Universidade de Aveiro Departamento de Química
2013/2014

INÊS NUNES CARDOSO

Caracterização de Diferentes Variedades de Arroz – Efeito de Diferentes Métodos de Cozedura

Characterisation of Different Rice Varieties – Effect of Different Cooking Methods

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Characterisation of Different Rice Varieties – Effect of Different Cooking Methods

Relatório de Estágio apresentado à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia – Ramo Biotecnologia Alimentar, realizada 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.

A todos os que fizeram, fazem e irão fazer parte da minha vida:

"*You don’t love someone because they're perfect, you love them in spite of the fact that they're not*"
Jodi Picoult

o júri

presidente

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agradecimentos

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Palavras-chave Arroz branqueado, caracterização, composição, métodos de cozedura, microondas, vapor.

Resumo

O arroz (*Oryza sativa*) é uma das principais culturas a nível mundial, sendo consumido por mais de metade da população do mundo. Embora as suas características e composição variem, de um modo geral o arroz branqueado é composto por amido, água, proteínas, lípidos, fibra dietética, vitaminas e minerais. O arroz vaporizado é processado de um modo diferente e representa uma percentagem significativa da produção total de arroz a nível mundial.

Devido ao estilo de vida atual e ao aumento do uso de fornos micro-ondas, congeladores e frigoríficos, os consumidores têm cada vez mais tendência a guardar restos de comida. O arroz branqueado é a forma de arroz mais consumida, sendo cozinhado por diferentes métodos. A composição do arroz tem uma grande influência nas suas características de cozedura e determina a preferência que os consumidores têm pelo arroz.

Este trabalho pretendeu caracterizar as diferentes variedades de arroz comercializadas pela empresa Novarroz em termos de tamanho, brancura e composição. As variedades escolhidas pertencem aos tipos aromático, agulha, agulha América do Sul, agulha vaporizado, carolino, médio, *risotto*, redondo e redondo vaporizado. Diferentes métodos de cozinhar arroz, tal como o forno micro-ondas e o vapor, foram testados e comparados com o método comum de cozedura em água fervente.

Fortes correlações foram detetadas entre os parâmetros de tamanho, brancura e composição analisados. Pode-se concluir que a composição do arroz tem uma grande influência nos tempos de cozedura e aparências registadas. Dos três métodos de cozedura testados, a pré-cozedura durante 10 minutes, seguida de congelamento e posterior descongelamento e cozedura usando um forno micro-ondas permitiu o método de cozedura mais rápido, do ponto de vista do consumidor. O arroz cozido obtido, com exceção de algumas variedades, era visualmente agradável. Uma grande variedade de arrozes cozinhou com uma aparência "pegajosa", mas alguns cozinharam com a aparência final "solta/não-pegajosa".

Keywords Milled rice, characterization, composition, cooking methods, microwave, steam.

Abstract

Rice (*Oryza sativa*) is one of the leading food crops in the world and it is consumed by more than half of the world's population. Although its characteristics and composition vary, in general, milled rice is composed of starch, water, proteins, lipids, dietary fibre, vitamins and minerals. Parboiled rice, a differently processed rice, represents a significant percentage of the total worldwide rice production.

Due to the current lifestyle and increasing use of microwave ovens, freezers and refrigerators, consumers tend to store food leftovers more than ever. Milled rice is one of the most consumed form of rice, being cooked with various different methods. Rice composition highly influences its cooking characteristics and determines rice preference by consumers.

This work intended to characterise the different rice varieties commercialised by the company Novarroz in terms of size, whiteness and composition. The varieties chosen belong to the types aromatic, *agulha*, *agulha* South America, *agulha* parboiled, *carolino*, medium, *risotto*, round and round parboiled. Different cooking methods, such as using a microwave oven and steam, were also tested and compared with the ordinary boiling method.

Strong correlations were found between the size, whiteness and composition parameters analysed. It can be concluded that rice's composition, such as resistant starch content, has a great influence on the cooking times and appearances registered. From the three cooking methods tested, the pre-cooking of rice for 10 minutes, followed by freezing and later de-frosting and post-cooking by using a microwave oven allowed the fastest cooking method, considering the consumer point of view. The obtained cooked rice, with exception of some varieties, was visually appealing. A wide range of rices cooked with a "sticky" appearance, but some were also found to provide a "non-sticky" end result.

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Abbreviations and Symbols

%	Percentage
AA/TS	Apparent-amylose-to-total-starch-ratio
approx.	approximately
BRC	British Retail Consortium
DWB	Dry weight basis
h	Hour/hours
HCl	Hydrochloric acid
IAPMEI	Institute for Support of Small and Medium Enterprises and Innovation
IFS	International Featured Standards
ISO	International Organization for Standardization
KCl	Potassium chloride
KOH	Potassium hydroxide
L/W	Length-to-width ratio
min	Minute/minutes
mm	Millimetres
NaOH	Sodium hydroxide
P/AA	Protein-to-apparent-amylose ratio
P/RS	Protein-to-resistant-starch ratio
P/TS	Protein-to-total-starch ratio
RS/AA	Resistant-starch-to-apparent-amylose ratio
RS/TS	Resistant-starch-to-total-starch ratio
Room temp.	Room temperature (20-25 °C)
SD	Standard deviation
SGS	Société Générale de Surveillance S.A
SME	Small and Medium Enterprises

Chapter 1 – Organization, Contextualization and Objectives

This report is divided into six chapters. Firstly, on this chapter, a brief introduction to rice consumption around the world is presented, contextualising the topic of my internship report, while also mentioning the organisation and objectives proposed. The second chapter addresses the current state of the art, focussing on rice composition, the relations/interactions between its components and, finally, its cooking. The third chapter describes the company and important concepts learned while working at their quality control laboratory. Afterwards, the fourth and fifth chapters, consist in all the methodologies used and in the discussion of the results obtained while also describing the optimisation of some of the methodologies. A brief conclusion is presented in chapter six.

Rice is one of the leading food crops in the world and is one of the most important staple foods[1], being consumed by more than half of the world's population[2]. This cereal provides 21 % of the energy supply, 14 % of the protein supply and 3 % of the fat supply at the worldwide scale.[3] In fact, rice is very rich in carbohydrates, specially starch,[4] contains a moderate amount of protein[4] with excellent biological value, amongst cereals, and high digestibility[5], lipids with high nutritional value[5], due to its lysine content, and is a good source of B complex vitamins[4] and minerals[3].

In 2012, the worldwide harvested area was 163.5 million hectares, of which India and China are responsible for 42.5 and 30.3 million hectares, respectively, and Portugal for 31.4 thousand hectares. In terms of unprocessed rice grains, in 2012, 17.7 million tons were produced worldwide, of which 7.0 million tons were produced by China, 3.2 million tons by India and 3.5 thousand tons by Portugal.[6] However, these numbers do not represent the real rice consumption in Portugal, since the Portuguese are the biggest rice consumers (*per capita*) in the European Union. In fact, Portugal imports annually 110 thousand tons of brown rice, while only exporting 20 thousand tons.[7]

It is estimated that about 20 % of the worldwide rice production is consumed as parboiled rice.[8] This rice is appreciated by consumers mainly for two reasons: it is firmer, less sticky and has an improved nutritional value when compared to non-parboiled rice, mostly because it retains more of its natural vitamins, such as the vitamin B1.[9] However,

parboiling can be achieved with different processing conditions, some more severe than others and therefore a grand variety changes may occur in parboiled rice.[10]

Rice characteristics such as size, appearance, industrial processing and composition highly influences rice cooking and eating quality.[11] Cooking practices are very diverse around the world and also influence the final appearance of cooked rice[12-13] with milled rice being the most consumer form of rice.[5, 14]

Nowadays, due to changes in lifestyle and lack of time, consumers tend to store rice leftovers for later consumption.[15] Moreover, there has also been an increasing tendency in the consumption of more firm and less sticky rice, since its grains remain separate for longer periods of time. Rice with such characteristics also enable an easier cooking experience due to being less prone to overcooking.[16]

The present internship report has the purpose of describing the work that was performed under the curricular internship at the company Novarroz – Produtos Alimentares, S.A., and at the University's laboratory for the Masters in Science in Food Biotechnology.

The company Novarroz – Produtos Alimentares, S.A is specialized in the processing and commercialization of rice and its by-products. Therefore, this company has a great interest in acquiring a deeper knowledge regarding its main product, in order to best serve the consumers, understand any possible complaints and necessities and optimize or develop new products to satisfy new market niches.

The main objective of this internship was the acquisition of work experience in an entrepreneurial environment of the food sector and therefore the knowledge of the company's organizational structure as well as its mission and policies. At Novarroz, more specifically the company's quality control laboratory, the objective was to learn and apply the methods used to assess the rice quality and commercial price, along with all the stages of rice processing until it is ready for the consumers. Another objective was the characterisation of the rice varieties commercialised by Novarroz using a wide range of parameters. These included size and whiteness parameters, analysed at the company, and composition and cooking parameters, analysed at the University. The last objective was

to compare different cooking methods in order to access their effects on cooked rice. The cooking methods considered included the traditional boiling method in excess water and two new cooking methods using a microwave oven or steam. These new methods are two-stepped: the first, designated as pre-cooking, is thought to be done industrially only while the second step, designated as post-cooking, would be the step done by consumers, at home.

Chapter 2 – State of the Art

2.1. Rice Species and Subspecies

Rice is a cereal from the *Gramineae* family and *Oryza* genus, comprised of 23 species from which only *Oryza glaberrima* and *Oryza sativa* are cultivated.[3]

The *Oryza sativa* species is by far the most presently cultivated and can be divided into three subspecies: *indica*, *japonica* and *javanica*. Rice belonging to the *indica* subspecies is the most commonly cultivated and is typically grown in tropical regions due to being drought tolerant whilst not tolerating colder temperatures. On the other hand, *japonica* rice is typically grown in regions with temperate climates, tolerating colder temperatures. However *japonica* rice is less tolerant to drought, insects and diseases. Another striking dissimilarity between these two subspecies is that *indica* grains are medium to long, narrow and flat, while *japonica* grains are short and wide.[3] Lastly, *javanica* rice varieties are short and wide just like *japonica*, but are generally grown in tropical regions. This subspecies may also be called tropical *japonica*.[17-18] Of all the subspecies, *indica* is the most common, constituting about 80 % of all cultivated rice, followed by *japonica*.[3]

2.2. Rice Grain Morphology

In general, the rice grain, depicted in **Figure 1**, is composed by four main components: the hull, the caryopsis coat (also known as bran), the endosperm and the embryo (also designated by germ).[5, 19-20]

The hull constitutes approximately 19 % of the total grain[11] and is composed by the two modified leaves designated palea and lemma. Bellow the hull is the caryopsis, which is composed by the caryopsis coat, the endosperm and the embryo. The caryopsis coat surrounds the endosperm and embryo and is composed, from the outside in, by the pericarp, the seed coat (also referred as tegmen) and the nucellus.[21] Under the caryopsis coat is the endosperm, which comprises the aleurone layer, the outermost layer of the endosperm tissue and the starchy endosperm.[19] The latter is composed by the subaleurone layer, surrounding an inner endosperm, featuring starch granules and some protein bodies. Finally, the embryo contains the embryonic leaves, or plumule, and an embryonic primary root, also known as radicule. The embryonic leaves and primary root

are joined by a very short stem referred as mesocotyl. The plumule is also surrounded by the coleoptile, which is in turn surrounded by the scutellum and the epiblast.[19]

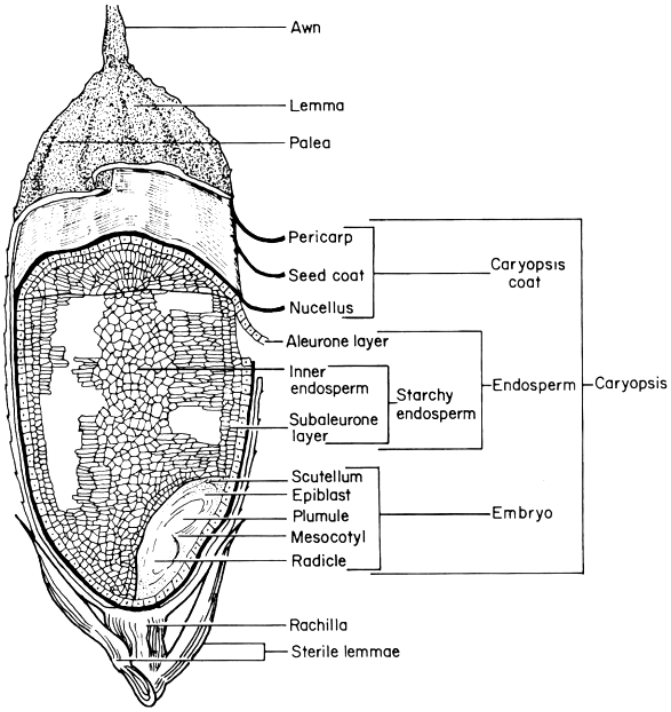


Figure 1 – Morphology of the rice grain.[19]

Rice receives different designations according to its physical state. All rice starts as paddy rice, which is rice in its natural unprocessed state. When the hull is removed, paddy rice yields de-hulled rice, also referred as brown rice. The consequent milling, at different extents, of brown rice yields semi-milled or milled rice, the latter being commonly known as white rice. The semi-milling process only removes the upper layers of bran and part of the germ, while the complete milling process completely removes the bran and germ.[22]

2.3. Rice and its Components

The knowledge of rice composition is of great importance to understand rice behaviour in cooking.[11]

Even though the rice variety has great influence on its composition, on average, it can be established that milled rice is composed by 75 % starch[3, 23], 12 % water[5], 10 %

protein[3, 24], 2 % lipids[5], 0.9 % dietary fibre[25], and even less quantities of vitamins and minerals[3].

The rice nutritional values do not only differ amongst different varieties, but also with the soil used, the environmental conditions felt throughout the plant development and, finally, with the industrial processing method to which rice is subjected. For example, brown rice, contains a higher amount of proteins[11], lipids and dietary fibre than milled rice due to the removal of bran and germ[26]. Dietary fibre is also removed, together with essential fatty acids, 80 % of the B1 vitamin, 67 % of the B2 vitamin, 90 % of the B6 vitamin, 50 % of the manganese, 50 % of the phosphorus and 60 % of the iron. However, rice after milling usually follows the same tendency, having low fat and low protein content and a higher protein digestibility.[5]

2.3.1. Starch

Starch is the major component of rice, being widely associated with all the other rice components such as proteins, lipids and minerals.[3, 27-28] This α-glucan is a semi-crystalline biopolymer that serves as a carbohydrate reserve in many plants[29], including cereals. It is mainly found in the grain's endosperm and occurs in the form of granules.[19] The rice starch granules are the smallest known to exist in cereal grains and are composed of amylose and amylopectin macromolecules.[23, 27, 30]

The starch's origin has great influence on its chemical composition, structure and properties, impacting several characteristics such as overall distribution of amylose and amylopectin, their structures, and size and shape of the granules.[28]

2.3.1.1. Amylopectin

Amylopectin is the main component of the rice starch, constituting more than 70 % of its total content.[27-28] It is a highly branched glucose polymer consisting of a backbone of α-1,4-linked glucosyl units and α-1,6 branches (**Figure 2**).[23, 31]

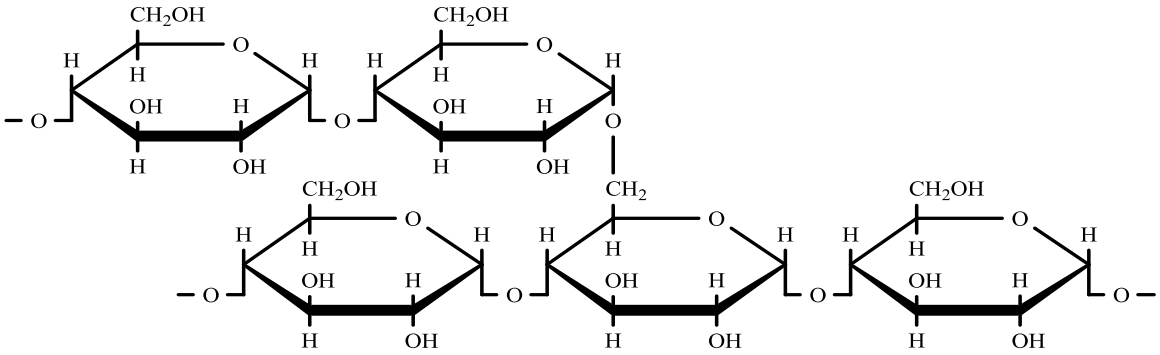


Figure 2 – Molecular structure of amylopectin.[27]

The amylopectin's structure consists of alternating crystalline and amorphous domains, due to its branched nature: when adjacent, its branches can form a double helix structure that is associated with crystallinity, while the branching points constitute the amorphous regions of its structure.[28, 30, 32]

2.3.1.2. Amylose

Amylose (**Figure 3**) is an essentially linear glucose polymer consisting of long chains of α-1,4-linked glucosyl units.[20, 23, 27]

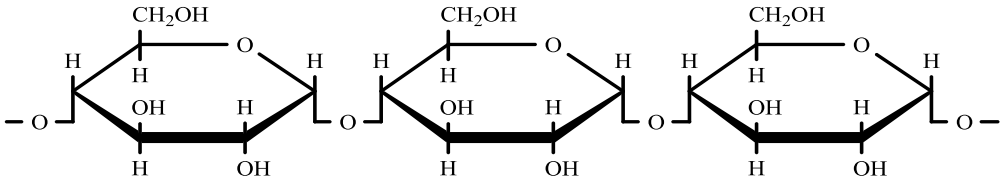


Figure 3 – Molecular structure of amylose.[27]

The amylose molecules usually form single helical structures, which are associated with the amorphous regions in the rice starch.[28, 30]

In general, rice starches contain up to 33 % of amylose[28]. According to this content, rice can be classified as waxy (0-2 %) or non-waxy (more than 2 %). Amylose content from non-waxy rice can still be sub-classified as very low (2-10 %), low (10-20 %), intermediate (20-25 %), or high (more than 25 %).[3, 33] The glutinous rice, also designated as waxy, is an example of rice with really low amylose content.[20] When cooked, this rice is sticky and

soft[34] as opposed to rices with higher amylose content, which are more firm. Thus, amylose is one of the major factors found to affect the eating quality of rice.[24] Rice's subspecies feature different tendencies regarding amylose content, with *japonica* rices being more prone to have lower amylose contents than *indica* rices.[3, 35]

Amylose has been found to form complexes with other rice components such as proteins and lipids, changing starch properties such as gelatinisation.[35]

2.3.1.3. Resistant Starch

Resistant start is defined as the sum of starch and products of starch degradation that escape digestion in the small intestine, followed by partial fermentation by the anaerobic bacteria that inhabit the colon.[31, 36-37] This particular type of starch can be composed of retrograded starch (both amylopectin and amylose fractions), physically inaccessible starch (if encapsulated within plant cell walls), chemically modified starch, starch complexed with other food components (such as protein and lipids) and non-digestible starch due to enzymatic inhibition (by phytic acid, for example).[31, 38-39]

Resistant starch can be formed, or its content can be increased, by heat treatments such as baking or cooking.[40] Besides baking, amylose and moisture content, amylose/amylopectin ratio, extent of starch gelatinisation, pH, processing time and temperature, number of heating-cooling cycles, freezing or drying also influence the formation/increase of resistant starch.[31, 41] However, Goñi *et al.* (1996) reported a decrease in the resistant starch content of rice when cooked: raw rice was found to contain 5 to 15 % of resistant starch (high content) while boiled rice contained 1 to 2.5 % (low content) or under 1 % (negligible content) depending if it was, respectively, cold or warm[36]. Åkerberg *et al.* (1998) also reported that sample preparation also influenced the resistant starch results: cooked whole rice was found to contain more resistant starch, about 4.6 %, than its cooked ground form (flour), which contained 1.6 %.[37]

2.3.1.4. Relation with Rice Properties

The white chalky appearance that occurs in the belly of some rice grains is a common feature for many rice varieties[42] and it can influence consumer preference between rices.[35,

[43-44] Chalkiness is caused by the loose-packed starch granules that characterise the amorphous regions of starch. This loose-packing is due to the existence of air spaces in between the granules, which consequently cause light to scatter, giving an opaque and white look to the grains. Although this chalky look can occur in all rice varieties, it is usually more abundant in rice belonging to the subspecies *japonica*.[45] One reason for this is, as Patindol *et al.* (2003) reported, is that chalky grains, besides containing less amylose, contain amylopectin majorly characterised for having shorter chains, i.e. more branching points and consequently a bigger amorphous domain.[46] Also, due to the loose-packed feature, chalky grains have been found to absorb more water and have shorter cooking times when compared to translucent grains. Chalky grains have also been found to be more fragile, less resistant, less hard and less cohesive, which affects the rice cooking quality.[43, 46]

Gelatinisation is the irreversible process of swelling of the starch granules and consequent collapse/disruption of the molecular order within the granule. In other words, it's the loss of crystallinity, in conjunction with water absorption followed by the rupture of the granular structure.[20] Starch granules experience gelatinisation when exposed to excess water and increasing temperature. In these conditions amylose is leached out of the starch granule, which consequently plays an important role during the retrogradation process that follows and on the cooking process.[20, 47] The temperature at which starch starts to experience the above mentioned changes is designated as gelatinisation temperature and usually varies from 55 up to 79 °C.[35] Amylose can for complexes with lipids, providing rigidity to the gelatinised starch[47] and consequently influencing rice cooking and eating quality[48]. These complexes are responsible for more significant variations in the gelatinisation than amylose alone, even in rices with low lipid content, such as milled rice. These complexes inhibit swelling and therefore may be responsible for increases in the gelatinisation temperature.[47] Because of the particular interaction between amylose and lipids, in general, the amylopectin content has a tendency to have a bigger influence on the swelling behaviour of starch. In fact, waxy starches with very low amylose content are less resistant to gelatinisation, i.e., have a lower gelatinisation temperature than non-waxy starches.[49]

Retrogradation of starch is a two-step process that takes place when the molecules of gelatinised starch start to reassociate in an ordered structure[50], i.e., start to crystallize, upon cooling. A two-step process occurs during retrogradation: firstly occurs the gelation and crystallization of amylose, followed by amylopectin crystallization. These crystallisation processes are fully reversible, in the case of amylopectin, and partially irreversible, in the case of amylose.[20, 39, 51] In other words, retrograded starch is the result of spontaneous changes that occur on subsequent cooling, ageing and/or drying of gelatinised starch.[20, 31] As mentioned before, amylose plays an important role in the overall retrogradation of starch by having a greater influence on its pasting properties.[47] More specifically, the solubilisation of amylose during gelatinisation results in a paste. In time, as the retrogradation process occurs, that paste becomes increasingly more opaque, gels and an increasing tendency to release water, also known as syneresis, occurs.[20] Just like in the gelatinisation process, amylose-lipid complexes influence the formation of gel because, since retrogradation depends on the amylose content, less amylose will be available to retrograde. A higher degree of amylopectin crystallization is found in waxy rice starch than in non-waxy, mainly due to their composition. It is mentioned in the literature[47] that amylopectin is more commonly the reason for starch retrogradation than amylose, which will, in fact, limit the extent of the retrogradation. Therefore, in general, the variation and extent of the retrogradation increases with the decreasing amylose content.[31] Amylose also tends to retrograde faster, taking up to 2 days, and at a larger scale than amylopectin, which takes up to 30-40 days.[39] This is a consequence of the amylose tendency to reassociate by forming hydrogen bonds with adjacent amylose molecules[52]. This reassociation, although allowing a higher degree of molecular organization, does not imply an increase in the starch's crystalline degree.[53]

2.3.2. Proteins and Amino acids

Protein is the second most abundant component of rice.[3, 5] It is found distributed throughout the rice grain with a higher concentration in the endosperm and germ[54] and decreasing, in the grain, from the outside in.[11] Rice protein is not only highly digestible[54],

but has excellent biological value, amongst cereals,[5] while also being considered important for the rice eating and cooking quality.[24]

Kennedy *et al.* (2003) reported, after an extensive analysis of almost 3000 *Oryza sativa* rice varieties, that protein content could vary from 4.5 to 15.9 % in milled rice.[3] Rice proteins include several different fractions.[55] Glutelins are the main storage proteins[56], accounting for 68 to 72 % of the overall content and are alkali-soluble. Globulins, the salt-soluble fraction, make up for 12 to 17 % of the total content and albumins, the water-soluble fraction, account for 10 to 12 %. Lastly, prolamins, the smallest fraction, are alcohol-soluble and constitute only 2 to 3 % of the rice protein content.[11, 20, 35] Oryzenin is one of the major glutelin proteins in rice, accounting for 70 % of the glutelin content.[57] Rice proteins are found as globoids, in the aleurone layer and in the germ, and as in protein bodies in the endosperm, the latter having a rich lipid core. Proteins can also be found bound to amylose granules.[35, 54]

Rice contains amino acids such as glutamic acid and aspartic acid, in high contents[5] (18 % and 10 % of the total protein content, respectively)[58], while also being quite deficient in the essential amino acid lysine[59] (approx. 4 % of total protein content)[5, 54].Nevertheless, the lysine content of rice protein is one of the highest amongst cereal proteins.[54] Brown rice has been found to have a lower percentage content of glutamic acid than polished rice, which indicates that this non-essential amino acid is more concentrated in the most interior layers of the grain. On the other hand, brown rice has a higher content in lysine than polished rice due to lysine being present in the outer and inner most layers of the grain.[58]

2.3.3. Lipids

Rice lipids, besides having a high nutritional value with about 80 % being unsaturated fatty acids[5], also impact the cooking and eating quality of rice through interaction with other rice components[48]. Its main location in the grain is the bran, hence the common designation of rice bran oil, constituting almost 20 % of the total lipid content[54]. Rice lipids can also be found in the aleurone layer[5, 11] and, in smaller quantities, in the endosperm[60].

The lipids present in rice can be classified in non-starch lipids[5] and starch lipids[48]. The non-starch lipids are the main lipids present in milled rice and are located as lipid bodies or spherosomes in the aleurone layer, while the starch lipids are present low concentrations and in complex with amylose.[5, 54, 61] The major non-starch fatty acids found in rice are the monounsaturated oleic acid, the essential polyunsaturated linoleic acid and the saturated palmitic acid.[5, 54, 62] Other unsaturated fatty acids existing in rice grains are the polyunsaturated linolenic acids and γ-linolenic acid, although these exist in much lower quantities.[63]

Rice contains three main types of lipids: neutral lipids, glycolipids and phospholipids, of which the major are, respectively, triacylglycerols and unsaturated fatty acids, steryglycoside, and phosphatidylcholine.[64]

Waxy rices have a lower starch lipid content, mainly due to having little or almost none amylose, while intermediate amylose rices have the highest lipid content.[54, 63] However, waxy rices have a higher content of non-starch lipids than non-waxy rices.[21, 5361]

2.3.4. Non-starch polysaccharides

Non-starch polysaccharides include water soluble polysaccharides, such as soluble dietary fibre, and insoluble dietary fibre[54], the latter being mainly composed of cellulose, hemicellulose[65-66], lignin[67] and pectic substances, all common plant cell wall materials[68]. Brown rice has a higher content of non-starch polysaccharides, of about 2.87 % in dry weight basis (DWB), while milled rice contains only 1.4 % (DWB).[67] As for dietary fibre, brown rice has been reported to contain 2.87 % while milled rice only contains 0.87 %, both in fresh food weight basis[25]. In fact, most of the dietary fibre is located in the hull and bran[5], while the endosperm has the lowest content[54].

During cooking, rice may lose some of its water soluble non-starch polysaccharides[69] but in general its presence is known to hinder the swelling of starch granules[65] which in turn, will decrease the water absorption during cooking[20]. This implies that a higher presence of non-starch polysaccharides will be responsible for longer cooking times[70].

2.3.5. Vitamins and Minerals

Rice is a good source of E[54], K[71] and B complex vitamins[4], which are mainly located in the bran and germ[11, 54]. The vitamin B1 (thiamine) content can ranges 0.117 to 1.74 mg/100 g (DWB), B2 (riboflavin) from 0.011 to 0.403 mg/100 g (DWB) and B3 (niacin) from 1.972 to 9.218 mg/100 g (DWB).[3, 5] Vitamins B1 and B2 can be found throughout the grain[11, 54] and vitamins B3 and B6 are mainly found in the bran layers[5]. Rice is reported as being a poor source of vitamins A[3], C (ascorbic acid) and D[4, 54].

Rice minerals are mostly located in the aleurone layer of the bran[5, 19] and on the germ.[11] These include calcium (0.07-0.25 %), magnesium (0.07-0.25 %), phosphorus (0.50-0.55 %) and potassium (0.15-0.23 %) along lesser amounts of iron, zinc, copper, manganese and sodium (0.09-0.17 %).[3, 5, 19]

2.4. Parboiled Rice

Parboiled, also designated as converted rice, has been subjected, either as paddy or as brown rice, to soaking in water, heat treatment (usually steaming), drying and finally to industrial processing. Parboiled rice is, therefore, rice whose starch has been fully gelatinised and hence the name parboiled (partially boiled).[16, 22, 72] All rice types can be parboiled but the consumer preferences vary according to country and rice type availability.[54]

Four main changes can take place in the rice grain during this process: diffusion of water or other compounds from or to the grain, carrying nutrients with it, starch gelatinisation followed by retrogradation and protein denaturation[73], the latter mainly occurring due to the high temperatures employed during parboiling.[16, 74-75]

Parboiling has several advantages: this process improves the nutritional value of milled rice, the milling recovery of paddy rice,[16] salvages poor quality or spoiled paddy rice, increasing the milling yield, meets the demand for firmer and less sticky rice[10] and makes rice less prone to overcooking[16]. On the other hand, parboiled rice tends to become rancid during storage[10], due to the high temperatures employed during its processing[16], may require longer cooking times (depending on the process and conditions used for parboiling)[16] and more energy is necessary to achieve a proper milling degree[76]. Parboiling

costs are also a drawback, since it represents an additional cost in water, energy and effluent treatments.[16, 77]

Prolonged parboiling decreases the vital constituents of rice such as proteins and minerals, not only due to the more extreme conditions used, but also due to leaching, therefore decreasing the rice nutritional value.[78] Ibukun (2008) reported that, as parboiling duration was increased, the nutritional value decreased with losses in crude protein content, calcium, iron, sodium and potassium. A bigger percentage of grain breakages after milling was also reported[78], eliminating one of the advantages of parboiling[16]

Parboiling is responsible for several changes in rice grains. In general, it can be responsible for changes in grain size[79], chalkiness[45], coloration[80] and composition[16]. The water diffusion coupled with the heat treatments employed during parboiling may result in thicker and longer rice grains, when compared with their non-parboiled counterparts.[79] Parboiling also reduces chalkiness[45], which is a direct consequence of the gelatinisation and retrogradation of starch granules and hardening of the endosperm, making the grains translucent.[54, 81] Thus, parboiled grains that still feature a white belly weren't fully parboiled.[76] Rice grains acquire a yellow amber coloration with parboiling, which increases with the severity of the parboiling process.[54, 80] As for composition, the gelatinised starch partially retrogrades[59] and the amylose content decreases due to amylose leaching during the processing steps of soaking and steaming.[82] Parboiled rice has a lower protein content due to protein denaturation[73], leaching[78] disruption of the protein bodies and increase in protein polymerization by disulphide bonds. The latter leads to the decrease in protein solubility and digestibility.[8, 54] As for lipid content, the disruption of the lipid spherosomes releases non-starch lipids that are consequently diffused into the outermost layers of the parboiled rice.[54, 82] Overall, parboiling causes the loss of vitamins A, C and of the B complex through leaching loss and thermal breakdown. However, parboiled rice still retains B complex vitamins than non-parboiled rice, due to inward diffusion from the bran into the endosperm.[54, 83] As for mineral content, parboiling may or may not affect the mineral composition of rice.[59, 78] Finally, parboiling removes the cooked non-parboiled rice volatiles, hence the different smell experienced during the cooking of these rices.[76]

There are several variations on the parboiling process and each one produces a slightly different parboiled rice. For example, a hot water soaking stage produces a more discoloured parboiled rice than a cold water soaking stage, but the latter taints paddy rice with off-flavours; pressure parboiling produces rice that is even more discoloured than both previously mentioned parboiling processes.[77]

2.5. Rice Cooking and Eating Quality

Rice composition has been found to have a major influence on its cooking and eating qualities, although it doesn't always explain its cooking characteristics.[24] Just as an example, differences in texture have been reported among rices with similar amylose contents[84]. Rice eating quality is usually evaluated, either by panellists or with instruments, according to its tenderness, hardness and cohesiveness. The latter can also be referred as stickiness and has been found to be negatively correlated with amylose content.[83] Amylose is heavily referenced as responsible for the cooking and eating quality of rice due to its gelatinisation and retrogradation, with lower values contributing to a reduction of the cooking time.[24, 47-48, 85] Its presence has been found to hinder water absorption, consequently hindering the volume expansion of rice during cooking, with waxy rices (low amylose content) expanding the least during cooking.[83] The resistance of the cooked grains to disintegration (also referred as loss of shape) has also been attributed to amylose content, with high-amylose rices being the most resistant and waxy being the least. This goes according with the higher tendency of low-amylose rices to stay moist and sticky after cooking[35] and might be correlated with the solid loss that occurs during cooking[86]. On the other hand, parboiled rice is more resistant to disintegration and leaching of components during cooking than its non-parboiled counterpart.[83] In fact, cooked parboiled rice is rarely sticky.[54] The degree of milling is another key factor affecting the gelatinisation: an increased milling degree will cause a decrease in the gelatinisation temperature. Therefore, the higher the milling degree, the faster rice will cook. In fact, for brown rice, the gelatinisation temperature will have a considerable increase mainly due to the presence of the bran's composition in lipids and proteins, which hinder water absorption by forming complexes with starch.[70, 87] The protein content is also responsible

for a higher cooking time, since a higher amount of disulphide bonds will decrease protein solubility[8, 54] and increase starch-protein interactions, while also decreasing the water absorption during cooking[57]. Cooking also reduces the protein's digestibility[54] and it has been suggested that the protein of cooked *indica* rice might be less digestible than the protein in cooked *japonica* rice.[8] Finally, lipids have also been reported to have a great influence in the cooking and eating quality of milled rice: when lipids complex with amylose they may hinder starch gelatinisation, therefore altering the way rice cooks.[48]

The shape and size of the grain has also been reported as influencing the cooking time, with the slimmest grains (higher lengths, smaller widths and higher length-to-width ratios) taking the least time. These grains have a bigger surface area and a smaller distance between the surface and the centre.[43, 88]

Storage of cooked rice is known to cause starch retrogradation, which increases the level of enzyme-resistant starch through amylopectin crystallization, also diminishing the solubility of the starch.[39] The aging of rice can also prolong its cooking time. A decrease in amylose leaching has been reported, which reflects an increase in starch's insolubility and a higher difficulty for rice to absorb water. This can be justified by the naturally occurring ordering of the starch structure during its aging.[89] Hardening of the rice texture and decreased stickiness also occur possibly due to the decreased capacity of the starch granules to rupture, which in turn limits the water absorption capacity of the grains, prolonging the rice cooking time.[90-91]

Cooking time is influenced by the gelatinisation time and a direct correlation has been reported between the two parameters.[35] Waxy rices, with a lower gelatinisation temperature, will have a higher degree of swelling, absorb more water and therefore cook faster. In contrast, non-waxy rice won't swell as much and will require a longer cooking time.[47, 49] The cooking time required to gelatinise the rice grains tends to be longer for higher protein rices.[83] This happens because the protein and cell-wall matrices that surround the starch granules inhibit starch swelling and solubilisation during cooking[92] by preventing water absorption[93].

Milled rices have a cooking time range of about 15 to 25 minutes, with the exception of some aromatic type rices that cook even faster due to their slim and long grains.[54]

Parboiled rices may have a higher or lower cooking time than their non-parboiled counterpart.[16] A common way of accessing the cooking time of rice is by checking the completion of gelatinisation of the rice grains.[94]

There is no standardised way of cooking rice, and each culture has its own preferred methods, eating habits, rice types, rice characteristics and textures.[12-13] Rice is highly used at the household level, where it is consumed as boiled, either with or without excess water (1:1 up to 4:1 water-to-rice ratio (v/v)[95]), fried and even steamed, the latter method being preferable for glutinous rice.[86, 96] Many different equipments may be used to achieve such results. These include using open pans, microwave or induction ovens, pressure cookers, and even electric rice cookers.[97] In some countries, washing and/or soaking rice is still a common practice. It is safe to say that this practice is more of a personal choice rather than a necessity (for most rice types) since, nowadays, rice is thoroughly cleaned from dust and impurities, such as stones, prior being marketed. Rice soaking and washing can, however, be used to reduce cooking time by increasing water absorption prior to cooking[98], but this is done at the expense of leaching nutrients, such as starch, proteins and B complex vitamins into the washing/soaking water.[13, 54, 96] In the same manner as soaking, boiling milled rice in excess water also results in nutrient leaching.[14] Boiling in the above mentioned conditions also increases the rice's tendency to disintegrate, resulting in mushy grains, that is, grains that have lost their shape.[83] Steamed milled rice, however, has been found to retain nearly all of its naturally occurring vitamins and minerals.[14] In fact, consumers from different countries have associated, in a study by Son *et al.* (2013), steaming with the preservation of vitamins and nutrients in rice, since rice isn't soaked, preventing the loss of nutrients by leaching.[13]

Changes in family lifestyle and the increased use of freezers, refrigerators and microwave ovens are an indication of the demands for convenient, easy to prepare foods, that are also suitable for frozen or chilled storage. Nowadays, the accelerated pace of modern life has also promoted an increase in the consumption of ready-to-eat rice, which is usually done by heating rice leftovers[15] in a microwave oven along with a small portion of water.[99] The domestic storage of rice leftovers is a special concern: rice must be refrigerated as soon as possible in order to prevent the growth of harmful bacteria such as

Bacillus cereus.[96, 100] Besides fully cooking rice and storing for later use, rice can be pre-cooked in advance and has been used for that purpose for years. More importantly, for this report, frozen rice has been reported as being used to supply chain restaurants that quickly heated it in microwave ovens for ready-to-eat rice.[54] Pre-cooking rice has been reported to affects starch digestibility through retrogradation[39], and consequently affects the cooking and eating quality of rice.

2.5.1. Microwave Oven

Microwaves are used for several food processing applications that include re-heating, cooking and thawing due to being rapid, convenient and cost effective. Common household microwaves operate at 2450Mhz. Microwave ovens work by applying an alternating electromagnetic field to the food being heated. This radiation, in the microwave spectrum, causes the food's polarized molecules, like water, to attempt to orient themselves according to the rapidly changing electric field. This in turn generates frictional heat: the molecules absorb energy from the field and then dissipate it into the surrounding food.[101-102] The microwave's biggest disadvantage is its limiting penetrating ability. So, in order to achieve a uniform heating, small quantities/volumes of food must be used. However, the microwave penetration increases dramatically when foods are being thawed, making it a useful technology for that purpose.[101-102] One of the advantages of using a microwave oven is the low cooking time required to cook rice. Kaasová *et al.* (2001) reported that microwave treatments increased progressively the gelatinisation of rice starch,[103] indicating the potential of this method for rice cooking. Moisture is reported has being uniform throughout rice portions cooked by microwave cooking, with exception of the top, where moisture content is slightly lower due to surface evaporation.[99]

2.5.2. Steam

Non-pressurised steam is one of the healthiest cooking methods used at the household level. Steam can be produced by pot of simmering water. Rice cookers also cook rice partially by steam.[104] Ghasemi *et al.* (2009) reported the effects of non-pressurised steaming rice that was first cooked in excess boiling water, stating that this

process could be used to achieve a perfectly cooked rice[12] Steaming decreased the hardness and increased the rice adhesiveness, which means that boiled and steamed rice grains were softer and stickier, than those cooked only by boiling water. It was also noted that steaming lead to the disruption of starch complexes and molecules adopted a more random orientation, which resulted in a well-expanded cooked rice[105]. It was also reported that this processed for cooking rice possibly resulted in a higher degree of gelatinisation since steam reaches higher temperatures than boiling water.[12] Son *et al.* (2013) reported that cooking glutinous rice through steam prevents the grains from turning pasty and sticking together too fast. This same study reported that steaming may be considered not convenient and time consuming, as opposed to boiling, but gives the desired stickiness some consumers, especially of Asian origin, prefer in rice.[13]

To the best of my knowledge, no more studies have reported the effects of microwaves and non-pressurised steaming on rice cooking and eating quality.

Chapter 3 – The Internship

3.1. The Company: Novarroz

The Company Novarroz – Produtos Alimentares, S.A. (**Figure 4**) is a family company founded in 1979 headquartered in Adães, Oliveira de Azeméis. This factory has the capability of operating 24h per day, employs 63 people and, for more than half a century, has had the privilege of working with experienced professionals.

Figure 4 – Novarroz factory (left) and logo (right).[106]

Novarroz is certified by ISO 9001, BRC Food and IFS by SGS, its technologically advanced manufacturing technique is attested by the EUREKA program, is currently adherent to the Portuguese project "*Compro o que é nosso*" (Buy what is ours) while also having the status of SME leader by IAPMEI.

This company's mission is to transform, commercialize and place on the market products with quality, particularly focussing on rice. The diversity of products has to be in accordance with the food safety guidelines, aiming to fully satisfy costumer and consumer needs. Novarroz' corporate policies establish a strategy that is developed based on six pillars and aims:

- Focus on customers and market;
- Guaranteed food quality and safety;
- Continuous improvement;
- Health protection and promotion of work safety;
- Respect for the environment; and
- Ethics and legality

In the Novarroz factory, rice from Portugal and around the world is dehulled, milled, oil-polished, packaged and sold. Rice by-products, such as broken rice and hulls are also sold. To accomplish all these operations this factory has at its disposal silos with storage capacity of 30 000 metric tons of paddy rice, a vertical modern mill with capacity to process 15 metric tons of rice per hour and silos with storage capacity of 800 metric tons of milled rice. As for packaging many options are used, such as complex polypropylene, vacuum, cartons, boil-in-bag bags and raffia bags.[106]

3.2. Standard Definitions Concerning Rice

The Portuguese *Decreto-Lei n.º 62/2000* defines the characteristics for rice and broken rice intended for human consumption. The methodologies for analysis, types of commercial grades, variety classification and technical standards concerning rice commercialization storage and labelling are also defined.[22]. According to these standards, together with the *Codex Standard for Rice*[107], rice can be defined regarding its physical state (**2.2.**), the length of its grains, the treatment to which it is subjected and type.

According to its length, rice grains can be round, medium or long. Round have a length inferior or equal to 5.2 mm and with a length-to-width ratio (L/W) inferior to 2. Medium grains have a length superior to 5.2 mm and inferior or equal to 6.0 mm and have a L/W inferior to 3. Finally, long grains can have a length superior to 6.0 mm and have a L/W superior to 2 or inferior to 3, or can have a length superior to 6.0 mm and a L/W superior or equal to 3.[22]

Regarding its treatment, rice can be parboiled, pre-cooked, glazed or oil-polished. Parboiled rice has been described in section **2.4.** Pre-cooked rice is subjected to a physical treatment that allows a substantial reduction of the cooking time. Glazed and oil-polished rice are both coated milled rice: the first is coated with a film of glucose and talcum powder, suitable for human consumption, while the latter is coated with white edible mineral oil, in accordance with the legislation in effect.[22]

Worldwide, there are several types of rice and each one of has specific characteristics. Some of the types acknowledged during the first months of the internship

were *agulha*, *carolino*, medium, round, *risotto*, glutinous, brown, parboiled, aromatic and wild (**Figure 5**).

Figure 5 – Rice types (from left to right) *agulha*, *carolino*, medium, round, *risotto*, glutinous, brown, parboiled, aromatic and wild.

The *agulha* and *carolino* rices are the most commonly cultivated in Portugal, belonging to the *indica* and *japonica* subspecies, respectively. The type *agulha* is longer, thinner and usually remains firm and non-sticky after cooking, as it is usual with *indica* rices. *Carolino*, on the other hand, is a genuine Portuguese rice that is shorter, wider, and gets sticky after cooking. This rice is very appreciated in Portuguese gastronomy due to its ease in absorbing flavours.[106, 108] The medium type has an appearance similar to *carolino*, round type rices have the shortest and widest grains and both have the characteristic stickiness of the japonica subspecies. Risotto rices may have similar dimensions to medium or round types, depending on the variety, but have a chalkier appearance. This rice is traditionally used in Italian gastronomy. Glutinous rice, also called sweet rice, has a unique white and opaque appearance and is usually used in sweet dishes. Its main characteristic is its very low amylose content. Brown rice is all rice that has been dehulled but not milled. It has a characteristic with a light brown coloration, depending on the variety, and requires longer cooking times than its milled counterpart. Parboiled rice has been described in **2.4.** The aromatic type includes rices such as *Basmati*, an extremely long and thin rice commonly in Indian gastronomy, and *Jasmine*, common in Chinese and Thai gastronomy. Aromatic rices are also recognized by their fragrant scent[109]. Wild rice is

actually a grass seed from the *Zizania* genus[110] characterized for having extremely long and thin grains, with a dark brown appearance and a nutty flavour. This seed is eaten in its brown un-milled form.

3.3. The Industrial Processing of Rice

The following industrial process describes only the stages conducted at Novarroz.

As soon as the paddy rice arrives at the factory it is weighted, still inside its cargo (**Figure 6 left**). A sample is then collected, from several points of the cargo, in order to achieve an overall representative sample. In the meantime, this sample is taken to the quality control laboratory, where analyses will be performed in order to assess the quality of the newly arrived rice. Such analyses include the moisture content and whiteness values and assessment of the percentages of impurities and defects **(Figure 6 centre)**. This assessment is done by selecting, either by hand or with the help of instruments, 100 grams of rice. Only after the laboratory has approved the sample, can the paddy rice be stored in the paddy rice silos (**Figure 6 right**) with the appropriate storage conditions in terms of moisture, temperature and pest control.

Figure 6 – Rice cargo being weighted (left), assessment of the percentages of impurities and defects (centre), and silos used for storage of the paddy rice cargo (right).

The first stage of processing consists in the removal, by cleaning machinery and de-stoners, of all impurities and foreign objects, such as stones, sticks and straws which are mixed with the grains.

The following stage, the dehulling of the grains, results in the separation of the brown rice and the hull, and is accomplished by a huller. Within this machine two things happen to the paddy rice: it goes through two rubber rollers spinning in different directions and at different velocities, whilst being sorted for any hull that may still be mixed. The resulting hull is stored and sold as a by-product to local industries.

The brown rice is then milled in the mill. Here, by friction with a spinning stone, the bran and germ are removed until the proper milling degree is achieved. The ground bran and germ are also stored and sold as by-products, to be used as animal feed.

Next, milled rice is polished with water mists (**Figure 7 left**) in order to remove dust and to acquire sheen, making it more appealing to the consumers. It is also in this stage that magnets remove metallic particles that may have passed through the first stage of processing.

In between the milling and polishing stages, and also after the polishing stage, rice is selected by graders and sifters (**Figure 7 centre**), wherein damaged and immature, or broken rice grains are removed according to colour and dimensions. In the packaging stage, broken rice can be reincorporated into the processed rice in percentages established in the legislation[22], while the remaining is used as animal feed. The resulting milled and selected rice is then stored in silos (**Figure 7 right**) until the time of packaging.

Figure 7 – Polisher (left), grade/sifter (centre) and silos of milled rice (right).

An additional stage may be performed before packaging: In the case of oil-polished rice, grains are mixed together with the appropriate amount of edible mineral oil, in accordance with the legislation in effect.

The last stage of the industrial processing is packaging. Here, packaged rice goes through metal detectors (**Figure 8 left**) and finally is warehoused in the storage area reserved for packaged goods (**Figure 8 right**), at controlled temperatures and moisture levels, until it is ready to be marketed.

Figure 8 – Metal detectors at the end of the packaging line (left) and storage area for packaged goods (right).

Chapter 4 – Methodologies and Materials

4.1. Samples

A total of 19 different rice varieties (samples) with different origins were provided by Novarroz – Produtos Alimentares, S.A. All the samples supplied for this study were milled and polished at the quality laboratory located at Novarroz, of which 3 had been previously parboiled before the aforementioned processing steps. The samples belong to different rice types, and were encoded according to their subspecies (**Table 1**), not being the same encodings presented in the report by Soares (2014) entitled "*Caracterização de variedades de arroz - Aspetos nutricionais*"[111].

All samples were kept in airtight bags with minimal exposure to light and air, during the course of this report. Each sample was checked in order to remove any contaminant variety prior to usage.

Table 1 – List of rice samples and corresponding types provided by Novarroz – Produtos Alimentares, S.A.

Sample	Type
*indica*1	Aromatic
*indica*2	
*indica*3	*Agulha* South America
*indica*4	
*indica*5	*Agulha*
*indica*6	
*indica*7	
*indica*8	
*indica*9	*Agulha* parboiled
*indica*10	
*japonica*1	*Carolino*
*japonica*2	
*japonica*3	Medium
*japonica*4	
*japonica*5	
*japonica*6	*Risotto*
*japonica*7	
*japonica*8	Round
*japonica*9	Round parboiled

4.2. Solutions, Standards and Reagents

Pepsin min. 700 U/g (*20895*) was obtained from *Riedel-de Haën*. α-Amylase from porcine pancreas Type VI-B ≥10 units/mg (*A3176*) and Amyloglucosidase from *Aspergillus niger* ~70 U/mg (10115) were obtained from *Sigma-Aldrich*. D-Glucose GOD-POD kit (*AK00161)* from *nzytech* was prepared and used according to the manufacturer's instructions.

Apparent Amylose standards with particle size inferior to 0.180 mm were provided by Novarroz and having the following concentrations: standard1 0.00 %, standard2 12.10 %, standard3 14.10 % standard4 14.25 % and standard5 22.80 %. Iodine solution for analysis of apparent amylose was prepared daily. Firstly, 2000±5 mg of potassium iodide were dissolved in approx. 20 mL of distilled water. Then, 200±1 mg of crystalline iodine were added and the mixture was stirred, in the dark, until full dissolution of the iodide crystals. Afterwards, the solution was transferred into a 100 mL volumetric flask and stored in the dark until needed.

All buffers were prepared beforehand and kept at 4 °C and all other reagents were of analytical grade.

4.3. Analysis of Grain Dimensions, Whiteness and Percentage of Chalky Area

Analysis of grain dimensions (length, width and L/W), whiteness (total and crystalline) and percentage (%) of chalky area, of the grain, were performed using the *AgroMay Statistic Analyzer S21* apparatus (*Analyzer S21* for short) (**Figure 9 left**) located at the Novarroz quality control laboratory. Another parameter for whiteness, designated by *Kett*, was analysed using the *Kett Electric Laboratory, model C-300-3* apparatus (*Kett* for short), also located at Novarroz (**Figure 9 right**).

The *Analyzer S21* is an inspector of cereal grains, mainly rice, which allows the quantification of the above mentioned parameters in rice, as well as other types of defects, through image processing and subsequent statistical analysis. The apparatus consists of two elements, the physical structure of the analyser and a software associated to a

computer. The physical structure is composed of a pump casing with a vertical dispenser, vibrating ramp, which allows the separation of the grains, and a high-speed camera that captures images of the sample's individual grains.[112]

Around 60-70 g of raw grains were placed in the vertical dispenser followed by pressing the button to capture the images and turning on the ramp vibrating system. Next, the vertical dispenser was opened, allowing the grains to fall on the ramp and, finally, the button to end capture is pressed. The recorded images were treated, according to the manual, discarding any overlapping grains.

The *Kett* is a commercial whiteness meter that instantly provides the whiteness value of a rice sample, which can be used to assess the milling quality and purity of the analysed sample. This whiteness value is measured by light reflectance and provided in a scale of 0 to 100.[113]

Firstly, the *Kett* was zeroed using an optical standard. The sample container was then filled with rice, closed and inserted into the *Kett*, as represented in **Figure 9 (right)**.

Figure 9 – *AgroMay Statistic Analyzer S21* apparatus (left) and *Kett Electric Laboratory*, *model C-300-3* apparatus (right).

4.4. Analysis of Moisture Content

Moisture content was determined using a *VirTis – Benchtop K* freeze drying apparatus and calculated as the loss in weight of the freeze-dried samples, according to the following **equation (a)**:

$$\%\ Moisture\ content = \frac{initial\ weigth - final\ weigth}{initial\ weigth} \times 100 \qquad \textbf{(a)}$$

Freeze-drying works in two stages. During the first stage pressure inside the container is lowered and enough heat is supplied in order to cause water sublimation in the frozen samples. During the second stage, pressure can be lowered and more heat is supplied in order to evaporate the remaining unfrozen water molecules from the sample.[114]

Whole rice grains from each sample were ground, passed through a 1.000 mm sieve, stored in a pre-weighted container, weighted and frozen. Prior to freeze drying, the caps were removed and stored in a desiccator, the container with sample was sealed using parafilm, which was then pierced through. Samples were placed in a freeze-drying container and dried for 3 days.

4.5. Analysis of Protein Content

Protein content was analysed by through nitrogen elemental analysis using a *TruSpec 630-200-200 CNHS Analyser*. For this, up to 10 mg of freeze dried samples were combusted in a furnace operating at 1075 °C and with an afterburner at 850 °C in order to convert the samples' nitrogen into nitrogen gas. Then, separation was achieved though gas chromatography followed by quantification with thermal conductivity.[115] Nitrogen content was converted into protein content by multiplying by the factor 5.95[116], as reported in the literature, followed by conversion into dry weight basis, using the previously determined moisture content.

4.6. Analysis of Total Starch Content

Analysis of total starch content was adapted from Teixeira (2013)[117] and Goñi *et al.* (1997)[118].

Around 5-7 whole raw grains per sample were ground, passed through a 0.500 mm sieve and approx. 30 mg were weighted into capped test tubes. Each sample was dispersed in 2 mL of KOH 2 M for 72 h at room temperature with constant stirring, followed by neutralisation with HCl 2 M. Afterwards, 3 mL of tris-maleate buffer 0.6 M pH 6.9 and 400 U of α-amylase were added and left stirring for 48 h at room temperature. A 200 µL aliquot was transferred, in triplicate, to eppendorfs followed by addition of 1 mL of sodium acetate

buffer 0.4 M pH 4.75 and 21 U of amyloglucosidase. The resulting reactional mixture was incubated for 24 h at 60 °C, followed by centrifugation for 1 min at 1000 rpm.

Glucose concentration of the centrifuged samples was spectroscopically quantified at 340 nm using the D-Glucose GOD-POD kit and a 96-well *Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer*. The obtained glucose concentration values were then converted into total starch content (%) by using a conversion factor of 0.9[118], as reported in the literature, followed by conversion into dry weight basis , using the previously determined moisture content.

4.7. Analysis of Resistant Starch Content

Analysis of resistant starch content was adapted from Goñi *et al.* (1996)[36] and the *Megazyme* Resistant Starch Assay Procedure[119].

Around 7-10 whole raw grains per sample were ground, passed through a 1.000 mm sieve and approx. 100 mg were weighted into capped test tubes. To each sample 2 mL of HCl-KCl 25 mM pH 1.6 and 14 U of pepsin were added and left stirring for 1 h at 40 °C. Then, the reactional mixture was neutralized with NaOH 3 M and tris-maleate buffer 0.6 M pH 6.9 was added until 3 mL were achieved. Next, 400 U of α-amylase were added and left stirring for 24 h at room temperature. The resulting solution was then adjusted to pH 4.75 with HCL 6 M followed by the addition of sodium acetate buffer 0.4 M pH 4.75 until 3 mL were achieved and finally by adding 168 U of amyloglucosidase. Incubation was ensured for 24 h at 60 °C. Afterwards, the tubes were centrifuged for 10 min at 3000 rpm, the supernatants were discarded and the residues were thoroughly washed, thrice, with 2.5 mL of ethanol 50 % (v/v) and dried by inversion of the test tubes. Next, dispersion in 2 mL of KOH 2 M was carried out for 30 min at room temperature with constant stirring and then the pH was adjusted to 4.75 with HCl 2 M. A 300 µL aliquot was transferred, in triplicate, to Eppendorfs followed by addition of 700 µL of sodium acetate buffer 0.4 M pH 4.75 and 21 U of amyloglucosidase. The resulting reactional mixture was incubated for 24 h at 60 °C, followed by centrifugation for 1min at 1000 rpm.

Glucose concentration of the centrifuged samples was spectroscopically quantified at 340 nm using the D-Glucose GOD-POD kit and a 96-well *Thermo Scientific™ Multiskan™*

GO Microplate Spectrophotometer. The obtained values were then converted into resistant starch content by using a conversion factor of 0.9[118] followed by conversion into dry weight basis, using the previously determined moisture content.

4.8. Analysis of Apparent Amylose Content

Analysis of apparent amylose content was performed in accordance with the method described in ISO CD 6647-2[120], with a few modifications.

Around 20-25 whole raw grains per sample were ground, passed through a 0.180 mm sieve and 100±0.5 mg were weighted into test tubes. Standards were also weighted and and 1 mL ethanol 96 % (v/v) plus 9 mL NaOH 1 M were added. Then, parafilm was used to seal the tubes and dispersion was ensured by shaking for 2-3 days at 180 rpm in an *Agitorb 200 ICP* orbital shaker. Afterwards the tubes were placed for 10 minutes in a boiling water bath and subsequently left to cool down until slightly warm to touch. Then, each tube's content was transferred into a 100 mL volumetric flask and the volume adjusted with water.

New test tubes were prepared in triplicate. In each, 0.5 mL of the prepared sample dispersion, 5 mL distilled water, 0.1 mL acetic acid 1 M, 0.2 mL iodine solution and 4.2 mL distilled water were added. Blank was prepared by substituting the prepared sample for NaOH 0.09 M. The reactional mixtures were homogenised right before reading the absorbance at 620 nm in a *Jenway 6405 UV/Vis* Spectrophotometer. Apparent amylose content was later converted into dry weight basis, using the previously determined moisture content.

4.9. Analysis of Ordinary Cooking Time

Analysis of ordinary cooking time was performed in accordance with the method described in the thesis by Sério (2013) entitled "*Potencialidades da espectroscopia NIR para análise de arroz comercial*" [121], with a few modifications.

About 10 tea infusers were filled with 2 g of whole raw grains each. A pot was filled with enough tap water to cover all the tea infusers, covered with its lid and placed over a 1500 watts *ok.* heating disk at the highest setting. As soon the water started to boil, the

tea infusers were submerged and the time count was started. After 10 minutes or more, depending on the sample, as different samples required different cooking times, the tea infusers were removed one at a time in 1 to 4 minute intervals and submerged in cold tap water for a few seconds, in order to stop the cooking. Immediately afterwards each the tea infuser was dried as best as possible and their content was placed in a plate identified with the corresponding cooking minute. In the end, the cooking was checked by counting the nuclei of 20 randomly selected grains when pressed between two petri dishes (**Figure 10**). The analysis was repeated in triplicate in the same conditions considering it is necessary that the last two portions of grains contain zero nuclei to ensure that the 100 % cooking stage is achieved.

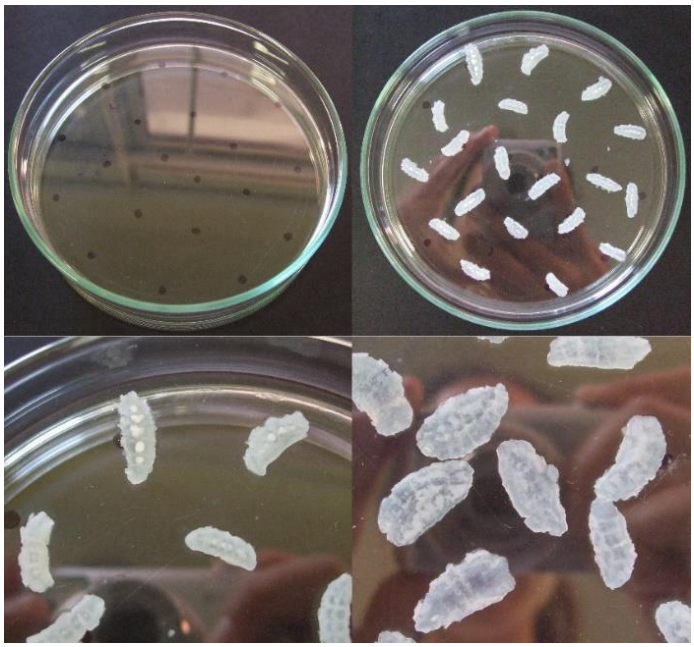


Figure 10 – Placing of grains in the petri dish (top left and right). Counting of the nuclei of 20 grains and appearance of the grains with (bottom left) and without (bottom right) nuclei.

For the calculation of the cooking time, the number of grains with nuclei obtained previously was converted into the number of grains without nuclei by subtraction from the total of 20 grains considered for the counting. The % of cooking was then calculated from the median of the previous value. Two equalisations were performed and the resulting equalised medians were plotted against the cooking time. By polynomial interpolation, the second order polynomial equation for the sample's cooking profile was obtained. Lastly, the cooking time for 80 % of cooked rice was obtained (**Appendix A**).

4.10. Analysis of Solids in the Drained Pre-cooking Water and Analysis of Post-Cooking Time Using a Microwave Oven or Steam

The following procedures are composed of a first and second part that were repeated in triplicate for each variation of the analysis. The post-cooking analysis (second part) was repeated until triplicate results were performed in the same conditions, considering it is necessary that the last two portions contain zero nuclei.

First Part: Pre-cooking and Obtaining the Pre-cooking Water

The volume of 25 g of whole raw grains was measured, corresponding to the measurement "1 part". A 1000 watts *Tristar* heating disk at the 3.5 setting was heated until the temperature was stable. Afterwards, a 400 mL beaker with 1 part distilled water covered with a watch glass was placed on the heating disk. As soon as the water began to boil, 2 parts of whole raw grains were added and the time count was started (**Figure 11 left**). The samples were cooked for the desired pre-cooking time (7, 10 or 13 minutes) followed by drainage of the leftover cooking water into a previously weighted petri dish (**Figure 11 top right**). The drained sample was placed in a second petri dish that had been covered with aluminium foil.

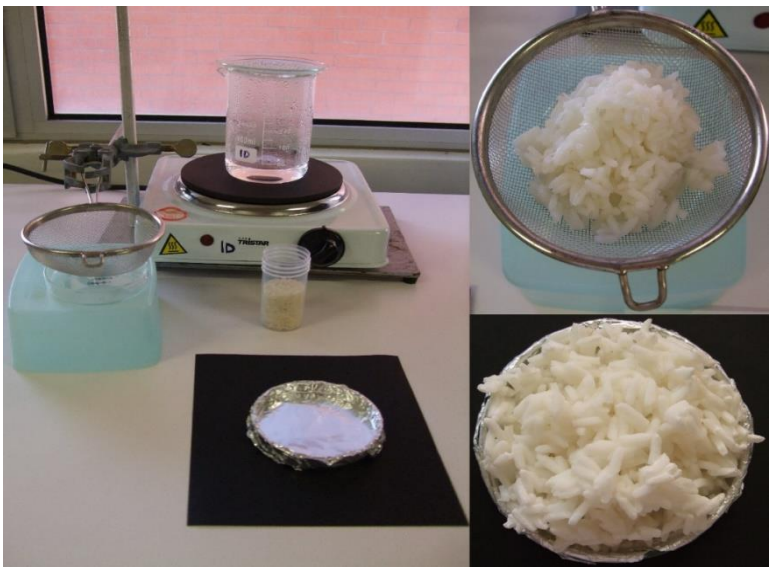


Figure 11 – Apparatus used to pre-cook and drain the samples (left), draining of a sample over a previously weighted petri dish (top right) and frozen sample before storage in a zip-lock bag (bottom right).

The petri dishes used for analysis of solids in the drained pre-cooking water were prepared in advance by thoroughly drying in a *Binder* drying oven with natural convection for 1 h at 105 °C and allowed to cool inside a desiccator. The above mentioned pre-cooking waters were then dried for 16 h at 105 °C, or until constant weight.

The petri dishes containing the pre-cooked rice were stored in a conventional horizontal freezer at -18 °C until completely frozen (**Figure 11 bottom right**). Afterwards, the samples were transferred into zip-lock bags and left inside the freezer for a maximum of 48 h.

Second Part: Post-cooking

A) With a Microwave Oven (300 watts)

One frozen sample was placed inside a larger petri dish (**Figure 12 left**). Immediately afterwards, 1 part of distilled water was poured over the frozen sample followed by placing it inside a *Samsung* 800 watts (*23 L MS23F301EAW*) microwave oven, covered with a microwave lid, at 300 watts (**Figure 12 right**). After 3 minutes, and at 1 minute intervals up until 11 minutes, the sample was mixed and a teaspoon-sized portion was removed, making sure that the microwave door isn't left open while the portion is collected. The portions were placed inside a tea infuser and submerged in cold distilled water for a few seconds, in order to stop the cooking. Afterwards, the tea infuser was dried as best as possible and its content was placed in a plate identified with the corresponding cooking minute. In the end, the cooking was checked by counting the nuclei of 20 grains when pressed between two petri dishes (**Figure 10**) and the microwave was allowed to cool down before the next sample was cooked.

The calculation of the cooking time was performed as described in **4.9.**

Lastly, samples were cooked according with the estimated time (mixing of the sample was always performed at the 3 minute mark) and visually checked for nucleus at the end of the pre-cooking and post-cooking stages, final appearance of the grains and if water absorption by the sample was complete or not. Observations were registered and can be found in **Appendix B**. Each sample was also photographed.

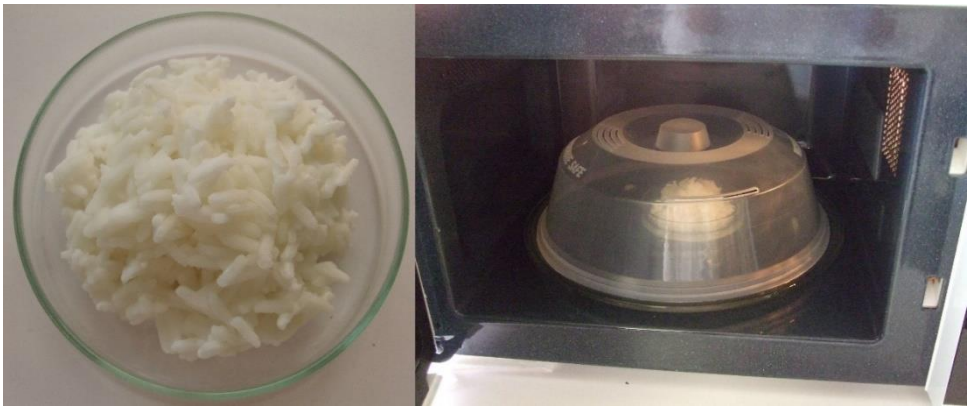


Figure 12 – Frozen sample (left) and microwave cooking apparatus (right).

B) With Steam

A 600 mL beaker containing 400 mL of distilled water, borosilicate glass beads and some weights was covered with a watch glass and was placed over a 1000 watts *Tristar* heating disk at the highest setting. When the water started to boil, a net containing one frozen sample was covered with the watch glass, placed inside the beaker and the time count was started (**Figure 13**). After 10 minutes or more, depending on the sample, and at 2-4 minute intervals, the sample was mixed and a tea spoon-sized portion was removed. The portions were placed inside a tea infuser and submerged in cold distilled water for a few seconds, in order to stop the cooking. Afterwards, the tea infuser was dried as best as possible and its content was placed in a plate identified with the corresponding cooking minute. In the end, the cooking was checked by counting the nuclei of 20 grains when pressed between two petri dishes (**Figure 10**).

The calculation of the cooking time was performed as described in **4.9.**

Lastly, samples were cooked according with the estimated time (without any mixing) and visually checked for nucleus at the end of the pre-cooking and post-cooking stages and final appearance of the grains. Observations were registered and can be found in **Appendix B**. Each sample was also photographed.

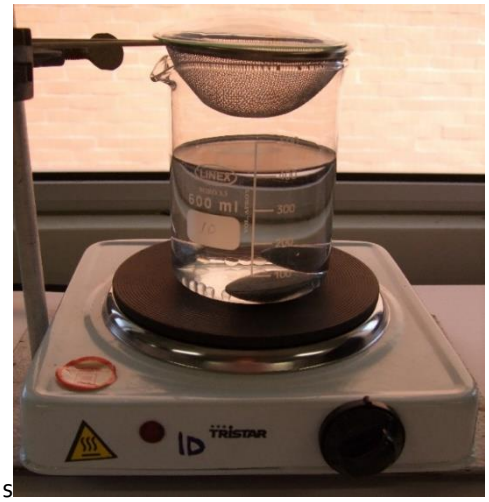


Figure 13 – Steam cooking apparatus.

4.11. Statistical Analysis

Microsoft Excel software was used for the statistical analysis of all the data obtained.

Whenever possible, experimental data was expressed as the means ± standard deviation (SD) and in dry weight basis. Composition parameters are presented in percentage of the grain, with the exception of moisture content. All results can be found in **Appendix B**.

The significance of differences among samples was analysed with the Student's *t*-test considering a significant level of 90%.

The relationship between different parameters was determined using Pearson's correlation and all correlation tables can be found in **Appendix C**.

Chapter 5 – Results and Discussion

5.1. Methodology Optimization

5.1.1. Analysis of Moisture Content

Analysis of moisture content was subjected to a small procedure optimisation.

Sério (2013) analysed his samples following the methodology described in ISO 712:2001[122], in which ground samples (5 g ± 1 g) with particle size inferior to 1 mm were dried in an oven at 130 °C until constant weight[121]. This methodology was used at first but it was sluggish, not very convenient, and only a limited quantity of samples could be dried at once. Therefore, a new alternative was tested: freeze-drying. This alternative might not be readily available at many laboratories but it easily allows the quantification of moisture content in many samples at once in just 3 days.

In **Table 2** the moisture content determined using both methods is shown. The moisture contents obtained with freeze-drying were very similar to the ones obtained by oven drying, therefore this method was chosen for the analysis of moisture content, as can be viewed in **4.4**.

Table 2 – Comparison of moisture contents obtained by oven drying and freeze-drying.

Samples	Oven drying (%)	Freeze-drying (%)
indica2	11.38	11.37
indica7	12.57	12.59
japonica3	12.30	12.27
japonica8	12.00	11.95

5.1.2. Analysis of Total Starch Content

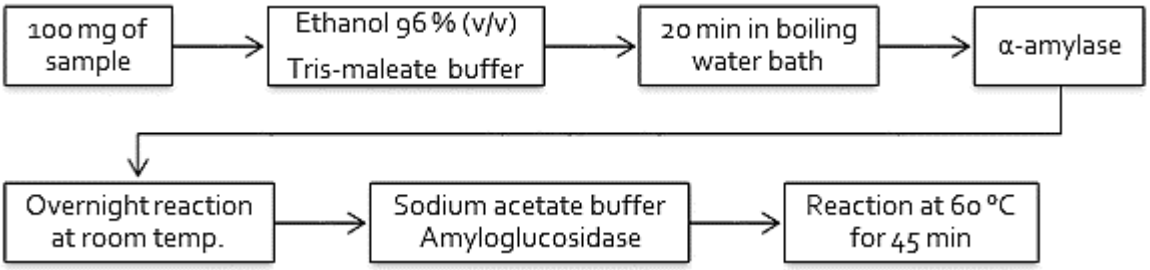
Starch is the most abundant component of rice[3], therefore it is of great importance establish a methodology for its quantification.

The critical step in this analysis is the solubilisation or gelatinisation of starch prior to its degradation to glucose, which isn't easily achieved. As Calixto *et al.* (1991) reported, raw starch granules are very slowly digested by enzymes[31], so any starch must always receive treatments that ease its quantification. Afterwards, an amyloglucosidase

(exogenous enzyme) can be used to hydrolyse the 1,4- and 1,6- linkages between the glucose subunits of the pre-solubilised/gelatinised starch.[123]

Sample particle size was kept the same throughout all the trials, that is, inferior to 0.500 mm. Buffer concentrations and pHs were also kept equal as they corresponded with the enzymes' requirements. The glucose concentration of the centrifuged samples was always quantified as described in **4.6.**

The first methodology and trial was conducted as described in Teixeira (2013)[117] (**Scheme 1**).



Scheme 1 – Simplified scheme of the first methodology, by Teixeira (2013)[117].

The results obtained with this first trial (**Table 3**) weren't as high as the expected averaged 75 % (approx. and in DWB)[23] mentioned in the literature and the standard deviations were higher than desired in half of the samples tested.

Table 3 – First trial: total starch content (DWB) obtained with the first methodology.

Samples	Total starch (%)
indica3	65.79±3.06
indica6	64.98±3.47
japonica1	65.48±1.46
japonica2	67.78±1.60

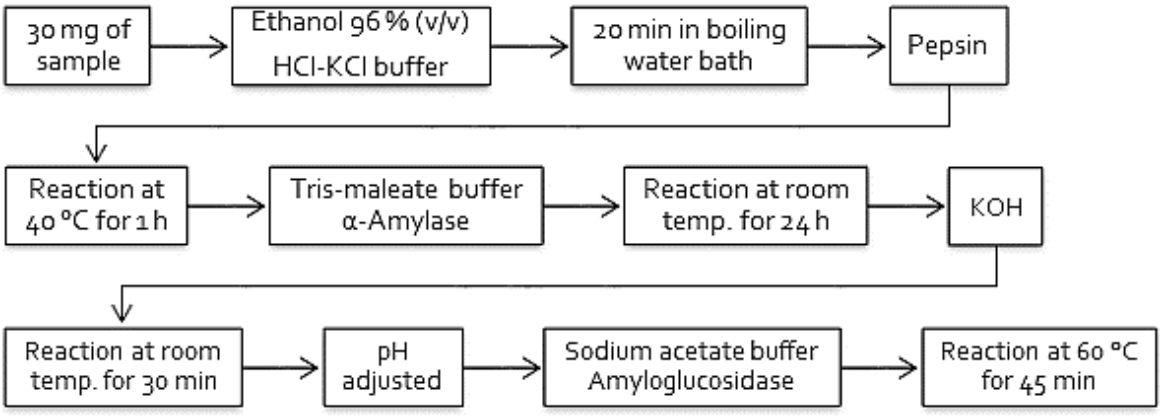
For this first methodology, three different modifications were tested in conjunction (second trial). One modification was the decrease in the quantity of sample used from 100 mg to approx. 30 mg, as this could aid the starch gelatinisation. The second and third

modifications were the increase in enzymatic quantities and reaction times, since the ones used in Teixeira (2013)[117] could be lower than the required for the amount of starch used. The quantity of α-amylase was kept the same, but its reaction time was increased from overnight (approx. 16 hours) to 24 hours. As for amyloglucosidase, its quantity and reaction time were increased from 8.4 U to 21 U and from 45 minutes to 90 minutes. As seen in **Table 4**, the decrease in sample quantity decreased the standard deviation of the obtained results, meaning that using 100 mg was hindering the crucial step of starch' gelatinisation. This modification was kept for the henceforth trials. The increase in enzymatic quantities and reaction times didn't increase much the results. Nevertheless, the 24 hour reaction time for α-amylase was kept as this is the endogenous enzyme[20, 32] for the hydrolysis of the starch's main chain. The amyloglucosidase quantity was also kept since it is required in excess conditions and, through calculations, it was verified that the quantity used by Teixeira (2013) was too low.

Table 4 – Second trial: total starch content (DWB) obtained with the modification of the first methodology.

Samples	Total starch (%)
indica3	66.97±2.11
indica6	67.21±2.45
japonica1	68.64±0.83
japonica2	68.83±0.26

The second methodology tested (third trial) (**Scheme 2**) was adapted from Goñi *et al.* (1996)[36], which reports a procedure for determining the resistant starch content.



Scheme 2 – Simplified scheme of the second methodology tested, adapted from Goñi *et al.* (1996)[36].

The results obtained with this more elaborate methodology still didn't reach the expected averaged 75 % (approx. and in DWB)[23], but a decrease in the standard deviation was obtained (**Table 5**). This may be due to the differences between the first and second methodologies, as the enzymatic reaction with pepsin and the dispersion step with potassium hydroxide were added. Goñi *et al.* (1996) found that adding the protein removal step increased only slightly, but significantly, the final values.[36] Such increase might be due to an improvement of the α-amylase accessibility by avoiding the starch and protein associations, the starch encapsulation by the protein matrix and the formation of glutinous lumps.[123-124] As for potassium hydroxide, it is the alkali used for starch dispersion/ solubilisation in some analyses of total starch content[35-36, 118, 123].

Table 5 – Third trial: total starch content (DWB) obtained with the second methodology.

Samples	Total starch (%)
indica3	61.43±0.31
indica6	65.54±0.04
japonica1	63.34±0.31
japonica2	68.61±0.35

Just like for the first methodology, the increase in enzymatic reaction times were tested by increasing the α-amylase reaction time from 24 hours to 72 hours and the amyloglucosidase reaction time from 45 minutes to 90 minutes. This didn't lead to a considerable increase in the final results, as can be seen in **Table 6**.

Table 6 – Fourth trial: total starch content (DWB) obtained with the first modification of the second methodology.

Samples	Total starch (%)
indica3	63.12±0.36
indica6	66.30±0.44
japonica1	62.12±0.28
japonica2	69.97±0.15

The next trial tested the removal of ethanol, as Goñi *et al.* (1996) reported that it could influence resistant starch results[36]. Moreels *et al.* (1987) reported that this was

indeed true, while also suggesting that at least ethanol content should be as low as possible to prevent enzyme denaturation[125]. Another modification tested together with ethanol removal was the increase in the dispersion time with potassium hydroxide from 30 minutes to 24 hours. A small increase in the final results was observed (**Table 7**). Nevertheless, ethanol was removed from the henceforth trials and a 24 hour dispersion time with potassium hydroxide was re-tested in the sixth trial.

Table 7 – Fifth trial: total starch content (DWB) obtained with the second modification of the second methodology.

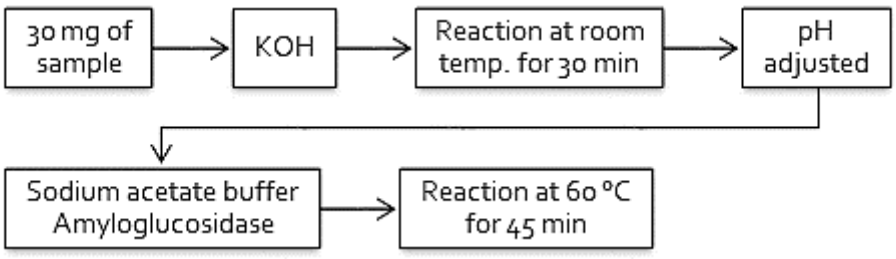
Samples	Total starch (%)
indica3	65.11±0.69
indica6	70.61±0.39
japonica1	68.02±0.06
japonica2	67.77±0.10

The last modification tested for this second methodology (sixth trial) was the dispersion with cold (4 °C) potassium hydroxide as both the *Megazyme* Total Starch Assay Procedure[126] and the *Megazyme* Resistant Starch Assay Procedure[119] use cold potassium hydroxide when analysing samples with high resistant starch content. However, it should be mentioned that, during the optimization of this analysis, the samples still hadn't been analysed regarding their resistant starch content. The article by Goñi *et al.* (1996) was the only literature source found that classified raw rice as a foodstuff with high resistant starch content (5 % to 15 %)[36]. The use of cold potassium hydroxide lead to a decrease in the obtained results as well as an increase in standard deviation (**Table 8**).

Table 8 – Sixth trial: total starch content (DWB) obtained with the third modification of the second methodology.

Samples	Total starch (%)
indica3	68.91±1.31
indica6	65.15±1.70
japonica1	68.46±0.50
japonica2	69.01±0.16

The third and final methodology tested (seventh trial) (**Scheme 3**) was based on the methodology employed by Goñi *et al.* (1997)[118].



Scheme 3 – Simplified scheme of the third methodology tested, adapted from Goñi *et al.* (1997)[118].

This methodology relies solemnly on potassium hydroxide to solubilise the starch and the final results weren't particularly high, as expected, not reaching near the averaged 75 % (approx. and in DWB)[23] but standard deviations weren't as big as in the first trial (**Table 9**).

Table 9 – Seventh trial: total starch content (DWB) obtained with the third methodology.

Samples	Total starch (%)
indica3	67.80±0.66
indica6	69.28±0.57
japonica1	69.00±0.61
japonica2	65.45±0.25

The first modification to this methodology was introducing a 24 hour α-amylase reaction in between the dispersion with potassium hydroxide and the reaction with amyloglucosidase. This originated the highest results of all the tested trials (**Table 10**). The higher standard deviation of the sample *japonica1* could be due to human error and wasn't regarded as meaningful. So, henceforth the 24 hour α-amylase reaction was kept whilst testing other modifications.

Table 10 – Eight trial: total starch content (DWB) obtained with the first modification of the third methodology.

Samples	Total starch (%)
indica3	63.43±0.20
indica6	74.78±0.52
japonica1	68.86±1.31
japonica2	72.78±0.47

Next the use of ethanol, to help with starch extraction, followed by gelatinisation for 20 minutes in a boiling water bath was tested. This was done before the amyloglucosidase reaction (and after the above mentioned 24 hour α-amylase reaction). The results obtained were lower, as can be seen in **Table 11** and again, the higher standard deviation of the sample *indica6* was regarded as human error. As mentioned during the fifth trial, ethanol was reported[36, 125] as being a source of enzyme denaturation and it is safe to say that this was what happened.

Table 11 – Ninth trial: total starch content (DWB) obtained with the second modification of the third methodology.

Samples	Total starch (%)
indica3	58.21±0.29
indica6	57.90±2.27
japonica1	61.02±0.34
japonica2	57.74±0.32

In lieu of the last obtained results, ethanol was removed from the procedure. Therefore, the tenth trial tested the use of a 20 minute boiling bath for gelatinisation, after the 24 hour α-amylase reaction and before the amyloglucosidase reaction. Results (**Table 12**) were higher than in the previous trial, which is another indication that ethanol influences negatively starch results. Nevertheless, the results weren't as high as in the seventh trial (**Table 10**). A possible explanation for the lower percentages obtained with the trials containing gelatinisation with a boiling water bath (all trials with exception of the seventh and tenth) is the formation of retrograded starch[31], hindering the enzymatic action over the overall starch.

Table 12 – Tenth trial: total starch content (DWB) obtained with the third modification of the third methodology.

Samples	Total starch (%)
indica3	69.32±0.42
indica6	72.23±0.37
japonica1	67.61±0.68
japonica2	66.87±0.64

Some final modifications were made to the reaction times employed in the seventh trial: increase of the potassium hydroxide dispersion time from 30 minutes to 24 hours, just like in the (fifth trial), increase of the α-amylase reaction time from 24 hours to 48 hours and increase of the amyloglucosidase reaction time from 45 minutes to 24 hours to ensure that both enzymatic reactions occurred in excess-type conditions. The obtained results (**Table 13**) were slightly higher than in the seventh trial and are around the averaged 75 % (in DWB) mentioned in the literature[23]. Therefore, the final procedure for the analysis of total starch content was attained and can be view in **4.6.**

Table 13 – Eleventh trial: total starch content (DWB) obtained with the optimised methodology.

Samples	Total starch (%)
indica3	70.46±0.34
indica6	78.49±0.87
japonica1	78.77±0.37
japonica2	78.45±0.64

It is possible to conclude that starch dispersion or solubilisation is indeed a key aspect in this analysis. Further efforts should still be made to improve and simplify even more this procedure while also improving the obtained results.

There is a last important observation to consider. Batey (1982) reported a reduction of 5 % in the glucose concentration obtained when re-analysing the final solutions of the total starch analysis after an overnight storage at 4ºC. A slightly smaller reduction was also observed when the final solutions were frozen.[123] Therefore, glucose concentration must be analysed on the same day when the amyloglucosidase reaction is stopped.

5.1.3. Analysis of Resistant Starch Content

The optimization of a direct analysis of resistant starch is important for the characterisation of rice samples since this is a key component of rice starch[31].

Some of the modifications and difficulties discussed in **5.1.2.** still apply for this optimisation, such as the solubilisation of starch. So, for simplicity's sake, they will only be addressed briefly in this topic.

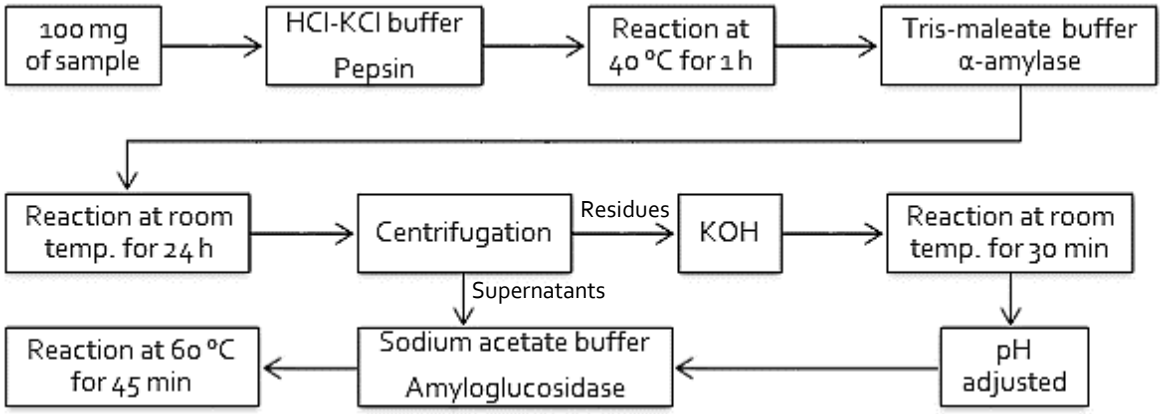
The key difference between total starch analysis and resistant starch analysis is the removal of digestible starch from the sample. It must be noted that an attempt was made to quantify both the resistant and digestible starch from the same procedure. However, as it will be discussed, the sum of both starches didn't give a good estimate of the total starch content, due to losses during the procedure or due to the procedure itself. In the end, the results obtained didn't justify the extra work required for the analysis of digestible starch.

Sample particle size was kept the same throughout all the trials, that is, inferior to 1.000 mm. Buffer concentrations and pHs were also kept equal as they corresponded with the enzymes' requirements. Enzymatic quantities were kept in the same proportion as used for the analysis of total starch (**4.6.**). The glucose concentration of the centrifuged samples was always quantified as described in **4.7.**

The first methodology and trial tested was adapted from Goñi *et al.* (1996) (**Scheme 4**). This methodology, as seen in **Scheme 2** (**5.1.2.**) has the protein removal step, important for the correct hydrolysis of the digestible starch. Furthermore, this step will prove to have a greater importance in the analysis of cooked samples, allowing a better simulation of the physiological conditions of the human stomach such as acidic pH and action of proteolytic digestive enzymes.[36]

Originally, this methodology didn't comprise the analysis of digestible starch. Therefore, an adaptation was made right from the start consisting of saving the supernatants into volumetric flasks and later reacting them with amyloglucosidase. The conditions used for this enzymatic reaction were the same for the analysis of both resistant and digestible starch, meaning that, both were reacted at the same time, in the same

water bath in order to prevent variations in the results. For this methodology the washing of the residue was done in triplicate with distilled water.



Scheme 4 – Simplified scheme of the first methodology tested, adapted from Goñi *et al.* (1996).[36]

As mentioned in **5.1.2.,** Goñi *et al.*(1996) reports a 5 to 15 % in resistant starch content for raw rice[36]. The results obtained for the first trial were higher than the aforementioned range and the standard deviations were too high (**Table 14**). This raised the possibility of an incomplete hydrolysis of digestible starch or the possibility that the residue wasn't properly washed during the centrifugation step. The sum of resistant starch and digestible starch yielded results lower than the ones obtained with the direct analysis of total starch (**Table 13**).

Table 14 – First trial: resistant starch and digestible starch contents (DWB) obtained with the first methodology.

Samples	Resistant starch (%)	Digestible starch (%)	Sum (%)
indica3	24.34±1.43	39.57±0.98	63.91
indica6	19.21±1.92	34.88±1.18	54.09
japonica1	24.20±0.61	26.27±0.81	50.47
japonica2	28.48±2.52	30.74±0.79	59.22

Therefore, the second trial included two modifications: reduction of the amount of sample used from 100 mg to 30 mg and increase in the reaction time of α-amylase from 24 hours to 72 hours, since harsh conditions, as the ones tested in **5.1.1.** can't be used for the solubilisation of starch. It can be concluded, from the results seen in **Table 15**, that in the

first trial there was indeed contamination of the resistant starch with its digestible counterpart. The results obtained were considered good with really good standard deviations, despite being lower than the reported by Goñi *et al.* (1996)[36]. The sum of both starches didn't yield values near those obtained for total starch. The use of the 24 hour reaction time with α-amylase was kept for the henceforth trials and the use of 30 mg of sample was kept until otherwise stated.

Table 15 – Second trial: resistant starch and digestible starch contents (DWB) obtained with the first modifications of the first methodology.

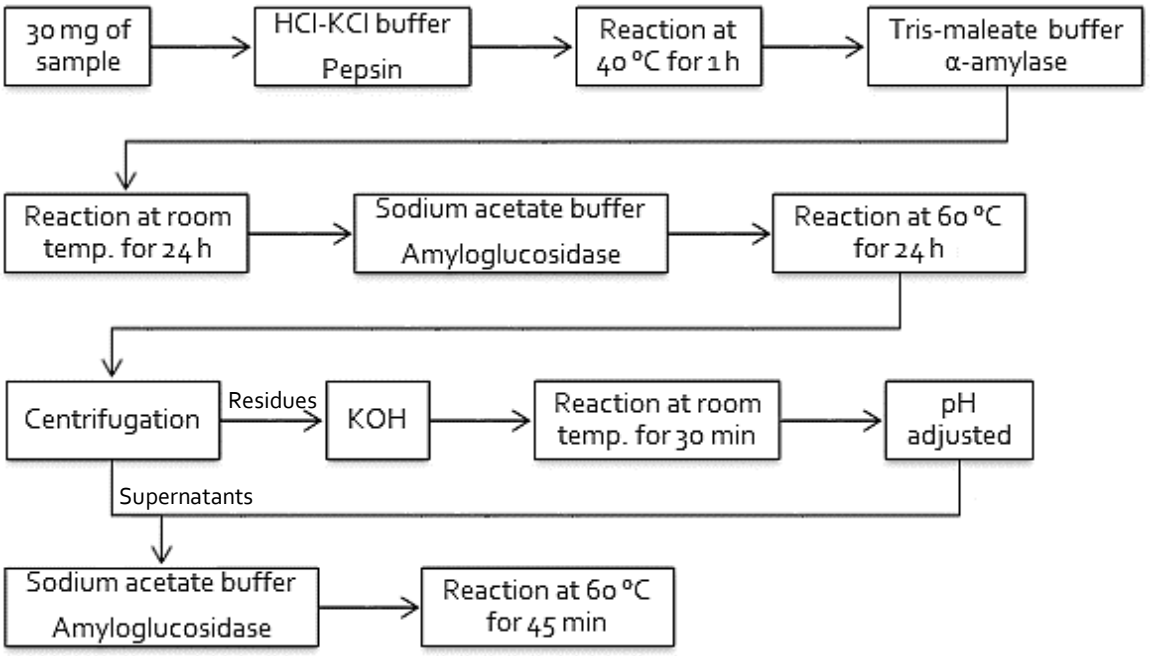
Samples	Resistant starch (%)	Digestible starch (%)	Sum (%)
indica3	5.57±0.05	63.92±0.42	69.39
indica6	6.17±0.02	62.78±0.25	68.95
japonica1	4.32±0.03	51.13±0.02	55.45
japonica2	4.64±0.04	47.69±0.16	52.33

The next trial tested if the results could be improved by increasing the dispersion time with potassium hydroxide from 30 minutes to 24 hours. As seen in **Table 16**, the content of resistant starch was, again, contaminated with digestible starch. Overall, the sums didn't differ much between the first 3 trials, so another methodology was considered in an attempt to overcome the contamination problem.

Table 16 – Third trial: resistant starch and digestible starch contents (DWB) obtained with the second modification of the first methodology.

Samples	Resistant starch (%)	Digestible starch (%)	Sum (%)
indica3	24.54±0.21	37.24±0.41	61.78
indica6	21.68±0.14	43.32±0.44	65.00
japonica1	20.61±0.11	44.11±0.27	64.72
japonica2	22.33±0.11	29.96±0.52	52.29

The second methodology was still based on the first one, but took into consideration the *Megazyme* Resistant Starch Assay Procedure[119] and the previously optimised procedure for the analysis of total starch (**5.1.1.**) (**Scheme 5**).



Scheme 5 – Simplified scheme of the second methodology tested, adapted from Goñi *et al.* (1996)[36] and *Megazyme* Resistant Starch Assay Procedure[119].

The *Megazyme* Resistant Starch Assay Procedure[119] solubilises the digestible starch by using an α-amylase together with an amyloglucosidase. This originates a supernatant wherein all the digestible starch is in the form of glucose, which in turn might help in the centrifugation step. The main problem with centrifuging samples only hydrolysed by α-amylase is that this enzyme leaves behind oligosaccharides and dextrines with bigger molecular weight than glucose. Thus, it is possible that only glucose and less heavy oligosaccharides were removed with the supernatant, leaving behind the heavier products of the hydrolysis together with the residue. For that reason, the reaction with α-amylase followed by a reaction with amyloglucosidase for 24 hours was tested. Ethanol 50 % (v/v) was also used for washing the residue in triplicate during the centrifugation, as described in the *Megazyme* Kit[119]. Ethanol is commonly used for the extraction of sugars[127] and, in fact, its use improved the removal of the supernatant by decantation since residues became less "fluffy" and settled easier at the bottom of the test tube, making it less likely to lose residue. As can be viewed in **Table 17**, the contamination of the residue was avoided and a good separation was achieved between resistant and digestible starch. However, the standard deviations were higher than before. It is safe to say that ethanol

didn't influenced these results, since its content in the test tube is extremely low to cause amyloglucosidase denaturation[125]: after decantation of the ethanolic supernatant, potassium hydroxide is added followed by sodium acetate buffer. The reason for higher standard deviations could be the use of very small quantities of sample, which improves the chances of error. The sum of both starches was, just like in the first 3 trials, bellow the values yielded by the optimised procedure for total starch (**Table 13**) even with the second amyloglucosidase hydrolysis. However, due to the high content of ethanol in the supernatant the amyloglucosidase didn't work properly during that last hydrolysis. It was then decided to remove the analysis of digestible starch from the procedure, and the supernatant was henceforth discarded.

Table 17 – Fourth trial: resistant starch and digestible starch contents (DWB) obtained with the second methodology.

Samples	Resistant starch (%)	Digestible starch (%)	Sum (%)
indica3	3.06±0.40	56.26±1.47	59.32
indica6	8.48±2.23	47.27±0.77	55.75
japonica1	3.05±0.17	56.22±0.44	59.27
japonica2	5.14±1.13	55.68±1.67	60.82

The fifth and last trial tested the use of 100 mg of sample, instead of the 30 mg, and the increase in the last reaction time of amyloglucosidase from 45 minutes to 24 hours, just like in the analysis of total starch. This increase lead to the decrease in the standard deviation (**Table 18**), when comparing with the previous trial. The standard deviations of the second trial were indeed even lower, but that might have happened due to using water to wash the residues. For this reason, the final procedure for analysis of resistant starch still requires that the residue is washed with ethanol 50 % (v/v), as can be viewed in **4.7.** The obtained results are below the 15 % mentioned in the literature[36].

Table 18 – Fifth trial: resistant starch content (DWB) obtained with the optimised methodology.

Samples	Resistant starch (%)
indica3	1.38±0.07
indica6	9.94±0.47
japonica1	5.96±0.53
japonica2	5.47±0.01

It can be concluded that, just like for the analysis of total starch, solubilisation of digestible starch is one of the key aspects in ensuring a good separation of the digestible and resistant fractions. Additional attempts should be made to improve and simplify this laborious and lengthy procedure.

5.2. Analysis of the Size Parameters

The results obtained regarding the size parameters of the analysed samples are summarised in **Chart 1**.

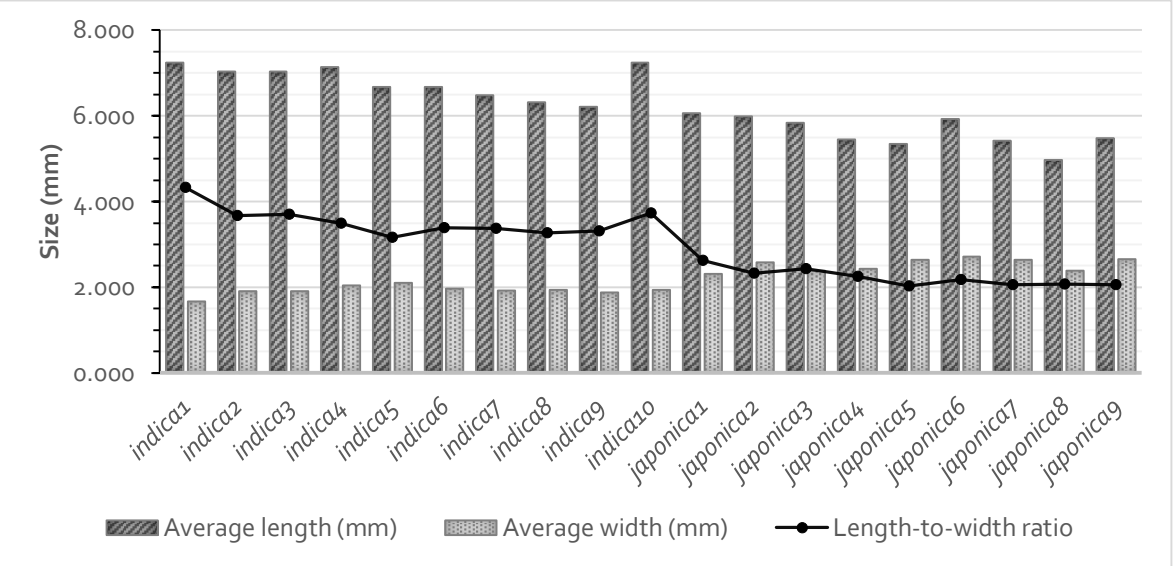


Chart 1 – Results obtained for the size parameters average length (mm), average width (mm) and length-to-width ratio.

The analysed samples varied quite a lot in the average length, with the shortest (*japonica8*) measuring just 4.977 mm and the lengthiest (*indica10*) measuring 7.252 mm. These measurements are quite characteristic of the samples' type as *japonica8* belongs to the round type and *indica10* belongs to the *agulha* type. However, *indica10* is a parboiled

sample and, as discussed in **2.4.**, parboiling can alter the grain's size[79], expanding it. The second lengthiest sample is *indica1* (7.242 mm) which is an aromatic type rice, characteristic for some of the lengthiest varieties[42]. As for average width, the samples range from 1.674 mm (*indica1*) to 2.714 mm (*japonica6*), which is also representative of their types: aromatic and *risotto*, a fairly wide type. The sample *indica1* also has the highest L/W (4.326). However, the lowest ratio belongs to *japonica5* (2.022), a sample belonging to the medium type. This sample is also the second shortest sample (5.353 mm).

As described in **3.2.**, the Portuguese *Decreto-Lei n.º 62/2000* defines rice grains according to their length, classifying them into four categories. According to these guidelines, the two parameters, length (in mm) and L/W, must be met.

The sample *japonica8* only meets one of the parameters, by having a length of 4.977 mm, which is inferior to 5.2 mm. However, its L/W is 2.080, which falls a little bit above the margin for the classification as a rice with round grains. Since this sample almost meets the last parameter, and no other classification is available, *japonica8* is classified has having round grains. It is noteworthy that this sample has indeed the appearance of a round grain type rice. The samples *japonica2, 3, 4, 5, 6, 7* and *japonica9* are classified has having medium grains, and indeed most of them belong to the very similar types, at least in size terms, *carolino*, medium and *risotto*. The parboiled round rice (*japonica9*) falls into the medium classification, probably due to the parboiling it was subjected to[79]. The sample *japonica1* (one of the *carolino* type samples) falls into the first category of long grains, which have a wider width than the long grains of the second category. The *indica* samples are, by exclusion, classified as having long grains of the second category.

5.2.1. Correlations

This and the henceforth correlation sub-chapters will be discussed based on the correlations considering all non-parboiled samples, unless otherwise stated.

Between the L/W and both the length and width parameters very high correlations were obtained (+0.94 and -0.96, respectively) (**Chart 2**). When considering just the

japonica samples, without the parboiled samples, an only moderate correlation is obtained between L/W and average width (-0.65).

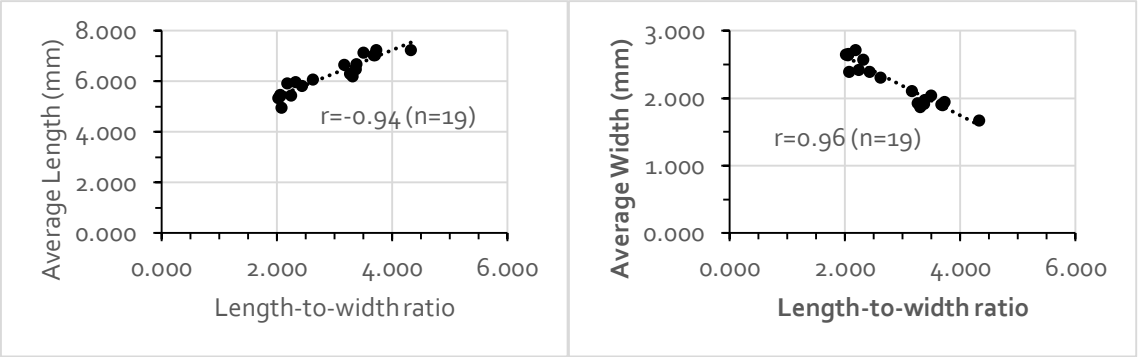


Chart 2 – Positive and negative correlations between average length (mm) and length-to-width, and between width (in mm) and length-to-width ratio, respectively.

A good negative correlation also exists between length and width (-0.83), which was also reported by Koutroubas *et al.* (2004)[42]. This is expected since longer grains tend to be the least wide. However, this correlation only occurs when considering all the samples at once, with or without parboiled samples.

5.3. Analysis of the Whiteness Parameters

Rice whiteness can be measured by four distinctive parameters: total whiteness, crystalline whiteness, *Kett* value and percentage of chalky area (**Chart 3**).

Total whiteness values ranged from 95.33 (*indica9*) to 148.03 (*japonica6*), while crystalline whiteness had a maximum value of 124.65, corresponding to the sample *japonica2*, and a minimum value of 93.12, corresponding to *indica9*. As for *Kett* value, it varied from 20.4 (*indica9*) to 51.2 (*japonica6*). It is important to mention that the three lowest values of total whiteness and *Kett* belong to the parboiled samples *indica9*, *indica10* and *japonica9* which have the characteristic yellow coloration of parboiled rice[80] and are, therefore, less white. Similarly the parboiled samples also gave the lowest values for percentage of chalky area (2.75 % for *indica9*, 4.19 % for *indica10* and 5.33 % for *japonica9*) which is a direct cause of parboiling[45]. The overall percentage of chalky area ranged from

2.75 % (*indica9*) to 40.69 % (*japonica6*) and the samples with highest percentages belong to *japonica* subspecies, as expected.

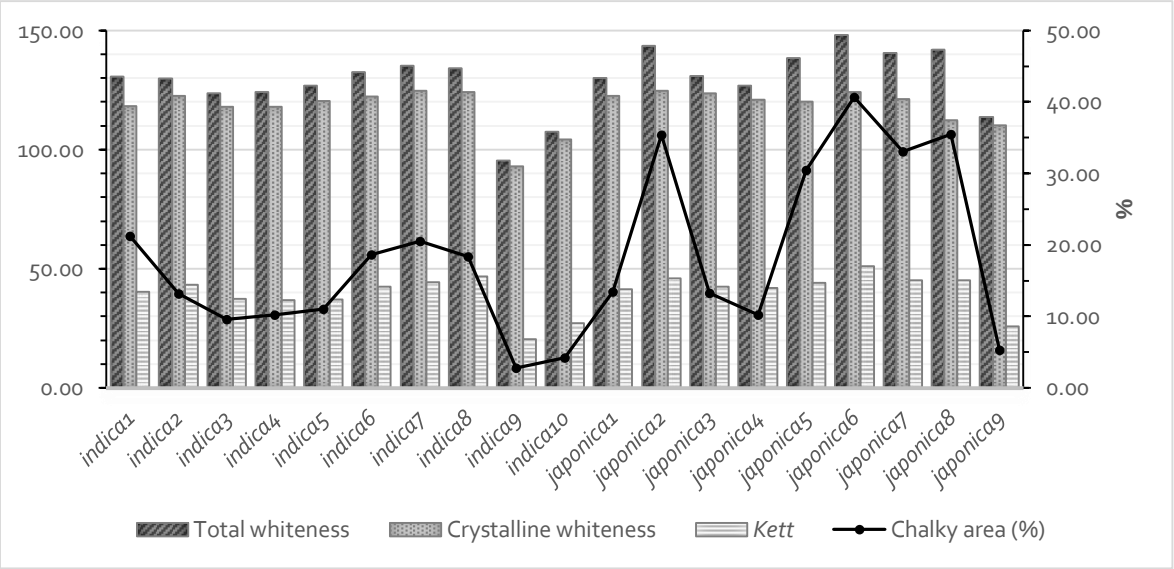


Chart 3 – Results obtained for the parameters total whiteness, crystalline whiteness, *Kett* and chalky area (in percentage)

5.3.1. Correlations

A very high correlation was obtained between the total whiteness and the percentage of chalky area (+0.97) . Total whiteness also correlates highly with *Kett* (+0.89) which, in turn, correlates highly with the chalky area (+0.78).These correlations were expected since all three parameters represent a measurement of the visually perceptible whiteness of the grains.

Total whiteness has a high positive correlation with crystalline whiteness (+0.83), when all samples are considered. However, this correlation is highly reduced (+0.18) when the parboiled samples are disregarded. In fact, by looking at the values of total and crystalline whiteness it can be seen that its values are very close to the total whiteness values for all 3 parboiled samples. This means that the crystalline whiteness accounts for almost all of the total whiteness in parboiled rices. This could be an indication that the yellow coloration characteristic of parboiled rices[80] is reflected in the total whiteness values. When comparing the correlation coefficients for parboiled and non-parboiled *indica* rices the correlation doesn't subside (+0.86). This can only be justified by the yellower appearance that some of the *agulha* samples naturally have. In fact, Sério (2013),

also analysed samples provided by Novarroz, and reported the same slight yellow coloration for the *agulha* and medium types of rice[121].

Total whiteness also correlates with the average length, width and L/W (-0.60, +0.65 and -0.62, respectively). Non-parboiled *indica* rices have a correlation coefficient of -0.66 between total whiteness and average length, meaning that shorter grains will have a higher total whiteness. On the other hand, non-parboiled *japonica* rices have a correlation coefficient of +0.73 between total whiteness and the average width, meaning that the widest grains will have a higher total whiteness. In fact *indica7* and *indica8*, the shortest of the *indicas*, and *japonica6* and *japonica7*, the widest of the *japonicas*, have higher total whiteness values for their subspecies (135.26 and 134.08, 148.03 and 140.64, respectively). When the parboiled samples are considered, no correlation is found between the size parameters and total whiteness, which is consistent with the impact of parboiling on the visual aspect of the grains.

Crystalline whiteness has a high positive correlation with *Kett* (+0.87) when considering all samples. Just like for the correlation between total whiteness and crystalline whiteness, this coefficient's value is highly reduced (+0.41) when the parboiled samples are disregarded. For the *indica* subspecies an even higher correlation was found (+0.88 for all *indicas* and +0.97 for non-parboiled *indicas*).

Between the percentage of chalky area and the crystalline whiteness exists a high positive correlation if all *indica* samples are considered (+0.81), i.e., chalkier *indica* samples will have a higher crystalline whiteness.

The total whiteness, crystalline whiteness and *Kett* were found to correlate, positively but moderately, with the resistant starch content in the grain (+0.56, +0.68 and +0.63, respectively) when considering all samples, parboiled and non-parboiled. These correlations couldn't be found when considering only the non-parboiled samples. This indicates that parboiled samples, which have lower values in all the whiteness parameters, are expected to also have a low resistant starch content. When considering the all the rices of the *indica* subspecies, high correlations were found between all the whiteness parameters and the resistant starch content (total whiteness, +0.79, crystalline whiteness, +0.78, *Kett*, +0.75, percentage of chalky area, +0.75). The same can be observed for

japonica rices, but with low to high correlations (+0.60, +0.61, +0.77 and +0.35 respectively), which could be due to the existence of only one parboiled *japonica* amongst the analysed samples. If the parboiled samples aren't considered, these correlations decrease.

The *Kett* value also only moderately correlates with the size parameters (length -0.54, width +0.54, L/W -0.54) and follows the same pattern of correlations as total whiteness: for non-parboiled *indicas*, a moderate correlation is found with average length (-0.63) and for non-parboiled *japonicas*, a correlation is found with the average width (+0.76). The correlations can no longer be found when the parboiled samples are considered.

No correlation was found between crystalline whiteness and the size parameters when considering all the non-parboiled samples. However, a high correlation exists with the average length when considering the non-parboiled *indica*, non-parboiled *japonica*, and all the *japonica* (-0.83, +0.87 and +0.71, respectively). *Indica* samples have negative correlations, while *japonica* samples have positive correlations: this indicates that the expected crystalline value is highly dependent on the subspecies in question: for a shorter non-parboiled *indica* rice, a higher crystalline whiteness value is expected while for shorter parboiled and non-parboiled *japonica* rices a lower crystalline whiteness is expected. The last correlation also indicated that the only parboiled *japonica* sample included in this study has a total whiteness similar to that of the non-parboiled *japonicas*.

The size parameters were also found to be moderately correlated with the chalky area (%) (length -0.56, width +0.62 and L/W -0.57). When looking at just the correlation coefficients for the non-parboiled *japonica* rices the average width is even more correlated with the chalky area (+0.74). This expected because, as it was mentioned in the state of the art, *japonica* rices tend to be chalkier[42]. This chalkiness is a consequence of their low amylose content and high content in short branched amylopectin, the latter being responsible for the bigger amorphous domain of this subspecies. The correlation isn't as strong for the *indica* subspecies. In fact, the incidence of chalkiness in *indica* type grains is very low, being attributed to a defective development of the grain[128].

5.4. Analysis of the Composition Parameters

The first composition parameter to be analysed was the moisture content. This parameter is also defined in the Portuguese *Decreto-Lei n.º 62/2000*, which states that the maximum moisture percentage allowed in rice for human consumption is 14 %[22]. As can be seen in **Chart 4,** the range goes from 10.72 % (*indica10*) to 12.59 % (*indica7*) which is around the value described in the literature[5] and below the 14 % defined by Portuguese law[22]. Amongst the samples, it is possible to observe that *japonica* samples tend to have the highest moisture content probably due to the grain's shape.

All of the following composition parameters have been converted into dry weight basis (DWB) to allow an easier comparison.

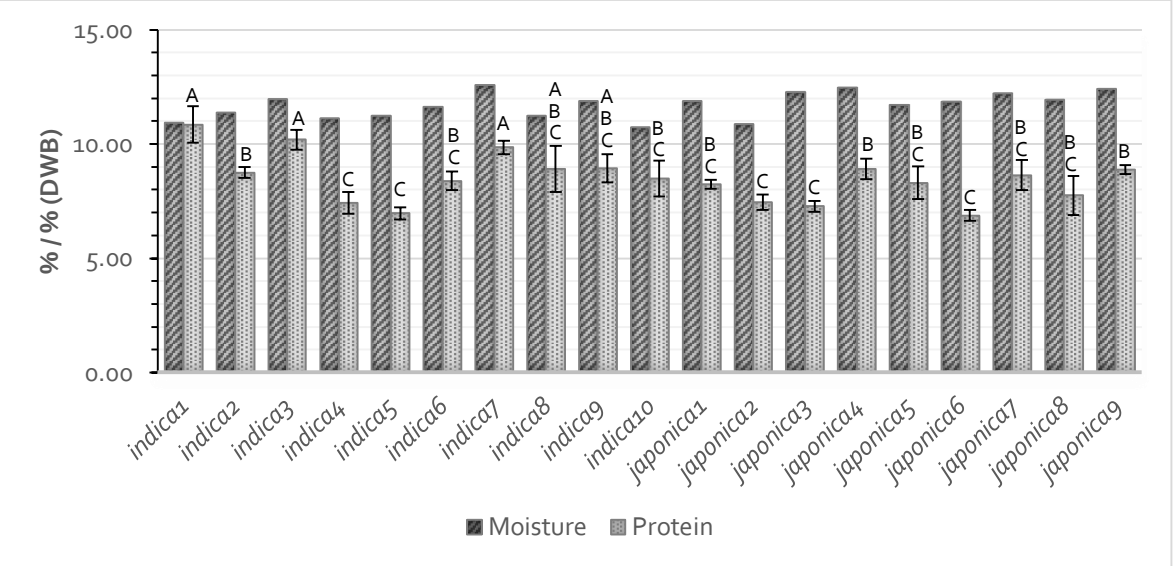


Chart 4 – Percentages obtained for the grain composition parameters moisture and protein (DWB).

Protein content ranged from 6.87±0.24 (*japonica6*) to 10.85±0.80 (*indica1*) indicating a medium content of protein amongst the analysed samples. In 1979, a protein content of 6 to 8 % was reported in 31 samples of Portuguese rices, with an average of 6.8 %[54]. This years' average was 8.48 %, considering all samples, and 8.42 % considering only the non-parboiled samples. The parboiled samples had a protein content higher than the averaged, therefore their non-parboiled counterparts would be expected to have an even higher protein content.[73]

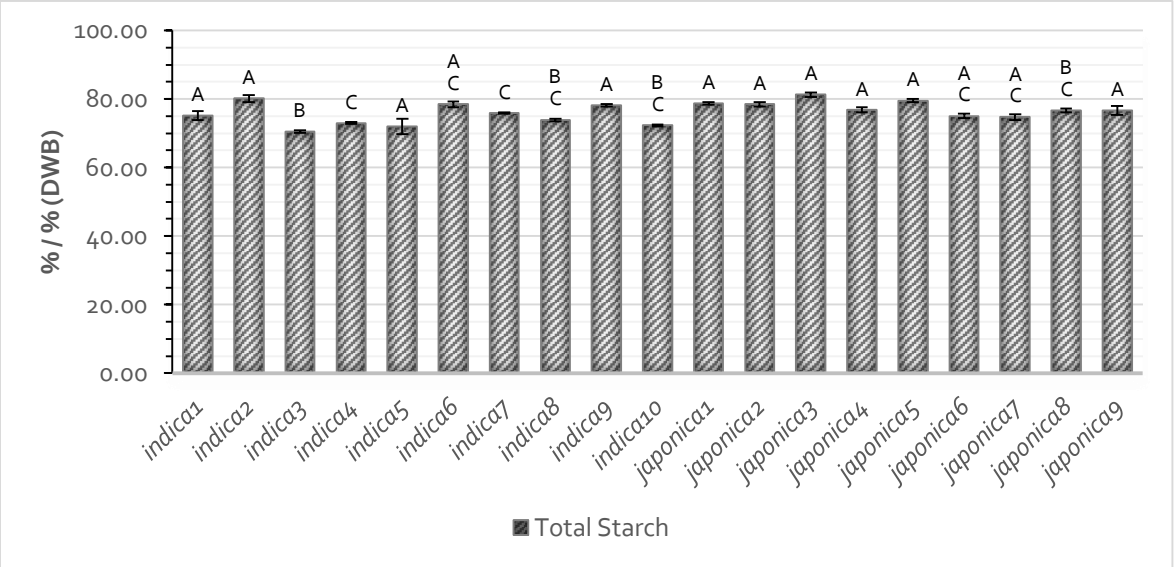


Chart 5 – Percentages obtained for the grain composition parameter total starch (DWB).

The lowest total starch content (**Chart 5**) was found in the sample *indica3*, with 70.46±0.34 %, and the highest in *japonica3*, with 81.21±0.64 %. Overall, the standard deviations obtained with the optimised method were good, but some samples had higher standard deviations. This possibility has been reported[123] and thus, a significance level of 90 % was used for the statistical analysis, instead of the customary 95 %. The overall total starch average the 75 % (in DWB) mentioned in the literature[23]. One reason for this difference could be that a lower degree of milling was performed on the samples. If this was the case, the grains will not only have a lower total starch content, but also have a higher protein content[5, 20].

A wide range of resistant starch values was obtained, as can be seen in **Chart 6**, with the highest belonging to the sample *indica6* (9.94±0.47 %) and the three lowest belonging to the parboiled samples *indica9*, *indica10* and *japonica 9* (0.53±0.01 %, 1.62±0.17 and 0.27±0.00 %, respectively). The obtained values do reach the range reported in the literature[36] of 5-15 % but are also lower, even in non-parboiled samples, which average 5.82 %. The extremely low resistant starch values of the parboiled samples could be a consequence of the parboiling process during which amylose is leached from the grain, thus, less amylose will remain on the grain to retrograde[59, 82]. The standard deviations obtained were considered good, although some samples had a higher values.

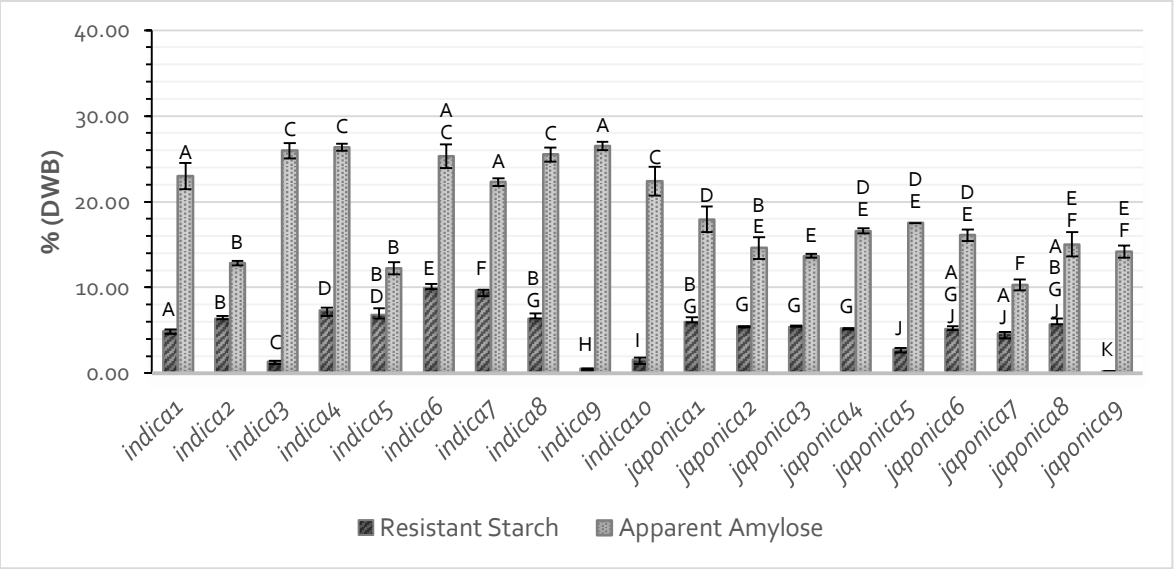


Chart 6 – Percentages obtained for the grain composition parameters resistant starch and apparent amylose (in DWB).

Amylose content in the analysed samples ranged from 10.30±0.63 % (*japonica7*) to 26.50±0.46 % (*indica9*). These values were obtained using the calibration curve obtained with the apparent amylose standards ($y = 0.013x + 0.077$, R^2 of 0.998). The samples can be divided into the three categories mentioned in **2.3.1.2.**: five samples can be classified has having high content (*indica3*, *indica4*, *indica6*, *indica8* and *indica9*), three as having an intermediate content (*indica1*, *indica7* and *indica10*) and the remaining (*indica2*, *indica5*, and all *japonicas*) as having low content of apparent amylose, which is coherent with the literature[3, 35].

In **Chart 7**, **Chart 8** and **Chart 9** it is possible to observe the ratios between the 4 composition parameters analysed.

The protein-to-total-starch ratio (P/TS) doesn't vary much between samples but, higher ratios can be observed for *indica1* and *indica3*, while lower ratios exist for *japonica2*, *japonica3* and *japonica6*. This shows that there is a differentiating trend between *indica* and *japonica* subspecies, just like Koutroubas *et al.* (2004) observed for *indica* and *japonica* samples[42].

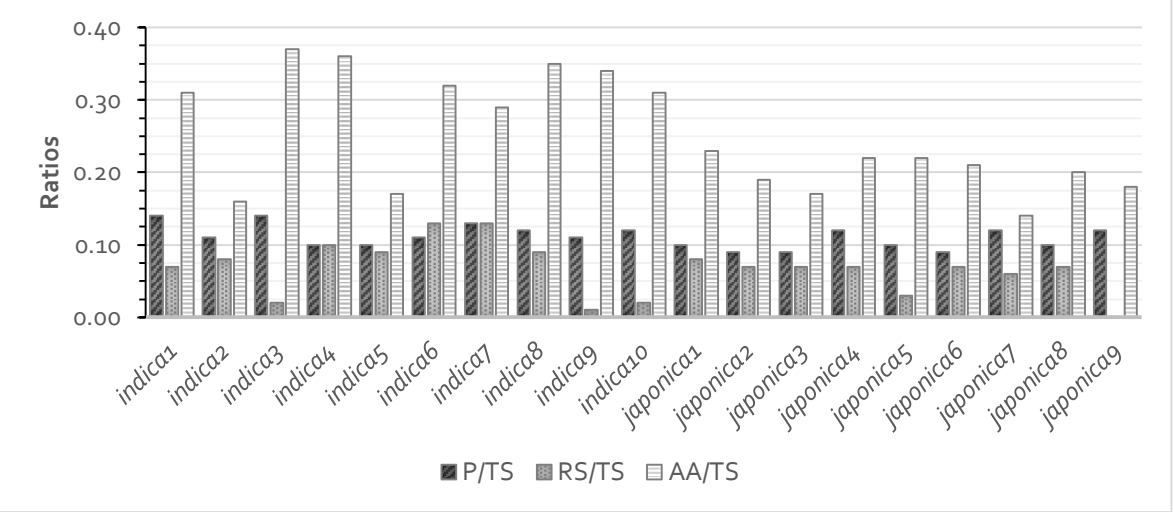


Chart 7 – Ratios between protein, resistant starch and apparent amylose with total starch (P/TS, RS/TS and AA/TS, respectively).

By considering the resistant-starch-to-total-starch ratio (RS/TS) lower ratios can be observed for the parboiled samples (*indica9*, *indica10* and *japonica9*), probably as a consequence of the parboiling. However, a low ratio is also observed for the non-parboiled sample *indica3*. The highest ratios were found for *indica6* and *indica7*.

The apparent-amylose-to-total-starch ratio (AA/TS) ratio also has a wide range of values with the lowest belonging to *japonica7*, *indica2*, *indica5*, *japonica3* and *japonica9* and the highest belonging to *indica3*, *indica4*, *indica6*, *indica9* and *indica10*. This shows that the *indica* subspecies is characterised for having higher ratios of amylose in relation with total starch[3, 31].

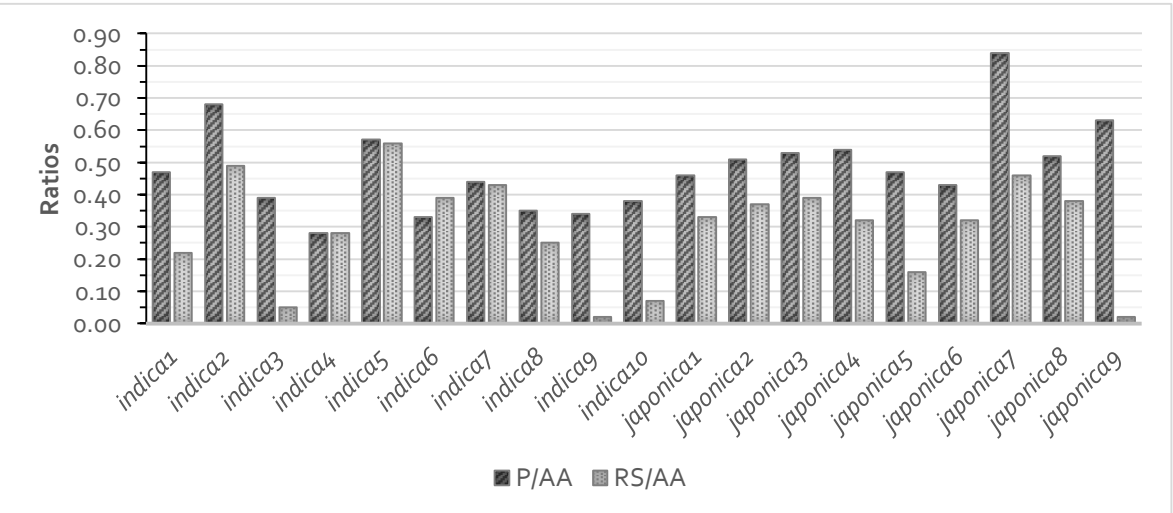


Chart 8 – Ratios between protein and resistant starch with apparent amylose (P/AA and RS/AA, respectively).

The ratio of protein-to-apparent-amylose (P/AA) also shows a wide range of values, with the lowest belonging to *indica4* and the highest to *japonica7*. This relation could be indicative of the complexation between amylose and proteins.

The ratio of resistant-starch-to-apparent-amylose (RS/AA) varies quite differently between samples and a clear trend isn't visible: some samples, like the non-parboiled *indica3*, and the parboiled *indica9*, *indica10*, *japonica9*, have a higher ratio, while samples like *indica2*, *indica5*, *indica7* and *japonica7* have lower ratios.

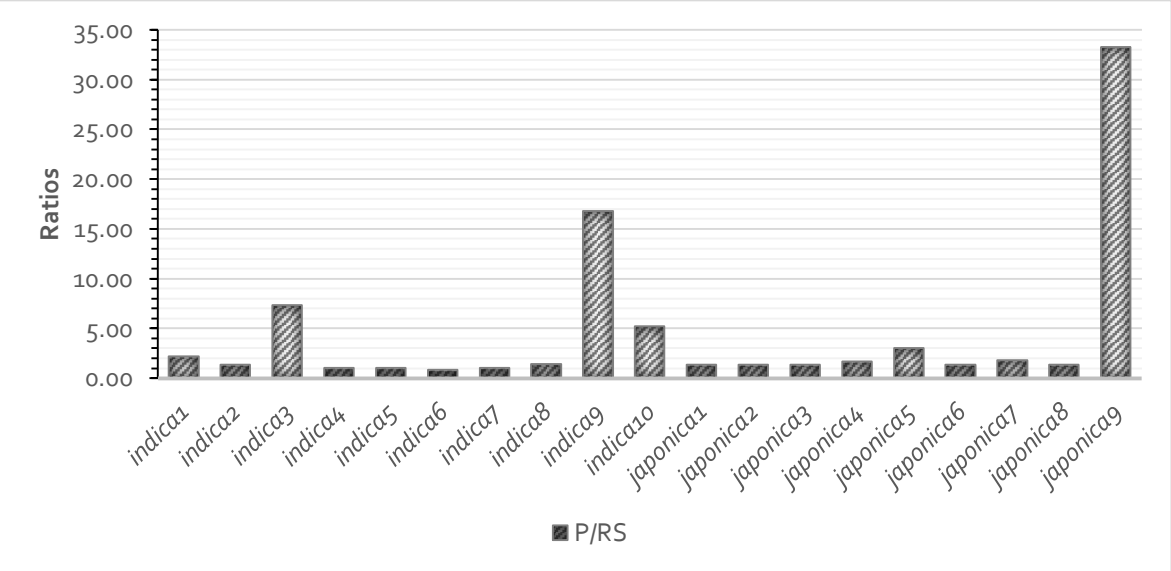


Chart 9 – Ratio between protein and resistant starch (P/RS).

Finally, the ratio that had the widest range of all was the protein-to-resistant-starch (P/RS), ranging from low ratios for most samples, and really high ratios for two of the parboiled samples: *indica9* and *japonica9*. This is suggests the influence of parboiling on the content of resistant starch and/or protein denaturation[40, 73].

5.4.1. Correlations

The average length was found to be negatively correlated with the moisture content (-0.50). This is a consequence of the grain's chalkiness: the higher the percentage of chalky area, higher will be the water absorption by the grain[43], even in the case of the atmospheric moisture.

For parboiled and non-parboiled *japonica* rices, the moisture content has been found to be negatively correlated with the total whiteness (-0.60 and -0.55, respectively) and with the percentage in chalky area (-0.61 and -0.53, respectively). This means that *japonica* rices with a lower moisture content have a higher total whiteness and are chalkier, which is coherent with the literature: a lower moisture content signifies that more air spaces will exist in between the starch granules, increasing the white opaque look of the grains also known as chalkiness[45].

Oko *et al.* (2012) reported a negative correlation between carbohydrate content and moisture content[5] so it should be expected that a correlation between total starch and moisture content would be observable. However, little to no correlation was found.

A moderate correlation has been obtained between the protein content and the parameters average width and L/W (-0.62 and +0.58, respectively). This indicates that thinner rices, such as the ones belonging to the *indica* subspecies, have a higher protein content. This has also been reported by Koutroubas *et al.* (2004)[42]. If the correlations regarding only *indicas* are examined, it is possible to see that the correlation coefficients obtained are even higher (-0.90 and +0.71, respectively). No such correlation can be found for *japonica* rices. When the parboiled samples are considered, the correlations aren't as strong, indicating the protein denaturation that these rices suffer during their processing[73].

Considering non-parboiled *indica* rices, a moderate correlation (+0.52) exists between protein content and the percentage of chalky area. This indicates that protein might be related with the occurrence of chalkiness in non-parboiled *indica* grains, probably interfering with the starch structure and increasing the amount of loose-packed granules[45]. An opposite observation can be made for non-parboiled *japonica* rices, since the correlation coefficient is negative (-0.56). For *japonica* rices, all and non-parboiled, protein is also correlated with *Kett* (-0.66 and -0.63, respectively).

A low, almost moderate correlation can be found between the protein content and the apparent amylose content (+0.48), indicating the relation showed by the P/AA[40].

Total starch has moderate correlations with total whiteness, crystalline whiteness and *Kett*, if only the non-parboiled *indica* samples are considered (+0.59, +0.54 and +0.60, respectively).

Resistant starch content was found as being moderately correlated with the average width and L/W of non-parboiled *japonica* rices (-0.60 and +0.62, respectively). This can only be justified by what was mentioned in the state of the art: waxy rices (low amylose content) were reported as having higher degrees of crystalisation, i.e., higher degrees of retrogradation[47]. However, no correlation was found between amylose content and resistant starch.

The size parameters also correlate, although only moderately, with the apparent amylose content in the grain (+0.55, for average length, -0.63, for average width, and +0.62, for L/W). This has also been verified by Koutroubas *et al.* (2004) and signifies that, the longer or narrower the grain, the higher the apparent amylose content. According to these results, samples belonging to the *japonica* subspecies are expected to have lower apparent amylose contents, while *indica* samples will have higher contents[42].

Calixo *et al.* (1991) reported a direct association between amylose content and the formation of retrograded starch, one of the fractions that compose resistant starch.[31] Several other studies[37, 51, 129] reported that higher amylose contents are correlated with a high resistant starch content. However, no such correlations were found in this report when considering all samples, or the *indica* and *japonica* subspecies separately.

Orford *et al.* (1987) reported that, at lower starch concentrations amylose retrogradation is favoured.[130] Therefore, the total starch content should be negatively correlated with the resistant starch content. However, the opposite was verified when considering the non-parboiled *indica* samples (+0.56). No correlation was found when considering all samples or only the *japonica* samples.

5.5 Analysis of the Cooking Parameters

5.5.1. Analysis of the Ordinary Cooking Time

The ordinary cooking times obtained for the analysed samples are summarised in **Chart 10**.

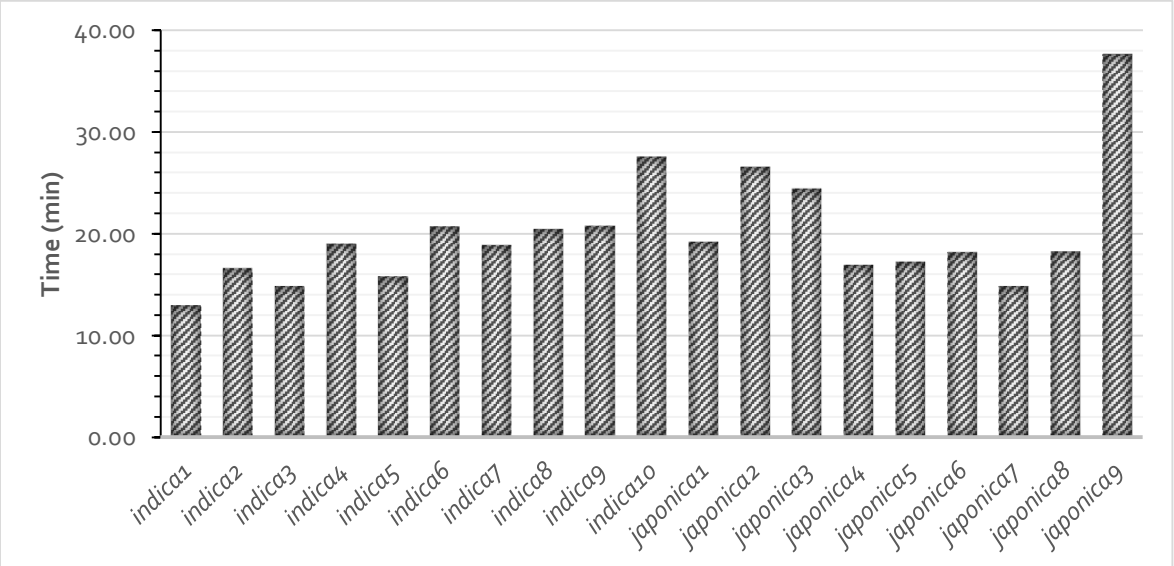


Chart 10 – Times (in minutes) obtained for the ordinary cooking time (80 % of cooked rice).

Sério (2013) calculated the ordinary cooking time for a final 90 % of grains cooked because it's at this percentage of cooking that the consumers are able to visually understand when the rice is ready to eat.[121] However, for this report 80 % was chosen instead of the 90 % in order to get a good margin to prevent the overcooking of rice. Another alteration was made in the calculations made: a median of the three cooking replicas was made instead of the average. The median has the advantage of not being influenced by any outlier values that can occur with such methodologies[131].

A wide range of ordinary cooking times (considering rice 80 % cooked) were obtained. The lowest value corresponds to the sample *indica1* which has the lengthiest and narrower grains of all the analysed samples. This time is characteristic of some aromatic rices with the mentioned characteristics[54]. The longest time belongs to the parboiled sample *japonica9*, taking 37.66 minutes. As mentioned, parboiling may increase the cooking times[16]. The second longest ordinary cooking time also belongs to another

parboiled sample (*indica10*) which takes 27.57 minutes to be 80 % cooked. The third longest time corresponds to the non-parboiled sample *japonica2* (26.56 minutes). This sample has a higher cooking time than the reported range for milled rices (15-25 minutes considering 100 % of cooked rice). This higher time could be a consequence of a lower milling degree[5, 20] or of a more compact starch structure. In fact, this sample has the highest crystalline whiteness of all (124.65). As the sample *indica1* is the fastest to cook, it would be expected to have a low apparent amylose content. However, that is not the case (22.98±1.55 %). One justification for this could be the shape of the grains: this sample has the lengthiest, narrowest grains and the highest L/W amongst all the analysed samples. Therefore, the water absorption by this grain will be easier due to the bigger surface area of the grain and a reduced distance between the surface and the centre of the grain.[43]

5.5.1.1. Correlations

The ordinary cooking time for 80 % of cooked grains was found to be moderately correlated with the protein and total starch content (-0.51 and +0.50, respectively). This is coherent with the literature, as protein hinders the water absorption during cooking, therefore increasing the cooking time required[57].

When considering only the non-parboiled *indica* samples, more correlations were found: the ordinary cooking time was found to correlate with length (-0.67), width (+0.50), L/W (-0.71), crystalline whiteness (+0.64), *Kett* (+0.50) and resistant starch (+0.69). The correlation with the size parameters indicates that shorter and wider grains will require a longer cooking time due to their lower surface area and higher distance between the surface and the centre of the grain[43, 88]. The resistant starch, just like protein, hinders the water absorption, therefore longer cooking times are required for higher resistant starch contents[43].

Considering the non-parboiled *japonica* samples, contrary correlations are found with the length and L/W (+0.55 and +0.53, respectively). A correlation between the ordinary cooking time and moisture, protein and total starch contents were also found (-0.59. -0.59 and +0.63, respectively). As discussed in the state of art, a higher protein

content hinders starch swelling and water absorption, and consequently prolonging the cooking time.

If all the *japonica* samples are considered, a correlation with the resistant starch is found (-0.64). This signifies that lower resistant starch contents are responsible for longer cooking times. However, it must be noted that the resistant starch content of the *japonica* samples analysed only varies from 0.27 % until 5.96 %, a value that is half of the values obtained for some of the *indica* samples. Still considering all the *japonica* samples, the ordinary cooking time is found to correlate with the total whiteness (-0.64) and with the *Kett* value (-0.78), meaning that samples with a whiter appearance will require shorted cooking times.

5.5.2. Analysis of the Solids in the Drained Pre-cooking Water

The percentages of solids in the drained pre-cooking waters have been summarised in **Chart 11**.

The maximum percentages of solids obtained for both drained pre-cooking times, 10 and 13 minutes, belong to the parboiled sample *japonica9* (2.68±0.11 and 2.27±0.01, respectively). The second and third highest percentages were obtained for the other two parboiled samples, *indica9* (2.01±0.10 % and 1.79±0.03 %, respectively) and *indica10* (2.00±0.17 % and 1.61±0.12 %, respectively). The highest percentages considering only non-parboiled samples are 1.77±0.26 % (*indica8*) for the 10 minute pre-cooking and 1.04±0.29 % (*indica6*) for the 13 minute pre-cooking. The minimum percentages obtained for both pre-cooking times belong to the sample *indica1* (0.98±0.06 and 0.03±0.01, respectively). This sample was also analysed for the pre-cooking time 7 minutes. With the increase in pre-cooking time, a decrease of solids in the drained water can be observed for all samples. The reduction between the 10 and the 13 minute pre-cooking time occurs for all the samples, varying from 0.22 to 1.71. This goes against the findings by Tamura *et al.* (2014), which reported that the quantity of leached solids increases with temperature and cooking time[132]. However, the methodology used was different: it must be noted that, at 13 minutes of pre-cooking, almost no water is left to be drained, and therefore, most of the solids that did in fact leach into the cooking water will sticky to the rice grains.

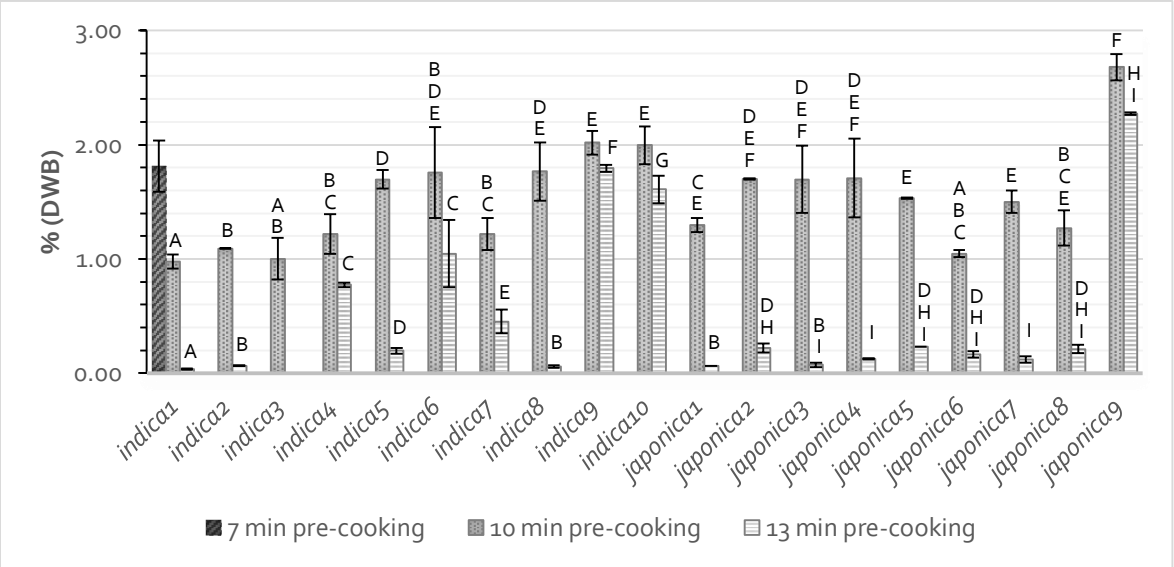


Chart 11 – Percentages of solids in the drained pre-cooking water for 7, 10 and 13 minutes of pre-cooking.

5.5.2.1. Correlations

The average length, average width and L/W, considering the non-parboiled *indica* samples, were found to be highly to moderately correlated with the percentage of solids in the drained pre-cooking water for the 10 minute pre-cooking time (-0.75, +0.58 and -0.76, respectively). If the non-parboiled *japonica* samples are considered, then the size parameters correlate with the pre-cooking time of 13 minutes (-0.43, +0.52 and -0.67, respectively). Just like before, this is a consequence of the grains' shape but it indicates that *japonica* samples will tend to have a higher leaching with higher cooking times and that *indica* samples start to leach their components earlier in the cooking process.

When all samples are considered, the parameters total whiteness, crystalline whiteness, *Kett* and percentage of chalky area were found to correlate highly to moderately with the percentage of solids in the drained 13 minute pre-cooking water (-0.77, -0.76, -0.86 and -0.56, respectively). The correlation with the 10 minutes of pre-cooking is lower (-0.53, -0.46, -0.60 and -0.41, respectively). However, if only the non-parboiled samples are considered, these correlations are no longer observable, meaning that these correlations are a consequence of the behaviour of the parboiled samples: parboiled samples with higher values (for either one of the four whiteness parameters) will have a lower leaching during the pre-cooking with 13 minutes. If all *indica* or *japonica* samples are considered, high correlations are found between the total whiteness (-0.84

and -0.71), crystalline whiteness (-0.83 and -0.73), *Kett* (-0.84 and 0.89), chalky area (-0.73 and -0.47) and the percentage of solids in the drained 13 minute pre-cooking. For the same *japonica* samples, the total whiteness and *Kett* have even higher correlation coefficients with the percentage of solids in the drained 10 minute pre-cooking water (-0.82 and -0.91, respectively). However, these correlations decrease when only the non-parboiled *japonica* samples are considered, indicating that, for parboiled samples, the higher the total whiteness or *Kett*, the higher percentage of solids will occur for the drained 10 minute pre-cooking waters. The correlation between for the chalky area mentioned indicates that non-parboiled *japonica* samples with higher percentages in chalky area will have a higher percentage of leaching.

When considering the all the non-parboiled and non-parboiled *indica* samples, the resistant starch content is found to be highly correlated with the percentage of solids in the drained 13 minute pre-cooking waters (+0.70 and +0.79, respectively). For non-parboiled *indicas* a moderate correlation is also found with the solids in the drained 10 minute pre-cooking waters (+0.52). For the latter, a correlation with protein content is also found (-0.60). A negative correlation occurs when considering all the *japonica* samples, correlating with both the 10 and 13 minute pre-cooking waters (-0.79 and -0.88, respectively). However, these correlations are no longer found when considering only the non-parboiled *japonica* samples, indicating that the percentage of solids for parboiled *japonicas* increases with the decrease in the resistant starch content of the samples.

A moderate but positive correlation is found between the solids of the 13 minute pre-cooking waters and the apparent amylose content (+0.57), which might be indicating what has been reported by Juliano (1979): since amylose is more soluble in boiling water than amylopectin, the solids in the drained cooking water have the tendency of being lower for low-amylose rices than for high-amylose rices. In other words, *japonica* samples have a lower tendency to suffer leaching than *indica* samples.[83] However this isn't' entirely true, since *japonica* rices are more sticky, it is harder to properly drain the leftover water, leaving the solids stuck around the cooked grains.

The ordinary cooking time also correlates highly and positively with the percentage of solids in the 10 and 13 minute pre-cooking waters (+0.80 and +0.71, respectively) when

all samples are considered. However, no correlation is found when considering all non-parboiled samples. This indicates that the higher the ordinary cooking time of the parboiled samples, the higher the solid percentages will be on any of the pre-cooking waters. Oko *et al.* (2012) also reported positive, but moderate, correlations between these two parameters[86].

Lastly, the percentages of solids correlate with each other (+0.74) only when all samples are considered.

5.5.3. Post-cooking Using a Microwave Oven or Steam

Before analysing the data, some observations must be noted.

Three different pre-cooking times were tested, the main being the 10 minute pre-cooking time. However, the sample *indica1* had already an ordinary cooking time so short that an adaptation for a 7 minute pre-cooking was tested just for that sample. The 13 minute pre-cooking time with post-cooking using a microwave oven lacks the cooking time for two samples: *indica1* wasn't analysed because, due to its short ordinary cooking time, it couldn't be successfully analysed with a pre-cooking time of 13 minutes; *indica3* was a sample of limited quantity.

Another important note is regarding the designations used in the **Registry of observations from the post-cooking analysis** (**Appendix B**). Many different expressions can be found throughout the literature so a clarification of the chosen words is given for the present report. The first classification designates if the cooked grains are "opened" or "closed". "Opened" grains, as can be seen in **Figure 14 (left)**, maintain their raw shape, while "closed" grains experience, with more or less extent, a division of the grain (**Figure 14 centre**). It is common for a sample to have both types of grains, however, the sample was classified as having "closed grains" only when all the observable grains were indeed "closed". The proportion of "opened" and "closed" grains wasn't registered as this would require a more time consuming statistical registry and less time would be available to analyse as much rice types as the ones considered for this report. As an alternative, a photographic registry was made and was given to the quality control laboratory at

Novarroz for future reference. The second classification designates if the cooked grains are "non-sticky", "sticky" or "mushy-prone". "Non-sticky" is used to describe cooked grains that don't clump together, i.e. remain separate and don't have a viscous feel. In the literature the words "fluffy", "flaky" or "dry"[133] are sometimes used instead of "non-sticky". "Sticky" is used to describe the opposite of "non-sticky", i.e., the cooked grains clump together with the help of the residual viscous water that remains on their surface. The literature also describes these grains as "clingy" or "moist"[133]. The last designation, "mushy-prone", is used to describe cooked grains that that have disintegrated, i.e. lost their shape and are no longer differentiable from the surrounding grains (**Figure 14 right**). This designation also implies that the grains are also "opened".

Figure 14 – Appearance of the grains and the corresponding designation used for its description: closed and non-sticky (left), opened and sticky (centre), opened, sticky and mushy-prone (right).

A key difference was noticed between post-cooking with a microwave oven and post-cooking with steam: the microwaves allows a homogenised cooking, while for steam it is essential that the rice is thoroughly mixed from time to time to allow the steam to reach all the grains in the sample and to prevent a final "mushy-prone" cooked rice. It is also important to note that, since the pre-cooked rice samples are used frozen, some extent of retrogradation is expected. This could be responsible for a higher overall cooking time. The frozen samples must always be heated for the same initial time in order to defrost. When using the microwave oven, the defrost time was of 3 minutes (in 300 watts) and when using steam, it was of 10 minutes. Finally, when using the microwave oven at 300 watts, a maximum of 11 minutes can be used to cook the samples. After this time the cooked grains become extremely dry and unappealing for consumption.

Initially, several different powers and water volumes were tested for the post-cooking analysis using a microwave oven. The powers tested were 100, 300, 450 and 800 watts. The same rice sample (*japonica8* pre-cooked for 10 minutes) was used throughout all the tests with or without 1 part of distilled water. By using the microwave with 800 watts without additional water, rice was found to become mushy and dry in 3 minutes. The same was observed when 1 part of water was used. By reducing the power for 450 watts, the rice took 2 more minutes, without water, and 3 more minutes with water, to reach a "mushy" and dry appearance. None of these tests allowed the required separation of the 20 grains for the assessment of the cooking time. Distilled water was henceforth added as a 1 part volume. Higher water volumes weren't considered because the samples had already absorbed water during the pre-cooking stage, and an excess of water increases the tendency of rice becoming "mushy". Next, the 100 watts were tested but the required time for cooking the sample *japonica8* reached 25 minutes. This time was too long, so the power was increased for 300 watts. This way, the cooking time of 6.57 minutes for a 10 minute pre-cooking was achieved.

The post-cooking times using a microwave oven obtained for the analysed samples are summarised in **Chart 12**.

As mentioned, the sample *indica1* was the only analysed for the 7 and 10 minutes of pre-cooking. The required post-cooking times for this sample were 5.73 and 4.21 minutes, respectively: an increase of 3 minutes in the pre-cooking time decreased the post-cooking time by 1.52 minutes. The cooked grains didn't differ in appearance between the two pre-cooking times tested, remaining "opened" and "sticky". However, the water that was added at the beginning of the post-cooking wasn't fully absorbed for the 10 minutes of pre-cooking but was fully absorbed for the 7 minutes. It is possible to conclude that the limit of absorption for *indica1* was exceeded for the 10 minutes of pre-cooking. A similar situation occurred for the sample *indica2* between the 10 and 13 minutes of pre-cooking. The opposite situation was also observed, i.e., incomplete water absorption for the 10 minutes of pre-cooking and complete water absorption for the 13 minutes of pre-cooking. This can be justified by the differences inherent to the samples' grains, i.e., the portion

used for the first post-cooking could have a lower percentage of chalky grains than the second post-cooking. Another situation was observed: incomplete water absorption for both the pre-cooking times tested. This happened for the parboiled sample *indica9*, and these observations are coherent with the difficulty of absorbing water that parboiled samples, especially the parboiled *agulhas*, have.

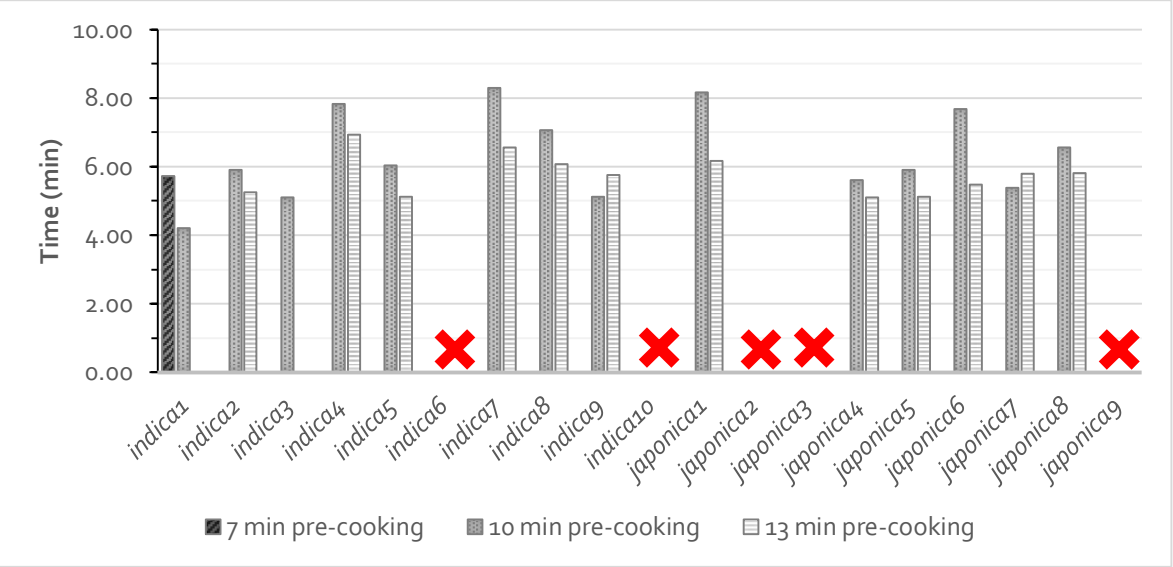


Chart 12 – Times (in minutes) obtained for the microwave post-cooking for 7, 10 and 13 minutes of pre-cooking (80 % of cooked rice).

For the 10 minutes of pre-cooking, the highest post-cooking time belongs to the sample *indica7* (8.29 minutes) and the shortest to *indica1* (4.21 minutes). The cooked appearance of the grains for both of these samples was "opened" and "sticky". The sample *indica3* should be highlighted due to its cooked appearance: disregarding the parboiled samples, this was the sample with the most "non-sticky" grains of all the samples (**Figure 15**). This sample also stands out from the remaining non-parboiled *indica* samples by having the lowest crystalline whiteness (117.86), percentage of chalky area (9.56 %), total starch (70.46±0.34 %), resistant starch (1.38±0.07 %), the third highest amylose content (25.94±0.89 %) and the second highest protein content (10.19±0.24 %). When re-analysing the ratios provided in **Chart 7**, **Chart 8** and **Chart 9**, it was observed that this sample had the lowest P/TS, RS/TS, RS/AA, and the highest AA/TS and P/RS. The composition of this sample could be a clue to finding more non-parboiled varieties that have "closed" and very "non-sticky" cooked grains. Some of the samples weren't able to cook with this

methodology (*indica6*, *indica10*, *japonica2*, *japonica3* and *japonica9*). Observing these post-cooking times, side by side with the ordinary cooking times, it can be seen that all of those samples require more than 20 minutes to cook through the ordinary boiling method. However, it should be noted that *indica8* and *indica9* also have cooking times of about 20 minutes, but don't correspond to the highest post-cooking times (for the 10 minute test). Both of these samples have significantly similar protein contents (8.91±1.02 and 8.94±0.61, respectively) but all other parameters differ between the two.

Figure 15 – Example of the photographic registry performed the post-cooking samples. Sample represented: *indica3*.

For the 13 minutes of pre-cooking, the highest post-cooking time belongs to the sample *indica4* (6.93 minutes) and the shortest to japonica4 (4.21 minutes). The required cooking time decreased within the range of 0.64-2.22 minutes. Again, no specific trend can be observed for this pre-cooking time. It should be noted that the sample *japonica7* had an increase in the required cooking time. No possible explanation could be found for this behaviour, other than that each replica cooked has its own set of grains. The same samples that weren't able to cook with 10 minutes of pre-cooking still didn't cook when the pre-cooking time was increased. Holm *et al.* (1988) has reported that, in many starchy foods, a portion of residual starch is not fully gelatinised during processing, usually due to limited water content or insufficient heating.[52] In this case, it is safe to rule out insufficient heating, as the main problem with microwave cooking is the water content present in or around the food. Therefore, it is possible to conclude that, for the varieties that didn't reach optimal cooking, two changes could be applied to the cooking process: either

increase the volume of water added at the beginning of the post-cooking step, or increase the pre-cooking time, which allows more time for the rice to absorb the pre-cooking water. The increase of the water ratio was tested for one of these samples (*japonica3*) with poor results: due to the excess of water, the grains became extremely "mushy" impeding the separation of the 20 grains required assessment of the cooking time (**Figure 16**). However, the increase in the pre-cooking time doesn't seem to be the reason since for most of the samples almost all of the pre-cooking water was absorbed.

Figure 16 – Appearance of the sample *japonica3* with 13 minutes of pre-cooking after cooking for 5 minutes in the microwave oven with a volume of water equal to two parts.

The post-cooking times using steam obtained for the analysed samples are summarised in **Chart 13**.

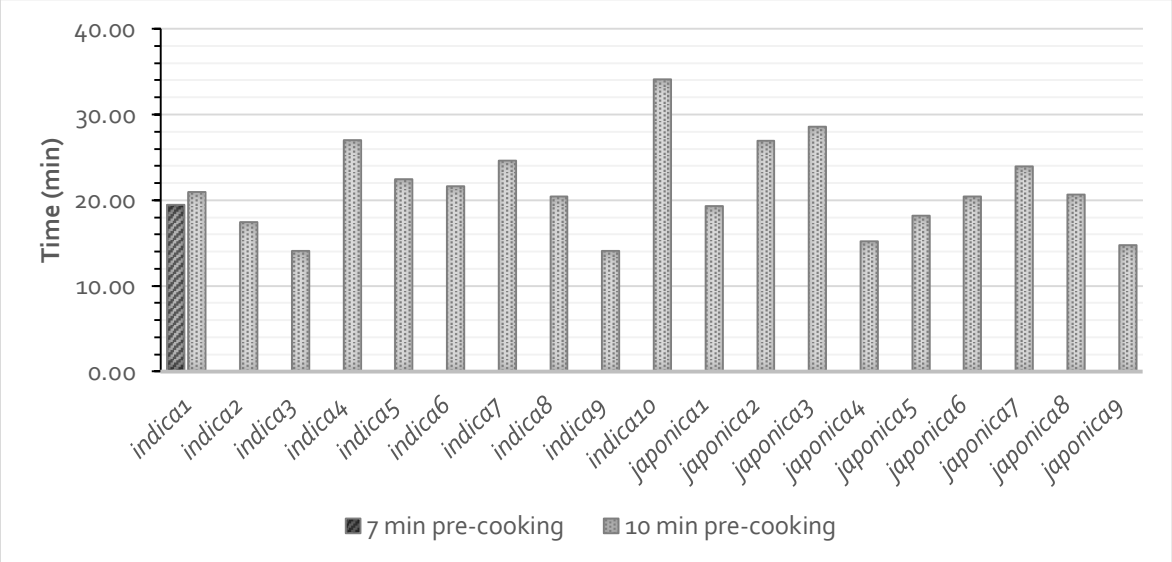


Chart 13 – Times (in minutes) obtained for the steam post-cooking for 7 and 10 minutes of pre-cooking (80 % of cooked rice).

Again, the sample *indica7* was the only sample analysed for the 7 and 10 minutes of pre-cooking. However, the post-cooking time increased from 19.48 to 20.95 minutes. The

major difficulty of this post-cooking process is the wide range of nucleus obtained for a certain time. In other words, a higher number of outliers is obtained due to the non-homogeneous cooking, making this post-cooking process not as precise as when using a microwave oven.

For the 10 minutes of pre-cooking the post-cooking times varied from 14.07 minutes (*indica3*) to 34.10 minutes (*indica10*). The cooked grains' appearance was, respectively, "closed" and "sticky", and "opened" and "non-sticky".

Some samples can be distinguished as far as the cooked grains' appearance. All parboiled samples had "non-sticky" grains while all the non-parboiled *japonica* samples had "sticky" grains. For the steam post-cooking, half of the *japonica* sample grains became "mushy-prone" while for the microwave cooking no such behaviour was observed (*japonica3*, *japonica5*, *japonica6* and *japonica8*). This is a consequence of the direct steam on the grains, which cause a higher leaching of the amylose, increasing the grains' tendency to disintegrate. As for the *indica* rices, the "mushy-prone" appearance was also observed for the steam post-cooking (*indica5* and *indica6*). The sample *indica5* cooked as "sticky" for both the pre-cooking times tested in the microwave, but the sample *indica6* was one of the samples that didn't cook in the microwave. One *indica* sample, the *indica8*, cooked to a "mushy-prone" appearance when pre-cooked for 10 minutes followed by post-cooking in the microwave. However, the sample didn't appear "mushy-prone" when the highest pre-cooking time was used. No possible explanation could be found for this behaviour, other than that each replica cooked has its own set of grains.

5.5.3.1. Correlations

Regarding the 10 minutes of pre-cooking time, the post-cooking time using the microwave oven is correlated with the resistant starch content (+0.63), with the ordinary cooking time (+0.88) and with the 13 minute pre-cooking time using a microwave oven (+0.75). When only the non-parboiled *indica* samples are considered, the correlation with the resistant starch content increases (+0.76), but if non-parboiled *japonica* samples are considered, the correlation coefficient decreases (+0.51). This indicates that the higher

resistant starch content of the *indica* samples directly influences the post-cooking time using a microwave and a 10 minute pre-cooking time.

Regarding the 13 minutes of pre-cooking time, the post-cooking time using the microwave is highly correlated with resistant starch (+0.62), amylose content (+0.71), ordinary cooking time (+0.61), with the solids drained for the same pre-cooking time (+0.66) and with the post-cooking time using steam with a 10 minute pre-cooking time (+0.64). The correlation with amylose content may be justified because, with the pre-cooking time of 13 minutes almost no water is left behind by the rice, therefore, the solids, which are mainly composed by amylose, remain stuck at the surface of the rice grains, covering them and are frozen together with the samples, and will therefore influence the post-cooking time. Oko *et al.* (2012) reported a negative correlation between amylose content and stickiness[86]. In fact, in the **Registry of observations from the post-cooking analysis** (**Appendix B**) it is possible to observe that all non-parboiled *japonica* varieties were classified as being "sticky" or "mushy-prone" after all the post-cooking processes.

Regarding the 10 minutes of pre-cooking time, the post-cooking time using steam is only moderately correlated with the ordinary cooking time (+0.59). A low correlation can also be found with resistant starch (+0.45). This correlation coefficient increases if only non-parboiled *indica* rices are considered (+0.73) as these samples were found to have higher resistant starch contents.

5.5.4. Comparison of the Cooking Methods

Between the ordinary cooking time and the steam post-cooking time (10 minutes pre-cooking), considering the 10 minutes of pre-cooking, all samples were found to require longer cooking times. Besides this expensive and time consuming disadvantage, there is a higher tendency for the disintegration of the grains, resulting in a "mushy", "sticky" and unappealing rice.

Between the ordinary cooking time and the microwave post-cooking time (10 minutes pre-cooking) the biggest increase in the cooking time is obtained for the sample *indica1* while the biggest decreases are obtained for *indica8* and *indica9*. However, when considering the 13 minutes of pre-cooking there is an overall increase in the cooking times

required, with the exception that the samples *indica8* and *indica9* still had a reduction in the cooking time.

As for the 7 minutes of pre-cooking, for both post-cooking methods, an increase in the cooking time occurs.

In can be concluded that the only method viable for cooking rice with a two-step cooking procedure is the use of a microwave oven. However, there are visible differences between using 10 or 13 minutes of pre-cooking, the best being the 10 minutes. One possible reason why the 10 minutes of pre-cooking is slightly faster may be due to the draining step performed between the pre- and post-cooking stages: as it was explained earlier, after 13 minutes there is almost no water left to drain and the solids (mainly amylose) which leached throughout the pre-cooking step will remain on top of the grains' surface, increasing the cooking time required. Since a low amylose content has been reported as a factor contributing to lower cooking times[24, 47-48, 85], then it is expected that the 10 minutes of pre-cooking, by enabling a lower amylose content in the overall sample, allows shorter cooking times. One possible alteration that could provide faster overall cooking times with the 13 minutes of pre-cooking would be to use a larger quantity of water in the pre-cooking step. This would prevent the amylose accumulation on the grains' surface. However, with this change another consideration must be made: the use of a higher quantity of water could decrease the quality of the final product by loss of the nutrients present in the pre-cooking water, which is discarded.

Chapter 6 – Conclusion

A methodology for the analysis of total starch content and of resistant starch in raw rice samples was achieved. However, further efforts should be made to improve these methodologies in order to simplify their use and obtain better results.

An extensive sample characterisation was made in this report and it is hoped that these results will allow Novarroz to gain more insight regarding the varieties commercialised. Differences were found between non-parboiled *indica*, non-parboiled *japonica* samples and parboiled samples: *indica* varieties were characterised for being lengthier, slimmer and having higher protein contents while *japonica* were characterised for their shorter and wider grains and also for their lower apparent amylose content. Parboiled samples had lower whiteness values, which is expected due to their yellow appearance and contained an extremely low resistant starch content. The latter wasn't expected since parboiling is known to promote starch retrogradation. Strong correlations were also found between the parameters considered for their characterisation. The analysis of the composition of these samples also allowed a better understanding of the components influence in the samples' cooking and eating quality. New methods of rice cooking were created for this report, using microwave and steam, and were used to access the samples' cooking time and appearance. A classification of the rice's appearance was also established and it is hoped that it is simple enough that future interns can use it after being familiarised with rice behaviour during cooking. The microwave and steam methods may, in the future, require some modifications depending on the equipments and apparatus used. However, it is also hoped that this report will make such adaptation easier. Between the three cooking methods, using the 10 minute pre-cooking together with a microwave oven post-cooking proved to be the fastest way of cooking rice of all types, when disregarding the pre-cooking times. A possible way of reducing even further the post-cooking times would be to use excess water together with higher pre-cooking times in order to prevent the amylose accumulation on the grains' surface. The sample *indica3* cooked with this method was highlighted as having the most "non-sticky" cooked grains and its whiteness and composition parameters could be a clue to finding other rice varieties that have a similar cooking behaviour. Some rice types appear more adequate for cooking with the tested methods than others, but further studies are needed in order to

access if the varieties used are representative or not of those types. Also, a more balanced selection of samples should be used in further studies, i.e., equal number of samples within each rice type.

In the future, the different cooked samples should be analysed throughout the pre-cooking and post-cooking processes to check the variations in composition, in an attempt to better understand rice cooking and eating quality. A more extensive characterisation of the samples regarding their composition should also be made. One parameter that could help to further understand the cooking behaviour of rice is its lipid content or the degree of crystallisation of starch.

With this internship I acquired irreplaceable work experience in the industrial food sector, which I consider to be a very valuable complement to my academic studies. The internships established between the university and companies, such as Novarroz, enable students to contact with the entrepreneurial environment, getting an insight that isn't possible with a thesis that only requires laboratory work. This internship was also advantageous for the company in several ways: new insights were given on the commonly employed methods of the quality control laboratory allowing their optimisation or adaptation, the rice databases were complemented with results for the year 2013 and it is hoped that the new findings will allow the company's further growth.

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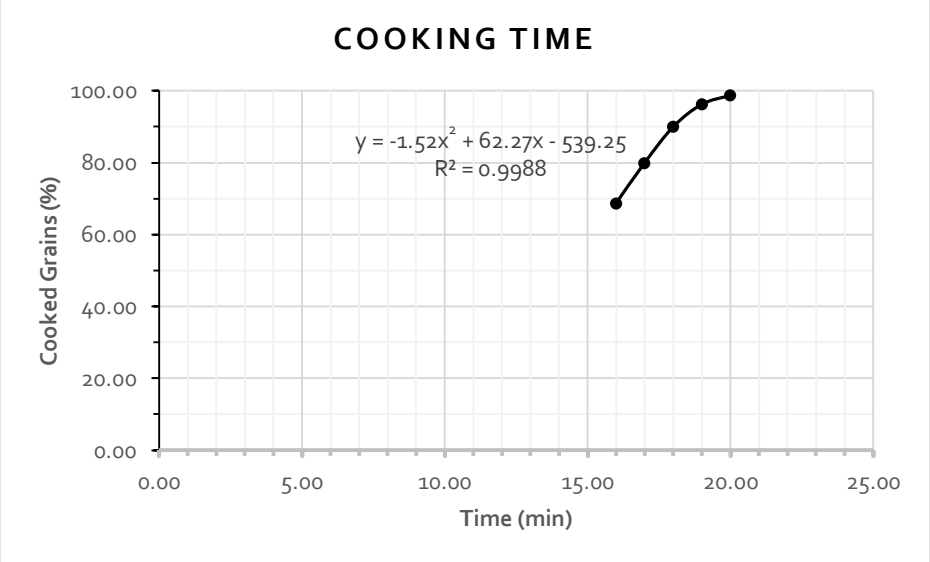
Appendix A

Calculations for the analysis of ordinary, microwave and steam cooking time (example).

Time (min)	Number of grains with nuclei			Number of grains without nuclei			Median	%	1st Eq.	2nd Eq.
15	11	10	10	9	10	10	10	50		
									62.5	
16	4	6	5	16	14	15	15	75		68.75
									75	
17	5	5	0	15	15	20	15	75		80
									85	
18	1	3	1	19	17	19	19	95		90
									95	
19	1	1	1	19	19	19	19	95		96.25
									97.5	
20	0	0	1	20	20	19	20	100		98.75
									100	
21	0	0	0	20	20	20	20	100		



Appendix B

Results obtained from the analyses performed (Part A). Means in columns followed by the same upper case letters are not significantly different (p<0.10).

Samples	Average length (mm)	Average width (mm)	Length-to-width ratio	Total white ness	Crysta lline white ness	*Kett*	Chalky area (%))	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)
indica1	7.242	1.674	4.326	130.63	118.33	40.4	21.22	10.93	10.85 ± 0.80^{A}	75.18 ± 1.30^{A}	4.94 ± 0.19^{A}	22.98 ± 1.55^{A}
indica2	7.038	1.914	3.677	129.89	122.43	43.4	13.21	11.37	8.75 ± 0.24^{B}	80.15 ± 1.00^{A}	6.34 ± 0.35^{B}	12.85 ± 0.27^{B}
indica3	7.043	1.902	3.703	123.54	117.86	37.4	9.56	11.96	10.19 ± 0.43^{A}	70.46 ± 0.34^{B}	1.38 ± 0.07^{C}	25.94 ± 0.89^{C}
indica4	7.137	2.041	3.497	124.10	118.07	36.8	10.24	11.13	7.43 ± 0.47^{C}	72.96 ± 0.29^{C}	7.37 ± 0.29^{D}	26.37 ± 0.42^{C}
indica5	6.671	2.109	3.163	126.91	120.39	37.2	11.02	11.25	6.96 ± 0.27^{C}	71.98 ± 2.25^{A}	6.82 ± 0.73^{BD}	12.23 ± 0.71^{B}
indica6	6.681	1.975	3.383	132.41	122.41	42.4	18.66	11.62	8.39 ± 0.40^{BC}	78.49 ± 0.87^{AC}	9.94 ± 0.47^{E}	25.30 ± 1.39^{AC}
indica7	6.479	1.921	3.373	135.26	124.60	44.3	20.52	12.59	9.86 ± 0.30^{A}	75.94 ± 0.23^{C}	9.63 ± 0.13^{F}	22.30 ± 0.46^{A}
indica8	6.316	1.932	3.269	134.08	124.09	46.7	18.37	11.25	8.91 ± 1.02^{ABC}	73.93 ± 0.37^{BC}	6.35 ± 0.63^{BG}	25.51 ± 0.82^{C}
indica9	6.220	1.877	3.314	95.33	93.12	20.4	2.75	11.88	8.94 ± 0.61^{ABC}	78.12 ± 0.37^{A}	0.53 ± 0.01^{H}	26.50 ± 0.46^{A}
indica10	7.252	1.945	3.729	107.49	104.38	27.1	4.19	10.72	8.50 ± 0.78^{BC}	72.29 ± 0.21^{BC}	1.62 ± 0.17^{I}	22.39 ± 1.66^{C}
japonica1	6.071	2.313	2.625	130.03	122.44	41.5	13.46	11.89	8.24 ± 0.19^{BC}	78.77 ± 0.37^{A}	5.96 ± 0.53^{BG}	17.94 ± 1.49^{D}
japonica2	5.985	2.574	2.325	143.55	124.65	46.1	35.35	10.86	7.45 ± 0.34^{C}	78.45 ± 0.64^{A}	5.47 ± 0.01^{G}	14.60 ± 1.28^{BE}
japonica3	5.833	2.394	2.437	130.83	123.61	42.5	13.24	12.27	7.28 ± 0.24^{C}	81.32 ± 0.64^{A}	5.37 ± 0.17^{G}	13.68 ± 0.24^{E}
japonica4	5.448	2.427	2.245	126.89	121.06	42.0	10.21	12.46	8.92 ± 0.45^{B}	76.80 ± 0.72^{A}	5.25 ± 0.00^{G}	16.60 ± 0.27^{DE}
japonica5	5.353	2.647	2.022	138.53	120.24	44.0	30.48	11.72	8.30 ± 0.71^{BC}	79.56 ± 0.49^{A}	2.75 ± 0.17^{J}	17.53 ± 0.00^{DE}
japonica6	5.927	2.714	2.184	148.03	124.04	51.2	40.69	11.85	6.87 ± 0.24^{C}	75.05 ± 0.65^{AC}	5.16 ± 0.31^{AGJ}	16.09 ± 0.65^{DE}
japonica7	5.424	2.643	2.052	140.64	121.23	45.3	33.03	12.21	8.64 ± 0.65^{BC}	74.74 ± 0.82^{AC}	4.69 ± 0.14^{AJ}	10.30 ± 0.63^{F}
japonica8	4.977	2.393	2.080	141.87	112.24	45.1	35.47	11.95	7.75 ± 0.86^{BC}	76.70 ± 0.62^{BC}	5.68 ± 0.66^{ABGJ}	15.02 ± 1.44^{EF}
japonica9	5.479	2.659	2.061	113.64	110.09	25.8	5.33	12.41	8.89 ± 0.20^{B}	76.73 ± 1.34^{A}	0.27 ± 0.00^{K}	14.18 ± 0.72^{EF}

Results obtained from the analyses performed (Part B). Means in columns followed by the same upper case letters are not significantly different (p<0.10).

Samples	Ordinary cooking time (min) (80% cooking)	Volume of 1 part (mL)	Solids in drained waters (DWB) 7 min	Solids in drained waters (DWB) 10 min	Solids in drained waters (DWB) 13 min	Microwave time (min) (80% cooking) 7 min	Microwave time (min) (80% cooking) 10 min	Microwave time (min) (80% cooking) 13 min	Steam time (min) (80% cooking) 7 min	Steam time (min) (80% cooking) 10 min
indica1	12.98	32.50	1.81±0.22	0.98±0.06^{A}	0.03±0.01^{A}	5.73	4.21		19.48	20.95
indica2	16.60	28.75		1.09±0.00^{B}	0.06±0.00^{B}		5.91	5.24		17.46
indica3	14.88	31.25		1.00±0.18^{AB}			5.11			14.07
indica4	18.99	30.00		1.22±0.17^{BC}	0.77±0.02^{C}		7.83	6.93		27.02
indica5	15.79	30.00		1.70±0.08^{D}	0.20±0.03^{D}		6.05	5.13		22.45
indica6	20.72	29.35		1.76±0.40^{BDE}	1.04±0.29^{C}		Doesn't cook	Doesn't cook		21.61
indica7	18.86	30.00		1.22±0.14^{BC}	0.45±0.10^{E}		8.29	6.56		24.65
indica8	20.46	29.35		1.77±0.26^{DE}	0.06±0.01^{B}		7.07	6.08		20.41
indica9	20.76	30.65		2.02±0.10^{E}	1.79±0.03^{F}		5.12	5.76		14.10
indica10	27.57	30.65		2.00±0.17^{E}	1.61±0.12^{G}		Doesn't cook	Doesn't cook		34.10
japonica1	19.19	28.75		1.30±0.06^{CE}	0.07±0.00^{B}		8.18	6.18		19.34
japonica2	26.56	28.75		1.70±0.00^{DEF}	0.22±0.04^{DH}		Doesn't cook	Doesn't cook		26.93
japonica3	24.44	29.35		1.70±0.30^{DEF}	0.07±0.02^{BI}		Doesn't cook	Doesn't cook		28.60
japonica4	16.94	28.75		1.71±0.34^{DEF}	0.12± 0.00^{I}		5.61	5.10		15.22
japonica5	17.27	28.75		1.53±0.01^{E}	0.23±0.00^{DHI}		5.90	5.13		18.16
japonica6	18.17	28.15		1.05±0.03^{ABC}	0.16±0.03^{DHI}		7.69	5.47		20.41
japonica7	14.83	28.75		1.50±0.10^{E}	0.12±0.03^{I}		5.38	5.79		23.97
japonica8	18.26	28.75		1.27±0.15^{BCE}	0.21±0.04^{DHI}		6.57	5.82		20.69
japonica9	37.66	29.35		2.68±0.11^{F}	2.27±0.01^{HI}		Doesn't cook	Doesn't cook		14.75

Ratios between the analysed composition parameters: protein, total starch, resistant starch and apparent amylose.

Samples	P/TS	RS/TS	AA/TS	P/AA	P/RS	RS/AA
indica1	0.14	0.07	0.31	0.47	2.19	0.22
indica2	0.11	0.08	0.16	0.68	1.38	0.49
indica3	0.14	0.02	0.37	0.39	7.36	0.05
indica4	0.1	0.1	0.36	0.28	1.01	0.28
indica5	0.1	0.09	0.17	0.57	1.02	0.56
indica6	0.11	0.13	0.32	0.33	0.84	0.39
indica7	0.13	0.13	0.29	0.44	1.02	0.43
indica8	0.12	0.09	0.35	0.35	1.4	0.25
indica9	0.11	0.01	0.34	0.34	16.81	0.02
indica10	0.12	0.02	0.31	0.38	5.23	0.07
japonica1	0.1	0.08	0.23	0.46	1.38	0.33
japonica2	0.09	0.07	0.19	0.51	1.36	0.37
japonica3	0.09	0.07	0.17	0.53	1.36	0.39
japonica4	0.12	0.07	0.22	0.54	1.7	0.32
japonica5	0.1	0.03	0.22	0.47	3.01	0.16
japonica6	0.09	0.07	0.21	0.43	1.33	0.32
japonica7	0.12	0.06	0.14	0.84	1.84	0.46
japonica8	0.1	0.07	0.2	0.52	1.36	0.38
japonica9	0.12	0	0.18	0.63	33.25	0.02

Registry of observations for *indica* samples from the post-cooking analysis using a microwave oven or steam: number of nucleus at the end of the pre-cooking and the post-cooking stages, final appearance of the grains and water absorption.

	Microwave post-cooking			Steam post-cooking	
Samples	**7 minutes**	**10 minutes**	**13 minutes**	**7 minutes**	**10 minutes**
indica1	Pre/post nucleus: 20/4 Grains: opened and sticky Water: absorbed	Pre/post nucleus: 20/2 Grains: opened and sticky Water: incomplete		Pre/post nucleus: 20/4 Grains: opened and sticky	Pre/post nucleus: 20/1 Grains: opened and sticky
indica2		Pre/post nucleus: 20/4 Grains: opened and sticky Water: complete	Pre/post nucleus: 20/2 Grains: opened and sticky Water: incomplete		Pre/post nucleus: 20/0 Grains: closed and sticky
indica3		Pre/post nucleus: 20/2 Grains: closed and non-sticky Water: incomplete			Pre/post nucleus: 20/2 Grains: closed and non-sticky
indica4		Pre/post nucleus: 20/3 Grains: closed and non-sticky Water: incomplete	Pre/post nucleus: 20/3 Grains: opened and sticky Water: complete		Pre/post nucleus: 20/3 Grains: opened and non-Sticky
indica5		Pre/post nucleus: 20/1 Grains: opened and sticky Water: incomplete	Pre/post nucleus: 15/3 Grains: closed and sticky Water: complete		Pre/post nucleus: 19/1 Grains: opened and sticky/mushy-prone
indica6					Pre/post nucleus: 20/2 Grains: opened and sticky/mushy-prone
indica7		Pre/post nucleus: 20/3 Grains: opened and sticky Water: complete	Pre/post nucleus: 20/3 Grains: opened and sticky Water: complete		Pre/post nucleus: 20/0 Grains: opened and sticky
indica8		Pre/post nucleus: 20/4 Grains: opened and sticky/mushy-prone Water: complete	Pre/post nucleus: 20/4 Grains: opened and sticky Water: complete		Pre/post nucleus: 20/4 Grains: opened and sticky
indica9		Pre/post nucleus: 20/3 Grains: closed and non-sticky Water: incomplete	Pre/post nucleus: 7/4 Grains: closed and non-sticky Water: incomplete		Pre/post nucleus: 7/0 Grains: opened and non-sticky
indica10					Pre/post nucleus: 8/0 Grains: opened and non-sticky

Registry of observations for *japonica* samples from the post-cooking analysis using a microwave oven or steam: number of nucleus at the end of the pre-cooking and the post-cooking stages, final appearance of the grains and water absorption.

	Microwave post-cooking			Steam post-cooking	
Samples	**7 minutes**	**10 minutes**	**13 minutes**	**7 minutes**	**10 minutes**
japonica1		Pre/post nucleus: 20/4 Grains: opened and sticky Water: complete	Pre/post nucleus: 19/4 Grains: closed and sticky Water: complete		Pre/post nucleus: 20/4 Grains: opened and sticky
japonica2					Pre/post nucleus: 20/4 Grains: opened and sticky
japonica3					Pre/post nucleus: 20/1 Grains: opened and sticky/mushy-prone
japonica4		Pre/post nucleus: 20/4 Grains: closed and sticky Water: complete	Pre/post nucleus: 14/1 Grains: closed and sticky Water: incomplete		Pre/post nucleus: 20/4 Grains: opened and sticky
japonica5		Pre/post nucleus: 20/4 Grains: closed and sticky Water: complete	Pre/post nucleus: 17/4 Grains: closed and sticky Water: complete		Pre/post nucleus: 20/1 Grains: opened and sticky/mushy-prone
japonica6		Pre/post nucleus: 20/4 Grains: closed and sticky Water: complete	Pre/post nucleus: 13/4 Grains: closed and sticky Water: complete		Pre/post nucleus: 20/0 Grains: opened and sticky/mushy-prone
japonica7		Pre/post nucleus: 20/3 Grains: closed and sticky Water: incomplete	Pre/post nucleus: 18/4 Grains: closed and sticky Water: complete		Pre/post nucleus: 20/2 Grains: opened and sticky
japonica8		Pre/post nucleus: 20/4 Grains: closed and sticky Water: complete	Pre/post nucleus: 18/3 Grains: closed and sticky Water: complete		Pre/post nucleus: 19/3 Grains: opened and sticky/mushy-prone
japonica9					Pre/post nucleus: 9/2 Grains: closed and non-sticky

Appendix C

Pearson correlations between the obtained results, considering all non-parboiled samples.

	Average length (mm)	Average width (mm)	Length-to-width ratio	Total whiteness	Crystalline whiteness	Kett	Chalky area (%)	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)	Ordinary cooking time (min) (80% cooked)	Solids in drained waters (%) (DWB) 10 min	Solids in drained waters (%) (DWB) 13 min	Microwave time (min) (80% cooked) 10 min	Microwave time (min) (80% cooked) 13 min	Steam time (min) (80% cooked) 10 min
Average length (mm)	1.00																	
Average width (mm)	-0.83	1.00																
Length-to-width ratio	0.94	-0.96	1.00															
Total whiteness	-0.60	0.65	-0.62	1.00														
Crystalline whiteness	0.09	0.12	-0.05	0.18	1.00													
Kett	-0.54	0.54	-0.54	0.89	0.41	1.00												
Chalky area (%)	-0.56	0.62	-0.57	0.97	0.00	0.78	1.00											
Moisture (%)	-0.50	0.30	-0.42	0.05	0.06	0.17	-0.03	1.00										
Protein (%) (DWB)	0.37	-0.62	0.58	-0.33	-0.12	-0.22	-0.25	0.11	1.00									
Total starch (%) (DWB)	-0.37	0.32	-0.35	0.27	0.32	0.36	0.16	0.14	-0.21	1.00								
Resistant starch (%) (DWB)	0.23	-0.33	0.25	-0.03	0.31	0.04	-0.14	-0.02	-0.15	0.16	1.00							
Apparent Amylose (%) (DWB)	0.55	-0.63	0.62	-0.40	-0.09	-0.31	-0.34	-0.19	0.48	-0.38	0.18	1.00						
Ordinary cooking time (min) (80% cooked)	-0.23	0.30	-0.33	0.28	0.47	0.31	0.15	-0.08	-0.51	0.50	0.30	-0.05	1.00					
Solids in drained waters (%) (DWB) 10 min	-0.39	0.29	-0.40	0.04	0.35	0.08	-0.06	0.02	-0.39	0.27	0.24	-0.20	0.53	1.00				
Solids in drained waters (%) (DWB) 13 min	0.31	-0.23	0.24	-0.18	-0.06	-0.28	-0.13	-0.06	-0.11	-0.10	0.70	0.57	0.19	0.13	1.00			
Microwave time (min) (80% cooked) 10 min	-0.08	0.16	-0.20	0.20	0.38	0.27	0.06	0.21	-0.47	0.12	0.63	0.17	0.88	0.02	0.50	1.00		
Microwave time (min) (80% cooked) 13 min	0.36	-0.42	0.40	-0.15	-0.01	-0.17	-0.14	-0.03	0.18	-0.32	0.62	0.71	0.61	-0.31	0.66	0.75	1.00	
Steam time (min) (80% cooked) 10 min	-0.01	0.14	-0.10	0.22	0.26	0.07	0.18	-0.16	-0.44	0.17	0.45	-0.14	0.59	0.28	0.28	0.44	0.74	1.00

Pearson correlations between the obtained results, considering all samples, non-parboiled and parboiled.

	Average length (mm)	Average width (mm)	Length-to-width ratio	Total whiteness	Crystalline whiteness	Kett	Chalky area (%)	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)	Ordinary cooking time (min) (80% cooked)	Solids in drained waters (%) (DWB) 10 min	Solids in drained waters (%) (DWB) 13 min	Microwave time (min) (80% cooked) 10 min	Microwave time (min) (80% cooked) 13 min	Steam time (min) (80% cooked) 10 min
Average length (mm)	1.00																	
Average width (mm)	-0.82	1.00																
Length-to-width ratio	0.94	-0.96	1.00															
Total whiteness	-0.34	0.44	-0.40	1.00														
Crystalline whiteness	-0.05	0.23	-0.16	0.83	1.00													
Kett	-0.24	0.30	-0.29	0.96	0.87	1.00												
Chalky area (%)	-0.46	0.52	-0.48	0.87	0.48	0.78	1.00											
Moisture (%)	-0.60	0.39	-0.51	0.06	0.06	0.05	0.00	1.00										
Protein (%) (DWB)	0.31	-0.55	0.52	-0.27	-0.16	-0.21	-0.28	0.12	1.00									
Total starch (%) (DWB)	-0.43	0.30	-0.38	0.16	0.12	0.17	0.16	0.27	-0.18	1.00								
Resistant starch (%) (DWB)	0.15	-0.18	0.14	0.56	0.68	0.63	0.29	-0.04	-0.20	0.13	1.00							
Apparent Amylose (%) (DWB)	0.55	-0.69	0.65	-0.42	-0.31	-0.31	-0.37	-0.24	0.45	-0.33	0.01	1.00						
Ordinary cooking time (min) (80% cooked)	-0.19	0.30	-0.28	-0.36	-0.31	-0.46	-0.28	0.05	-0.20	0.19	-0.36	-0.10	1.00					
Solids in drained waters (%) (DWB) 10 min	-0.29	0.24	-0.30	-0.53	-0.46	-0.60	-0.41	0.11	-0.14	0.14	-0.41	-0.10	0.80	1.00				
Solids in drained waters (%) (DWB) 13 min	0.11	-0.10	0.11	-0.77	-0.76	-0.86	-0.56	0.05	0.13	-0.12	-0.55	0.34	0.71	0.74	1.00			
Microwave time (min) (80% cooked) 10 min	-0.08	0.22	-0.23	0.34	0.40	0.37	0.17	0.18	-0.47	0.05	0.65	0.05	0.64	-0.14	-0.09	1.00		
Microwave time (min) (80% cooked) 13 min	0.36	-0.39	0.39	-0.08	0.00	-0.08	-0.13	-0.03	0.17	-0.31	0.45	0.63	0.53	-0.25	0.27	0.68	1.00	
Steam time (min) (80% cooked) 10 min	0.26	-0.04	0.12	0.14	0.14	0.12	0.13	-0.43	-0.37	-0.12	0.31	-0.05	0.18	0.00	-0.08	0.50	0.64	1.00

Pearson correlations between the obtained results, considering only non-parboiled *indica* samples.

	Average length (mm)	Average width (mm)	Length-to-width ratio	Total whiteness	Crystalline whiteness	Kett	Chalky area (%)	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)	Ordinary cooking time (min) (80% cooked)	Solids in drained waters (%) (DWB) 10 min	Solids in drained waters (%) (DWB) 13 min	Microwave time (min) (80% cooked) 10 min	Microwave time (min) (80% cooked) 13 min	Steam time (min) (80% cooked) 10 min
Average length (mm)	1.00																	
Average width (mm)	-0.38	1.00																
Length-to-width ratio	0.76	-0.88	1.00															
Total whiteness	-0.66	-0.28	-0.13	1.00														
Crystalline whiteness	-0.83	0.14	-0.53	0.86	1.00													
Kett	-0.63	-0.27	-0.15	0.90	0.88	1.00												
Chalky area (%)	-0.35	-0.59	0.24	0.89	0.54	0.71	1.00											
Moisture (%)	-0.40	0.09	-0.30	0.28	0.43	0.23	0.14	1.00										
Protein (%) (DWB)	0.20	-0.90	0.71	0.28	-0.05	0.28	0.52	0.30	1.00									
Total starch (%) (DWB)	-0.04	-0.21	0.11	0.59	0.54	0.60	0.48	0.03	0.06	1.00								
Resistant starch (%) (DWB)	-0.49	0.34	-0.47	0.61	0.64	0.40	0.46	0.20	-0.41	0.56	1.00							
Apparent Amylose (%) (DWB)	-0.01	-0.25	0.14	0.02	-0.16	0.05	0.23	0.14	0.36	-0.27	-0.07	1.00						
Ordinary cooking time (min) (80% cooked)	-0.67	0.50	-0.71	0.44	0.64	0.50	0.18	0.24	-0.43	0.28	0.69	0.33	1.00					
Solids in drained waters (%) (DWB) 10 min	-0.75	0.58	-0.76	0.35	0.50	0.29	0.13	-0.12	-0.60	0.03	0.52	-0.04	0.66	1.00				
Solids in drained waters (%) (DWB) 13 min	-0.02	0.41	-0.31	-0.13	-0.06	-0.24	-0.09	0.29	-0.35	0.12	0.79	0.49	0.60	0.28	1.00			
Microwave time (min) (80% cooked) 10 min	-0.57	0.57	-0.71	0.30	0.55	0.29	0.04	0.43	-0.43	0.07	0.76	0.18	0.90	0.38	0.72	1.00		
Microwave time (min) (80% cooked) 13 min	0.02	-0.15	0.10	0.04	-0.11	0.00	0.26	0.30	0.25	-0.27	0.56	0.91	0.76	-0.27	0.80	0.94	1.00	
Steam time (min) (80% cooked) 10 min	-0.18	0.33	-0.29	0.19	0.14	-0.05	0.22	-0.07	-0.45	0.03	0.73	0.11	0.42	0.27	0.56	0.67	0.77	1.00

Pearson correlations between the obtained results, considering only all the *indica* samples, non-parboiled and parboiled.

	Average length (mm)	Average width (mm)	Length-to-width ratio	Total whiteness	Crystalline whiteness	Kett	Chalky area (%)	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)	Ordinary cooking time (min) (80% cooked)	Solids in drained waters (%) (DWB) 10 min	Solids in drained waters (%) (DWB) 13 min	Microwave time (min) (80% cooked) 10 min	Microwave time (min) (80% cooked) 13 min	Steam time (min) (80% cooked) 10 min
Average length (mm)	1.00																	
Average width (mm)	-0.19	1.00																
Length-to-width ratio	0.75	-0.79	1.00															
Total whiteness	0.08	0.02	0.06	1.00														
Crystalline whiteness	0.11	0.15	-0.01	0.99	1.00													
Kett	0.02	0.00	0.03	0.98	0.97	1.00												
Chalky area (%)	-0.07	-0.31	0.19	0.89	0.81	0.87	1.00											
Moisture (%)	-0.56	0.02	-0.39	0.11	0.11	0.12	0.17	1.00										
Protein (%) (DWB)	0.11	-0.89	0.65	0.13	0.03	0.16	0.38	0.31	1.00									
Total starch (%) (DWB)	-0.30	-0.25	-0.03	0.02	-0.04	0.10	0.23	0.23	0.08	1.00								
Resistant starch (%) (DWB)	-0.15	0.31	-0.28	0.79	0.78	0.75	0.75	0.19	-0.25	0.30	1.00							
Apparent Amylose (%) (DWB)	-0.15	-0.28	0.07	-0.24	-0.29	-0.21	-0.03	0.17	0.34	-0.15	-0.21	1.00						
Ordinary cooking time (min) (80% cooked)	-0.13	0.30	-0.32	-0.48	-0.45	-0.43	-0.44	-0.18	-0.34	0.01	-0.16	0.27	1.00					
Solids in drained waters (%) (DWB) 10 min	-0.50	0.36	-0.56	-0.56	-0.55	-0.53	-0.46	-0.17	-0.49	0.06	-0.21	0.12	0.76	1.00				
Solids in drained waters (%) (DWB) 13 min	-0.11	0.11	-0.18	-0.84	-0.83	-0.84	-0.73	0.06	-0.18	0.04	-0.55	0.48	0.75	0.69	1.00			
Microwave time (min) (80% cooked) 10 min	-0.28	0.58	-0.58	0.38	0.43	0.40	0.23	0.32	-0.41	-0.06	0.75	0.06	0.60	0.07	-0.06	1.00		
Microwave time (min) (80% cooked) 13 min	0.09	-0.07	0.12	0.14	0.11	0.12	0.27	0.25	0.22	-0.29	0.34	0.77	0.57	-0.29	0.21	0.81	1.00	
Steam time (min) (80% cooked) 10 min	0.39	0.28	0.05	0.01	0.03	-0.05	-0.04	-0.45	-0.35	-0.31	0.22	-0.03	0.60	0.25	0.21	0.70	0.63	1.00

Pearson correlations between the obtained results, considering only non-parboiled *japonica* samples.

	Average length (mm)	Average width (mm)	Length-to-width ratio	Total whiteness	Crystalline whiteness	Kett	Chalky area (%)	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)	Ordinary cooking time (min) (80% cooked)	Solids in drained waters (%) (DWB) 10 min	Solids in drained waters (%) (DWB) 13 min	Microwave time (min) (80% cooked) 10 min	Microwave time (min) (80% cooked) 13 min	Steam time (min) (80% cooked) 10 min
Average length (mm)	1.00																	
Average width (mm)	0.00	1.00																
Length-to-width ratio	0.76	-0.65	1.00															
Total whiteness	-0.06	0.73	-0.53	1.00														
Crystalline whiteness	0.87	0.29	0.47	-0.06	1.00													
Kett	0.10	0.76	-0.43	0.91	0.13	1.00												
Chalky area (%)	-0.20	0.74	-0.64	0.99	-0.18	0.85	1.00											
Moisture (%)	-0.36	-0.30	-0.06	-0.55	-0.24	-0.35	-0.53	1.00										
Protein (%) (DWB)	-0.42	-0.20	-0.16	-0.56	-0.24	-0.63	-0.44	0.42	1.00									
Total starch (%) (DWB)	0.26	-0.45	0.50	-0.49	0.20	-0.58	-0.52	-0.15	-0.15	1.00								
Resistant starch (%) (DWB)	0.34	-0.60	0.62	-0.15	0.01	-0.06	-0.23	0.03	-0.25	-0.12	1.00							
Apparent Amylose (%) (DWB)	0.18	-0.25	0.31	-0.26	-0.01	-0.17	-0.27	-0.14	0.00	0.32	-0.09	1.00						
Ordinary cooking time (min) (80% cooked)	0.55	-0.22	0.53	0.01	0.41	-0.04	-0.08	-0.59	-0.59	0.63	0.35	0.01	1.00					
Solids in drained waters (%) (DWB) 10 min	-0.02	-0.19	0.10	-0.51	0.24	-0.59	-0.48	-0.01	0.37	0.50	-0.16	-0.23	0.40	1.00				
Solids in drained waters (%) (DWB) 13 min	-0.43	0.52	-0.67	0.65	-0.35	0.45	0.72	-0.61	-0.16	-0.14	-0.47	0.13	0.04	-0.06	1.00			
Microwave time (min) (80% cooked) 10 min	0.71	-0.27	0.71	0.12	0.25	0.22	0.00	-0.55	-0.65	0.16	0.51	0.52	0.84	-0.79	-0.32	1.00		
Microwave time (min) (80% cooked) 13 min	0.29	-0.46	0.53	0.04	-0.14	-0.11	0.01	-0.20	-0.15	-0.09	0.63	-0.22	0.29	-0.48	-0.52	0.55	1.00	
Steam time (min) (80% cooked) 10 min	0.35	0.05	0.22	0.24	0.33	0.12	0.17	-0.33	-0.55	0.33	0.24	-0.64	0.70	0.29	-0.12	0.03	0.60	1.00

Pearson correlations between the obtained results, considering only all the *japonica* samples, non-parboiled and parboiled.

	Average length (mm)	Average width (mm)	Length-to-width ratio	Total whiteness	Crystalline whiteness	Kett	Chalky area (%)	Moisture (%)	Protein (%) (DWB)	Total starch (%) (DWB)	Resistant starch (%) (DWB)	Apparent Amylose (%) (DWB)	Ordinary cooking time (min) (80% cooked)	Solids in drained waters (%) (DWB) 10 min	Solids in drained waters (%) (DWB) 13 min	Microwave time (min) (80% cooked) 10 min	Microwave time (min) (80% cooked) 13 min	Steam time (min) (80% cooked) 10 min
Average length (mm)	1.00																	
Average width (mm)	-0.05	1.00																
Length-to-width ratio	0.76	-0.68	1.00															
Total whiteness	0.06	0.21	-0.11	1.00														
Crystalline whiteness	0.71	-0.04	0.53	0.50	1.00													
Kett	0.17	0.00	0.10	0.93	0.68	1.00												
Chalky area (%)	-0.09	0.42	-0.36	0.95	0.27	0.78	1.00											
Moisture (%)	-0.38	-0.15	-0.16	-0.60	-0.40	-0.45	-0.61	1.00										
Protein (%) (DWB)	-0.43	-0.03	-0.27	-0.66	-0.46	-0.63	-0.56	0.50	1.00									
Total starch (%) (DWB)	0.28	-0.47	0.51	-0.21	0.24	-0.11	-0.35	-0.19	-0.20	1.00								
Resistant starch (%) (DWB)	0.29	-0.57	0.56	0.60	0.61	0.77	0.35	-0.28	-0.49	0.07	1.00							
Apparent Amylose (%) (DWB)	0.20	-0.29	0.33	-0.06	0.10	0.07	-0.14	-0.18	-0.07	0.33	0.08	1.00						
Ordinary cooking time (min) (80% cooked)	0.17	0.17	0.01	-0.64	-0.45	-0.78	-0.48	0.01	0.09	0.20	-0.64	-0.12	1.00					
Solids in drained waters (%) (DWB) 10 min	-0.13	0.20	-0.22	-0.82	-0.53	-0.91	-0.66	0.30	0.54	0.12	-0.79	-0.24	0.85	1.00				
Solids in drained waters (%) (DWB) 13 min	-0.17	0.37	-0.36	-0.71	-0.73	-0.89	-0.47	0.30	0.42	-0.16	-0.88	-0.14	0.85	0.87	1.00			
Microwave time (min) (80% cooked) 10 min	0.71	-0.27	0.71	0.12	0.25	0.22	0.00	-0.55	-0.65	0.16	0.51	0.52	0.84	-0.79	-0.32	1.00		
Microwave time (min) (80% cooked) 13 min	0.29	-0.46	0.53	0.04	-0.14	-0.11	0.01	-0.20	-0.15	-0.09	0.63	-0.22	0.29	-0.48	-0.52	0.55	1.00	
Steam time (min) (80% cooked) 10 min	0.37	-0.12	0.33	0.50	0.55	0.48	0.38	-0.44	-0.64	0.35	0.52	-0.48	-0.08	-0.29	-0.49	0.03	0.60	1.00