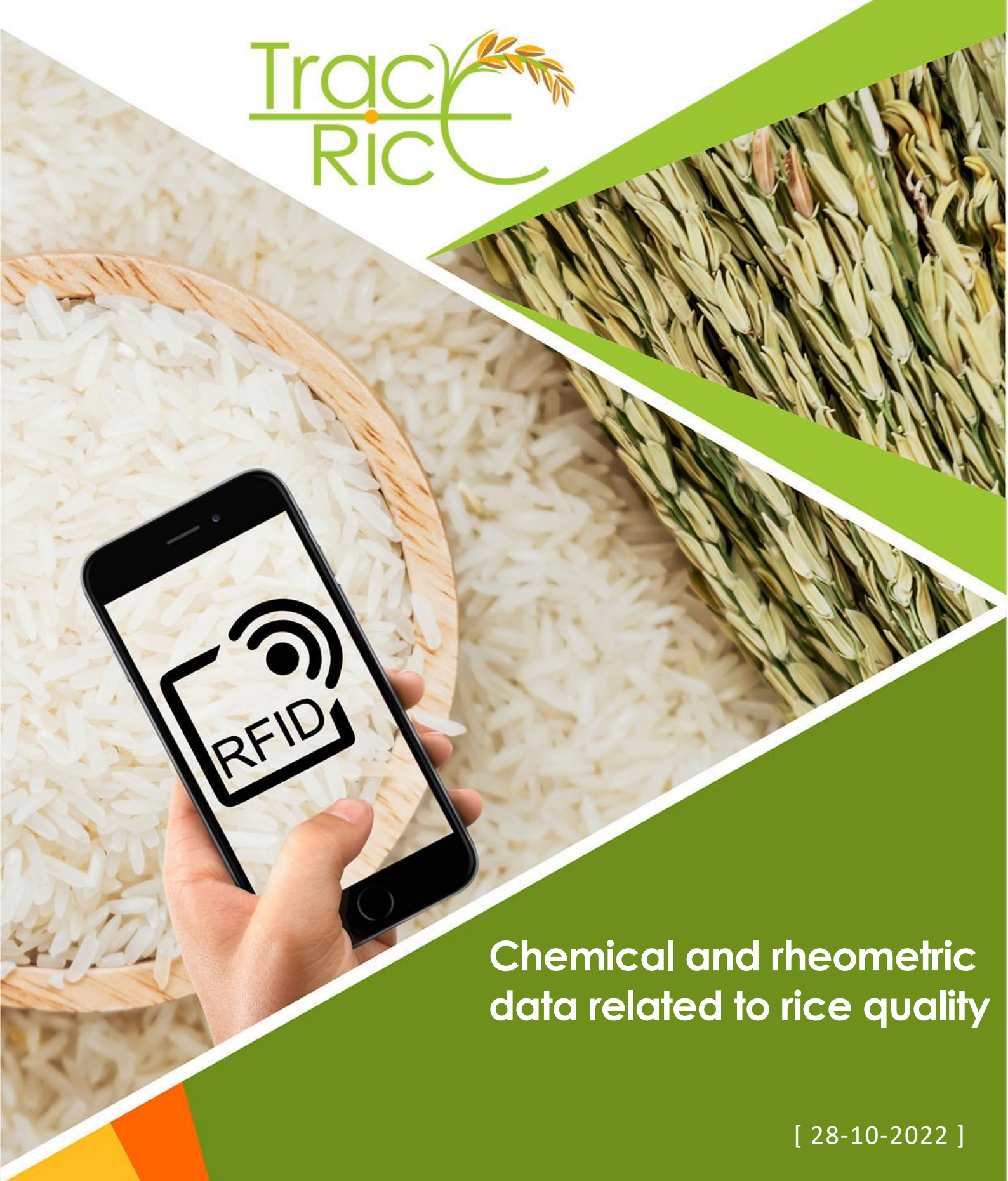

Chemical and rheometric data related to rice quality

[28-10-2022]

TRACE-RICE with Grant nº 1934, is part of the PRIMA Programme supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation

PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

http://trace-rice.eu

Project Acronym: TRACE-RICE

Project Coordinator: Carla Moita Brites

Project Duration: September 2020 – August 2024 (48 months)

Deliverable No.: D1.4

Dissemination level: Confidential

Work Package: WP1 - TEST SOLUTIONS FOR AUTHENTICITY, ORIGIN AND TRACEABILITY

Task: Task 1.3 – EVALUATION OF QUALITY: CHEMICAL RHEOMETRIC, SENSORY ANALYSIS AND COLLECTION OF SPECTRAL DATA

Lead beneficiary: IBET

Contributing beneficiaries: INIAV

Due date of deliverable: 30 August 2022

Actual submission date: 28 October 2022

Written by: Ana Margarida Rodrigues, Cristiana Pereira, supervised by MR Bronze

HISTORY OF CHANGES			
Date	Beneficiary	Version	Change
30-08-2022	IBET	-	Task 1.3 Leader informs the Project Coordinator that due to COVID pandemics problems, D1.4 is delayed
20-10-2022	IBET	1	Version 1 of D1.4 sent to Project Coordinator
27-10-2022	INIAV	2	Revised version of D1.3 (v2) including INIAV activities
28-10-2022	INIAV	3	Final version approved by project coordinator

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1. Purpose

The main goal of Task 1.3 is to use instrumental analysis to characterize the quality of rice grains, namely to identify compounds responsible for the aroma, flavour and taste of rice.

Analysis include:

- Collect and harmonize data of well-established quality parameters
- Characterization of volatile organic compounds (VOCs) using solid-phase microextraction followed by gas chromatography-mass spectrometry (SPME-GC-MS)
- Characterization of compounds responsible for sweet taste by high performance liquid chromatography (HPLC)
- Characterization of compounds responsible for bitter taste using spectrophotometry (saponins and phenolic saponins), GC-MS (benzaldehyde quantification), and LC-MS (lipid deterioration products and phenolic compounds).

Spectral data collection using Fourier-transform infrared spectroscopy (FTIR) will be used to build models for the prediction of different parameters related to rice quality, safety and authenticity.

The data produced in this task will be correlated with the genetic data and results obtained from the sensory panels.

2. Rice well-established quality parameters

The rice quality evaluation is based on determinations of basic composition, amylose content, viscosity profiles, texture and sensory analysis following CEN/TC338 and ISO TC34/SC4 standards. The basic chemical composition of rice flours namely protein, lipid, fibre and starch contents were determined by NIR transflection MPA equipment (Bruker Optics, Germany Bruker) with the cereals B-FING package calibration provided by Bruker Company (Massachusetts, USA). For amylose content determinations were according EN ISO 6647-2:2020 and texture analysis based on ISO 11747 (Determination of rice kernel resistance to extrusion after cooking) and on ISO 14864 (Rice - Evaluation of gelatinization time of kernels during cooking). Viscosity profiles were assessed using appropriate viscosimeter and NWP-new work proposal will be performed. Gelatinization and pasting profiles of white and brown rice flours were determined through a rapid visco analyzer (RVA, Newport Scientific, Warriewood, Australia), with the software Thermocline for Windows (TCW) according to AACC method 76-21. Peak, minimum (through) maximum and final viscosities, were obtained through curve resulting from a cycle of heating, cooling and stirring a suspension of flour in water. For viscosity analyses, inter-laboratories tests with 7 laboratories were performed for validating and harmonizing the protocols (see attached reports). The variability of the physicochemical properties of the 22 selected rice varieties were evaluated. The values obtained in white and brown rice flours for these parameters from different varieties are presented in tables 1 and 2.

Table 1 - Basic composition of rice flours

Rice Samples	Starch (%)	Protein (%)	Lipids (%)	Fibre (%)	Ash (%)
White rice flour	73.63 - 76.87	6.33 - 8.35	0.68 - 1.29	-	0.34 - 0.81
Brown rice flour	66.36 - 70.61	6.88 - 9.33	2.46 -3.51	0.96 - 2.35	0.82 -1.41

Table 2 - Physicochemical rice flour parameters

Rice Samples	Amylose (%)	Gelatinization Temp. (ºC)	Peak1 (cP)	Trough1 (cP)	Breakdown (cP)	Finalvis (cP)	Setback1 (cP)	PeakTime (cP)	Pasting Temp. (ºC)	Setback2 (cP)
White rice flour	16.43 - 31.09	60.12- 73.35	1776-4375	1160-2300	198-2146	2749-4198	- 1125 - 1834	5.49 - 6.42	68.27 - 90.10	1018 - 2563
Brown rice flour	8.85 - 19.21	61.80 -74.30	1308-2814	948-1513	108-1376	2174-4385	68 -2630	5.67- 6.33	87.40 - 91.55	1095 - 3216

It is possible to differentiate the amounts of nutrients in the different fractions (bran, white flour, and brown rice flour), statistical analysis still undergoing for identification of groups of varieties with different quality characteristics.

Concerning the sensory analysis the development of an adequate lexicon for Portuguese vocabulary was performed for training a panel of 12 expert rice sensory assessors.

3. Untargeted SPME-GC-MS characterization of volatile compounds

Volatile organic compounds (VOCs) present in cooked rice were analysed by SPME-GC-MS. These compounds are often responsible for flavour and aromatic traits of rice varieties.

For the characterization of VOCs in rice samples, brown and white rice grains of 22 varieties were used (44 samples in total). Preliminary tests were carried out to access the best conditions for sample preparation and maximize the extraction of VOCs. These included the selection of most suitable cooking method (pan, headspace vial), the rice:water (w/v) ratio (1:2, 1:1 and 2:1, w/v) and incubation temperature (40 °C, 90 °C).

After optimization, the following conditions were used: rice sample was weighed (0.5 g) in a 20 mL headspace vial and 1 mL of Ultra-pure water was added. Each vial was pre-incubated at 90 °C for 30 min, allowing the rice to cook and avoiding the loss of VOCs that occurred in a traditional cooking method (pan). The fiber (DVB/CAR/PDMS 50/30 µm) was exposed to the headspace for 15 min at 90 °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 splitless 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 splitless 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 analysed in triplicate.

The data acquisition (Figure 1) was made using Shimadzu software GC-MS solution (version 2.10) when analysed with 5-MS column, 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.

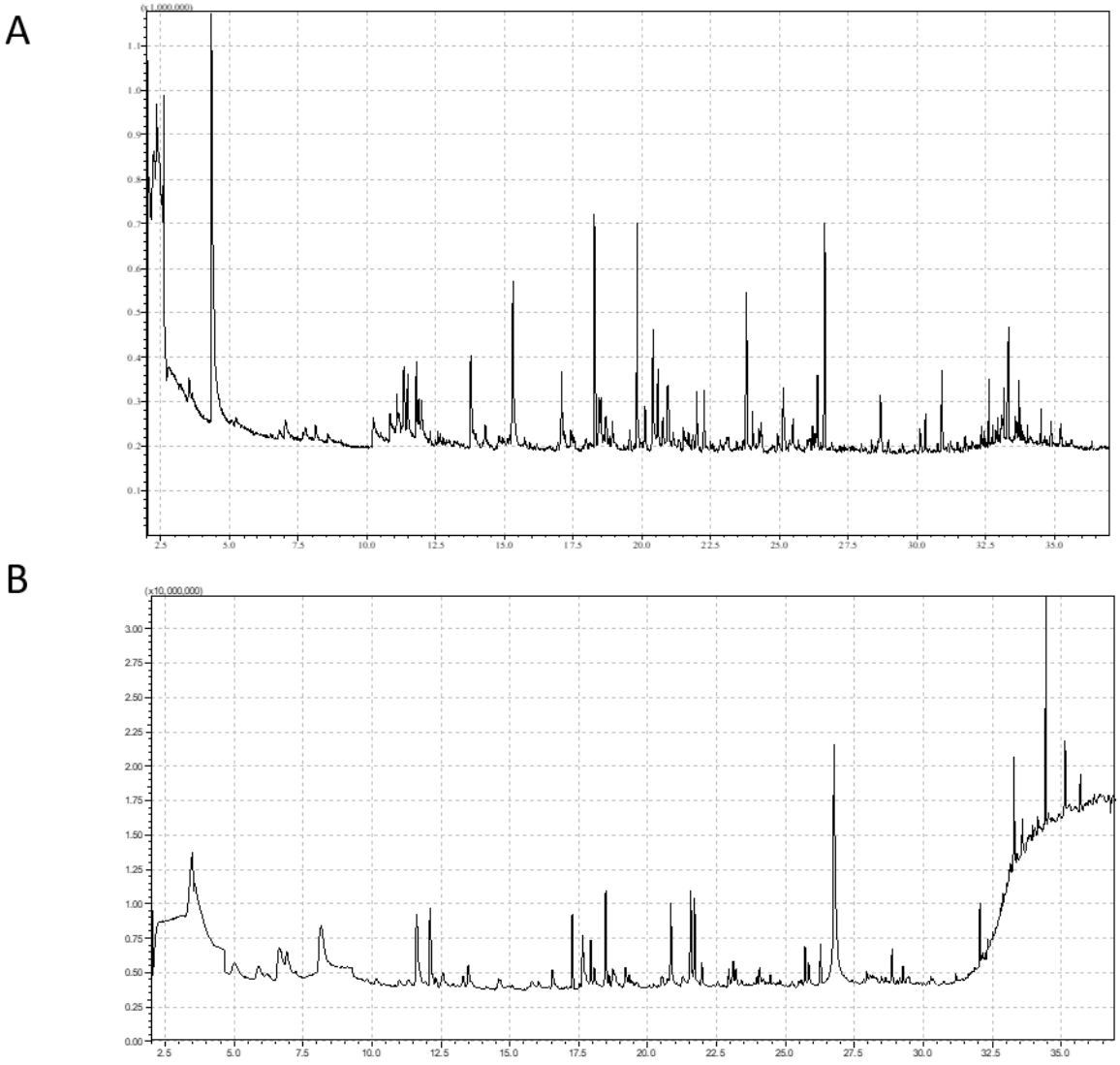

Figure 1 - Chromatographic separation of the volatile organic compounds in cooked white rice sample (Giza 177) using a Teknokroma Sapiens-5MS GC (A), and Teknokroma Sapiens-WAX.MS (B) capillary column.

Brown and white rice samples of the selected 22 rice varieties are currently being analysed as previously described. To the date of the preparation of this report all rice samples were analysed (in triplicate) using both GC capillary columns. Up to 80 VOCs have been identified in cooked rice samples including alcohols, aldehydes (e.g., hexanal as one of the major VOC identified in all samples and associated with a ”green” aroma), alkanes, ketones, and olefins. Data treatment is still undergoing.

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

In order to address objective 5 of TRACE-RICE project, which is to build predictive mathematical models that can be applied to speedily differentiate rice varieties and evaluate significant chemical parameters for rice quality, spectral data was collected using Fourier-transform infrared spectroscopy (FTIR). FTIR is a rapid and cost-effective method that requires minimal or no sample preparation. In this task spectral data from raw and cooked rice samples will be used to build a spectral database of brown and white rice samples of the 22 selected varieties. Briefly, rice samples were milled by bead-beating at 25 Hz for 60 s using a mixer mill (Retsch Mixer Mill MM 400) with stainless steel beads. Cooked rice samples (cooked in a 20 mL headspace vial as previously described for SPME-GC-MS analysis) were also homogenized by bead-beating at 25 Hz for 60 s.

Spectral data was collected 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} (Figure 2). Recordings were performed in six replicates. Figure 2 highlights the differences between Lusitano white rice, brown rice and cooked white rice samples.

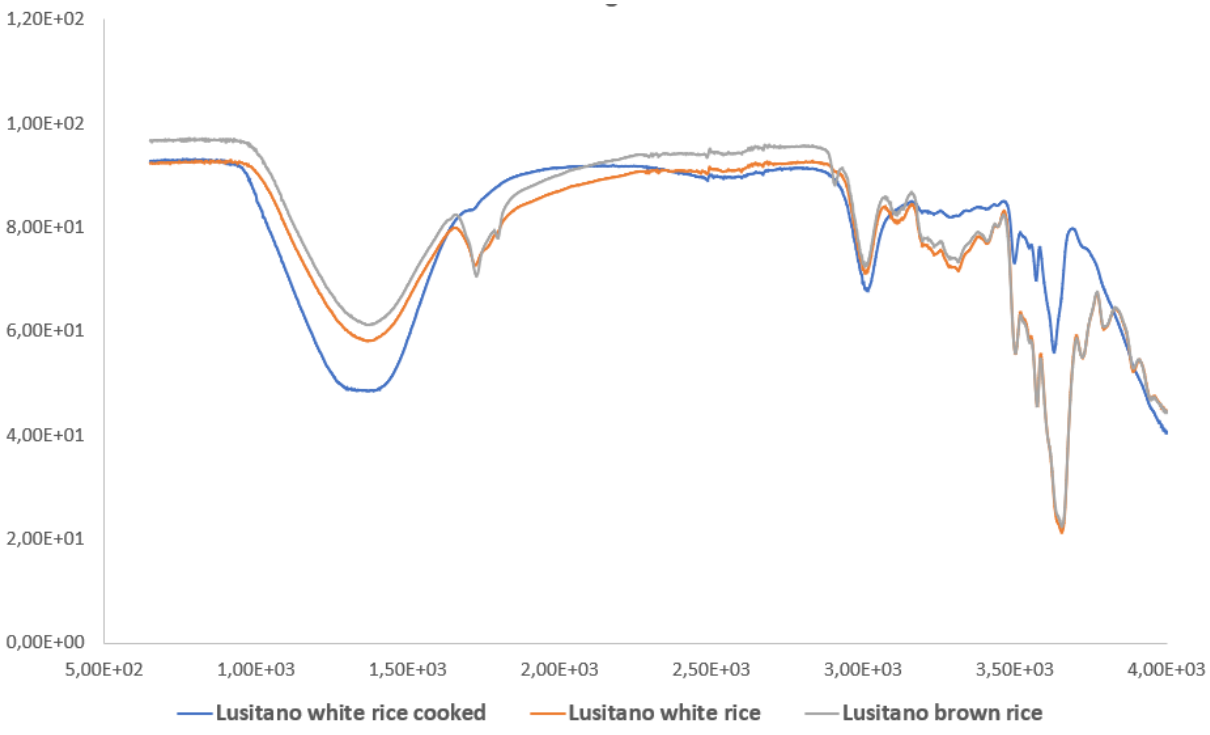

Figure 2 - FTIR absorption spectra of Lusitano white rice (blue), brown rice (orange) and cooked white rice (gray) samples.

To the date of the preparation of this report 22 samples of rice flour and 5 samples of cooked rice were analysed (in sixplicate). Data treatment is still undergoing.

5. Collection of spectral data using near-infrared spectroscopy (NIR)

The 22 selected rice varieties (brown and white raw rice flours) containing approximately 25 cm^3 of rice flour were loaded in a circular sample cup and pressed slightly to obtain a similar packing density. Sample spectra were collected using an NIR transflection MPA equipment (Bruker Optics, Germany). For each rice sample, a successive scans were performed, over a wavenumber range (12,000–4000 cm^{-1}), at 16cm^{-1} of resolution. For each rice sample, two spectra were obtained to spectral database of different varieties and being controlled by the OPUS software.

The data processing is still undergoing with chemometric techniques (PLS and back-propagation ANN algorithms) with the main objective of developing suitable and robust models for evaluation and prediction of rice quality parameters using NIR spectra.

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

For the analysis of phenolic compounds, including the quantification of ferulic acid, p-coumaric acid, and total phenolic content (TPC), two extraction protocols were evaluated. The first extraction protocol[1] allowed to obtain three fractions (free, soluble conjugate, and insoluble-bound phenolic compounds) and the second extraction protocol[2] two fractions (free and soluble conjugated phenolic compounds). Extractions were performed by TRACE-RICE partner INIAV and as part as a doctoral research project on the valorization of rice byproducts. Due to the high content of phenolic compounds, brown rice flour and bran were used for the optimization of the extraction protocol. The obtained fractions were analysed 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] with minor modifications.

The HPLC system used was a Thermo Vanquish UHPLC system (Thermo Scientific, San Jose, USA), equipped with an autosampler, pump, photodiode-array detector (PDA), and electrochemical detector (ED) (Dionex Ultimate 3000 RS Electrochemical Detector, (Dionex, Sunnyvale, CA, USA). Chromatographic separation of compounds was carried out on Luna 5 um C18 100A (250 x 4 mm; Phenomenex) column. The Dionex® ED performed signal measurements by integrated voltammetry at potentials between -1.0 V and 1.0 V with a scan time of 1.00 s. The obtained results were acquired at a frequency of 50 Hz using an analogue/digital converter. The photodiode array detector was programmed for scanning between 192 and 680 nm at a speed of 1 Hz with a bandwidth of 5 nm. The detection was monitored using three individual channels, 280, 320, 360 and 520 nm. The injection volume was 10.00 µL and total time of analysis was 65 min. A binary gradient elution (A- 0.5% formic acid in Milli-Q® Water 95% and B - 0.5% formic acid in acetonitrile 90% and 9.5% Milli-Q® Water) was as follows 0–5 min, 94.4% A; 5–15 min, 94.4-80% A; 15-22 min, 80-60% A; 22-32 min, 60%; 32-45 min, 60-0% A; 45-50 min, 0% A; 50-55 min, 0-94.4% A; 55-65 min, 94.4% A (initial conditions). The flow rate was systematically controlled and set at 0.6 mL/min. Ferulic acid and p-coumaric acid were monitored at 320 nm (Figure 3) and phenolic compounds in general at 280 nm. The data acquisition system was

[1] Shao Y et al. (2014) J Cereal Sci 59: 211- 218
[2] Sumczynski D et al. (2017) Food Chem 218: 107–115
[3] Oliveira-Alves SC et al. (2017) Food Chem 232: 295-305

the Chromquest version 7.2.10 (Thermo Scientific, Chromeleon, Chromatography Data System — Surveyor, San Jose, CA, USA).

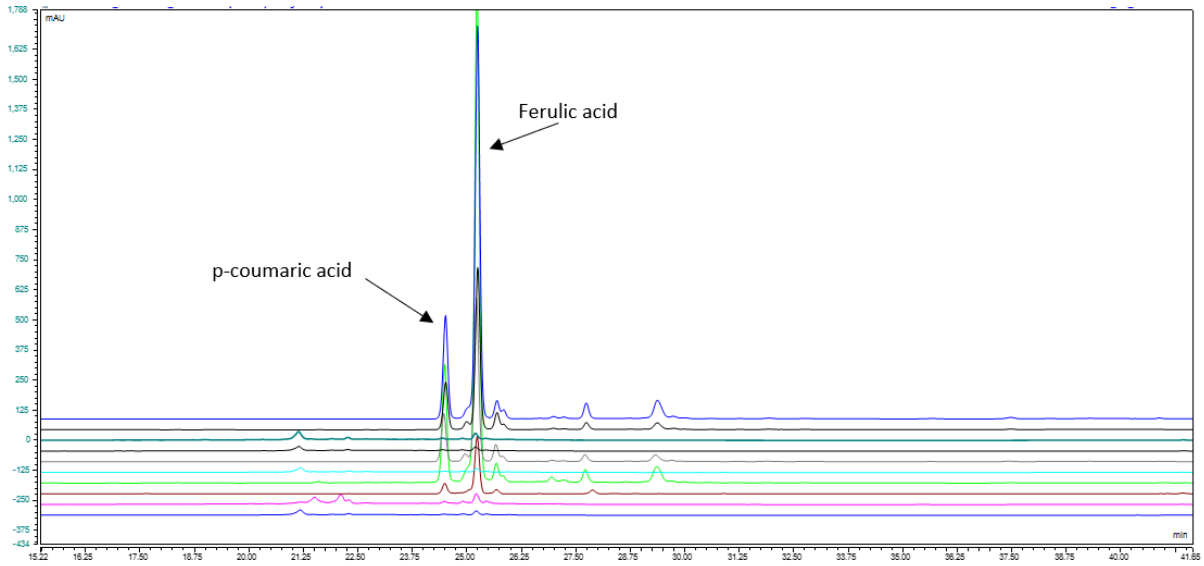

Figure 3 - Chromatographic separation of p-coumaric and ferulic acid (at 320 nm) in the different fractions obtained during extraction optimization using brown rice bran test sample.

Overall, the first protocol allowed to extract higher amounts of phenolic compounds (up to 2-fold) and was selected for the evaluation of phenolic compounds in the 22 varieties of brown rice (currently ongoing). Also, the first protocol allows to collect soluble conjugated phenolic compounds which offers an advantage over the second protocol. For the identification of individual phenolic compounds that show a significant variation between varieties, samples will also be analysed on a LC-MS/MS system for metabolite identification.

7. Other chemical analysis

The colorimetric method for the analysis of saponins and phenolic saponins is currently being optimized. This method is based on a vanillin-sulphuric acid assay to determine total saponin content on plant material[4]. Readings will be performed at 540 nm using a Ultrospec 3000 (UV/Vis Spectrophotometer.

Other chemical analyses include the quantification of benzaldehyde by GC-MS and lipid deterioration products by LC-MS, on the 22 rice varieties. Protocol optimization for these analyses will be conducted in the upcoming weeks.

[4] Lee AV et al. (2018) Technologies 6: 84

TRACE-RICE

TRACE-RICE Consortium

Instituto Nacional de Investigação Agrária e Veterinária, I.P.

iBET Instituto de Biologia Experimental e Tecnológica

ITQB Universidade Nova de Lisboa

Casa do Arroz

ernesto morgad desde 1920

Mater Dynamics

BGI www.bgi.pt

Grupo Desarrollo

iata Instituto de Agroquímica y Tecnología de Alimentos

Alexandria University