29^{a}$
japonica15 G24h_w	Germinated Brown Round	6,53	$78,58 \pm 1,68^{a}$	$12,73 \pm 0,08^{abh}$	$10,00 \pm 0,07^{ab}$
japonica15 G48h_w	Germinated Brown Round	7,23	$74,21 \pm 0,80^{ab}$	$12,58 \pm 0,66^{ah}$	$9,33 \pm 0,49^{ac}$
japonica15 G72h_w	Germinated Brown Round	6,90	$70,22 \pm 3,40^{acd}$	$12,14 \pm 0,33^{ach}$	$8,53 \pm 0,24^{de}$
japonica15 G24h_3.0	Germinated Brown Round	6,72	$77,85 \pm 2,10^{ad}$	$11,08 \pm 0,15^{cdi}$	$8,62 \pm 0,12^{cd}$
japonica15 G48h_3.0	Germinated Brown Round	7,49	$75,57 \pm 6,37^{aeh}$	$10,85 \pm 0,34^{di}$	$8,20 \pm 0,26^{de}$
japonica15 G72h_3.0	Germinated Brown Round	6,96	$68,04 \pm 1,58^{bdefj}$	$8,09 \pm 0,13^{ef}$	$5,50 \pm 0,09^{fhi}$
japonica15 G24h_4.0	Germinated Brown Round	6,72	$77,99 \pm 3,36^{ag}$	$8,07 \pm 0,14^{ef}$	$6,29 \pm 0,11^{gk}$
japonica15 G48h_4.0	Germinated Brown Round	7,78	$66,46 \pm 3,04^{bchi}$	$7,67 \pm 0,33^{e}$	$5,10 \pm 0,22^{h}$
japonica15 G72h_4.0	Germinated Brown Round	6,79	$64,01 \pm 1,53^{cfij}$	$8,10 \pm 0,48^{ef}$	$5,18 \pm 0,31^{fh}$

ANNEX E2 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

Sample	Type	Resistant starch (%) (grain)	Resistant starch (%) (in total starch)	Glycemic index	Protein (%)
japonica14 G0h	Brown Risotto	$3,17 \pm 0,07^{ilm}$	$2,29 \pm 0,06^{jkp}$	$81,26 \pm 1,05^{ae}$	$9,75 \pm 1,10^{beg}$
japonica14 G24h_w	Germinated Brown Risotto	$4,83 \pm 0,29^{acdf}$	$3,33 \pm 0,20^{cdif}$	$86,81 \pm 0,72^{hj}$	$9,91 \pm 0,32^{beg}$
japonica14 G48h_w	Germinated Brown Risotto	$5,81 \pm 0,35^{bc}$	$3,80 \pm 0,23a^{ilo}$	$89,38 \pm 1,49^{cghm}$	$9,89 \pm 0,29^{beg}$
japonica14 G72h_w	Germinated Brown Risotto	$4,76 \pm 0,16^{acdf}$	$2,85 \pm 0,10f^{hkp}$	$94,96 \pm 0,25^{fikl}$	$9,92 \pm 0,20^{beg}$
japonica14 G24h_3.0	Germinated Brown Risotto	$4,44 \pm 0,12^{ajk}$	$3,01 \pm 0,08f^{gkl}$	$85,53 \pm 0,39^{gj}$	$10,95 \pm 1,62^{bg}$
japonica14 G48h_3.0	Germinated Brown Risotto	$4,06 \pm 0,14^{fghik}$	$2,66 \pm 0,09^{ckp}$	$87,81 \pm 2,51^{gh}$	$10,50 \pm 0,48^{bgh}$
japonica14 G72h_3.0	Germinated Brown Risotto	$2,17 \pm 0,05^{l}$	$1,27 \pm 0,03^{m}$	$97,57 \pm 1,84^{k}$	$10,81 \pm 0,21^{gi}$
japonica14 G24h_4.0	Germinated Brown Risotto	$2,99 \pm 0,06^{il}$	$2,01 \pm 0,04^{mp}$	$88,58 \pm 0,71^{cgh}$	$10,35 \pm 0,83^{bcfg}$
japonica 14 G48h_4.0	Germinated Brown Risotto	$3,72 \pm 0,37^{dik}$	$2,42 \pm 0,24^{jkp}$	$88,82 \pm 0,89^{cgh}$	$10,45 \pm 0,63^{bcg}$
japonica 14 G72h_4.0	Germinated Brown Risotto	$3,92 \pm ,10^{fgik}$	$2,54 \pm 0,06^{dkp}$	$92,56 \pm 0,91^{dlm}$	$10,68 \pm 0,04^{bdg}$
japonica15 G0h	Brown Round	$4,30 \pm 0,19^{adfm}$	$3,42 \pm 0,15^{acdf}$	$83,82 \pm 1,83^{aj}$	$8,42 \pm 0,24^{abe}$
japonica15 G24h_w	Germinated Brown Round	$4,74 \pm 1,03^{abdf}$	$3,73 \pm 0,81^{abf}$	$76,40 \pm 1,78^{b}$	$8,07 \pm 1,05^{abe}$
japonica15 G48h_w	Germinated Brown Round	$4,94 \pm 0,36^{acf}$	$3,67 \pm 0,27^{abf}$	$88,85 \pm 1,38^{cghm}$	$7,38 \pm 0,50^{ae}$
japonica15 G72h_w	Germinated Brown Round	$7,13 \pm 0,82^{e}$	$5,01 \pm 0,58^{e}$	$93,62 \pm 1,20^{df}$	$7,24 \pm 0,66^{ae}$
japonica15 G24h_3.0	Germinated Brown Round	$5,54 \pm 0,42^{bcj}$	$4,31 \pm 0,32^{ae}$	$78,15 \pm 0,46^{be}$	$7,92 \pm 0,47^{aceh}$
japonica15 G48h_3.0	Germinated Brown Round	$4,98 \pm 0,47^{acf}$	$3,76 \pm 0,36^{agio}$	$78,81 \pm 0,61^{be}$	$7,78 \pm 0,19^{ace}$
japonica15 G72h_3.0	Germinated Brown Round	$5,42 \pm 0,21^{ac}$	$3,69 \pm 0,14^{aghio}$	$90,07 \pm 0,92^{cdh}$	$7,99 \pm 0,27^{acdeh}$
japonica15 G24h_4.0	Germinated Brown Round	$5,65 \pm 0,53^{bcj}$	$4,40 \pm 0,41^{beo}$	$75,54 \pm 1,80^{b}$	$6,92 \pm 0,94^{a}$
japonica15 G48h_4.0	Germinated Brown Round	$4,70 \pm 0,20^{acdg}$	$3,12 \pm 0,13^{cdfij}$	$83,57 \pm 0,49^{aj}$	$7,69 \pm 0,64^{aef}$
japonica15 G72h_4.0	Germinated Brown Round	$5,19 \pm 0,17^{ach}$	$3,32 \pm 0,11^{cdfi}$	$91,82 \pm 0,91^{cdi}$	$8,22 \pm 0,38^{acdefi}$

ANNEX E3 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

Sample	Type	Reducing sugars (mg/100 g dry matter)	GABA (mg/100 g dry matter)
japonica14 G0h	Brown Risotto	$44{,}04 \pm 1{,}49^{ac}$	$6{,}10 \pm 1{,}62^{ag}$
japonica14 G24h_w	Germinated Brown Risotto	$42{,}78 \pm 1{,}23^{a}$	$12{,}97 \pm 2{,}37^{abdfg}$
japonica14 G48h_w	Germinated Brown Risotto	$48{,}44 \pm 2{,}68^{ac}$	$17{,}45 \pm 4{,}64^{bcdef}$
japonica14 G72h_w	Germinated Brown Risotto	$56{,}05 \pm 4{,}59^{acde}$	$17{,}61 \pm 0{,}54^{bcdef}$
japonica14 G24h_3.0	Germinated Brown Risotto	$53{,}93 \pm 1{,}77^{acd}$	$16{,}37 \pm 6{,}53^{abdfg}$
japonica14 G48h_3.0	Germinated Brown Risotto	$59{,}46 \pm 3{,}37^{acdek}$	$20{,}41 \pm 0{,}40^{df}$
japonica14 G72h_3.0	Germinated Brown Risotto	$81{,}08 \pm 3{,}08^{bfghj}$	$20{,}95 \pm 0{,}62^{bdf}$
japonica14 G24h_4.0	Germinated Brown Risotto	$42{,}52 \pm 0{,}29^{ac}$	$14{,}63 \pm 1{,}77^{abd}$
japonica 14 G48h_4.0	Germinated Brown Risotto	$54{,}73 \pm 5{,}64^{acde}$	$14{,}07 \pm 1{,}21^{abd}$
japonica 14 G72h_4.0	Germinated Brown Risotto	$53{,}28 \pm 6{,}83^{acd}$	$18{,}04 \pm 1{,}97^{bde}$
japonica15 G0h	Brown Round	$53{,}37 \pm 6{,}86^{acd}$	$9{,}27 \pm 0{,}08^{ae}$
japonica15 G24h_w	Germinated Brown Round	$62{,}61 \pm 12{,}70^{acdf}$	$20{,}24 \pm 0{,}41^{bdf}$
japonica15 G48h_w	Germinated Brown Round	$52{,}65 \pm 1{,}98^{acd}$	$23{,}62 \pm 0{,}31^{cdf}$
japonica15 G72h_w	Germinated Brown Round	$69{,}04 \pm 10{,}04^{dfgij}$	$18{,}39 \pm 3{,}32^{bde}$
japonica15 G24h_3.0	Germinated Brown Round	$56{,}57 \pm 6{,}16^{acde}$	$17{,}78 \pm 1{,}60^{bde}$
japonica15 G48h_3.0	Germinated Brown Round	$75{,}32 \pm 1{,}57^{efgij}$	$29{,}76 \pm 4{,}20^{f}$
japonica15 G72h_3.0	Germinated Brown Round	$78{,}61 \pm 12{,}54^{fghjk}$	$43{,}62 \pm 3{,}17^{h}$
japonica15 G24h_4.0	Germinated Brown Round	$59{,}42 \pm 10{,}64^{acik}$	$29{,}59 \pm 3{,}48^{f}$
japonica15 G48h_4.0	Germinated Brown Round	$61{,}93 \pm 6{,}64^{cjk}$	$19{,}52 \pm 0{,}55^{bdef}$
japonica15 G72h_4.0	Germinated Brown Round	$61{,}08 \pm 11{,}24^{aikj}$	$14{,}80 \pm 3{,}85^{abd}$

ANNEX E4 – Chemical and nutritional characterization of brown and germinated rice samples, in dry matter.

Sample	Type	Soluble phenolic compounds(mg/100 g dry matter)	Insoluble phenolic compounds (mg/100 g dry matter)
japonica14 G0h	Brown Risotto	$47,52 \pm 2,29^{adefg}$	$896,28 \pm 14,16^{a}$
japonica14 G24h_w	Germinated Brown Risotto	$37,43 \pm 0,96^{b}$	$841,98 \pm 34,22^{b}$
japonica14 G48h_w	Germinated Brown Risotto	$46,49 \pm 1,20^{adefg}$	$816,77 \pm 6,45^{bc}$
japonica14 G72h_w	Germinated Brown Risotto	$43,14 \pm 1,81^{abg}$	$810,57 \pm 26,90^{bc}$
japonica14 G24h_3.0	Germinated Brown Risotto	$41,17 \pm 1,78^{ab}$	$806,54 \pm 6,39^{bc}$
japonica14 G48h_3.0	Germinated Brown Risotto	$44,65 \pm 2,45^{adgh}$	$814,50 \pm 32,91^{bc}$
japonica14 G72h_3.0	Germinated Brown Risotto	$44,71 \pm 2,44^{adgh}$	$828,88 \pm 7,07^{b}$
japonica14 G24h_4.0	Germinated Brown Risotto	$39,27 \pm 1,54^{bhl}$	$843,59 \pm 8,19^{ab}$
japonica 14 G48h_4.0	Germinated Brown Risotto	$45,01 \pm 2,91^{adegh}$	$764,63 \pm 33,05^{c}$
japonica 14 G72h_4.0	Germinated Brown Risotto	$36,71 \pm 2,67^{b}$	$799,65 \pm 11,36^{bc}$
japonica15 G0h	Brown Round	$66,69 \pm 3,03^{i}$	$671,78 \pm 29,37^{d}$
japonica15 G24h_w	Germinated Brown Round	$54,60 \pm 3,81^{j}$	$654,08 \pm 12,47^{de}$
japonica15 G48h_w	Germinated Brown Round	$49,62 \pm 1,89^{gjk}$	$594,16 \pm 2,42^{ef}$
japonica15 G72h_w	Germinated Brown Round	$51,12 \pm 2,23^{djk}$	$615,11 \pm 10,89^{def}$
japonica15 G24h_3.0	Germinated Brown Round	$48,72 \pm 2,59^{gjk}$	$637,47 \pm 11,27^{def}$
japonica15 G48h_3.0	Germinated Brown Round	$51,69 \pm 2,19^{ejk}$	$615,26 \pm 8,71^{def}$
japonica15 G72h_3.0	Germinated Brown Round	$45,70 \pm 1,50^{akl}$	$638,02 \pm 5,74^{def}$
japonica15 G24h_4.0	Germinated Brown Round	$49,98 \pm 1,66^{gjk}$	$635,97 \pm 2,48^{def}$
japonica15 G48h_4.0	Germinated Brown Round	$52,97 \pm 1,37^{fj}$	$586,08 \pm 9,20^{f}$
japonica15 G72h_4.0	Germinated Brown Round	$46,98 \pm 2,20^{afk}$	$636,44 \pm 47,17^{def}$

ANNEX F – Correlations values for brown and germinated rice samples with all parameters analysed.

a) **All ungerminated and germinated samples**

VARIABLE	M	TS	RS	RST	AM	AMT	GI	PR	SUG	SPC	IPC	GABA
M	**1.00**											
TS	0,09	**1.00**										
RS	-0,44	0,35	**1.00**									
RST	-0,37	0,61	0,95	**1.00**								
AM	0,33	0,29	0,15	0,20	**1.00**							
AMT	0,33	0,61	0,23	0,37	0,93	**1.00**						
GI	-0,05	-0,83	-0,17	-0,41	-0,11	-0,39	**1.00**					
PR	0,29	-0,64	-0,68	-0,79	0,06	-0,18	0,46	**1.00**				
SUG	-0,10	-0,70	-0,32	-0,48	-0,28	-0,49	0,61	0,48	**1.00**			
SPC	0,71	-0,24	-0,47	-0,50	0,38	0,22	0,14	0,58	0,25	**1.00**		
IPC	-0,14	0,74	0,49	0,66	-0,03	0,26	-0,48	-0,90	-0,48	-0,61	**1.00**	
GABA	-0,30	-0,61	-0,40	-0,49	-0,26	-0,44	0,52	0,46	0,69	0,00	-0,49	**1.00**

Strength of Pearson's correlation

0 < r < 0.19 – very weak

0.2 < r < 0.39 – weak

0.40 < r < 0.59 – moderate

0.60 < r < 0.79 – strong

0.80 < r < 1.0 – very strong

Moisture (%) – M
Total starch (%) - TS
Resistant starch (%) - RS
Resistant starch in total starch (%) - RST
Amylose (%) - AM
Amylose starch in total starch (%) - AMT
Glycemic index - GI
Protein (%) – PR
Reducing sugars (mg/100g dry matter) - SUG
Soluble phenolic compounds (mg/100g dry matter) – SPC
Insoluble phenolic compounds (mg/100g of dry matter) – IPC
GABA (mg/100g of dry matter) - GABA

b) Ungerminated and germinated japonica14 samples

VARIABLE	M	TS	RS	RST	AM	AMT	GI	PR	SUG	SPC	IPC	GABA
M	**1.00**											
TS	0,50	**1.00**										
RS	-0,23	0,14	**1.00**									
RST	-0,14	0,33	0,98	**1.00**								
AM	0,29	0,39	0,54	0,58	**1.00**							
AMT	0,41	0,61	0,48	0,57	0,97	**1.00**						
GI	-0,57	-0,96	-0,16	-0,34	-0,40	-0,60	**1.00**					
PR	-0,46	-0,33	-0,41	-0,43	-0,69	-0,68	0,32	**1.00**				
SUG	-0,31	-0,72	-0,40	-0,51	-0,38	-0,52	0,66	0,37	**1.00**			
SPC	0,88	0,69	-0,05	0,07	0,46	0,60	-0,74	-0,66	-0,41	**1.00**		
IPC	0,39	0,46	-0,39	-0,28	0,40	0,49	-0,35	-0,10	-0,11	0,45	**1.00**	
GABA	-0,43	-0,60	-0,42	-0,49	-0,40	-0,50	0,59	0,41	0,70	-0,58	-0,19	**1.00**

Strength of Pearson's correlation

0 < r < 0.19 – very weak

0.2 < r < 0.39 – weak

0.40 < r < 0.59 – moderate

0.60 < r < 0.79 – strong

0.80 < r < 1.0 – very strong

Moisture (%) – M
Total starch (%) - TS
Resistant starch (%) - RS
Resistant starch in total starch (%) - RST
Amylose (%) - AM
Amylose starch in total starch (%) - AMT
Glycemic index - GI
Protein (%) – PR
Reducing sugars (mg/100g dry matter) - SUG
Soluble phenolic compounds (mg/100g dry matter) – SPC
Insoluble phenolic compounds (mg/100g of dry matter) – IPC
GABA (mg/100g of dry matter) - GABA

VARIABLE	M	TS	RS	RST	AM	AMT	GI	PR	SUG	SPC	IPC	GABA
M	**1.00**											
TS	0,31	**1.00**										
RS	-0,49	-0,22	**1.00**									
RST	-0,36	0,28	0,87	**1.00**								
AM	0,30	0,62	-0,02	0,26	**1.00**							
AMT	0,35	0,77	-0,11	0,25	0,98	**1.00**						
GI	0,02	-0,72	0,38	-0,01	-0,06	-0,25	**1.00**					
PR	0,46	-0,04	-0,58	-0,61	0,13	0,13	0,06	**1.00**				
SUG	-0,23	-0,55	0,24	-0,04	-0,41	-0,48	0,45	0,14	**1.00**			
SPC	0,60	0,08	-0,20	-0,17	0,25	0,22	0,24	0,01	0,26	**1.00**		
IPC	0,62	0,68	-0,25	0,07	0,46	0,56	-0,22	0,26	-0,31	0,15	**1.00**	
GABA	-0,75	-0,50	0,46	0,21	-0,34	-0,43	0,31	-0,30	0,68	-0,13	-0,59	**1.00**

c) Ungerminated and germinated japonica15 samples

Strength of Pearson's correlation

0 < r < 0.19 – very weak

0.2 < r < 0.39 – weak

0.40 < r < 0.59 – moderate

0.60 < r < 0.79 – strong

0.80 < r < 1.0 – very strong

Moisture (%) – M
Total starch (%) - TS
Resistant starch (%) - RS
Resistant starch in total starch (%) - RST
Amylose (%) - AM
Amylose starch in total starch (%) - AMT
Glycemic index - GI
Protein (%) – PR
Reducing sugars (mg/100g dry matter) - SUG
Soluble phenolic compounds (mg/100g dry matter) – SPC
Insoluble phenolic compounds (mg/100g of dry matter) – IPC
GABA (mg/100g of dry matter) - GABA