Trace Rice


# [Physico-chemical, sensory, and health-related parameters of processed rice]

[29-10-2022]

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http://trace-rice.eu

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# Executive summary

The purpose of this task is to develop innovative rice-based products maintaining or improving the technological, nutritional, and textural properties. Focus has been put on improving nutritional quality of a traditional products such as sushi, and the technological and healthy properties of brown rice. Firstly, the effect of sushi seasoning made with different kinds of vinegar, sugar, and salt on the technological quality and digestibility of sushi was evaluated. Secondly, the combination of pre-soaking with enzymatic treatment can be an alternative to modify the technological properties, bioactive compounds and digestibility of brown rice. Specifically, enzymatic treatment with proteases or cell wall enzymes combined with soaking was carried out in two ways: i) cooking in excess of water; ii) cooking without excess of water (all the water is consumed during cooking).

# TABLE OF CONTENTS

Executive summary ____ 3
TABLE OF CONTENTS ____ 4
1. INTRODUCTION ____ 5
2. Using seasonings for varying the technological and nutritional quality of cooked rice ____ 6
2.1.1. Unraveling seasonings impact on cooked rice quality: Technological and nutritional implications for sushi ____ 6
3. Technological improvement of brown rice. ____ 10
3.1. Introduction ____ 10
3.2. Material and methods ____ 11
3.2.1. Material ____ 11
3.2.2. Activities ____ 11
3.2.3. Brown rice pre-treatment ____ 11
3.2.4. Rice cooking ____ 12
3.3. Results ____ 13
3.3.1. Effect of enzymatic treatment in rice grains before cooking ____ 13
3.3.2. Brown rice cooked with excess of water ____ 17
3.3.3. Brown rice cooked without excess of water (adjusted water) ____ 20
4. List of samples selected from items 2 and 3. ____ 22
4.1. Biocompounds and digestibility of selected rice samples ____ 22
4.2. HPLC-DAD-electrochemical detector and LC-MS analysis of phenolic compounds ____ 25
4.3. Untargeted SPME-GC-MS characterization of volatile organic compounds ____ 25
4.4. Collection of spectral data using Fourier-transform infrared spectroscopy (FTIR) ____ 27
5. Conclusions ____ 27
6. References ____ 27

# 1. INTRODUCTION

Nowadays, rice is one of the agricultural products with the greatest development in terms of crops and consumption. Rice is a staple food and supplies 20% of the world's energy supply (Zhang & Rahman, 2022). There are hundreds of rice-based products on the global market: crackers, cookies, baby food, desserts, drinks, vinegar, snacks, breakfast cereals, etc. (TRACERICE D3.1. Market analysis of rice-based products and election of consumers targets). However, its main consumption is as cooked white rice as a complement to other foods or in ready-to-eat products.

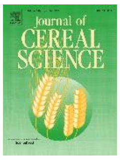
Rice is an important source of fiber, minerals, proteins, vitamins, antioxidants, and other biomolecules that may have a positive effect on human health (Burlando & Cornara, 2014; Goufo & Trindade, 2014). Its composition depends mainly on the processing, white rice is the type that contains the highest carbohydrate content and the lowest content of the rest of the macro components, and bioactive compounds compared with brown rice (Mir et al., 2020). Numerous investigations have related the consumption of foods rich in carbohydrates, such as white rice, to an increase in the glycemic index and thus to an increase in the prevalence of type 2 diabetes (Chang et al., 2014; Jukanti et al., 2020). The glycemic index of rice depends on the variety mediated by its amylose content and the processes (Kaur et al., 2016). For this reason, in recent years one of the challenges of the research and rice industry has been to obtain products with a lower glycemic index. For this purpose, different strategies have been studied: i) selection of different varieties (Frei et al., 2003; Rathinasabapathi et al., 2015); ii) use of different processes such as germination, extrusion, puffing, parboiling, etc. (Sivakamasundari et al., 2022).

Another strategy to reduce the glycemic index of rice and rice-based products is the consumption of brown rice. The bran of brown rice contains vitamins, minerals, fiber, GABA (gamma-aminobutyric acid), PUFA (polyunsaturated fatty acids), γ-oryzanol, etc., which could contribute to blood glucose lowering (Panlasigui & Thompson, 2006; Sen et al., 2020; Xia et al., 2019). Nevertheless, white rice is still preferred by consumers, due to its shorter cooking time, shelf life, and its taste, appearance, and texture when compared to brown rice (Mir et al., 2020; Saleh et al., 2019). In recent years, numerous studies have been conducted to increase the consumption of brown rice by trying to develop new products or modifying the processes of traditional products to increase their acceptability. Bioprocess techniques have been used (fermentation, germination, etc.) focused mainly on increasing the bioavailability of micronutrients but paying attention to improving the sensorial characteristics of the products (Xia et al., 2019).

Despite all the research carried out in recent years, the application of innovation to traditional rice or rice-based foods with lower glycemic index is still needed. Therefore, the development of innovative products was studied maintaining or improving the technological, nutritional, and textural properties of traditional products such as sushi, but was also evaluated the effect of enzymatic treatment before cooking brown rice to check its impact on technological or nutritional properties.

# 2. Using seasonings for varying the technological and nutritional quality of cooked rice

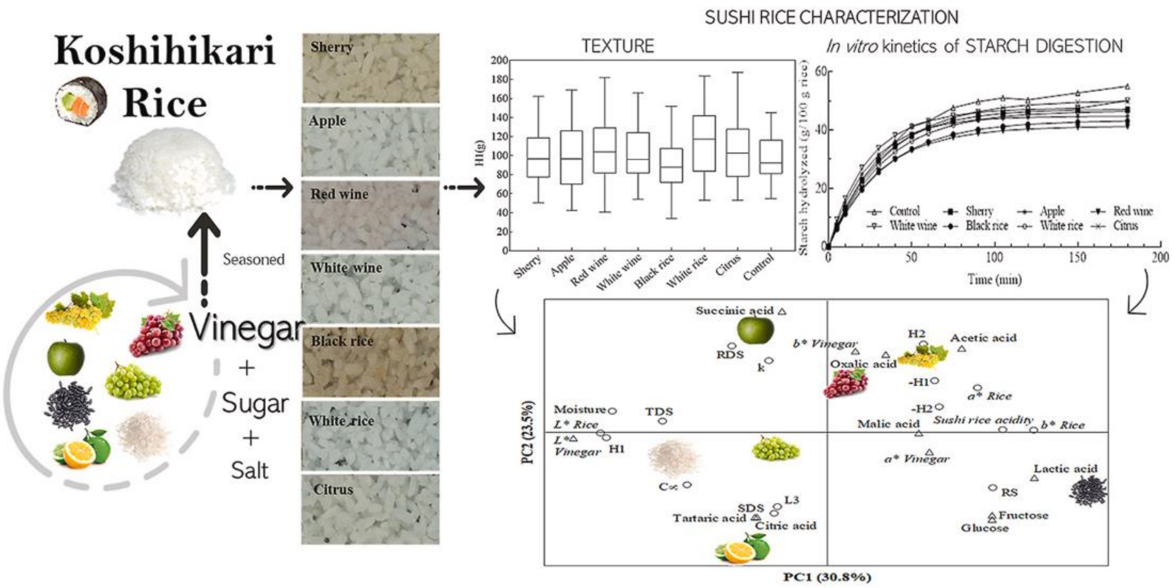
Sushi consumption is becoming a global phenomenon, owing to the growing interest in both Asian cuisines and a healthy diet (Hsin-I Feng, 2012). Sushi is cooked white rice seasoned with rice vinegar, sugar, and salt. This seasoning is crucial to the quality of the sushi because of the flavor and texture of the rice. On the other hand, the blood glucose lowering effect of acetic acid (the most important component of vinegar) has been described (Budak et al., 2014). Therefore, it could be hypothesized that the seasoning of sushi rice could impact the digestibility and texture of cooked white rice. Since vinegar of different origins are available on the market and the effect of their use on the quality and digestibility of cooked white rice is not known, it was decided to conduct a study testing different seasonings and check the effect on cooked white rice for sushi. The results were published in the Journal of Cereal Science in 2021[1].




### 2.1.1. Unraveling seasonings impact on cooked rice quality: Technological and nutritional implications for sushi

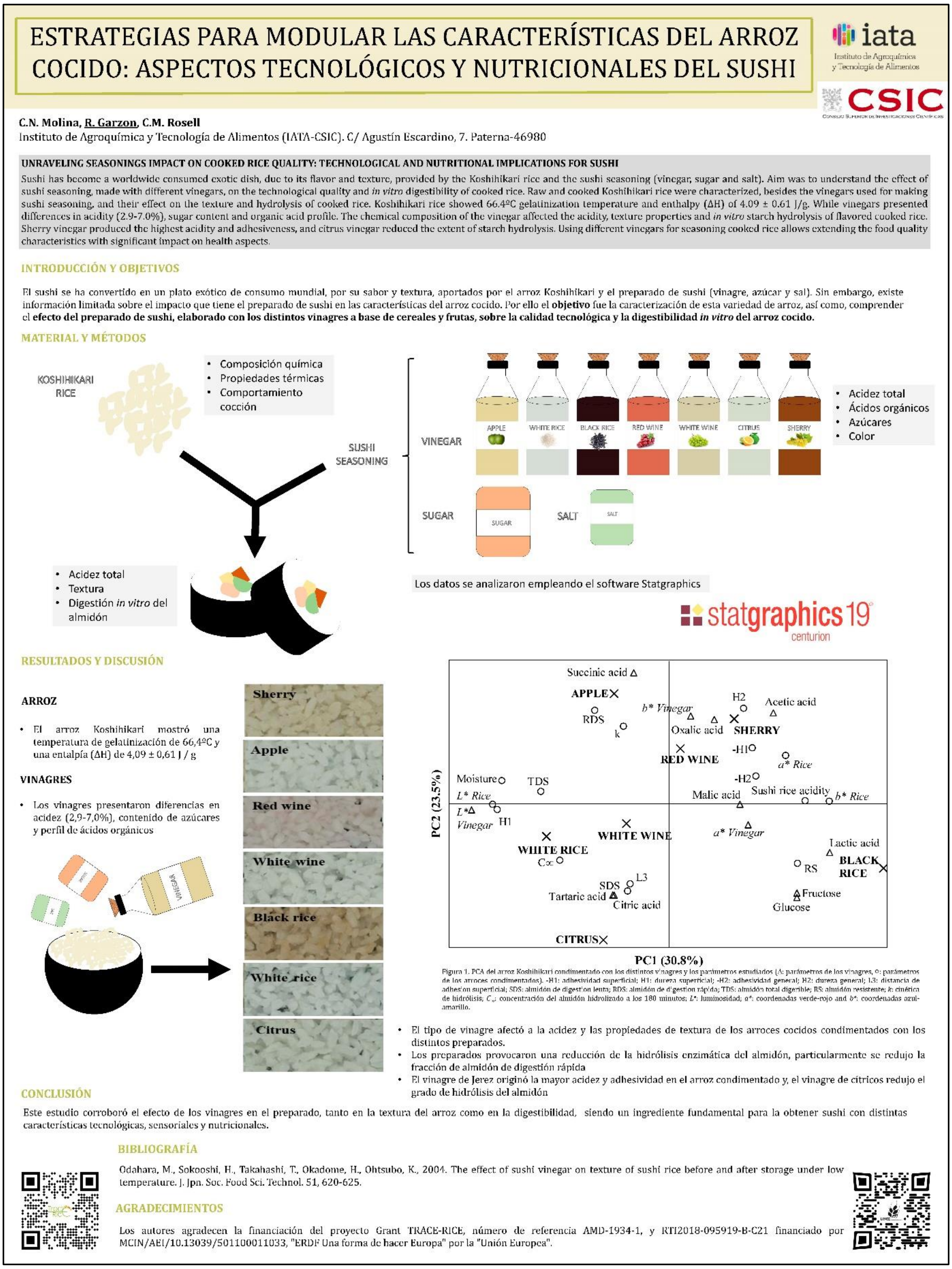
*Sushi has become a worldwide consumed exotic dish, due to its characteristic flavor and texture, provided by the Koshihikari rice and the sushi seasoning (vinegar, sugar, and salt). Nevertheless, it is not known how the nature of vinegar could impact the sushi rice characteristics. Therefore, the objective of the study was the characterization of this rice variety, as well as the study of the effect of sushi seasoning made with different kinds of vinegar, sugar, and salt on the technological quality and digestibility of sushi. Koshihikari variety was characterized as well as the different kinds of vinegar (acidity, color, and organic acids profile) and the effect of the seasoning on the texture and enzymatic hydrolysis of cooked rice were studied. The Koshihikari variety had medium amylose (15%), low fat (0.6%) and high protein content (8%). While the types of vinegar presented differences in acidity (2.9-7.0%), sugar content and organic acid profile. With the double compression method, it was possible to compare and obtain differences in the texture profiles of the rice with sushi seasonings and the control, with apple vinegar being higher in general hardness and superficial adhesiveness. The sushi seasonings reduced the enzymatic hydrolysis of starch, particularly, the RDS and $C\infty$. Overall, the use of different vinegars in obtaining the sushi seasoning allows modifying the technological properties of rice (texture, color, acidity), as well as the behavior of starch hydrolysis.*

[1] Molina, C. N., Garzón, R., & Rosell, C. M. (2022). Unraveling seasonings impact on cooked rice quality: Technological and nutritional implications for sushi. Journal of Cereal Science, 104, 103442. Doi: 10.1016/j.jcs.2022.103442




## *Results dissemination*

Molina, C. N., Garzon, R. & Rosell, C.M. 2021. Estrategias para modular las características del arroz cocido: aspectos tecnológicos y nutricionales del sushi. XXXIII Jornadas Técnicas de la Asociación de Técnicos Cerealistas. Zamora, Spain.



# ESTRATEGIAS PARA MODULAR LAS CARACTERÍSTICAS DEL ARROZ COCIDO: ASPECTOS TECNOLÓGICOS Y NUTRICIONALES DEL SUSHI

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**UNRAVELING SEASONINGS IMPACT ON COOKED RICE QUALITY: TECHNOLOGICAL AND NUTRITIONAL IMPLICATIONS FOR SUSHI**

Sushi has become a worldwide consumed exotic dish, due to its flavor and texture, provided by the Koshihikari rice and the sushi seasoning (vinegar, sugar and salt). Aim was to understand the effect of sushi seasoning, made with different vinegars, on the technological quality and *in vitro* digestibility of cooked rice. Raw and cooked Koshihikari rice were characterized, besides the vinegars used for making sushi seasoning, and their effect on the texture and hydrolysis of cooked rice. Koshihikari rice showed 66.4ºC gelatinization temperature and enthalpy (ΔH) of 4.09 ± 0.61 J/g. While vinegars presented differences in acidity (2.9-7.0%), sugar content and organic acid profile. The chemical composition of the vinegar affected the acidity, texture properties and *in vitro* starch hydrolysis of flavored cooked rice. Sherry vinegar produced the highest acidity and adhesiveness, and citrus vinegar reduced the extent of starch hydrolysis. Using different vinegars for seasoning cooked rice allows extending the food quality characteristics with significant impact on health aspects.

## INTRODUCCIÓN Y OBJETIVOS

El sushi se ha convertido en un plato exótico de consumo mundial, por su sabor y textura, aportados por el arroz Koshihikari y el preparado de sushi (vinagre, azúcar y sal). Sin embargo, existe información limitada sobre el impacto que tiene el preparado de sushi en las características del arroz cocido. Por ello el **objetivo** fue la caracterización de esta variedad de arroz, así como, comprender el **efecto del preparado de sushi, elaborado con los distintos vinagres a base de cereales y frutas, sobre la calidad tecnológica y la digestibilidad *in vitro* del arroz cocido.**

## MATERIAL Y MÉTODOS



Los datos se analizaron empleando el software Statgraphics

## RESULTADOS Y DISCUSIÓN

**ARROZ**

- El arroz Koshihikari mostró una temperatura de gelatinización de 66,4ºC y una entalpía (ΔH) de 4,09 ± 0,61 J / g

**VINAGRES**

- Los vinagres presentaron diferencias en acidez (2,9-7,0%), contenido de azúcares y perfil de ácidos orgánicos





Figura 1. PCA del arroz Koshihikari condimentado con los distintos vinagres y los parámetros estudiados (Δ: parámetros de los vinagres, ○: parámetros de los arroces condimentados). -H1: adhesividad superficial; H1: dureza superficial; -H2: adhesividad general; H2: dureza general; L3: distancia de adhesion superficial; SDS: almidón de digestion lenta; RDS: almidón de digestion rápida; TDS: almidón total digerible; RS: almidón resistente; *k*: cinética de hidrólisis; $C_{\infty}$: concentración del almidón hidrolizado a los 180 minutos; $L^*$: luminosidad; $a^*$: coordenadas verde-rojo and $b^*$: coordenadas azul-amarillo.

- El tipo de vinagre afectó a la acidez y las propiedades de textura de los arroces cocidos condimentados con los distintos preparados.
- Los preparados provocaron una reducción de la hidrólisis enzimática del almidón, particularmente se redujo la fracción de almidón de digestión rápida
- El vinagre de Jerez originó la mayor acidez y adhesividad en el arroz condimentado y, el vinagre de cítricos redujo el grado de hidrólisis del almidón

## CONCLUSIÓN

Este estudio corroboró el efecto de los vinagres en el preparado, tanto en la textura del arroz como en la digestibilidad, siendo un ingrediente fundamental para la obtener sushi con distintas características tecnológicas, sensoriales y nutricionales.

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## AGRADECIMIENTOS

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Molina, C.N. & Garzon, R. 2022. Blog: Foods in health information (FSTA). Sushi rice: A traditional food with room for innovation. [https://www.ifis.org/blog/sushi-rice-a-traditional-food-with-room-for-innovation](https://www.ifis.org/blog/sushi-rice-a-traditional-food-with-room-for-innovation).

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# Sushi rice: A traditional food with room for innovation

By Carla N. Molina & R. Garzon on 19-Aug-2022 10:41:52

Rice is mainly composed of starch, which is the main factor responsible for the appearance of rice once cooked (Pinciroli M. et al., 2015). Starch is a polysaccharide composed of two polymeric fractions called amylose and amylopectin.

- Amylose is a mostly linear polymer made up of glucose units linked with α-1,4-bonds, which are folded into a helix as a result of the angles in the bonds between the glucose molecules, while amylopectin is a polymer with a larger number of glucose molecules linked by linear α-1,4 and α-1,6 bonds, which give rise to branching.
- Amylopectin constitutes the major fraction of starch, the ratio of amylose to amylopectin being normally 25%/75% (Pinciroli M. et al., 2015).

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## What kind of rice is used to make sushi?

There are so many types of rice and each one has its own characteristics. For that reason, it is important to know the varieties of rice and more specifically the one use to make sushi. Within the genus Oryza, there are two species of rice: Oryza sativa L., which is from Asia, and Oryza glaberrima Steud. from Africa. The first one is widely cultivated in the five continents and is divided into three subspecies: Indica, Japonica and Javanica.

While Indica subspecies are characterized by long or semi-long, fine and spongy grains, Japonica subspecies are identified by their short, round and hard grains. Javanica subspecies were incorporated

# 3. Technological improvement of brown rice.

## 3.1. Introduction

Rice quality is conditioned by different factors, such as variety, area and crop characteristics, post-harvest handling (drying, transport, storage and handling) and also for the cooking processing. Milling quality includes physical properties (kernel size, shape, milling recovery, degree of milling and appearance of the grain) and physicochemical properties such as amylose content, crude protein, gelatinization temperature, etc. On the other hand, rice culinary quality is controlled mainly by the physicochemical quality, but also by the texture of the cooked grain, flavor, aroma and others that are related to consumers preferences.

Brown rice is considered more nutritious because it comprises the bran and germ containing fibers and vitamins. However the acceptability is limited due to the presence of both of them, bran and germ, that are closely to the following factors (Xia et al., 2019): i) hard texture perception and chewing palatability; ii) required a prolonged cooking time, particularly due to the fact that the bran layers slower the absorption of water during cooking; iii) greater susceptibility to oxidation; iv) presence of anti-nutritional factor (e.g. phytic acid). Several methods have been studied to reduce cooking time, improve the texture, shape or color of brown rice, including partial milling heating and cooling (Mohapatra & Bal, 2006), germination (Patil & Khan, 2011), ultrasonic (Cui et al., 2010), presoaking with low (Thirumdas et al., 2016) or high pressure (Yu et al., 2015), and hydrothermal (Sareepuang et al., 2008) treatments, etc. Another approach has been focused in the use of enzymes, these are a useful tool for improving the processing behavior or modifying the properties of cereal-based foods.

Enzymes act as catalyst to bring about a specific biochemical reaction, so it is possible to act only on one of the grain parts, while the other parts are slightly affected. Endogenous and added enzymes have an important effect on the quality of cereals, used to facilitate processing and achieve improved and uniform product quality. Amylases and proteases have long been used in baking, while those that degrade the cell wall are gaining increasing importance. Although enzymes act at the molecular level, they can induce remarkable changes in both the microstructure and functional properties of cereal-based foods. Since lower acceptability of brown rice is largely due to the bran, the most interesting enzymes to study for the modification of this part are proteases and wall degrading enzymes. Proteases are enzymes that act by breaking the bonds that join the amino acids of proteins, assisting their digestion. The purpose of rice protein hydrolysis is to make the protein more digestible to increase its protein level or to allow easier hydration and dispersion in some foods. The characteristics of the hydrolyzed product will vary according to the degree of hydrolysis, so the lower the degree of hydrolysis the milder the flavor, but the lower the solubility. The use of cell wall degrading enzymes (cellulases, xylanases, hemicellulases, β-glucanases, etc.) have been used to increase availability of bioactive molecules in bran layer before the polishing step with successful results (Singh et al., 2016). Many of the applications of these enzymes have been focused on the modification of rice straw (Sarnklong et al., 2010; Vlasenko et al., 1997; Zhu et al., 2006). In a few studies, they have been used to modify the properties of rice bran fiber (Liu et al., 2021), but their potential to improve the cooking quality of whole brown rice grain has not been explored.

Generally, enzyme treatment requires that the enzymes be suspended in liquid and kept for certain time and temperature. Soaking is a treatment that has been traditionally performed for a long time. Soaking combined or not with other techniques (germination, high pressure, etc.) have a beneficial effect in improving the bioactive components (Komatsuzaki et al., 2007; Liang et al., 2008; Munarko et al., 2021) of brown rice and modifying its technological properties such as texture (Han & Lim, 2009; Sareepuang et al., 2008; Yu et al., 2015).

Probably the combination of pre-soaking with enzymatic treatment can be an alternative to modify the technological properties, bioactive compounds and digestibility of brown rice. Therefore, the **objective of this study was to find a suitable enzymatic treatment that would reduce the cooking time of rice and at the same time improve the technological and nutritional properties of rice.** For this purpose, enzymatic treatment with proteases or cell wall enzymes combined with soaking was carried out in two ways: i) cooking in excess of water; ii) cooking without excess of water (all the water is consumed during cooking).

## 3.2. Material and methods

### 3.2.1. Material

Brown rice (Arrocerias Pons, Valencia, Spain) was used to analyze the effects of the soaking and enzymes treatments on the grains and to see the change in the behavior of the rice properties. The enzymes (Novozymes, Denmark) used were:

- Alcalase 2.4 LFG, broad-spectrum protease
- Flavourzyme, exoprotease to release aminoacids
- Shearzyme Plus, a blend of xylanases, cellulases and beta-glucanases.
- Novozym 28251, a mixture of cellulases and xylanases.
- Celluclast, cellulase.
- Ultimase BWL 40, cellulase and xylanase.
- Phytaflow FG, phytase.

### 3.2.2. Activities

The characterization of the changes resulting in the treated rice was performed in two stages: after treatment of each sample and after cooking, as shown in Figure 1.

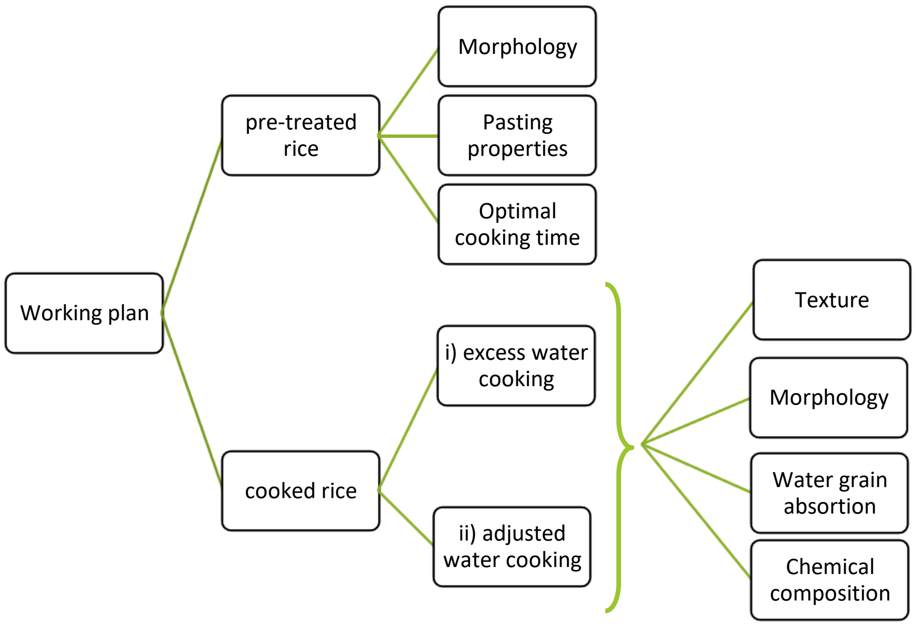



*Figure 1. Work plan of the research.*

### 3.2.3. Brown rice pre-treatment

Preliminary studies have been carried out at different pH (5.0 and 7.0), but no differences were observed in the final properties. Therefore, to make the application easy for the industry, it was carried out in water. Rice (60 g) was soaked in water or water + enzyme (120 mL) for three different times (1, 2 and 18 h) at 50ºC. In the case of enzyme-treated samples, 200 mg of enzyme/100 g of rice were added to the soaking water.

The characterization of the enzymatically treated rice samples before cooking consisted of:

#### *Optimal cooking time*

To determine the optimum cooking time of the samples obtained after each treatment, the method of Bhattacharya and Sowbhagy (1971) was followed with a slight modification in the volume of cooking water: the water/rice ratio was kept in excess.

For cooking the sample, 10 g of rice, previously washed, were added to a beaker with 330 mL of boiling distilled water. The rice was cooked for 10 min. After this cooking time, 10 grains were removed every minute and pressed between two dishes to check the degree of starch gelatinization. The minute in which no white kernel was observed on the cooked rice grains is the optimum time, which corresponds to complete gelatinization as shown in Figure 2. For this test, two replicates per sample were performed.

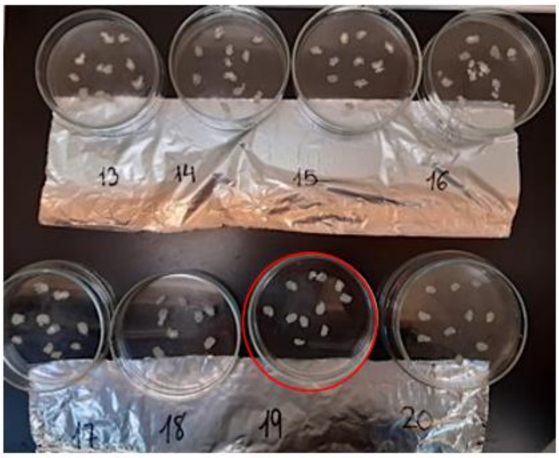



*Figure 2. Optimal cooking time determination.*

#### *Pasting properties*

First of all, rice grains after treatment were milled and their moisture were calculated to stablish the quantity of solid used in the analysis. The pasting properties of the rice flour were analyzed by employing Rapid Visco Analyzer (RVA 4500 Perkin Elmer, Hägersten, Sweden), using the standard 1 heating and cooling profile. The test was performed in duplicate, and the parameters recorded were: pasting temperature, peak viscosity, trough, breakdown, final viscosity, setback and time of peak viscosity.

#### *Morphology*

To observe the changes in morphology after treatment, whole grains were scanned in a plate at a resolution of 600 dpi.

### 3.2.4. Rice cooking

For cooking the brown rice, two different assays were carried out:

- With excess of water: rice was cooked with the same proportion of water used to determine the optimum cooking time (10 grams rice in 330 mL of water). The rice was added when the water was boiling and the cooking time for each sample was that obtained at the optimum cooking time.

- Without excess water: to hold the possible bioactive compounds generated (not lost in the extra water), the pretreated rice was cooked in an electric rice cooker with a ratio of 1:1.6 (rice: water distilled) suggested by Li et al. (2019) to allow the rice to absorb all the water. Rice was cooked during the optimal cooking time.

#### *Chemical composition*

The chemical composition of brown rice treated samples were determined by the following standard methods: moisture (ISO 712:2009); protein (ISO 16634:2:2016); minerals (ISO 2171:2007); total fat (ISO 2171:2007) and soluble, insoluble, and total dietary fiber (AACC 32-07.01). All the determinations were made at least in triplicate.

#### *Texture*

The texture of cooked brown rice treated samples were performed with a texturometer (TA. XT-Plus, Stable Micro Systems, London, U.K). Each rice grain was compressed with a 10 mm aluminum probe, two times: first low compression (25% (LC)), followed by a high compression test at 90% (HC). Surface hardness and stickiness were measure at LC (25%), while general grain hardness and stickiness were determined in the second plot curve (HC; 90%).

#### *Water absorption*

During rice cooking stage, swelling of the grains occurs because of water absorption. The volume of water retained by the grains will depend on the type of treatment applied to the sample, so the absorbed water of 100 of rice during cooking was calculate.

## 3.3. Results

### 3.3.1. Effect of enzymatic treatment in rice grains before cooking

To perform the enzymatic treatment, rice grains were soaked with the enzymes. This would be a pretreatment prior to cooking and may affect the properties of the grain.

## *Pasting properties of pre-treated rice grains*

The pasting properties of the rice samples were analysed with the previously milled sample. Figure 4 shows the heating and cooling curves of the samples, in which the phenomena of gelatinization (heating stage) and gelation (cooling stage) of the starch are recorded. The apparent viscosity profile of the samples was modified by both enzyme pretreatment and incubation time (Figure 3). In general, the peak viscosity of the samples treated for 18 hours was higher than those incubated for shorter time. That is, the longer the incubation time, the higher the swelling capacity of the starch granules, likely due to the modification of the external layers. The effect of the enzymes was dependent on their action, with the enzyme alcalase (protease) causing the highest viscosity peaks, followed by ultimase (cellulase and xylanase).

## *Grain morphology after treatment*

As observed in Figure 4 and Figure 5, the appearance of the grain changed with incubation times, and the extent of modification increased with treatment time. Differences were observed even after 1 hour soaking.

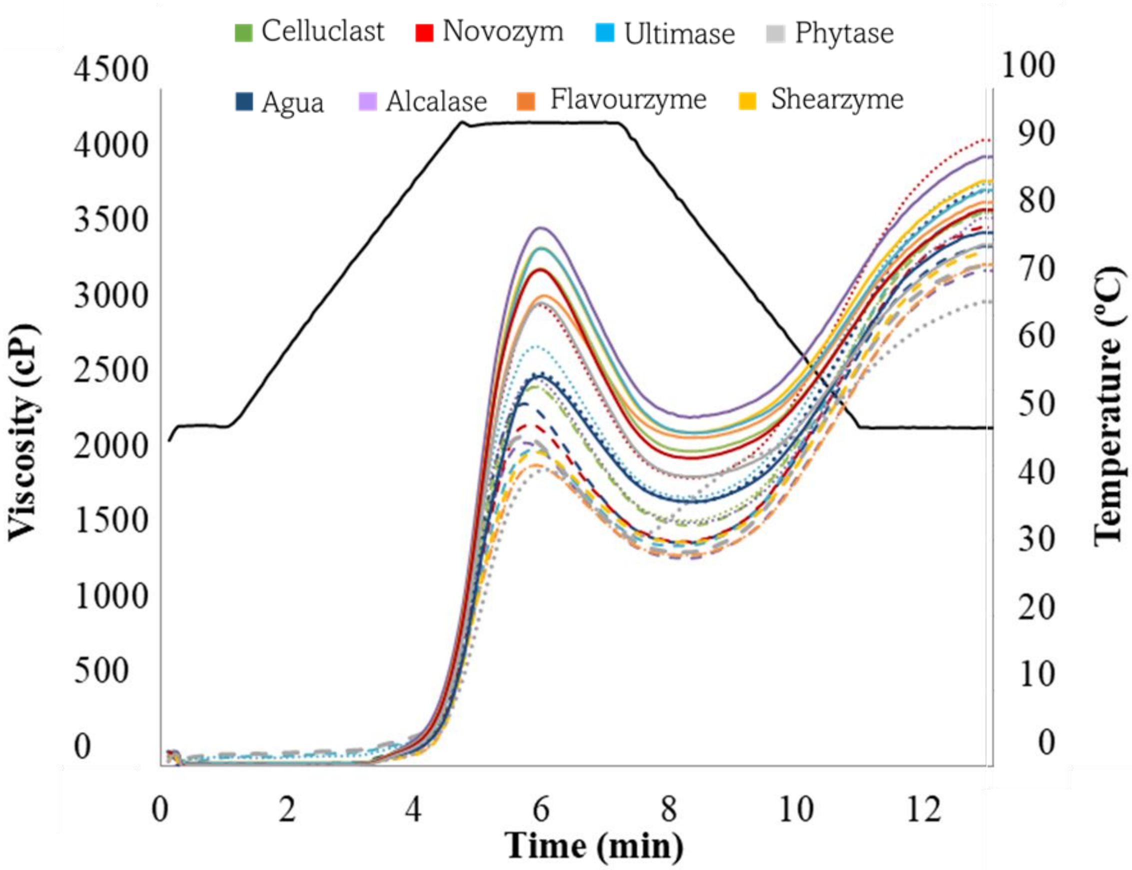



*Figure 3. Apparent viscosity profiles of rice flours obtained after conditioning in the presence of enzymes. The black line indicates the temperature gradient used in the recording. Type of line represent the treatment time: 1 h, striped lines; 2 h dotted lines and 18 h continuous lines.*

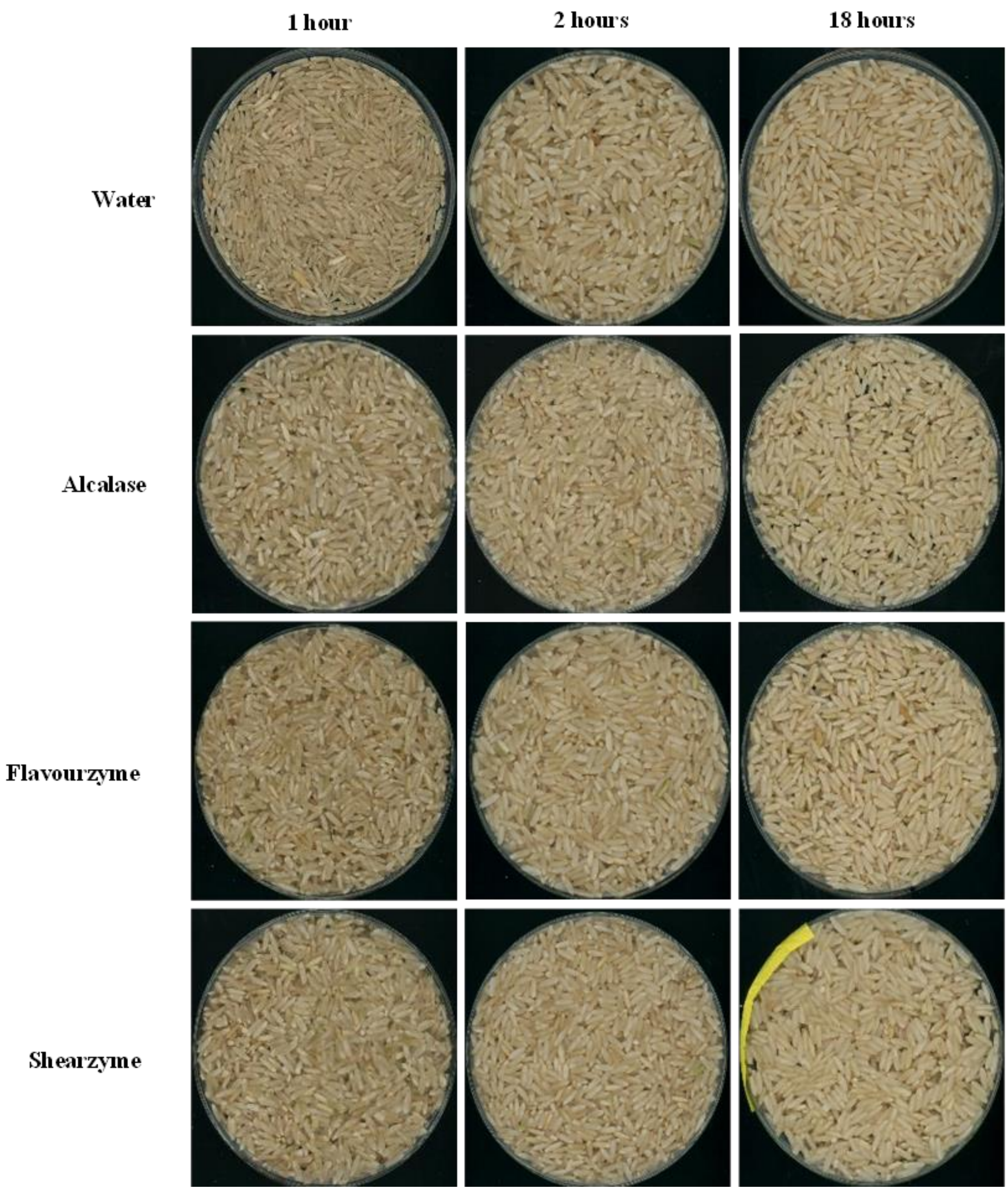



*Figure 4. Rice aspect after treatments with water, alcalase, flavourzyme and shearzyme.*

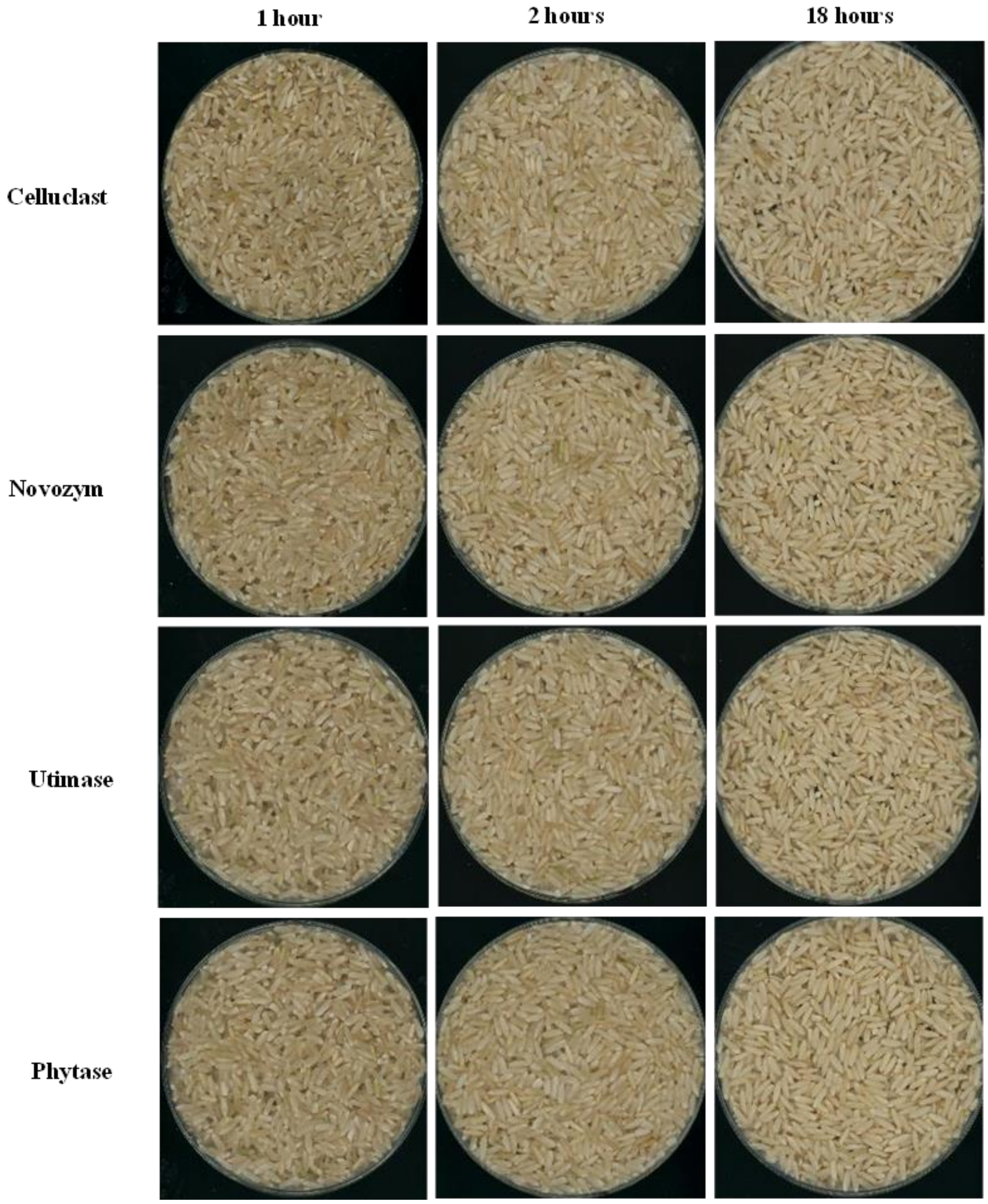



*Figure 5. Rice aspect after treatments with celluclast, novozym, ultimase and phytase.*

## Optimal cooking time of treated brown rice.

The optimum cooking time (OCT) of each sample was influenced by the enzymatic treatment applied (Figure 6), which indicated that the enzymatic treatment affected the external structure of the rice grain, favoring the access of the cooking water to the grain, and accelerating starch gelatinization.

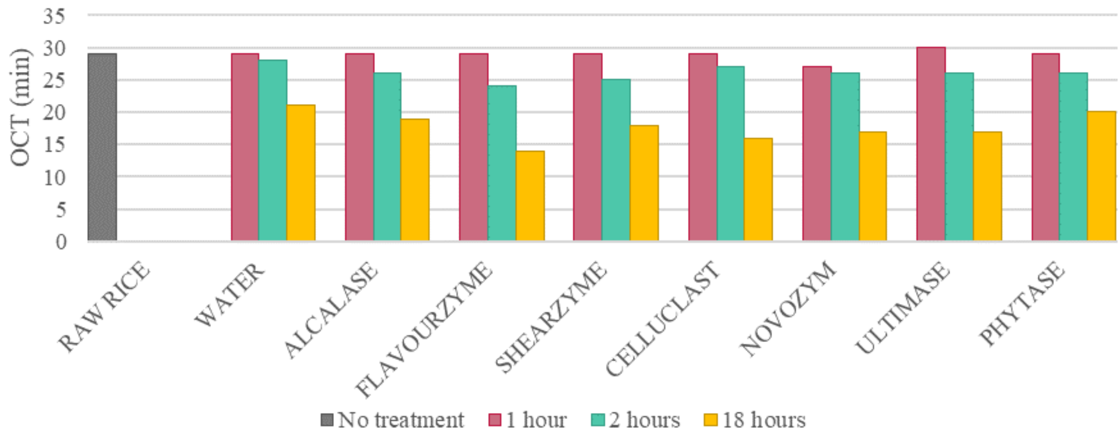



*Figure 6. Optimal cooking time of treated brown rice.*

### 3.3.2. Brown rice cooked with excess of water

Soaked rice in the presence of proteases, xylanases, cellulases and phytases were cooked to check their impact on texture. Results showed that soaking was not influenced by the pH of the conditioning solution, but by the soaking time of the grains. Rice grains (Figure 7 and Figure 8) have a maximum liquid absorption capacity that does not increase with longer soaking time. The apparent viscosity profile showed significant differences for all the samples according to the applied enzymatic treatment. In addition, the cooking time of the samples decreases considerably with long soaking times. The double compression method applied for the evaluation of the texture allowed to verify that the hardness was influenced by the enzymes and by the soaking time (Figure 9). The presence of enzymes during conditioning modifies the technological behavior and composition of cooked rice, but despite the use of enzymes with very diverse activities, the study has not allowed the selection of specific enzyme(s) that improve the technological and/or nutritional properties of cooked rice. Likely, the excess of cooking water masked the impact of previous soaking and dilute the nutrients.

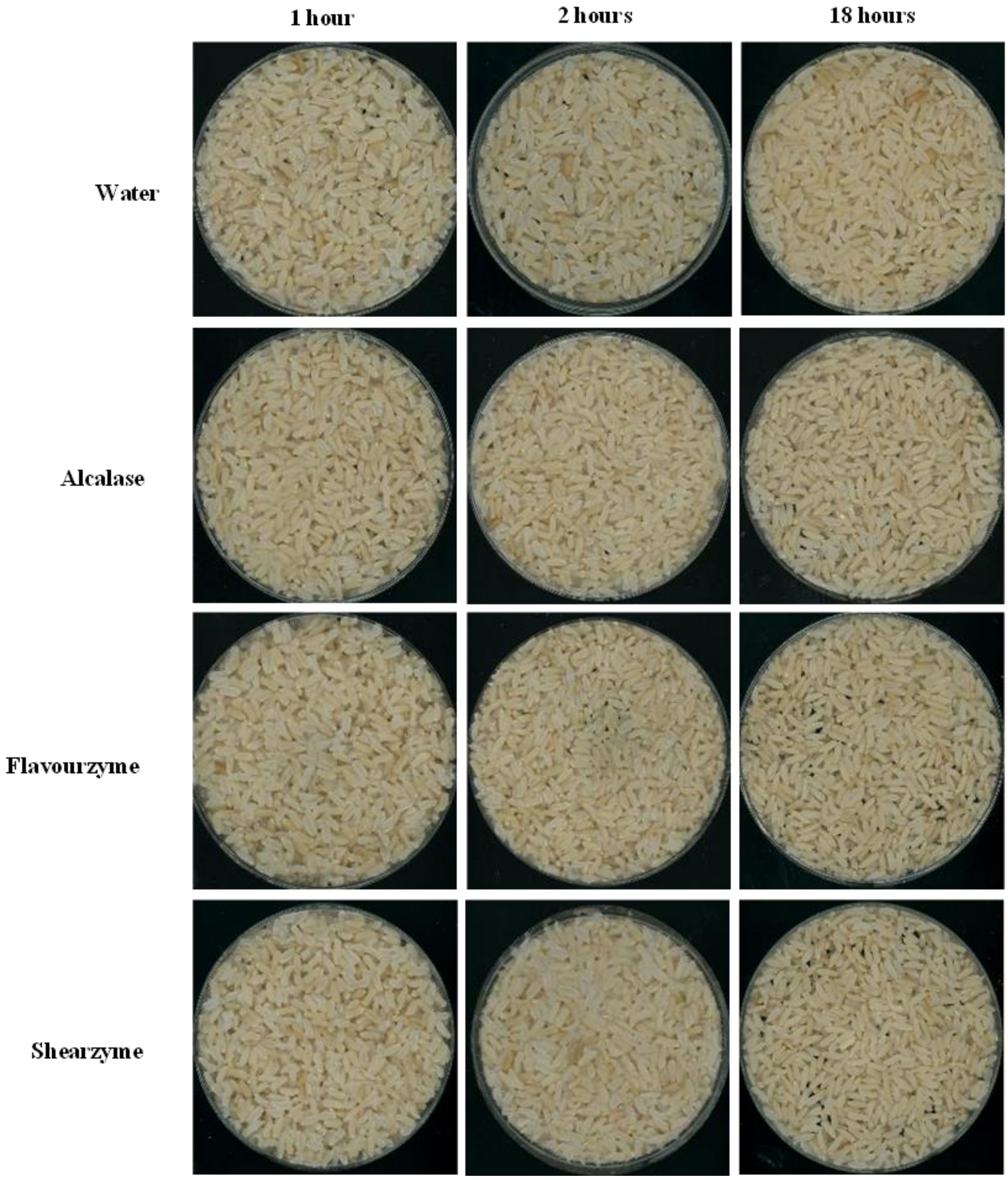



*Figure 7. Rice cooked in excess of water for the control and the samples after treatments with alcalase, flavourzyme, and shearzyme.*

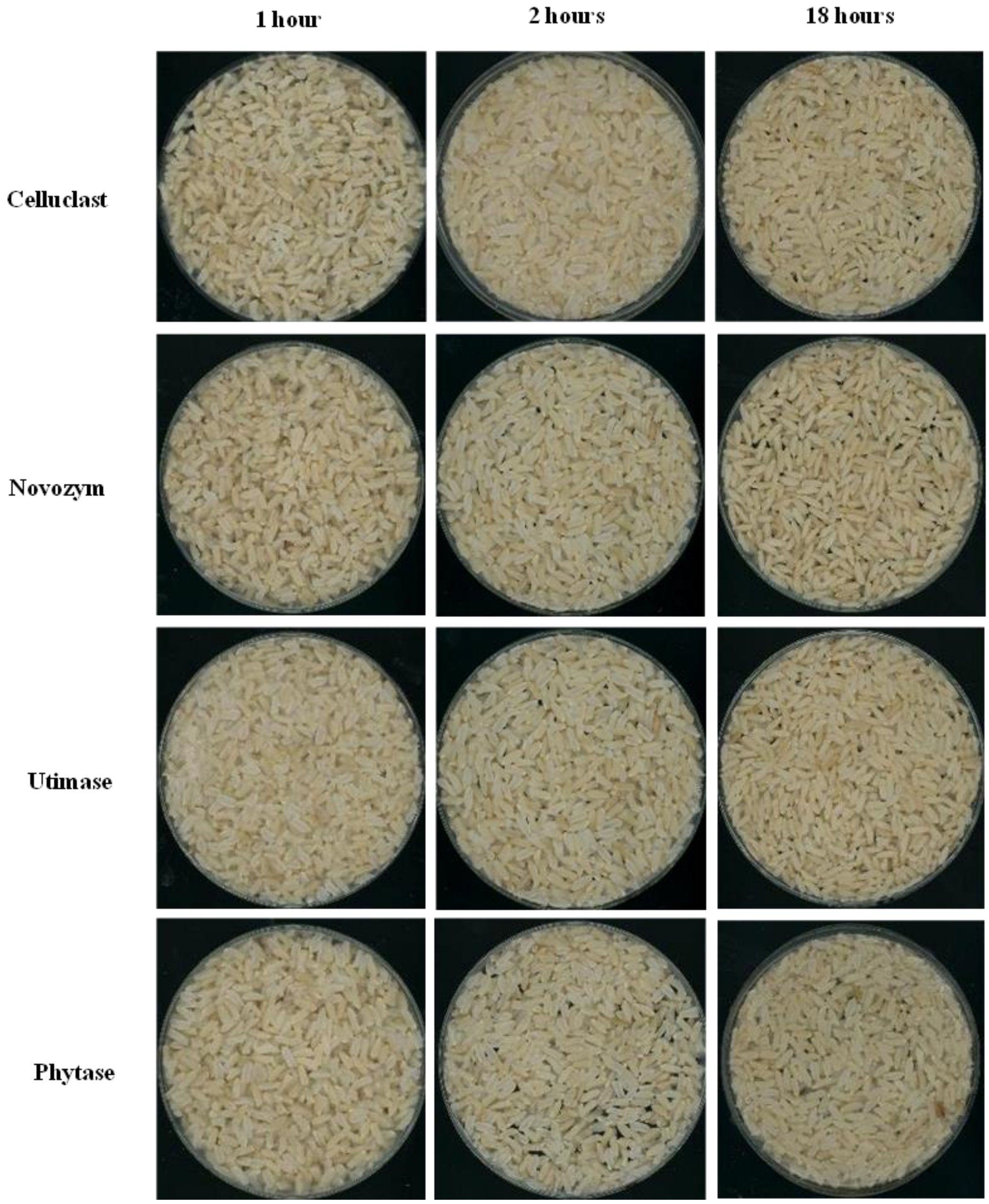
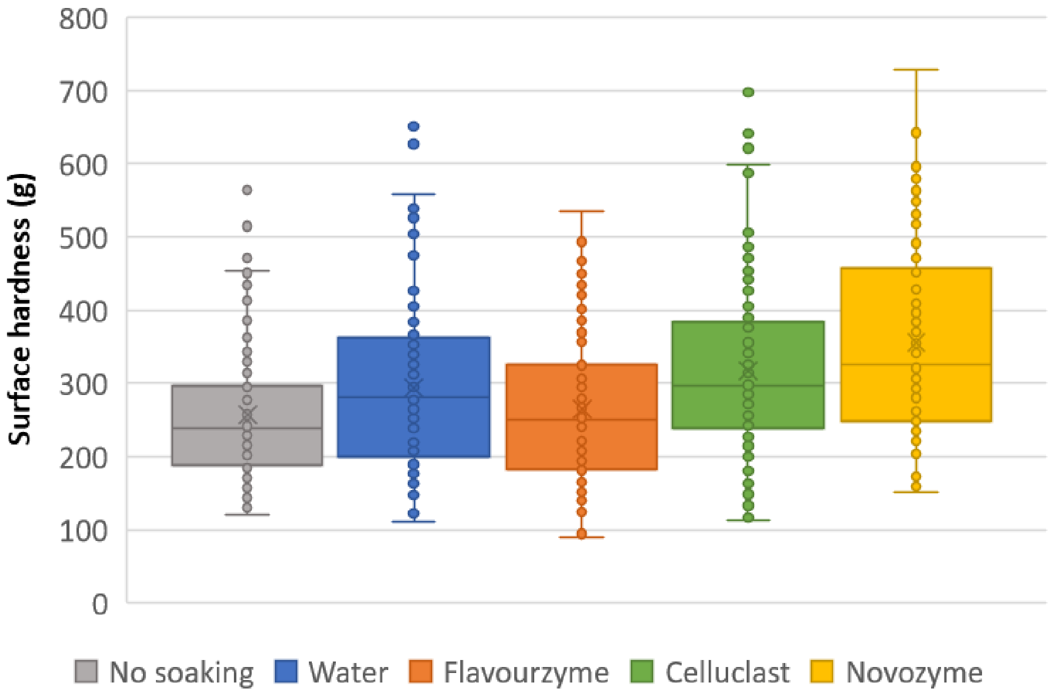



*Figure 8. Rice cooked in excess of water after treatments with celluclast, novozym, ultimase, and phytase.*


*Figure 9. Surface and general cooked grain hardness of no soaked, soaked with water or with enzyme cooked grains with excess of water.*

### 3.3.3. Brown rice cooked without excess of water (adjusted water)

A second batch of soaked rice but cooking with the optimum water was carried out. For this purpose, three enzymes were selected: flavourzyme (exoprotease), novozym (xylanase) and celluclast (celullase) to perform the soaking during 18 h. Then treated rice were cooked in an electric rice cooker using the optimum cooking time 3.3.1) depending on the enzyme used. The Figure 10 shows the surface and general texture data of cooked rice grains. The overall grain hardness was affected by the treatment. Softer cooked rice was obtained with celluclast or novozym enzymes. Composition (Figure 11) indicated that the treatment with enzymes (flavourzyme and celluclast) affected mainly proteins.



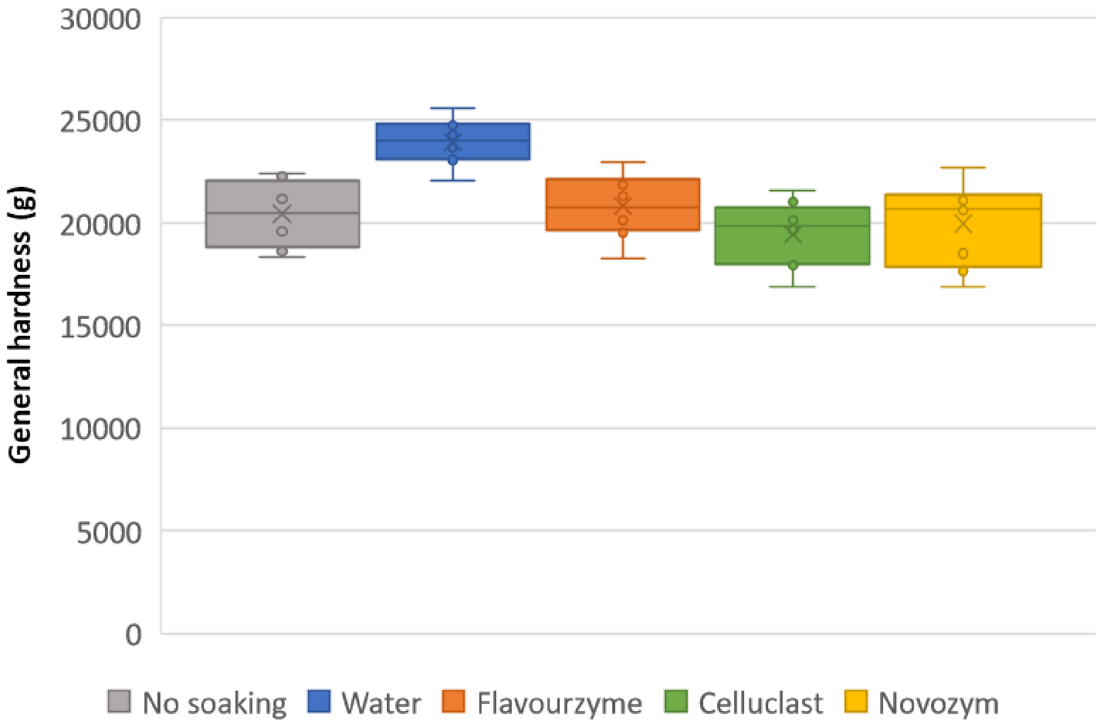




*Figure 10. Surface and general hardness of no soaked, soaked with water or with enzyme cooked grains without excess of water.*

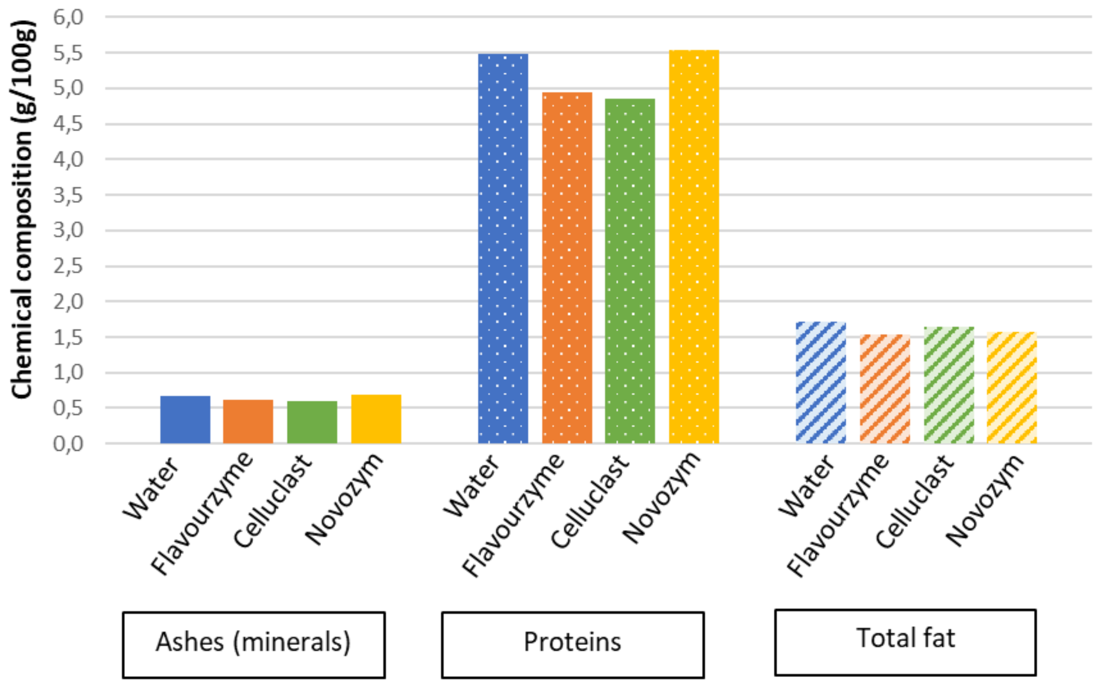



*Figure 11. Chemical composition of no soaked, soaked with water or with enzyme cooked grains without excess of water.*

# 4. List of samples selected from items 2 and 3.

The treatments performed on both sushi rice and enzymatically treated brown rice showed no significant differences in the chemical composition of macro compounds. Therefore, in collaboration with INIAV, the study of some specific components such as γ-oryzanol and the digestibility of the samples were performed.

The samples agreed to be analyzed were:

**Brown rice subjected to enzymatic treatment during soaking:**

1. Brown rice soaked for 18 h with water.
2. Brown rice soaked for 18 h with Novozym 28251 (200 mg/100 g rice in the soaking solution)
3. Brown rice soaked for 18 h with Flavourzyme (200 mg/100 g rice in the soaking solution)

**Sushi rice treated with different seasoning solutions:**

1. Sushi rice cooked and without seasonings.
2. Sushi rice treated with seasoned solution without vinegar
3. Sushi rice treated with Red wine vinegars.

## 4.1. Biocompounds and digestibility of selected rice samples

The brown rice samples had approximately 23.5 mg/100 g of gamma-oryzanol (Table 1). The amount of gamma-oryzanol depends on both the variety and the processing of the rice and can range from 8.5 to 21.5 mg/100 g (Cho et al., 2012). The resistant starch content of the samples varied as a function of enzymatic treatment or seasoning (Table 2). The highest values were obtained in the case of white rice without seasoning and brown rice soaking in water. The digestion of the samples resulted in significant differences (Table 3 and Figure 12). The slowly digestible starch (SDS) content of the novozym-treated sample was higher than that of the brown rice soaked in water. In addition, this sample reduced the estimated glycaemic index, as did the sample seasoned with red wine vinegar compared with the no treated brown rice or no seasoned white sushi rice.

Table 1. Results of gamma-Oryzanol (GO) in treated rice samples.

| Rice Sample | GO (mg/100 g) | Mean GO (mg/100 g) | Standard error | (%RSD) |
|---|---|---|---|---|
| Sushi rice treated with seasoned solid without vinegar | n.d. | n.d. | n.d. | n.d. |
| Sushi rice treated with red wine vinegar | n.d. | n.d. | n.d. | n.d. |
| Sushi rice cooked and without seasonings | n.d. | n.d. | n.d. | n.d. |
| Brown rice soaked 18h with Flavourzyme (200 mg /100 g rice) | 26.18<br>22.48<br>22.78 | 23.81 | 1.19 | 8.64 |
| Brown rice soaked 18h with Novozym (200 mg /100 g rice) | 25.91<br>22.51<br>21.09 | 23.17 | 1.43 | 10.68 |
| Brown rice soaked 18h with water | 25.57<br>22.30<br>22.79 | 23.55 | 1.02 | 7.47 |

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*Table 2. Results obtained for resistant starch in samples.*

| Rice Sample | Resistant Starch (g/100 g) | (%SD) |
|---|---|---|
| Sushi rice treated with seasoned solid without vinegar | 0.119 | 0.002 |
| Sushi rice treated with red wine vinegar | 0.064 | 0.006 |
| Sushi rice cooked and without seasonings | 0.179 | 0.032 |
| Brown rice soaked 18 h with Flavourzyme (200 mg /100 g rice) | 0.375 | 0.080 |
| Brown rice soaked 18 h with Novozym (200 mg /100 g rice) | 0.370 | 0.084 |
| Brown rice soaked 18 h with water | 0.478 | 0.013 |

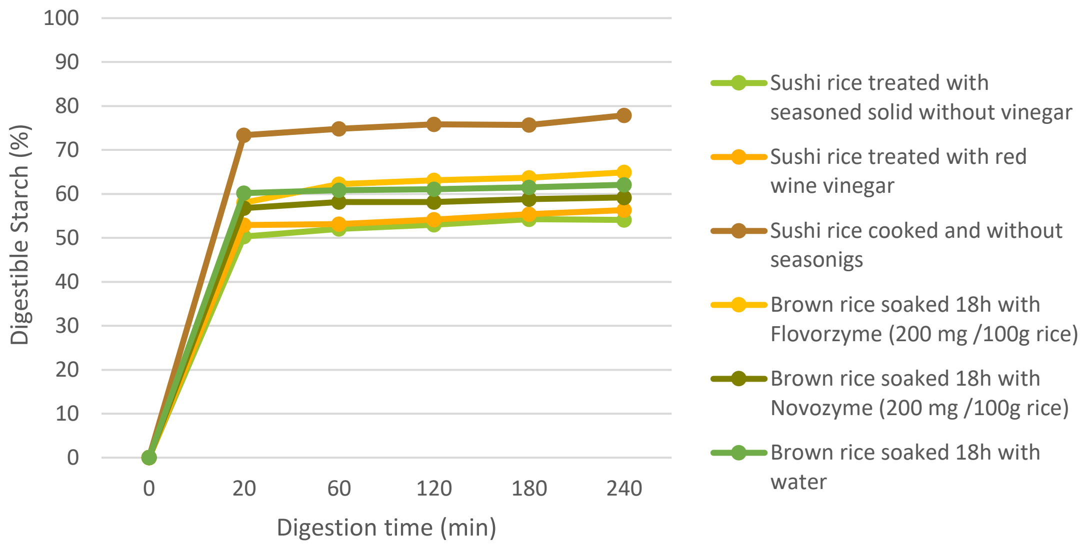


*Figure 12. Hydrolysis of starch of rice samples: starch determined at 20 min to measure rapidly digestible starch (RDS); slowly digested starch (SDS) is measure at 120 min (SDS=starch value at 120 min - starch value at 20 min); total digestible starch (TDS) and resistant starch (RS) of samples is measure at 240 min.*

*Table 3. Parameters of hydrolysis rice grains digestibility.*

| | Starch (g/100g) | | | | | Estimated Glycemic Index (GI) |
|---|---|---|---|---|---|---|
| **Sample** | **Rapidly Digestible Starch (RDS)** | **Slowly Digestible Starch (SDS)** | **Total Digestible Starch (TDS)** | **Resistant Starch (RS)** | **Total Starch (TDS+RS) (g/100 g)** | |
| Sushi rice treated with seasoned solid without vinegar | 50.31 ± 1.11 | 2.72 ± 0.08 | 54.11 ± 0.27 | 0.16 ± 0.01 | 54.27 ± 0.30 | 71.53 |
| Sushi rice treated with red wine vinegar | 52.93 ± 0.83 | 1.23 ± 0.85 | 56.33 ± 1.82 | 0.23 ± 0.015 | 56.56 ± 1.84 | 72.52 |
| Sushi rice cooked and without seasonings | 73.39 ± 1.19 | 2.45 ± 0.10 | 77.89 ± 1.38 | 0.26 ± 0.007 | 78.14 ± 3.93 | 85.30 |
| Brown rice soaked 18 h with Flavourzyme (200 mg /100 g rice) | 58.01 ± 2.00 | 5.10 ± 1.01 | 64.92 ± 0.71 | 0.43 ± 0.022 | 65.35 ± 3.45 | 77.41 |
| Brown rice soaked 18 h with Novozym (200 mg /100 g rice) | 56.81 ± 0.34 | 1.36 ± 0.14 | 59.22 ± 0.49 | 0.41 ± 0.024 | 59.63 ± 0.50 | 74.85 |
| Brown rice soaked 18 h with water | 60.18 ± 0.62 | 0.86 ± 0.44 | 62.08 ± 1.20 | 0.44 ± 0.024 | 62.52 ± 1.18 | 76.63 |

## 4.2. HPLC-DAD-electrochemical detector and LC-MS analysis of phenolic compounds

The extraction protocol for phenolic compounds[2] optimized in Task 1.3 by INIAV team is currently being applied to the six processed rice samples selected from items 2 and 3. After phenolic compounds extraction, the three obtained fractions (free, soluble conjugate, and insoluble-bound phenolic compounds) will be analyzed by high performance liquid chromatography-diode array detector-electrochemical detector (HPLC-DAD-ECD) using a well-established method for routine analysis of phenolic compounds at IBET[3] . This analysis included the quantification of ferulic acid, p-coumaric acid, and total phenolic content (TPC). For the identification of individual phenolic compounds that show a significant variation between varieties, samples will also be analyzed on a LC-MS/MS system for metabolite identification.

## 4.3. Untargeted SPME-GC-MS characterization of volatile organic compounds

Volatile organic compounds (VOCs) present in the six processed rice samples selected from items 2 and 3 were analyzed by SPME-GC-MS at IBET.

Each rice sample was weighed (0.5 g) in a 10 mL headspace vial and pre-incubated at 40 °C for 3 min. The fiber (DVB/CAR/PDMS 50/30 µm) was exposed to the headspace for 40 min at 40 °C and desorbed on the chromatograph injector for 8 min at 250 °C.

The analysis of VOCs was carried out by GC-MS, in two different conditions. (1) VOCs analysis was performed using a GC-MS equipment (QP 2010 Plus, Shimadzu, Kyoto, Japan) coupled with an autosampler AOC-5000 and a Teknokroma Sapiens 5MS column (30 m × 0.25 mm ID, film thickness 0.25 µm) and an AOC-5000 Shimadzu autosampler. Helium was used as a carrier gas, and after the spitless injection, runs were performed in a column flow at 2 mL/min, using a temperature gradient for the separation of sample components: column oven was initially maintained at 40 °C during 5 min, followed by a gradual increase of 5 °C/min until 170 °C and programmed to rise to 230 °C at the rate of 30 °C/min; at the end the temperature was kept for 4 min. (2) VOCs were also analyzed through a GC-MS QP 2010, Shimadzu coupled to an autosampler AOC-5000 Plus, and a TeknoKroma Sapiens Wax MS (60 m, 0.25 mm (i.d.), 0.25 µm column). Carrier flow was performed at 4 mL/min, with an injection mode in spitless and detector at 250 °C. The gradient temperature used was the same above mentioned. For both conditions, the ionization energy was 70 eV, a scan range of 29–300 m/z, while the detector and ionization temperatures were at 250 °C. Each sample was analyzed in triplicate.

The data acquisition was made using Shimadzu software, GC-MS solution, version 2.10 when analyzed with 5-MS column (Figure 13), while a different version (4.50 SP1) was used for the analysis performed with a Wax column. Compounds were identified by matching their mass spectra with those available in the NIST 21, 27, 107, 147, and Wiley 229 libraries, and by comparing the RIs to those reported in the literature.

[2] Shao, Y., Xu, F., Sun, X., Bao, J., & Beta, T.(2014). Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). Journal of Cereal Science, 59, 211- 218. DOI: 10.1016/j.jcs.2014.01.004

[3] Oliveira-Alves, S. C., Vendramini-Costa, D. B., Betim Cazarin, C. B., Maróstica Júnior, M. R., Borges Ferreira, J. P., Silva, A. B., Prado, M. A., & Bronze, M. R. (2017). Characterization of phenolic compounds in chia (Salvia hispanica L.) seeds, fiber flour and oil. Food chemistry, 232, 295–305. DOI: 10.1016/j.foodchem.2017.04.002

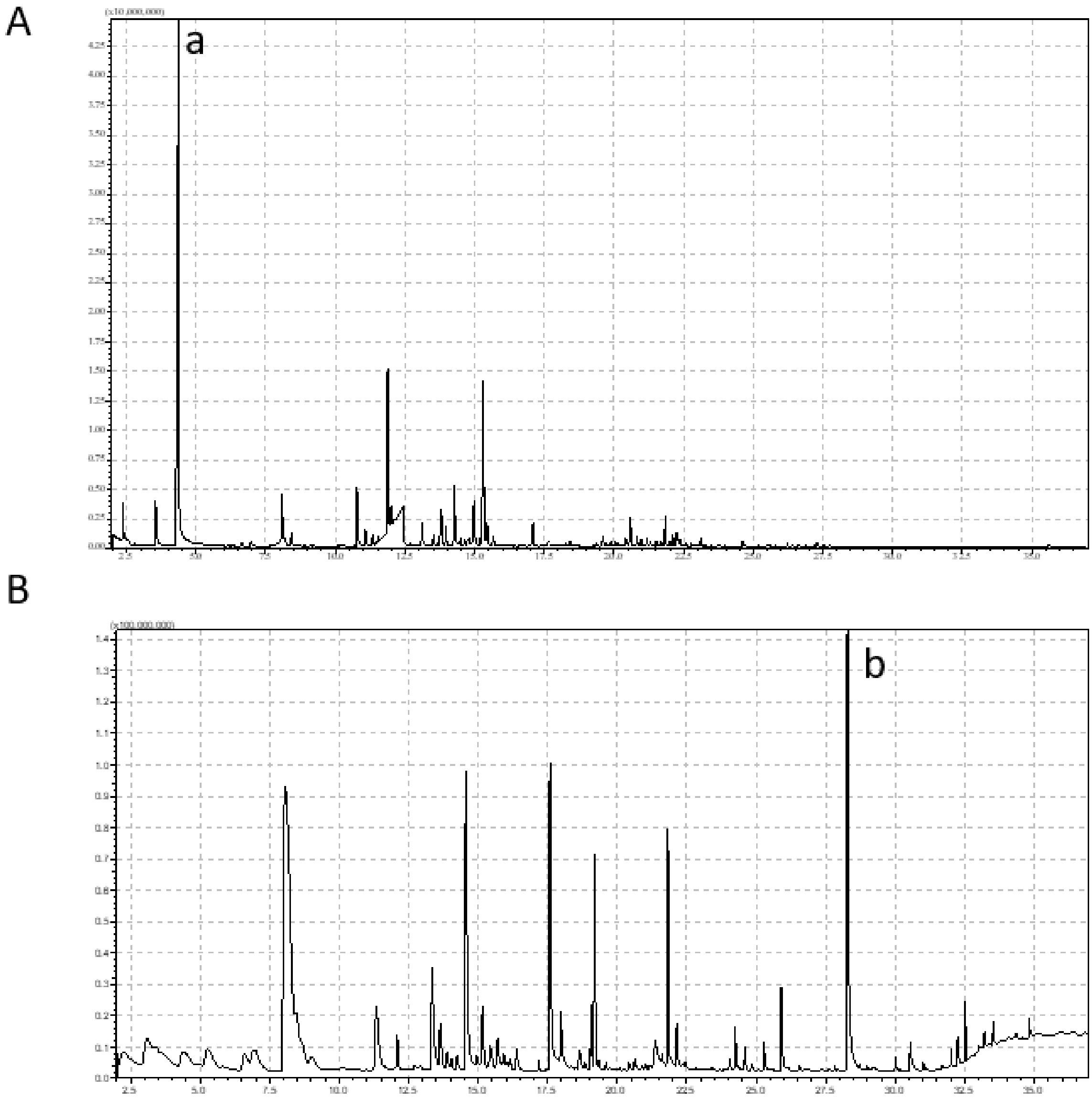


*Figure 13 - Chromatographic separation of the volatile organic compounds in sushi rice cooked without seasoning using a Teknokroma Sapiens-5MS (A) and a TeknoKroma Sapiens Wax MS (B) GC capillary column. a – hexanal; b – octanoic acid methyl ester*

All cooked sushi rice and brown rice samples were analyzed (in triplicate) using both GC capillary columns. Data analysis is currently being conducted. The major classes identified in these rice samples include alcohols, aldehydes (e.g., hexanal the major VOC identified in Figure 13A and associated with a ”green” aroma), alkanes, ketones, organic acids (e.g., octanoic acid methyl ester as the major VOC putatively identified in Figure 13B) and olefins.

### 4.4. Collection of spectral data using Fourier-transform infrared spectroscopy (FTIR)

Spectral data of the selected samples from items 2 and 3 were collected at IBET using a Thermo Scientific FTIR spectrometer (San Jose, USA) Class 1 Laser Product Nicolet 6100 using an ATR accessory with a diamond crystal. The acquisition of the spectra was performed using the software OMNIC version 7.3 (Thermo Electron Corporation). The background spectrum of the air was collected before each sample spectrum acquisition. The crystal was cleaned using water and acetone and dried with a soft tissue. For the sample spectrum acquisition, the different flours were placed in the ATR crystal and the spectra were recorded with 32 scans between 4000–650 $cm^{-1}$ and with a resolution of 4 $cm^{-1}$. Recordings were performed in six replicates. Spectral data will be added to the spectral database collected in TRACE-RICE WP1.

## 5. Conclusions

Sushi seasoning made with different vinegars affected the color, texture and reduced the extent of starch hydrolysis in cooked white rice. Its application is an alternative to obtain rice with different technological and nutritional characteristics.

It was confirmed that soaking brown rice grains at 50ºC reduces the optimum cooking time and increases the hardness of the cooked grains. The presence of enzymes during conditioning modifies the technological behavior and composition of cooked rice, but despite the use of enzymes with very diverse activities, the study has not allowed the selection of the enzyme(s) that improve the technological and/or nutritional properties of cooked rice.

Studies conducted by INIAV confirmed the reduction of the glycemic index in both sushi rice and brown rice obtained after soaking with enzymes. They are clean alternatives to modulate the hydrolysis of starch in these products.

Studies currently being conducted by IBET will provide additional information of the changes in the chemical profile of processed sushi rice and brown rice.

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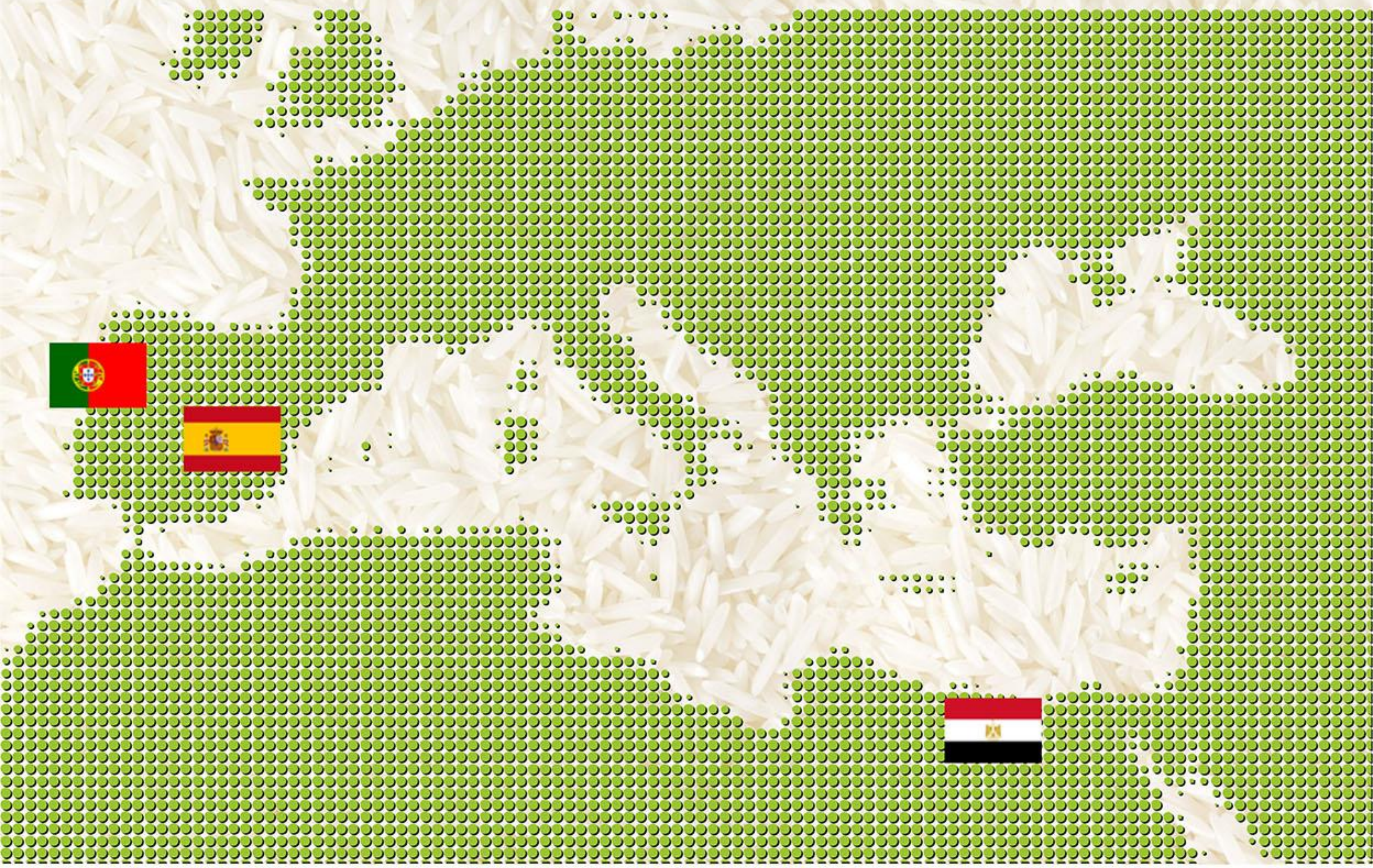
Trace Rice



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