



List of bioaccessible compounds

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Project Coordinator: Carla Moita Brites

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Deliverable No.: D1.5 List of bioaccessible compounds

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

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Written by: Ana Margarida Rodrigues, Maria do Rosário Bronze, Cristiana Pereira, Manuela Lageiro



HISTORY OF CHANGES			
Date	Beneficiary	Version	Change
30-11-2021 30-07-2022	IBET	-	Task 1.3 Leader informs the Project Coordinator that due to COVID pandemics and difficulties in RH retention/recruitment, D1.5 is delayed
24-10-2022	INIAV	1	Version 1 of D1.5 sent to INIAV
28-10-2022	IBET	2	Revised version of D1.5 (v2)
29-10-2022	INIAV	3	Final Version approved by project coordinator

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1. Purpose

The main goal of Task 1.3 is to use chemical analysis to characterize the quality of rice grains, namely to identify compounds responsible for the sensory characteristics as aroma, flavour and taste of rice. As rice is an important source of bioactive compounds that are responsible for antioxidant, anticancer, antidiabetic, and antiinflammation activity¹. Those bioactive compounds include different families of compounds as phenolic compounds namely flavonoids and cinnamic acids as coumaric acid, also carotenoids, phytosterols, lipids as γ -oryzanol, and phytic acid. Higher levels of these compounds are found mostly in the outer layer of brown rice grains (bran/germ fraction). γ -Oryzanol, phytic acid and γ -aminobutyric acid were selected for quantification in selected rice varieties.

Although it is important to determine the presence of all these compounds present in the rice varieties under study, it is crucial to assess if these compounds are indeed absorbed after ingestion and can be metabolized to effectively reach the target organs without undergoing degradation. To achieve this goal, *in vitro* digestion assays will be performed using cooked rice samples, as prepared for the human sensory panel evaluation at TRACE-RICE partner INIAV.

2. γ -Oryzanol, phytic acid and γ -aminobutyric acid

The importance of valorization of brown rice is due to the higher nutritional value of the bran and their impact on the pathological mechanisms associated with diabetes^{2,3}. It is also aimed to discuss the value of selected rice bran compounds, namely γ -oryzanol, phytic acid and γ -aminobutyric acid, and their quantification by HPLC methods, to provide important insights into the nutritional quality of different rice varieties.

γ -Oryzanol

γ -Oryzanol is found mainly in the lipid portion of the rice bran at a concentration ranging from 59.4 to 912.0 mg/100 g, depending on factors such as the extraction method and rice bran origin. Almost 95% total γ -oryzanol content is essentially composed of four principal ferulate compounds: 24-methylenecycloartanyl ferulate, cycloartenyl ferulate, campesterol ferulate, and β -sitosterol⁴. The γ -oryzanol activity related to diabetes is associated with its antioxidant and hypocholesteolemic properties⁵. The latter comes from its ability to inhibit the hydrolytic function of the enzyme cholesterol esterase (CEase), essential to produce free cholesterol in the lumen. As for its potential antioxidant activity, the component of γ -oryzanol standing out with the greatest antioxidant activity *in vitro* is 24-methylenecycloartanyl, due to the presence of a hydroxyl group on the phenolic ring⁶. The administration of γ -oryzanol reduces blood glucose levels as well as the symptoms of diabetic neuropathy in diabetic rats⁷. According to Kobayashi et al.⁸, γ -Oryzanol can be absorbed in its form or it gives rise to ferulic acid and phytosterols metabolites.

¹ Verma DK & Srivastav PP (2020) Trends Food Sci Technol 97: 355-365

² Mohan V et al (2014) Diabetes Technol Ther 16: 317-325

³ Panlasigui L & Thompson L (2006) J Food Sci. Nutr 57: 151-158

⁴ Xu Z et al. (2001) J Agric Food Chem 49: 2077-2081

⁵ Rungratanawanich W et al. (2020) In: Academic Press, Cambridge, MA, USA, 201-208

⁶ Huang CJ (2003) University and Agricultural and Mechanical College, LA, USA, 1-159.

⁷ Ghatak SB & Panchal SS (2012) J Pharmacogn 22: 1092-1103.

⁸ Kobayashi E et al. (2019) Nutrients 11: 104.

Phytic acid

Phytic acid (PA), also known as phosphorus phytate or *myo*-inositol-1,2,3,4,5,6- hexaphosphate, is an organic acid essentially found in seeds and cereal brans that represents about 65 to 73% of the total phosphorus content in rice. In rice grain, PA is present in the bran⁹. As for its bioavailability, it has been demonstrated that, after ingestion, up to about 66% of PA is degraded and absorbed in the stomach and intestine¹⁰. PA dephosphorylation is metabolized to various inositol phosphate derivatives, which are more easily absorbed¹⁰. Despite being considered an anti-nutrient, due to the formation of complexes (with calcium and magnesium) during the digestion process, PA has been studied for its hypolipidemic and antioxidant effects, the latter mainly through the suppression of oxidative reactions catalyzed by Fe⁹. PA benefits for diabetes are related to the prevention of hyperglycemia through the reduction of starch digestibility. In fact, animal studies show the reduction of blood glucose when PA is added to diet. It is also thought that PA reduces starch digestion rate, binding to the salivary and pancreatic amylase enzyme through phosphate bonds or by binding with minerals that catalyze the activity of amylase, such as calcium, restricting its activity^{11 12 13}.

γ-Aminobutyric acid (GABA)

γ-Aminobutyric acid (GABA) is produced by the decarboxylation of L-glutamic acid by glutamate decarboxylase during the germination process of whole rice. Rice bran contains a large amount of glutamic acid, allowing the use of this by-product in the synthesis of GABA by fermentation. Thus, increased levels of GABA can be obtained by reinforcing the germination status of the grain¹⁴. The amount of GABA in rice bran ranges from 10.7 - 58.0 mg/100 g (before germination) and 90.0–350.0 mg/100 g (after 10–12 h of rice germination)¹⁵. GABA is also a neurotransmitter found in the central nervous system with two important functions: a tranquilizer and a hypotensive. Protective actions of GABA have been associated with the prevention of Alzheimer's disease, reduction of blood pressure, and prevention of diabetes¹⁶. As for its antidiabetic properties, GABA is known to control insulin secretion by pancreatic β-cells through glucagon inhibition, thereby contributing to the increase of β-cell mass and insulin production¹⁷. Overall, studies have shown that regular consumption of GABA rich foods can contribute to the increase of insulin secretion and decrease blood glucose¹⁸.

3. Bio-accessibility of phenolic compounds in cooked rice samples

In this Task (Task 1.3), the bio-accessibility of cooked rice samples will be analysed using a standardized in vitro digestion method¹⁹. Simulated salivary fluid, gastric fluid and simulated intestinal fluid electrolyte stock solutions will be used to mimic the digestive processes in the mouth, stomach and intestine, respectively. The pH and duration of digestive phases will be adjusted accordingly for each process, namely 2 min at pH 7 for the salivary; 2h at pH 3 for the gastric and 2 h at pH 7 for the

⁹ Canan C et al. (2011) J Food Compos Anal 24: 1057–1063

¹⁰ Schlemmer U (2001) Arch Anim Nutr 55 255–280

¹¹ Dilworth L et al. (2005) West Indian Med J 54: 102–106

¹² Lee S-H et al. (2006) Nutr Res 26: 474–479

¹³ Sanchis P et al. (2018) Sci Rep 8: 1–13

¹⁴ Dat LQ et al. (2019) J Sci Technol 57: 137–143

¹⁵ Oh S-J et al. (2019) Food Chem 271: 187–192

¹⁶ Adisakwattana S et al. (2009) J Enzym Inhib Med Chem 24: 1194–1200

¹⁷ Soltani N et al. (2011) Proc Natl Acad Sci 108: 11692–11697

¹⁸ Tian J et al. (2011) PLoS ONE 6: e25338

¹⁹ Minekus M et al. (2014) Food Funct 5: 1113–1124

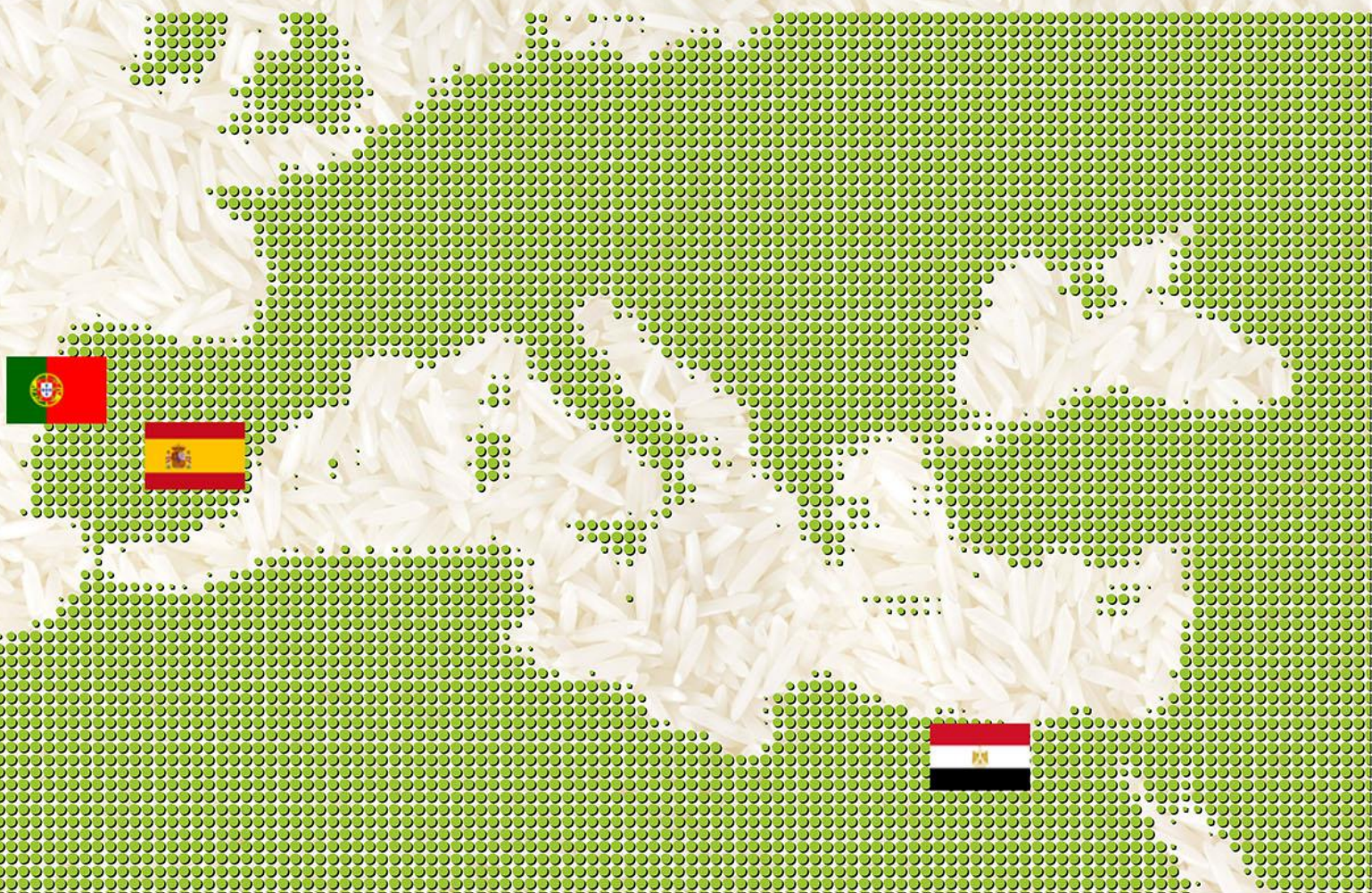
intestinal. Prior to the experiment, the concentration of bile salts and activity of all digestive enzymes will be determined¹⁹ to ensure the effectiveness of the in vitro digestion process.

The rice samples to be analyzed in this Task will be provided by INIAV according to the results from the human sensory panel analysis. Sample preparation will be performed by IBET in close collaboration with TRACE-RICE partner INIAV. Salivary, gastric and intestinal digested fractions will be characterized by chromatographic techniques at IBET to identify the main metabolites and bio-accessible compounds. Reports from the literature show a decrease in the levels of phenolic acids p-coumaric acid and ferulic acid in raw rice bran after artificial gastrointestinal digestion²⁰. However, different phenolic compounds may have different binding affinity towards proteins and susceptibility to break down by the digestive enzymes²¹. This highlights the importance of assessing the bio-accessibility of phenolic compounds in cooked rice samples, and how it may be affecting the health benefits associated with these compounds.

²⁰ Peanparkdee M et al. (2020) Food Chem 329: 127157

²¹ Nignpense BE et al. (2022) Food Biosci 47: 101706

Trace Rice



TRACE-RICE Consortium



IBET
Instituto de Biologia
Experimental e Tecnológica



UNIVERSIDADE
NOVA
DE LISBOA



Grupo Desarrollo



iata

Instituto de Agroquímica
y Tecnología de Alimentos

