Universidade de Aveiro Departamento de Química
2018

Paulo David Martins Brites

Desenvolvimento sustentável de biomateriais ativos utilizando subprodutos da indústria do arroz

Sustainable development of active biomaterials based on rice industry byproducts

Universidade de Aveiro Departamento de Química
2018

Paulo David Martins Brites

Desenvolvimento sustentável de biomateriais ativos utilizando subprodutos da indústria do arroz

Sustainable development of active biomaterials based on rice industry byproducts

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, realizada sob a orientação científica da Doutora Cláudia Sofia Cordeiro Nunes, Estagiária de Pós-Doutoramento do CICECO – Instituto de Materiais de Aveiro da Universidade de Aveiro e da Doutora Paula Celeste da Silva Ferreira, Equiparada a Investigadora Coordenadora do Departamento de Engenharia de Materiais e Cerâmica da Universidade de Aveiro

This work was developed within the scope of project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013)

This was work was financed by national founds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement

Júri

Presidente	Professor Doutor João Filipe Colardelle da Luz Mano Professor Catedrático do Departamento de Química da Universidade de Aveiro
Arguente	Professora Doutora Dulcineia Maria de Sousa Ferreira Wessel Professora Adjunta no Instituto Politécnico de Viseu
Orientadora	Doutora Cláudia Sofia Cordeiro Nunes Estagiária de pós-doutoramento do CICECO – Instituto de Materiais de Aveiro da Universidade de Aveiro

Acknowledgements

I would like to thank Dr. Cláudia Nunes and Dr. Paula Ferreira, my supervisors, who were restless throughout the year, counselling and guiding me with inexhaustible patience.

I would like to thank Eng. Célia Miranda for providing data in DTA/TGA analysis, from the Department of Materials and Ceramic Engineering of University of Aveiro, and Dr. Manuela Marques for her help getting the data from Elemental Analysis, from the Department of Chemistry of University of Aveiro

I would also like to thank my laboratory colleagues for the vital help they gave me throughout the year: Ana, Mariana, Zélia, Joana, Mirtha, Idalina, Fernando, Manuel, Sónia, Soraia, Angélica, Andreia and Guido; as well as Dr. Manuel António Coimbra for his valuable guidance and advice in the weekly reunions throughout the year.

Last but not least, I would like to thank my mother, the main pillar for my success, and my sisters and father whom I love with all my heart; my closest friends, Diogo and António, who never failed to support me when I most needed; and the rest of my family who has been supporting me from day one.

Special thanks to Caçarola® for providing the rice husks and rice bran for this work.

palavras-chave

Cascas de arroz, farelo de arroz, tratamento hidrotermal, sílica, quitosana, compostos fenólicos, biofilmes

resumo

Os bioplásticos aparecem hoje em dia como uma alternativa ecológica para substituir os plásticos baseados em combustíveis fósseis, que têm um impacto ambiental progressivamente maior. A quitosana tem sido proposta para a produção de bioplásticos para embalagens alimentares devido às suas características antimicrobianas e antioxidantes, para além da sua capacidade para a formação de filmes. Por outro lado, milhões de toneladas de subprodutos da indústria do arroz, nomeadamente o farelo e as cascas, são produzidos anualmente. O farelo de arroz é rico em compostos funcionais como o gama-oryzanol que tem capacidade antioxidante e de filtro UV. As cascas de arroz, para além de conterem compostos fenólicos, com propriedades antioxidantes, são também ricas em sílica.

O objetivo deste trabalho foi desenvolver processos sustentáveis para a produção de biomateriais ativos para embalagem alimentar. Para isso, estudou-se um método de extração que alia a pressão à temperatura, em que o único solvente utilizado é água, o tratamento hidrotermal, para extrair os compostos dos subprodutos do arroz. Diferentes temperaturas de extração foram testadas, sendo que no caso do farelo de arroz se escolheu o extrato obtido a 220 ºC e no caso das cascas o obtido a 200 ºC. Estes extratos apresentaram os maiores rendimentos de extração (37% no caso do farelo e 6% no das cascas), concentração de compostos fenólicos relativamente elevada (0,34 mgGAE/g para o farelo e 0,16 mgGAE/g para as cascas) e ainda uma elevada quantidade de hidratos de carbono (41% no de farelo e 25% no das cascas). Estes extratos aquosos foram adicionados à formulação dos filmes de quitosana num rácio de quitosana:extrato de 1:0,1 e 1:0,5 no caso do farelo e num rácio de 1:0,1 para as cascas.

Os filmes, obtidos por evaporação de solvente, foram caracterizados em relação à sua atividade antioxidante, humidade, solubilidade, hidrofobicidade da superfície e propriedades mecânicas. Os filmes com extrato das cascas de arroz apresentaram uma maior resistência em meio ácido, apresentando uma solubilidade de apenas 19 % (tendo o filme de quitosana uma solubilidade de 33%). As propriedades mecânicas foram afetadas com a incorporação dos extratos, havendo uma diminuição na resistência à tensão e flexibilidade nos filmes com maior concentração de extrato de farelo e com extrato de cascas. Ao nível da hidrofobicidade, a incorporação dos extratos não teve um efeito significativo neste parâmetro. Os filmes com o extrato das cascas de arroz e de farelo apresentaram uma atividade antioxidante superior ao filme controlo de quitosana (12,6% superior no caso do farelo e 6,5% no caso das cascas).

Os resultados demonstram que os subprodutos da indústria do arroz têm potencial para serem usados na produção de biofilmes com características interessantes para aplicações alimentares, nomeadamente como embalagens ativas impedindo a oxidação dos produtos alimentares.

keywords

Rice husks, rice bran, hydrothermal treatment, silica, chitosan, phenolic compounds, biofilms

abstract

Bioplastics emerge nowadays as an ecological alternative to replace fossil fuels-based plastics, which have a progressively larger environmental impact. Chitosan has been proposed as an alternative to the production of bioplastics in food packaging due to its antimicrobial and antioxidant characteristics, as well as its film forming capacity. On the other hand, millions of tons of rice industry byproducts, namely rice bran and rice husks are produced yearly. Rice bran is rich in functional compounds, such as gamma-oryzanol with antioxidant and UV light filter characteristics. Rice husks, besides having antioxidant properties from phenolic compounds, are rich in silica.

The objective of this work was to develop a sustainable process to produce bioactive materials for food packaging. For that, an extraction methodology combining temperature and pressure and using water as solvent, hydrothermal treatment, was used to extract the valuable compounds from rice byproducts. Different extraction temperatures were tested and, in the case of rice bran, the extract obtained at 220 ºC was chosen, whereas for the rice husks was the one obtained at 200 ºC. These extracts had the greatest extraction yields (37% for bran and 6% for husks), a relatively high concentration of phenolic compounds (0.34 mgGAE/g for bran and 0.16 mgGAE/g for husks), as well as a high concentration of carbohydrates (41% for the bran and 25% for the husks). These aqueous extracts were added to the formulation of the chitosan films with a mass chitosan:extract ratio of 1:0.1 and 1:0.5 for the bran and 1:0.1 for the husks.

The films, obtained by solvent casting, were characterized in relation to their antioxidant activity, humidity, solubility, surface hydrophobicity and mechanical properties. The films with the incorporation of rice husks extract presented a higher resistance in acid medium, having a solubility of only 19% (the chitosan film had a solubility of 33%). The mechanical properties of the films were affected by the incorporation of the both extracts, having a decrease in the resistance to the tension and flexibility in the films with the incorporation of the highest bran extract and husks extract in comparison with the control film. On the other hand, the incorporation of extracts did not affect significantly this parameter. All the films with the extract incorporation presented a higher antioxidant activity than the control chitosan film (12.6% higher for the bran extract and 6.5 % higher for the husks extract).

The results show that byproducts from rice industry have high potential to be used for biofilms production with interesting characteristics for food packaging, namely as active packaging inhibiting the food products oxidation.

Table of contents

Index of figures

Index of tables

Abbreviations

ABTS	2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
a.u.	Arbitrary units
COD	Chemical oxygen demand
DA	Degree of acetylation
DTA	Differential Thermal Analysis
FCR	Folin-Ciocalteu reagent
FTIR	Fourier-transform infrared spetroscopy
GAE	Gallic acid equivalents
GC-FID	Gas chromatography-Flame ionization detector
HC	Hydrochar
HT	Hydrothermal treatment
MM	Molecular mass
RB	Rice bran
RBA	Rice bran ashes
RH	Rice husks
RHA	Rice husks ashes
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
WE	Water extract

Chapter 1 Introduction

The increasing costs of fossil resources extraction, as well as their predicted dwindling, has raised the interest in renewable and sustainable alternatives with the potential of replacing petroleum as a source of fuels, chemicals and materials (1,2). Bioplastics, as an alternative to petroleum-based plastics, have been widely studied and used. All kinds of biomass, including polysaccharides such as starch, cellulose and chitosan, and other polymeric structures such as proteins and lipid-based polymers have been used to produce bioplastics.

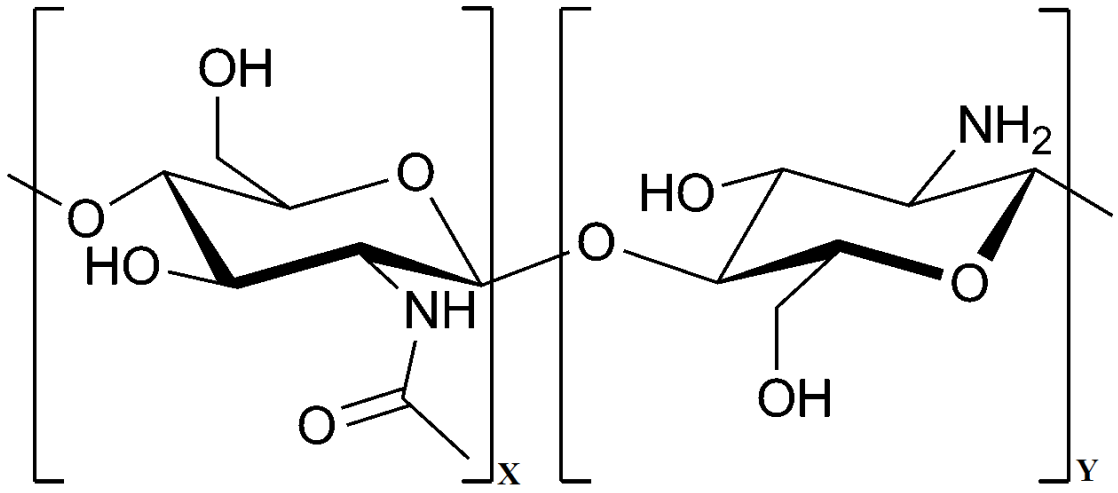
Polysaccharides appear as one of the main solutions as they are one of the most abundant fraction of biomass. They also have renewable and recyclable nature, biodegradable character, and excellent film forming ability (3). Various kinds of films can be made using polysaccharides. Chitin and chitosan (resulting of the partial deacetylation of chitin) appear as one of the most promising for food grade films, because of their natural characteristics, such as their biocompatibility, antimicrobial and antifungal activity, biodegradability and an excellent film forming ability, as well as a low cost of production (4–6). Chitosan film properties can be enhanced for food grade purposes using different approaches, such as chemical modifications or nanocomposite formation, which can allow greater hydrophobicity and mechanical resistance.

1.1. Chitosan

1.1.1. Structure

Chitin is a high-molecular-weight linear polymer, constituted by *N*-acetyl-2-amino-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) units linked by β(1→4) bonds, which is mainly extracted from the exoskeleton of crustaceans, since it is their main component (7).

Chitosan is obtained by partial deacetylation of chitin. This means that chitosan contains two types of β(1→4) linked structural units, *N*-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) (7). Chitosan and chitin are distinguished by the degree of acetylation (DA), for DA values higher than 50% the polymer is named chitin, and for DA values lower than 50%, it is recognized as chitosan (Figure 1) (8).



X > 50% → Chitin
Y > 50% → Chitosan

Figure 1.1. Structure of chitin and chitosan (adapted from Battegazzore *et al.* (9)).

As chitosan is obtained from a natural product by chemical modification, at a low cost of production (6), it does not have a defined and unique structure, and its properties will largely depend on DA and molecular weight. It has however many characteristics and properties, that make this polymer a very interesting resource, being easier to apply in a various number of fields than its acetylated form, which is insoluble in aqueous media.

1.1.2. Biological properties

Chitosan is easily dissolved in diluted acidic aqueous solutions and easily processed, as opposed to chitin, which is insoluble in most solvents and difficult to process. It has been getting a lot of attention in various scientific and industrial fields, such as biotechnology, medicine, packaging, wastewater treatment, cosmetics and food science (10)**.** Furthermore, chitosan has unique physicochemical and biological properties such as antimicrobial and antifungal activity, biodegradability and biocompatibility which are attained with lower DA and molecular mass (MM) (6), and an excellent film forming ability (4,5). In addition, these characteristics, alongside with the great barrier properties materialized on the ability to retard the mass transfer rate of moisture, oxygen, aromas, oils and solutes, make chitosan a promising coating material. This polymer has also good

flexibility and has the potential to form food grade films (3). All these characteristics will allow chitosan to take part in the formation of active, edible and biodegradable films, which can improve the quality and nutritional stability of food products, extending their shelf life and limiting their contamination (3). Although, chitosan has some limitations to be used for packaging, presenting lower tensile strength, flexibility and barrier properties, especially when compared with widely used materials like polyethylene or propylene, which present excellent mechanical and barrier properties for plastic production (11).

Antimicrobial activity

Chitosan anti-microbial activity will vary, depending on the DA, MM, target micro-organism, pH of the medium and other additives or food components. Lower DA, lower MM, and lower medium pH allowed a greater antimicrobial activity. The main responsible for this activity is believed to be the effective number of protonated amine (-NH_3^+) groups (12,13).

Three mechanisms have been proposed for this activity. One of the mechanisms suggests that when the medium conditions are slightly acidic, the amine groups of chitosan are protonated and interact with the anionic groups at the surface of Gram-negative bacteria causing the formation of an impermeable layer around the cell, which will not allow the transportation of essential solutes to its interior. This process can be reversible if pH is raised (14).

Other mechanism suggests that chitosan can inhibit the growth of microorganisms by acting as a chelating agent of essential metals, nutrients and oligo-elements for the organisms, which will then be unavailable, inhibiting their growth. The third mechanism involves the inhibition of ribonucleic acid (RNA) and proteins synthesis by permeation to the cell nucleus, causing disturbances, which will lead to cell death (12)**.**

Antioxidant activity

Chitosan with a low DA shows a great antioxidant activity, allowing metals complexation (15). There are two ways in which the antioxidants can inhibit the radical chain reactions. It can be through direct elimination of the ROS and/or by chelation of metallic ions such as Fe^{3+} or Cu^{2+}. Chitosan could be a potential natural antioxidant to stabilize food products and prolong their shelf life because it can chelate metallic ions, being its main line of action as an antioxidant (16). It is also believed that chitosan can

act as an antioxidant because of the amine and hydroxyl groups bonded in the positions C2, C3 and C6, which are believed to react with unstable free radicals to form more stable macromolecules (17). As referred before, the antioxidant properties of chitosan depend largely on its characteristics. Lower DA induces a higher antioxidant activity. Lower molecular mass (MM) means higher ability to eliminate free radicals and to complex metallic cations, such as Fe^{3+} and Cu^{2+}, and thus higher antioxidant activity (18,19).

1.1.3. Applications

Chitosan has various potential applications due to its versatility in terms of properties. The applications vary from medical areas to food industry. In medicine, for example, this polymer is used to heal wounds, like burns, as it can form biocompatible, water and oxygen permeable films directly on the wound. The advantage of using chitosan is its capacity of being destroyed gradually by lysozyme (naturally presented in the skin) action, eliminating the necessity to remove it, and thereby lowering the probability of tissue lesion (20). This polymer can also form nanoparticles and microspheres as well as hydrogels, films and pills, making for a great system of controlled drug delivery (6)**.** Other uses have been tested in the medical sciences area like the use of chitosan as a non-viral vector for the transport of genes. The main advantage of this method is that the polymer does not originate endogen recombination, oncological effects or immunologic reactions. Chitosan matrices are also a promising material to create regenerative tissue engineering systems due to its controlled biodegradability and porosity. The matrices are used for the culture, support and organization of damaged tissues.

Chitosan is also widely used in the industrial area. It can facilitate biocatalysis, being used to immobilize enzymes, cells, organelles and other complex structures (6), being also used in the field of residual water treatment, to remove a great variety of compounds, such as negatively charged contaminants (nitrates and phosphates), organic compounds and heavy metals from residual waters (21,22).

The food industry is where some of the most important applications of chitosan appear, due to its biological properties. Its use as a preservative, antioxidant, dietetic fiber and emulsifier agent has been described (23). Many studies suggest chitosan as a technologic aider as a clearing agent, acidity and enzymatic browning controller in fruit juices (24) and to inhibit the browning in white wine (25). It is also used as a substitute of nitrites in meat products (26,27) and as an emulsifier in milk products (28). Chitosan

coatings, which are semi-permeable and biodegradable as stated before, can retard the ripening, water loss and reduce the deterioration of food products, by creating a modified atmosphere similar to that used in storage, but at a lower cost and with no effect on the taste of food products (29). Highly perishable foods such as bread, strawberries, eggs, tomatoes and cabbages have been subjected to storage using chitosan films with satisfactory results in prolongation of shelf life**.** (30–33)

Despite these beneficial characteristics and applications, films based solely in chitosan can present brittle characteristic, high hydrophilicity and weak barrier properties, which is something unwanted in food grade films. To contour these problems, chitosan can be chemically and structurally altered to obtain the desired characteristics, such as higher flexibility and hydrophobicity of the film.

1.1.4. Methods to improve chitosan properties

Many different approaches can be taken to improve the characteristics of chitosan-based films. Some strategies involve changes in the polymer's chemical structure (reticulation and grafting) and some involve the addition of inorganic and organic compounds which can interact with the polymer to confer new characteristics and/or to improve the mechanical properties of the films.

Chemical changes

Because of the high number of free amine groups, chitosan is very reactive and can easily be converted to various derivatives, with different properties and possible applications (Figure 1.2). Chitosan can be modified by two different mechanisms: 1) the crosslinking (covalent or ionic), and 2) the grafting (34). The most important reactions, which occur mainly in the amine groups, are the formation of Schiff bases and the N-acetylation (35).

In crosslinking, the polymeric chains are inter-connected by molecules, leading to the formation of a covalent three-dimensional net. These molecules usually have a lower molecular mass than the polymer and have at least two functional groups which will allow the connection between two chains of chitosan (34). Covalent reticulation will allow the enhancement of the structural stability and promote physical, chemical and mechanical changes in the chitosan. Modifications on the solubility, hydrophilicity, absorption

capability, porosity and permeability may occur as consequence of the changes in morphology, and in mechanical and rheological properties (34).

There are many possible reticulation agents, but the most used to reticulate chitosan are dialdehydes, such as glyoxal and glutaraldehyde (36,37). The aldehyde groups form covalent bonds with the amine groups from the chitosan, forming a Schiff base. Dialdehydes allow direct reactions in aqueous mediums, which is an advantage taking bioavailability in account. However, they are chemically synthetized, presenting some toxic effects. Glutaraldehyde, for example, is neurotoxic, and glyoxal is mutagenic (34,38). Genipin is currently used in substitution of dialdehydes, because it is a natural, non-toxic compound which reacts spontaneously with amine groups (39–41).

In grafting, instead of forming a three-dimensional net, the molecule connects to one chain of the polymer, instead of two. In these case, more molecules can connect forming low molecular mass chains.

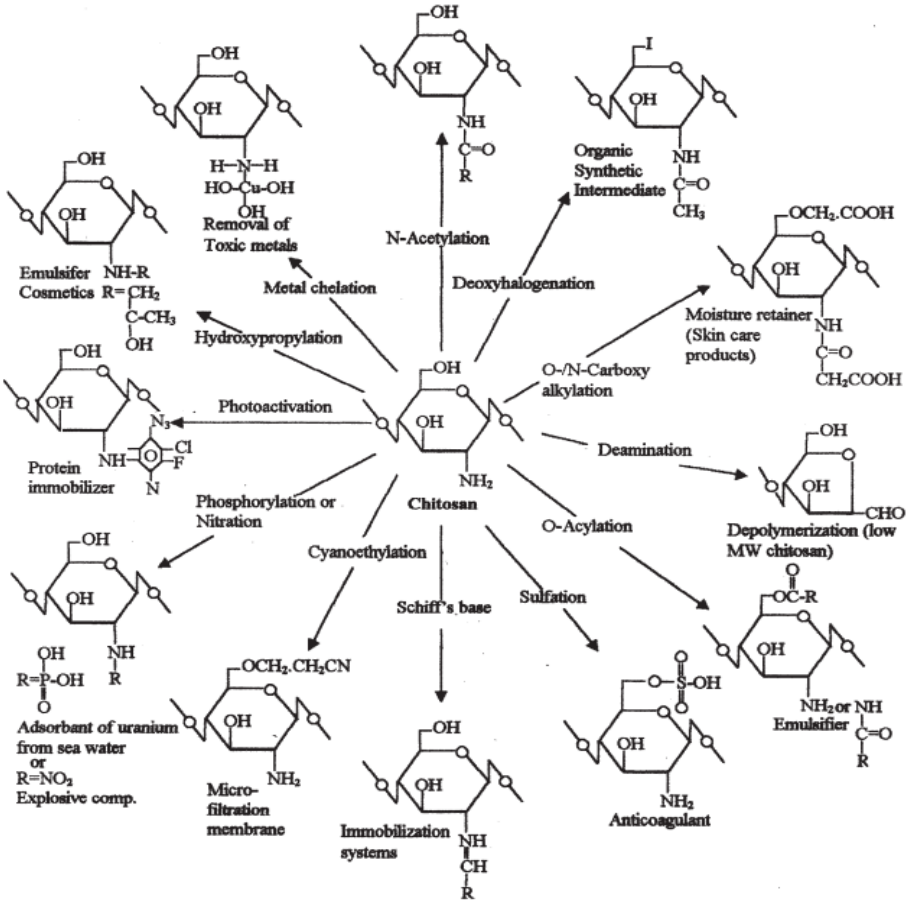


Figure 1.2. Chitosan derivatives (adapted from Tharanathan *et al*. (42)).

Depending on the molecules that are bonded to the polymer, many different results and applications for the novel material can be obtained, such as moisture retainers, emulsifiers, anticoagulants, immobilization systems, microfiltration membranes, adsorbents of uranium from sea water, protein immobilizers, removers of toxic metals and intermediates for organic synthesis (42), as shown in figure 1.2.

Plasticizers incorporation

Natural chitosan films are rigid and fragile so there is the need to add a plasticizer during their preparation, and it is normally applied simultaneously with other approaches. Many compounds can act as plasticizers, such as esters, water, oligosaccharides, glycols, phenols, ketones and ethers (43,44). Plasticizers enhance the elongation capacity of the films, and consequently their flexibility, by lowering the breaking stress. Besides the changes in the mechanical properties, plasticizers also affect barrier properties by altering the permeability to water vapour (43). These compounds allow for a greater mobility of the polymer chains by interacting with it, enlarging the intermolecular space and acting as internal lubricants, reducing the friction forces between chains, such as hydrogen bridges, Van der Waals or ionic forces, and they also lower the glass transition temperature (43–45). Mechanical properties of chitosan films have been studied with the addition of polyols (glycerol, sorbitol and polyethylene glycol) and fatty acids (stearic and palmitic acid) as plasticizers. Polyols were proved to be efficient in lowering the break stress and in raising the rate of deformation of the films, while fatty acids failed to enhance the mechanical properties of the films (46).

Nanocomposite formation

In spite of being film forming material, natural biopolymers have some limitations during processing, like having high water sensitivity, low thermal stability, and weak mechanical and water vapour barrier properties that delay their use in industrial scale (47,48). It is possible to improve physical and chemical properties of these biopolymers through nanocomposite formation (49). The formation of organic-inorganic composites through incorporation of fillers like clays, silicates, metal nanoparticles and carbon nanotubes, is an effective approach to enhance the physical and mechanical limitations of chitosan (48–50) because the particles will interact with the polymer. In the intercalated state, the inorganic material is dispersed as lamellar structures and the polymer chains penetrate between the layers. In the exfoliated structure there is a complete delamination

of the inorganic material and individual layers are dispersed throughout the polymer matrix leading to improvement in mechanical properties (increased flexibility and film resistance) (51) (Figure 1.3).

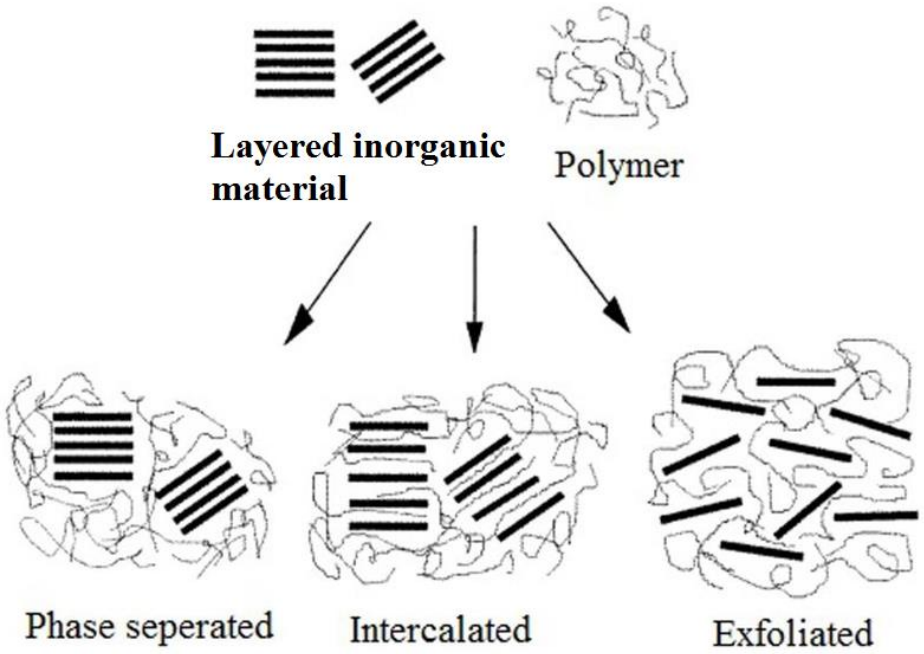


Figure 1.3. Structure of composites from the interaction of a layered inorganic material and polymer matrix (adapted from Rane *et al.* (51)).

Regarding food grade films, many studies report preparation and characterisation of nanocomposites containing chitosan. Silica is often used to form nanocomposites with chitosan because it can confer the previously brittle and permeable material the desirable characteristics for food storage. The most common method for the formation of silica-chitosan composites has been *in situ* sol-gel, which allows to obtain nano-sized hybrid organic-inorganic particles with uniform dispersion, which interact with the polymer resulting in the enhancement of the mechanical, barrier and optical properties (50,52).

Intercalated chitosan-silica nanocomposites with enhanced thermal and mechanical properties have been prepared through solution-mixing processing technique (53,54). In these, the interfacial interaction between the silicate layers and the polymer matrix has a critical importance in their unique properties. Oliveira *et al.* (55) used physical mixing of the chitosan solution with the spherical silica particles, and simple casting to obtain chitosan-silica films. The addition of silica nanoparticles has proved to be effective in

increasing film elasticity and elongation, and a slight rise of surface hydrophobicity and steam permeability.

However, food grade amorphous silica is normally obtained from silica rich sand, through pyrolysis at high temperatures (1000 ºC), having a high energy spend, or through a wet route, involving the precipitation of sodium silicates with sulfuric acid, which means that strong bases and acids are required (56). Alternative sources of silica are constantly being searched, and rice husk (RH) is seen nowadays as an alternative source.

Compound incorporation

Many different compounds have been used to improve several characteristics in chitosan films. The incorporation of essential oils and phytochemicals (eugenol and thymol) has been widely used to increase the antioxidant activity of films (57). The antioxidant and antimicrobial activity of chitosan films has been increased with the incorporation of polyphenols, although at a cost of decreased mechanical properties (tensile strength and elongation at break) (58). Cinnamon bark oil and soybean oil also increased the antimicrobial activity in chitosan films, also showcasing loss of tensile strength. Polysaccharide and phenolic compounds-rich grape pomace extracts have been used to improve chitosan films characteristics: the incorporation of oil obtained proved to increase the films antioxidant activity, while the waxes improved mechanical properties, increasing the flexibility and decreasing the stiffness. The use of grape seed oil has proven to rise the hydrophobicity of chitosan films because of the triacyl glycerides present in its composition. The lipids present in this oil also acted as plasticizers, increasing the films flexibility and decreasing the brittleness (59). The incorporation of phenolic compounds from red wine has also been tested in chitosan films cross-linked with genipin, and the antioxidant activity was doubled, and the solubility was decreased (60).

Hemicellulose polysaccharides like arabinoxylans have also been successfully incorporated in chitosan films, with increase of mechanical properties (tensile strength and elongation at break), increasing the functionality of edible films with the polysaccharides prebiotic properties (61).

1.2. Rice industry by-products

In 2012, 729 million tons of rice were produced worldwide, and with its processing, rice husks and rice bran were generated as byproducts (62) (Figure 1.4). The rice husks are produced during the first stage of milling, when the rough rice is milled. The rice bran is produced during the second phase of milling, the whitening process, when the bran is removed from the rice (63). These byproducts are normally wasted in low value applications like incineration for energy in the case of rice husks, and animal feed in the case of brans. They have although several compounds that could be valued, namely to films formulation.

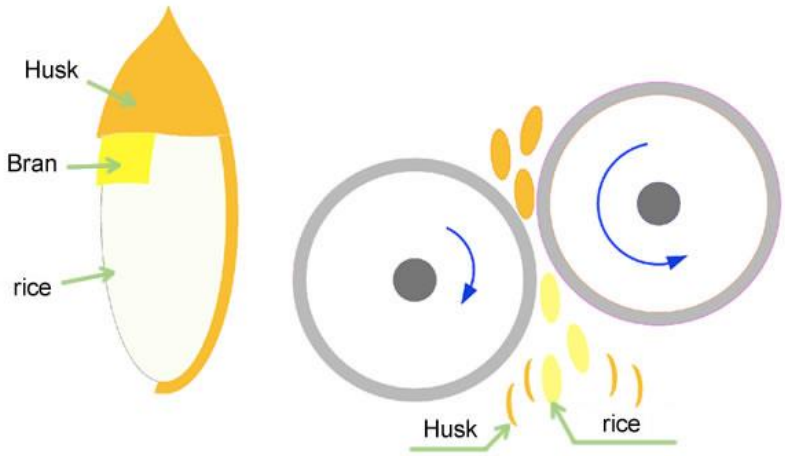


Figure 1.4. Schematic representation of the rice grain and milling process (adapted from Rice Knowledge Bank (63)).

1.2.1. Rice husk

Rice husk (RH) is abundantly available worldwide as an agricultural by-product of the rice industry, comprising a fifth of the weight of rice produced. More than 150 million tons of RH were wasted (62). RH is widely used in diverse areas such as bio-fertilizing, animal husbandry, pest control and sorbent, and as building material. Very often it is still treated as a waste product of rice milling, being burned in open air or just dumped on wasteland (64).

RH are a complex lignocellulosic material with a high mineral content, and it is composed by 35.62% cellulose; 11.96% of hemicellulose; 15.39% of lignin; 18.71% of

ash; 2.33% of crude protein; 0.29% of crude fat; 6.18% of moisture (65–67), as well as having a total phenolic compounds concentration of 600 mgGAE/100 g (68).

RH acid hydrolysates have been used as a raw material to scale up ethanol production (69,70). For the valorisation of this byproduct, the antioxidant activity of RH have been enhanced through far-infrared radiation which released bound phenolic compounds (71). Lignocellulolytic cellulases and xylanase enzymes have been produced from *Aspergillus heteromorphus* through solid-state fermentation of microwave-alkali pre-treatment of rice husks (72). Furthermore, RH has also proposed to remove radioactive cesium from aqueous solutions (73).

Silica from rice husk

As RH can be used as a renewable fuel, having in account its high calorific value (4012 kcal kg^{-1}) (74), during the combustion there is the production of rice husk ashes (RHA), containing more than 90% of silica (75). The abundant presence of silica in RHA has attracted attention of many researchers due to its possible industrial exploitation. Many studies regarding the extraction of silica from RH have been published in the literature in the past years (76–78). Many ways to extract silica have been reported: 1) burning RH at 600-800 ºC in a pure oxygen atmosphere, obtaining a homogenous size distribution of nanometric silica particles (79); 2) burning at 700 ºC under air, it can be obtained silica with a high specific surface (80); and 3) dissolution of the RH in a strong NaOH solution, followed by the silica precipitation in acid (81,82).

1.2.2. Rice bran

The rice bran is a byproduct of rice milling which contains the pericarp, aleurone and sub-aleurone fractions of the rice kernel. About 76 million tons of rice bran is produced worldwide every year. This byproduct contains many bioactive compounds, such as phytosterols (83).

Rice bran is composed by 42-45% of carbohydrates; 10% of fiber; up to 27% of total fats; up to 14% of protein; 7-10% ashes and 7-9.5% moisture (84), as well as having a total phenolic compounds concentration of 260 mgGAE/100 g (68). Regarding carbohydrates, the major ones in brans are celluloses, hemicelluloses and starch. The quantity of starch depends on the milling phase. Normally, it is obtained 5-35% but in an efficient two-stage milling operation, values of 5-15% are more likely. In waxy varieties,

amylose values are normally low, although values as high as 5.7% have been reported, 10-20% in short- and medium-grain varieties, and 20-35% in long-grain varieties (85). Hemicelluloses make up 8.7-11.4% of the bran. The water-soluble hemicelluloses have an arabinose:xylose ratio of 1:8, as well as galactose and protein. The alkali-soluble bran hemicelluloses contain arabinose, 37%; xylose, 34%; galactose, 11%; uronic acid, 9%; protein, 8%; and traces of glucose (86). The content in cellulose ranges between 9.6 and 12.8%. Lignin content of bran ranged from 7.7 to 13.1% (86). Glucose, fructose, sucrose and raffinose make up 3-5% of the rice bran in the form of free sugars, being concentrated in the aleurone layer (85). It was also reported that nonreducing sugars are more abundant than reducing sugars (86).

Protein content is influenced by variety, environment, and nitrogen fertilization (86). Regarding protein composition, it has been reported to be albumin, 37%, globulin, 36%, prolamin, 5% and glutein, 22% (85). Serine, alanine and glutamic acid are the main free amino acids (87), rice bran also contains various enzymes, including enzymes of microbial origin (88). Lipase is one of the most important factors for the non-utilization of this byproduct as a foodstuff because it will degrade the triacylglycerols into free fatty acids, which will decrease the shelf life of rice bran and make it unsuitable for human consumption (86).

Palmitic, oleic and linoleic acids are the three major fatty acids present, making up about 90% of the total fatty acids of rice bran (Table 1.1). Bran lipids are distinguished in three groups: glycerolipids, sterol lipids and sphingolipids. Cholesteryl esters and cholesterol have also been identified in the bran (86).

Table 1.1. Fatty acid composition of Rice Bran Oil (adapted from Sounders *et al.* (86)).

Fatty acid	**Composition (%)**
Palmitic (C16:0)	15.9
Stearic (C18:0)	1.7
Oleic (C18:1)	40.7
Linoleic (C18:2)	37.9
Linolenic (C18:3)	1.4
Arachidic (C20:0)	0.6

Rice bran possesses significant levels of various micronutrients such as phytosterols, oryzanol and tocotrienol, giving it interesting properties (89). The gamma oryzanol present in this byproduct has been reported to have a high antioxidant activity. This is actually not one compound, but a mixture of ferulate, esters of sterols and triterpene alcohol (Figure 1.4) (90). Its antioxidant activity helps lowering plasma cholesterol, decreasing serum cholesterol and its absorption, and decreasing platelet aggregation. It has also been used to increase muscle mass and to cure hyperlipidemia and menopause disorders. Oryzanol has been used as a nutraceutical in the preparation of functional food as well as in cosmetics.

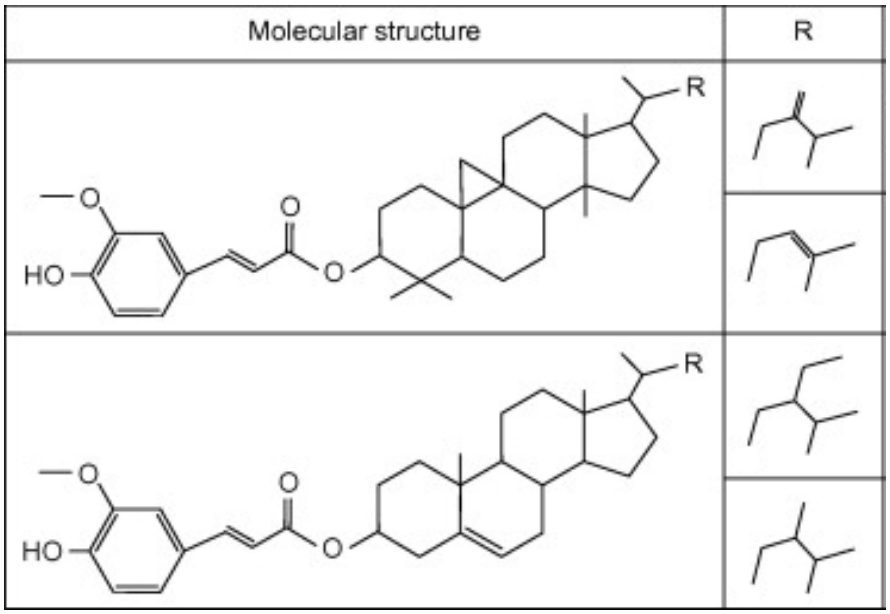


Figure 1.5. Chemical structure of the main components of gamma oryzanol (adapted from Lerma-García *et al.* (91)).

General health benefits of rice bran

Several compounds in the composition of rice bran have a high antioxidant activity, which can help prevent chronic diseases. The incorporation of this byproduct in functional foods has shown to improve insulin sensitivity, reduce the risk of coronary heart disease and decrease of blood cholesterol levels (92–95). The oryzanol components, referred before, can also act as a protective agent against UV light, being inclusively incorporated in sunscreens. Ferulic acid esters of gamma oryzanol can stimulate hair growth, acting therefore as anti-ageing agents (89). Nutraceuticals developed from the fiber fraction of rice bran have been used to control type I and type II Diabetes Mellitus

(96). Bone loss has been reduced in women suffering from postmenopausal osteoporosis rice bran supplemented food (89). Therefore, the presence of these essential nutrients makes rice bran suitable for the development of food products.

It has been used as a substitute to cereals in breakfast/dinner recipes, with a good acceptability (97). It has also been used to decrease bread and cookie spread and to increase muffin volume (98). Cookies moisture, crude protein, fat and mineral contents, as well as average width, thickness and spread factor have been enhanced with the addition of rice bran (99). Defatted rice brans, with unique functional and nutritional properties and low-fat content have also been used to enhance ready-to-eat breakfasts, as well as corn flakes and tortilla chips, with satisfactory results (100,101). Stabilized rice bran has been incorporated in frozen pizzas as a source of dietary fibre. The physical, chemical, rheological and sensorial characteristics of the pizzas were studied, and it increased the levels of crude fat, ash and dietary fibre contend of pizza dough (102).

1.3. Green extraction methodology

Taking in account a circular economy as well as a green route to diminish the carbon footprint of the extraction processes of the various compounds from rice husk and rice bran, it is important to explore new ways to obtain them. A promising technique for silica extraction from the rice husk, as well as the previously referred compounds from rice bran, is hydrothermal extraction.

1.3.1. Hydrothermal treatment

In the last few years, hydrothermal treatment (HT) has arisen as a promising new technology for the conversion of wet lignocellulosic biomass into highly functional materials and fuels (103). In this process, subcritical water reacts with the fibrous components of the biomass, degrading and reconstructing it (104). The HT treatment causes hydrolysis, dehydration, decarboxylation, condensation, polymerization and aromatization of biomass (105)**,** generating two main products: an insoluble and carbon-rich hydrochar (HC), and the aqueous extract, as well as small amounts of gases (106)**.**

The published studies regarding HT mainly focus on the biochar characterization for energetic purposes, with few works regarding the liquid phase characterization a valorisation. The physicochemical properties of the formed hydrochar, make it of great potential for various applications. It is a homogenous, hydrophobic, energy-dense solid,

with a porous structure containing micro to nano-sized carbon spheres and oxygen-containing functional groups (104). This material has been tested as a solid fuel (103–106), soil supplement (107) and as feedstock for pellets (108), among others. The aqueous extract formed on the process is mainly composed of source-related organic acids and other intermediate products (furfurals, phenols and sugars, as well as various nutrients originally present in the biomass) (109).

The invasive aquatic plant *Elodea nuttallii* was submitted to HT by Poerschmann *et al.* (110) and its biochar and aqueous extracts were analysed. The author tested HT at 200 and 240 ºC for a 14 h holding time. The water extract dissolved organic carbon (DOC) concentration and chemical oxygen demand COD were analysed. It was possible to conclude that extracts obtained from HT at 240 ºC have a better biodegradability, because the BOD/COD ratio is higher than the 200 ºC counterparts. Normally, the organic carbon content (OC) is mostly transferred to the biochar, and just a minor part of DOC. The recovered OC was higher in the 240 ºC biochar than at 200 ºC. Although, this recovery was smaller than what was previously recorded with brewer spent grain as substrate (111). This low recovery of OC is justified by high ash content of the plant, which is associated with its high capability of accumulating inorganic species. The relatively high BOD_{10}/COD-ratios indicated a high probability of suitability of both extracts for biogas production. A similar test has been conducted using brewer's spent grain. This study revealed that compounds such as phenols and fatty acids were present in the water extracts (111).

Other studies have been conducted where the characterization of both the biochars and the aqueous extracts were made. Wu *et al.* (112) analysed the HT products of loblolly pine at 180, 200, 220, 240 and 260 ºC using NMR techniques. It was concluded that HT is indeed a valid carbon sequestration method, in which the solid residue (>50% fixated carbon) obtained at 260 ºC reached a higher heating value (HHV) of 30.74 MJ/kg (1.53 times that of untreated pine). The water extract consisted mostly of condensed polyaromatic structures with a high content of carbonyl and carboxyl groups. The authors concluded, based on the HHV results, lignin content, structural components and energy consumption, that 240 ºC is the optimal temperature for biochar production.

Kalderis *et al.* (113) used HT to obtain hydrochars from rice husks for potential use as fuel. The tests were conducted at 200 and 300 ºC. The HC formed presented low surface area, meaning that the HCs didn't form porous structures. The most promising HC for usage as fuel was obtained at 300 ºC for a holding time of 6 h. It presented a predicted higher heating value (HHV) of 17.8 MJ kg^{-1}, and a fixed carbon content of 46.5%, the highest values of all the hydrochars obtained. In this study, the water extracts were not considered.

Chapter 2 Objective

The main aim of this work is to develop an active biomaterial which can be an alternative to synthetic plastics in food packaging. It is proposed for this, the searching of raw materials from rice industry byproducts following the current environmental and energy trend of the circular economy.

The work is divided in several specific objectives:

1) To study the use of hydrothermal treatment as an alternative green methodology to extract valuable compounds from byproducts of the rice industry (rice husks and rice bran);
2) To characterize the composition of each extract and select the ones to be further applied as active components in food packaging;
3) To evaluate the potential of applying the selected extracts on the design of an active food packaging material having chitosan as matrix;
4) To characterize the mechanical properties, antioxidant inhibition, solubility of films and surface hydrophobicity of the resulting materials in comparison with pristine chitosan films.

Chapter 3 Experimental section

In this chapter, it is described all the experimental procedures involved since the collection of the rice industry byproducts; to their characterization; extraction, and application on films preparation.

3.1. Samples

The rice husks and bran were obtained from Caçarola® (Oliveira de Azeméis) and stored at -20ºC until use.

The samples moisture was determined by weight loss after drying at 105 ºC. Small aluminium foil shaped boxes were put in an oven at 105 ºC during 12-16 h. After cooling in a desiccator, the boxes were weighted. The samples were placed in the tinfoil boxes and rigorously weighted. Then were placed in the oven at 105 ºC for a minimum of 16 h. After this time, the boxes with the dry samples were placed to cool down in a desiccator for 30 min and then weighted. The moisture was calculated in triplicate using the Equation 1:

$$\%\, moisture = \left(\frac{m_{sample\ i} - m_{sample\ f}}{m_{sample\ i}}\right) \times 100\% \quad \text{Equation 1.}$$

where $m_{sample\ i}$ is the initial mass of the sample, and $m_{sample\ f}$ is the mass of the sample after drying at 105 ºC.

3.2. Extraction from rice husks and rice bran

3.2.1. Hydrothermal treatment

The samples were weighted and mixed with distilled water (1:4 w/v) and placed in an autoclave. The temperatures tested were of 180, 200 and 220 ºC for 1 h holding time. Some samples were extracted sequentially, which means that they were subjected to a sequence of increasing temperatures, being removed the water extract for each temperature and added a new volume of distilled water previously to new temperature treatment. For the sequential extraction, a bigger recipient was used, and these samples include a "S" on their name. The typical procedure for the sequential extraction involved the mixture of 30 g of rice husks or rice bran with 120 mL of distilled water and the heating to 180 ºC, using a ramp of 5 ºC/min and a holding time of 1 h. After the autoclave was cooled down, the supernatant (water extract) was filtrated and stored at 4 ºC, leaving the solid fraction (hydrochar) intact. Distilled water was added to compensate the liquid removed and the mixtures were then heated to 200 ºC with the same heating ramp and

holding time. The process was repeated, and the mixtures were finally heated to 220 ºC. The water extracts (WE) were filtered with filter paper.

For the non-sequential extraction, smaller autoclaves were used. For each temperature (180, 200 and 220 ºC), 26 g of rice husks or rice bran were weighted and mixed with 104 mL of distilled water. The different mixtures were heated to 180, 200 and 220 ºC for 1 hour, and the WE were filtrated (113,114).

The different WE were frozen, lyophilized and stored in a desiccator for posterior characterization and use in the films formulation. The water extracts are identified according to extraction conditions described in Table 3.1.

Table 3.1. Denomination of the water extracts obtained in this work: H and B stand for husks and bran, respectively, followed by the indication of the extraction temperature. The samples obtained by sequential method present a "S" on their name.

HT Extraction	**Non-sequential**			**Sequential**		
Temperature (ºC)	**180**	**200**	**220**	**180**	**200**	**220**
Rice husks	H180	H200	H220	H180S	H200S	H220S
Rice bran	B180	B200	B220	B180S	B200S	B220S

3.2.2. Silica extraction from rice husks through a conventional method

To evaluate the total silica content of the byproducts, 10 g of rice husks were weighted and mixed with 100 mL of NaOH 5%. The mixture was transferred to a plastic boiling flask and boiled for 30 minutes at 100 ºC. The solution was left overnight to optimize the extraction and formation of sodium silicates. It was then filtered, and the filtrate was transferred to a plastic cup. It was then neutralized with HNO_3 10% through drop-by-drop addition, under agitation, until a gel was formed. The gel was left overnight and was filtered, being thoroughly washed with distilled water to remove the excess of acid. It was then dried at 110 ºC, for 8 h, and weighted after grounding. The silica was weighted and characterized by Fourier-transform infrared spectroscopy (FTIR) and thermogravimetric-differential thermic analysis (TGA-DTA). It was then calcinated to obtain a greater degree of purity (115).

3.3. By-products and water extracts characterization

3.3.1. Lipid content

The determination of the byproducts lipid content was performed with a Soxhlet extraction method. About 20 g of each sample were weighted into a paper cartridge and placed in the Soxhlet extractor. The solvent used for the extraction was n-hexane (150 mL). The extraction was done at 80 ºC (boiling temperature) for about 6 hours. After, the solvent was evaporated using a rotary evaporator to obtain the lipidic fraction. The flask with the lipids was weighted and the fat percentage was determined by the Equation 2:

$$\%\, fat = \left(\frac{m_{sample\ lipids}}{m_{sample\ i}}\right) \times 100\% \qquad \text{Equation 2}$$

where $m_{sample\ lipids}$ is the mass of the lipidic fraction obtained, and $m_{sample\ i}$ is the initial mass of the sample.

3.3.2. Total protein content

Elemental analysis was performed using an Elemental Analysis System – LECO CHNS-932, which allows for the determination of carbon, hydrogen, nitrogen and sulphur in solid and liquid samples. The operating temperatures of the combustion furnace was 1075 ºC and the afterburner temperature was 850 ºC. Thermal conductivity was used to detect the nitrogen, and the nitrogen-to-protein conversion factor of rice products (5.75) (116) was used to estimate the protein content. This characterization was made for each byproduct and water extract.

3.3.3. Total polysaccharide content and composition

The composition in monosaccharides of polysaccharides was determined by Gas Chromatography – Flame Ionization Detector (GC-FID) after acid hydrolysis and derivatization to alditol acetates. The polysaccharides of each byproduct and freeze-dried water extract (1 mg) were pre-hydrolysed with 200 µL of 72 % H_2SO_4 at room temperature for 3 h. Then, a hydrolysis was made at 100 ºC for 150 min with 1 M H_2SO_4. The hydrolysate was cooled down and 200 µL of 1 mg/mL 2-deoxyglucose were added as internal standard. To 1 mL of this mixture were added 200 µL of 25% NH_3 and 100 µL of a 3 M NH_3 solution containing 150 mg/mL of $NaBH_4$, for the reduction of monosaccharides to alditols, at 30 ºC for 1 h. For the decomposition of the excess $NaBH_4$,

50 µL glacial acetic acid was added 2 times. The acetylation of the alditols was made by the addition of 0.45 mL of 1-methylimidazole and 3 mL of acetic anhydride to 0.3 mL of the previous solution. This solution was kept at 30 ºC for 30 min. The alditol acetates were then extracted with dichloromethane and the organic phase was rinsed several times with water. The solvent was then evaporated in a centrifuged evaporator. The quantification of each monosaccharide was made by injection of 2 µL of sample dissolved in acetone in a gas chromatograph, Perkin Elmer - Clarus 400, equipped with a DB-225 capillary column (30 m length, 0.25 mm internal diameter with 0.15µm of film thickness) coupled to a FID detector, using H_2 as eluent. The injector temperature was set to 220 ºC, and detector temperature to 230 ºC. The program of temperature used has a total time of 9 min with an initial temperature of 200 ºC, with a rise of 40 ºC/min until 220 ºC and a holding time of 7 min, and a second rise of 20 ºC/min until 230 ºC with a final temperature of 230 ºC for 1 min (117,118). The monosaccharides identification was performed by retention time comparing with standards.

Uronic acids determination

The uronic acid analysis was performed for rice bran and rice husks, according to the modified m-phenylphenol colorimetric method (117,118). The hydrolysis was performed simultaneously with the neutral sugar analysis and during this stage an aliquot of 0.5 mL of hydrolysate of each sample was taken after 1 h of incubation. To this volume, 3.0 mL of distilled water were added. The calibration curve was prepared using rising concentrations of galacturonic acid standard solution (0.0 mg/mL; 0.019 mg/mL; 0.038 mg/mL; 0.058 mg/mL; 0.077 mg/mL). Three tubes (two replicas and a white) for sample were prepared, with 0.5 mL of sample. The tubes were placed in an ice bath, were 3.0 mL of 50 mM sodium borate in concentrated sulfuric acid were added. The tubes were vigorously agitated, sealed and put in a boiling (100 ºC) bath for 10 min. After, were cooled down in an ice bath. The following step was the addition, in the dark, of 100 µL of MFF (0.15% m-phenylphenol (m/v) in 0.5% NaOH (m/v) to two of the three tubes of each sample. The tubes were then stirred and left to react in the dark for 30 min. The absorbance was read at 520 nm. The uronic acid content of the samples was determined using the galacturonic acid calibration curve.

3.3.4. Total phenolic compounds

The total phenolic content of the water extracts was determined by Folin-Ciocalteu method. This method involves the use of Folin-Ciocalteu reagent (FCR), which is a mixture of heteropoly, phosphomolybdic and phosphotungstic acids. It is the simplest method to determine the content of phenolic and polyphenolic antioxidants. The method relies on electron transference from the phenolic compounds in alkaline medium to form a blue chromophore, where the maximum absorption (750 nm) depends on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer in the range of 690 to 710 nm. A microplate method adapted from the colorimetric Folin-Ciocalteu method (119) was carried out. Initially, 60 µL of water were dispensed in the well, followed by 15 µL of Folin-Ciocalteu reagent and 15 µL of sample, diluted accordingly, or standard solution to construct the calibration curve. The solutions were left to react for 5 min and afterwards 150 µL of 7% Na_2CO_3 solution was added. The microplate was incubated at 30 ºC for 60 minutes, and the absorbance was measured at 760 nm in a BioTek Eon™ spectrophotometer. The calibration curve was constructed using a solution of gallic acid in a range of 0.05 to 0.250 mg/mL as standard, which allowed the quantification of phenolic compounds in the samples.

3.3.5. Morphological analysis

Scanning Electron Microscopy (SEM) was used to reveal the morphology of the initial samples. The SEM equipment used was a Hitachi® model SU-70. This microscope can achieve magnifications from 30 to 800000 times with acceleration voltage in the range of 0.1 to 30 kV.

3.3.6. Fourier Transform Infrared spectroscopy (FTIR)

For the FTIR analysis of the byproducts and freeze-dried water extracts, single reflection diamond ATR reflection system (Perkin Elmer Spectrum BX) was used. The spectra were acquired at the absorbance mode between 4000 and 500 cm^{-1} (mid infrared region) with a resolution of 4 cm^{-1} and 32 scans per sample. Five replicates were collected for each byproduct and freeze-dried water extract.

3.3.7. Differential Thermal (DTA) and Thermogravimetric Analysis (TGA)

Thermal analysis: Differential Thermal Analysis (DTA) and ThermoGravimetric Analysis (TGA) were performed to understand the chemical and structural changes on samples (byproducts and for each of the water extracts) upon temperature increase. The equipment used was *Setaram*™ model *Labsys TG-DSC16.* The analyses were performed by heating the powder from room temperature up to 800 ºC using a heat rate of 10 ºC/min under flowing air.

3.4. Chitosan films

3.4.1. Chitosan films preparation

For the films preparation, 1.5%(m/v) chitosan (Aldrich, average molecular mass and ≥75% degree of acetylation (DA), according to the manufacturer) solution was obtained through dissolution on an aqueous acetic acid solution 5% (v/v), with agitation, for 16 h at room temperature. The solution was then filtrated through a porous plate. A mass of 0.375g of glycerol was added as plasticizer agent per 100 mL of chitosan solution and the mixture was heated in a 50 ºC bath for 10 min with agitation for homogenisation. After, the solution was degasified under vacuum. This solution (31 g) was then transferred to a 12 x 12 cm plexiglass plate (144 cm^2 of useful area). The plate with the solution was placed in a stove in a levelled support for 16h at 35 ºC for film formation through solvent evaporation.

For the preparation of chitosan films with water extract, the extract was initially dissolved, making a solution with a concentration of 20 mg/mL. The solution was added with different chitosan:WE ratios. (1:0.5 and 1:0.1, w:w). The addition of the WE were made before heating the mixture at 50 ºC for plasticizer homogenization, being the following protocol the same as for the control films.

A similar method was used to make chitosan films with silica, with the addition of silica dissolved in water. The amount of silica added was equivalent to the silica expected to exist in the rice husks water extracts. For the equivalent of 1:0.5, a proportion of 1:0.15 of silica was prepared, and for the equivalent of 1:0.1, a proportion 1:0.03 of silica was prepared, taking into account that the rice husk water extract has around 30% silica.

3.4.2. Films characterization

Humidity

The determination of films humidity is accomplished by drying until constant weight. Small aluminium foil shaped boxes are put in an oven at 105 ºC during 12-16 h. After cooling in a desiccator, the boxes were weighted. The film samples were cut into 4 cm^2 squares and placed in the tinfoil boxes and rigorously weighted. The films were placed in the oven at 105 ºC for a minimum of 16 h. After this time, they were placed to cool down in a desiccator for 30 min and then weighted. The film humidity was calculated using Equation 3.

$$\%\, humidity = \left(\frac{m_{film\,i} - m_{film\,f}}{m_{film\,i}}\right) \times 100\% \quad \text{Equation 3}$$

where, $m_{film\,i}$ is the mass of the initial film and $m_{film\,f}$ is the mass of film after dried. The films humidity determination was made in triplicate.

Solubility

The film solubility was determined through the films difference in mass before and after being immersed in a matrix with water a set period of time. A square of 4 cm^2 of film is first weighted and then put in 30 mL of a water and sodium azide solution (0.02 % (w/v), to inhibit microbiological growth, and left at room temperature in an orbital shaker (80 rpm) for 7 days. The films were removed from the solution and put in an oven at 105 ºC for 16 h. After cooled down for 30 min in a desiccator, the films were weighted. The films solubility was determined by the Equations 4 and 5.

$$m_{dried\ film\,i} = m_{film\,i} \times \left(1 - \left(\frac{\%humidity}{100}\right)\right) \quad \text{Equation 4}$$

$$Solubility = \left(\frac{m_{dried\ film\,i} - m_{dried\ film\,f}}{m_{dried\ film\,i}}\right) \times 100\% \quad \text{Equation 5}$$

where $m_{film\,i}$ is the film initial mass, % humidity is the value determined with the procedure above, $m_{dried\ film\,i}$ is the initial mass of the dried film and $m_{dried\ film\,f}$ is the mass of film after it was immersed in the matrix and dried. All solubility determination trials were made in triplicate.

Antioxidant activity (ABTS)

The antioxidant activity of the films was determined by an adaptation of the ABTS method. A solution of 7 mM ABTS (Sigma-Aldrich) in 2.45 mM potassium persulfate (Fluka) was prepared and left to react for 12-16 h in the dark at room temperature to form $ABTS^{+\bullet}$. From the $ABTS^{+\bullet}$ solution, 1 mL is diluted in 80 mL of distilled water and its absorbance was measured at 734 nm in a *Jenway 6405* UV/Vis spectroscope. The concentration of the solution was adjusted with distilled water in order to have an absorbance between 0.7 and 0.8. A square of 1 cm^2 of film was put in 3 mL of the $ABTS^{+\bullet}$ solution and left to react in the dark for 24 hours. The films antioxidant ability was measured using the Equation 6.

$$\%\ inibition = \left(\frac{abs_{control} - abs_{sample}}{abs_{control}}\right) \times 100\% \quad \text{Equation 6}$$

where $abs_{control}$ is the value of absorbance of $ABTS^{+\bullet}$ solution (without film) and abs_{sample} is the value of absorbance of $ABTS^{+\bullet}$ solution with the film. Each sample has 3 replicas including the blank solution (the control).

Mechanical properties

The mechanical properties of films are very important to reveal the behaviour of a material when subjected to different stress/strain conditions, which are critical for their applications. To determine these characteristics uniaxial extension tests are preformed, which originate a stress/strain curve from which different mechanical parameters can be obtained, such as stress at breaking point, yield strength (elasticity limit), Young modulus and strain at breaking point.

The mechanical properties of the films were evaluated through tension until rupture tests following ASTM D 882 and 883 practices. The texturometer used was a TA-HDi, Stable Micro Systems. This method allows calculating deformation percentage, tension at rupture point and Young's modulus. The first lets us know the distance the film increases in its length from initial size until breaking due to the applied contrary forces (expressed in percentage), giving the information on plastic part of the films. The tension at rupture allows us to know what force needs to be applied to the films extremities, in opposite directions, to rupture. Young's modulus is a value used to express film elasticity,

corresponding to the slope of stress-strain curves i.e it is achieved by dividing the tensile stress (MPa) by the extensional strain, in the elastic portion of the physical stress–strain curve (initial linear part). Tensile stress is the force applied which causes the film to stretch until immediately before its breaking point, causing film stretching. The extensional strain is a dimensionless value that expresses a proportion between the distance in the stretched film and its original length. It is found by dividing the difference in length before and after the film stretching (elastic phase) by its initial length.

The films were stored before the test in a chamber with magnesium nitrate (55% relative humidity) to control their humidity for 5 days. The films were cut to the desired width and length (1 x 9 cm) and stored again in the chamber with controlled humidity. The films were tested in a room with controlled temperature and humidity, 26 ºC and 60±5 %, respectively. The films thickness was measured immediately before the tests with a digital micrometer (*Digimatic Micrometer MDE-25PJ*, Mitutoyo). The tests were performed onto the 5 cm^2 of exposed film secured by the machine vertical grip system. The tests used a rate of 1.0 mm/s extensional deformation until sample rupture. Six strips of each sample were tested.

Contact angle (surface hydrophobicity)

The contact angles between ultra-pure water and the films were determined using the system *OCA 20*, Dataphysics. The films were stored in a chamber with magnesium nitrate for humidity control (55% relative humidity). The films were cut into strips of 1x9 cm. The test used 3 µL of ultra-pure water drops automatically dosed via syringe. The contact angles were obtained using the *SCA20_M4* Dataphysics software. For each sample 6 strips of film were tested, and, in each strip, 10 measurements were performed.

3.4. Statistical analyses

The results from the humidity, solubility, mechanical properties, contact angle and antioxidant activity were statically analysed using F-tests and t-student tests (Microsoft Excel 2016), to inquire if the samples were significantly different. The samples were considered significantly different when the significance level was $p \leq 0.05$.

Chapter 4. Water extraction of rice husks and rice bran by hydrothermal treatment

This chapter presents the characterization of the as-received rice byproducts, and the studies concerning the hydrothermal extraction of the samples at three different temperatures (180, 200 and 220 ºC), the effect of a simple extraction at each temperature and of sequential extractions of samples.

4.1. Initial byproducts characterization

An overall characterization of the byproducts, rice husks and rice bran, was performed (Table 4.1). Both rice husks and rice bran showed to have a high carbohydrate content, 62% and 66%, respectively. This was expected because the husk is a lignocellulosic material, composed by cellulose, hemicellulose and lignin, and the bran is mainly constituted by starch, cellulose and hemicellulose (65–67,86). Lipid content analysis revealed to be higher in rice bran (17%) than in rice husks (1%), which is in accordance with the bibliography. Bran is reported to have an oil content ranging between 10 and 23%, and the husks less than 1% (65–67, 86). Rice bran revealed also a higher content of protein (12%) in comparison with husks (2%), which also are according to the bibliography, reporting 9 to 17% of crude protein in bran and 2% in husks (65–67, 86). Regarding dry basis moisture, rice bran and rice husks showed similar results, around 7%. The ash content was higher in the husks (24%) than in the bran (13%), and the values are slightly higher than the ones reported in the bibliography (19% in husks and 10% in bran) (65–67) (84). These values are slightly different, since these are natural matrixes with different production conditions, which can be heterogeneous in composition, but overall the results were concordant with those reported in the bibliography.

Table 4.1. General composition of rice bran and rice husks in mass fraction percentage (% w/w).

By-product	Moisture	Carbohydrates	Lipids	Protein	Ash	Total
Rice husks	7.30±0.03	62.4±4.7	0.77	1.6 ± 0.3	23.5	95.6
Rice bran	7.0±0.5	66.4±5.7	17.33	12 ± 1.2	13	115.7

As the carbohydrate content was high, the specific monosaccharide content was determined in rice husks and rice bran. Both byproducts presented a high molar percentage of glucose, 70 mol% in rice bran and 51 mol% in rice husks (Table 4.2). This glucose residues could be from starch in rice bran and from cellulose in rice husks (65–67,88). In terms of molar percentage of xylose, rice husks presented higher values (34 mol%) than rice bran (8 mol%), and the opposite was verified for arabinose content (4%

for rice husks and 9% for rice bran). This means that the arabinoxylans present in the husks have an arabinose:xylose ratio of 1:8, which is in accordance with the bibliography, that reported the same ratio and percentages of 4 and 34% (120) and the bran of 1:1 (86). The byproducts revealed similar content in uronic acids (9 mol% and 11 mol%, for husks and bran, respectively) and mannose (1 and 2 in molar%, respectively).

Table 4.2. Monosaccharide content (molar %) of rice husks and rice bran.

Byproduct	**Molar %**					**Total (%, w/w)**
	Ara	Xyl	Man	Glc	UA	
Rice husks	4.4 ± 0.7	34.3 ± 4.2	0.9 ± 0.6	51.1 ± 3.6	9.4 ± 1.5	62.4
Rice bran	9.1 ± 0.7	8.3 ± 0.7	1.6 ± 0.4	69.8 ± 2.2	11.3 ± 0.8	66.4

Figure 4.1 shows the SEM micrographs of the rice husks and bran after milling with liquid nitrogen. The rice husks present large irregular sized particles (Figure 4.1a). The surface is pretty smooth and in the particles side view, it is possible to distinguish the overlapped layers (Figure 4.1b) associated to the lignocellulosic structure (65–67,86). These layers seem to be porous as shown in Figure 4.1c. The rice bran presents smaller particles than husks (Figure 4.1 d-f), with a smoother surface possibly due to the presence of the high fat and protein content in its composition.

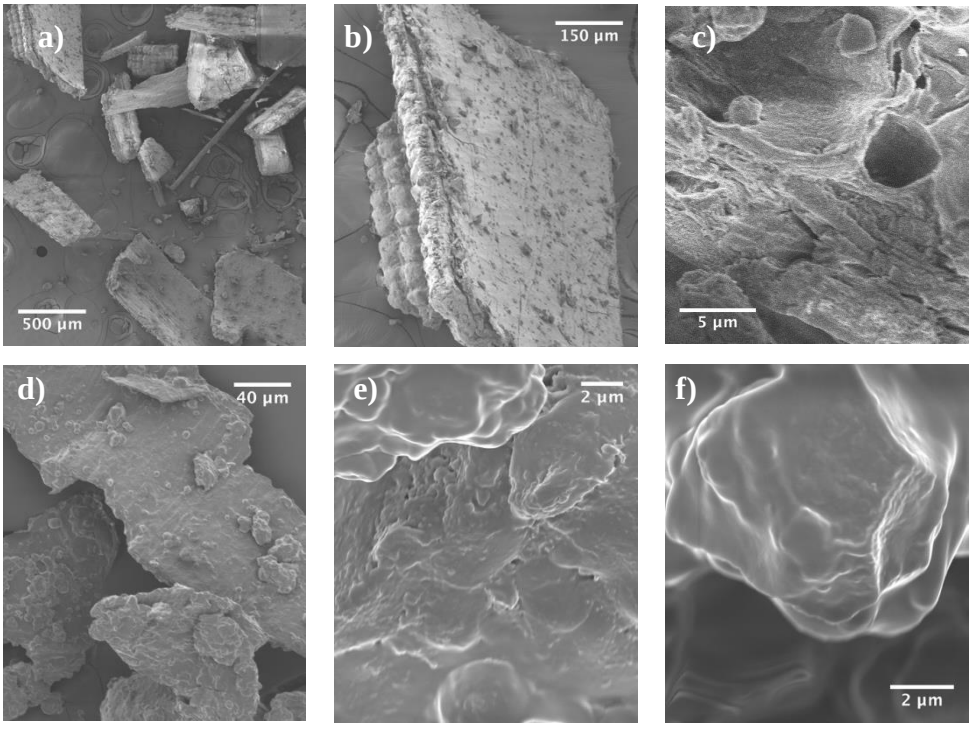


Figure 4.1. SEM micrographs of rice husks (a, b, and c) and rice bran (d, e, and f).

A FTIR analysis was performed for the ashes of both samples to confirm the presence of silica (Figure 4.2). It is possible to observe that, as expected, the rice husk ashes are rich in silica displaying the typical peaks at 1042 and 800 cm^{-1} corresponding to the stretching and bending of the Si-O-Si bonds, respectively (121–123). On the other hand, it is confirmed that rice bran has a lower silica content than husks as the spectrum does not show clear peaks at the mentioned wavenumbers.

Figure 4.2. FTIR spectra for rice bran and rice husk ashes, and purified silica.

4.2.1. Characterization of water extracts obtained by hydrothermal treatment

Water extracts of the two byproducts were obtained by hydrothermal treatment using different temperatures (180, 200 and 220 ºC), individually or sequentially. The lyophilised extracts were characterized by TG-DTA to study their thermal behaviour and to estimate the quantity of inorganic materials. It was observed that the extracts had an ash percentage between 20 and 30%. Having in consideration the constitution of rice husk ashes (75), up to 90% of these ashes are silica. The composition of the extracts is presented in Table 4.3. The extracts obtained from rice husks with the greatest extraction yield were the ones obtained with the highest temperatures: 200 ºC (H200, 5%), and 220 ºC (H220, 6%). In bran, both extracts obtained with 220 ºC, non-sequentially (B220) and sequentially (B220S), reveal the greatest extraction yields among rice bran extracts (37 and 24%, respectively). The rice bran extracts have a higher extraction yield, in

comparison with rice husks, because bran is composed by a higher quantity of soluble compounds, which were extracted to the liquid phase by the temperature/pressure treatment. All the other extracts showed yields below 4%, except the extract from bran at 200 ºC (B220), which had 9% of recovery. In all the extracts, both sequentially and non-sequentially extracted at 220 ºC had the utmost yield, meaning that the high temperature treatment potentiates the extraction of compounds.

Table 4.3. Extraction yields conditions; proportion of monosaccharides (mol%); total quantity of sugars, and protein content of the water extracts obtained by hydrothermal treatment at different temperatures and conditions.

Byproduct	**Extract**	**$\eta_{(\%)}$**	**Molar %**					**Total**	**Protein**
			Ara	Xyl	Man	Glc	Gal	**(µg/mg)**	**(%)**
Husks	H180	1.57	3.3±0.5	5.8±0.3	1.7±0.1	85.5±0.5	3.7±0.4	435±66	8.5±0.05
	H200	4.79	9.9±0.2	36.5±1.5	0.6±0.5	42.0±0.8	11.1±0.0	245±13	3.6±0.04
	H220	5.59	7.9±6.3	5.8±0.4	1.8±0.4	80.3±5.6	4.2±0.6	715±232	8.2±0.3
	H180S	0.61	4.5±0.1	6.2±0.3	2.8±0.1	81.6±0.4	4.9±0.4	645±294	8.9±0.1
	H200S	2.10	8.3±0.1	62.3±3.7	0.3±0.5	24.1±2.5	5.0±0.6	702±268	5.8±0.5
	H220S	4.41	8.3±0.5	65.4±7.0	0.3±0.4	20.9±6.6	5.2±0.5	715±238	3.4±0.1
Bran	B180	2.56	2.0±0.1	0.8±0.13	1.3±0.1	94.6±0.2	1.4±0.1	971±119	8.5±0.2
	B200	8.85	3.2±0.2	1.3±0.0	1.1±0.6	93.1±0.7	1.4±0.1	697±156	9.3±0.3
	B220	37.18	3.9±1.1	5.7±1.4	1.4±0.5	87.5±3.1	1.5±0.1	406±31	11.4±0.5
	B180S	2.46	2.0±0.1	1.1±0.1	3.9±0.1	89.5±0.1	3.4±0.1	529±110	12.1±0.02
	B200S	3.16	4.8±0.1	2.1±0.1	2.2±0.1	89.5±0.1	1.4±0.0	510±207	9.1±0.8
	B220S	24.42	5.0±0.0	6.4±0.0	1.2±0.0	85.9±0.0	1.5±0.0	378±76	11.3±0.1

Regarding the relative molar % of neutral sugars, it is possible to observe that all the extracts obtained from rice bran are mainly composed of glucose (86-95 mol%), which should arise from the starch (85), with minor content of Ara, Gal, Man, and Xyl (< 5 mol%). At 220 ºC, slightly higher proportion of Xyl was observed (6 mol%), which could be related with the extraction of arabinoxylans. In rice husks sequential extraction, the extraction at 180 ºC (H180S) has a high quantity of glucose (82-86 mol%), while the ones obtained at higher temperatures have a superior quantity of xylose (65-71 mol%) and arabinose (8 mol%). The high Glc content should arise from the starch that can be solubilized with low temperature processing, whereas xylose and arabinose, should result from arabinoxylans (65–67), which are mainly extracted at temperatures above 180 ºC.

In the non-sequential extraction at 200 ºC, mainly arabinose and xylose appear, probably due to the extraction of arabinoxylans and xylans. The sequential extract at 220 ºC shows 85 mol% of glucose. This means that arabinoxylans could be extracted concomitantly with polysaccharides rich in Glc, as cellulose-like materials. The presence of these polysaccharides, such as arabinoxylans may enhance mechanical properties (tensile strength and elongation at break) and may give prebiotic characteristics (61).

Among the husks, the extracts obtained at 180 ºC, sequentially and non-sequentially, as well as the one obtained non-sequentially at 200 ºC, presented the highest absolute quantity of protein (8.9, 8.5 and 8.2%, respectively). Most of the protein is extracted at 180 ºC, being the percentage of protein extraction progressively lower in the sequential extractions. Protein may be useful in enhancing the mechanical properties of the films (to increase stress at break and decrease strain at break) (124). As expected, rice bran extracts have greater quantity of protein than the ones of husks (12% in bran and 2% in husks, Table 4.1). The extracts of bran obtained sequentially and non-sequentially at 220 ºC, as well as the one obtained sequentially at 180 ºC, had the highest percentage of protein (11.3, 11.4 and 12.1%, respectively). The bran, having higher concentration of protein, does not show the same behaviour in extraction as husks, with similar values being obtained in the different sequential extraction temperatures.

FTIR analysis was performed to evaluate the presence of silica in the extracts. The spectra obtained for the WE are represented in Figure 4.3, as well as the pure silica spectrum. The spectra from the different water extracts present similar profile. From the analysis of the patterns, it is not clear the presence of silica on the obtained extracts from both matrices and under different extraction conditions. It cannot be ruled out the presence of silica, but the typical peaks (at 1042 and 800 cm^{-1}) (121–123) are completely overlapped by the sugar fingerprint zone (125), characterized by a strong peak at 1080 cm^{-1}. It is known that rice bran does not contain silica, based on the FTIR analysis made on rice bran (Figure 4.2). The peaks at 3374 and 1637 cm^{-1} correspond to the bending and stretching of OH groups from the water adsorbed and the remaining organic matter (mainly sugars, based on the composition of the extracts), and the ones at 2920 and 1463 cm^{-1} correspond to the bending and stretching of CH_2, which also correspond to the sugars.

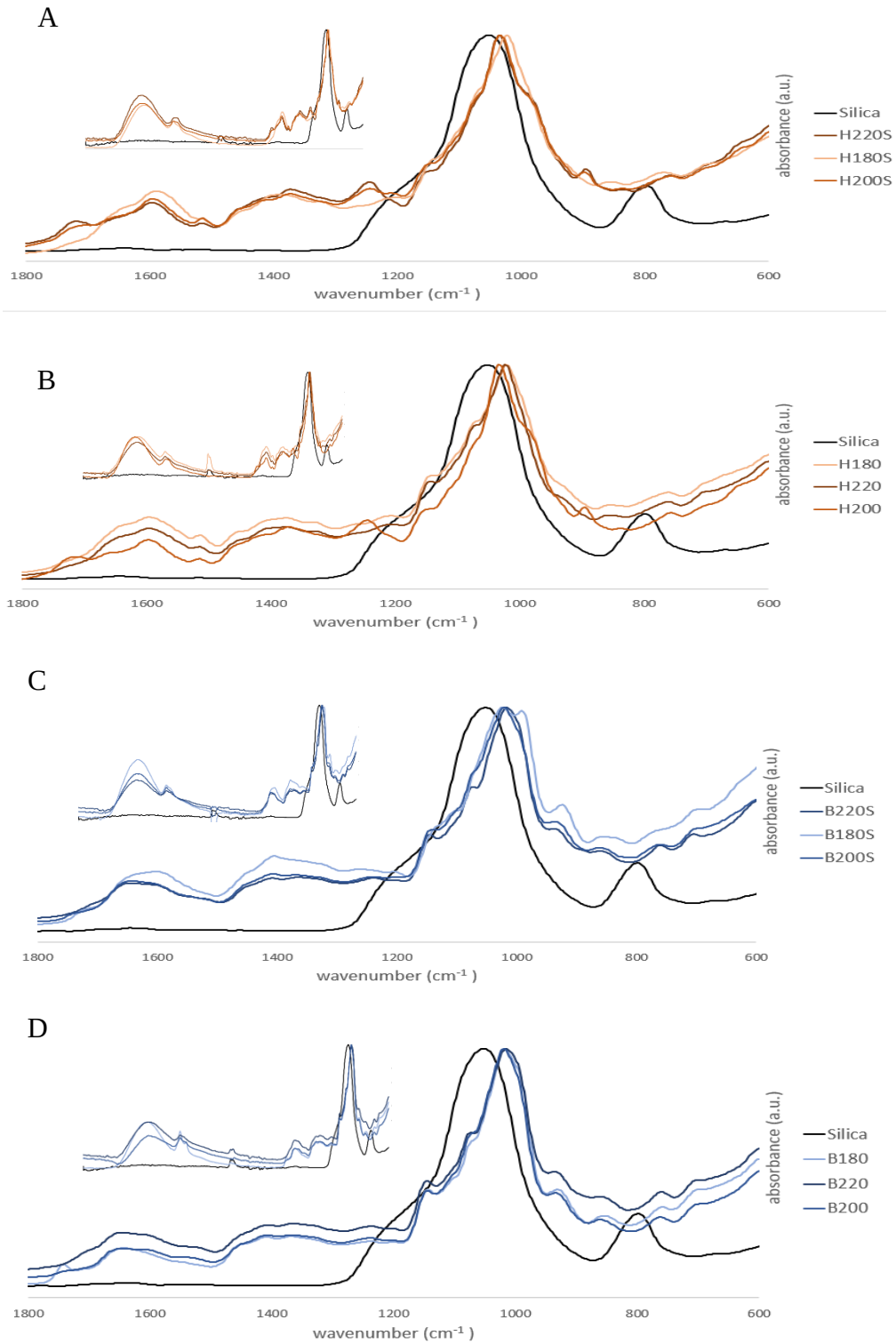


Figure 4.3. FTIR spectra for the sequentially and non-sequentially extracted WE from rice husks (A and B, respectively) and rice bran (C and D, respectively). Pure silica FTIR spectrum is also displayed for comparison purposes. For each condition, the spectra in the full range of wave-numbers are displayed in the insets.

The total phenolic compound content was determined in the water extracts of both samples, to know their potential antioxidant activity. The results obtained for the total phenolics in the water extracts are presented in the Figure 4.4. The water extracts obtained from the hydrothermal extraction of rice husks presented, generally, greater quantity of phenolic compounds, when compared with those obtained from rice bran ($p \leq 0.05$). The presence of antioxidant compounds such as phenolic compounds was expected, as these compounds are present in both rice husks and bran, with emphasis on husks (68). The water extract obtained sequentially at 220 ºC had the greatest quantity of phenolic compounds ($p \leq 0.05$), followed by H200, indicating a greater antioxidant activity (126). Within the sequential extraction, there is a slight rise in total phenolics with a rise in temperature, indicating that temperature potentiates the extraction.

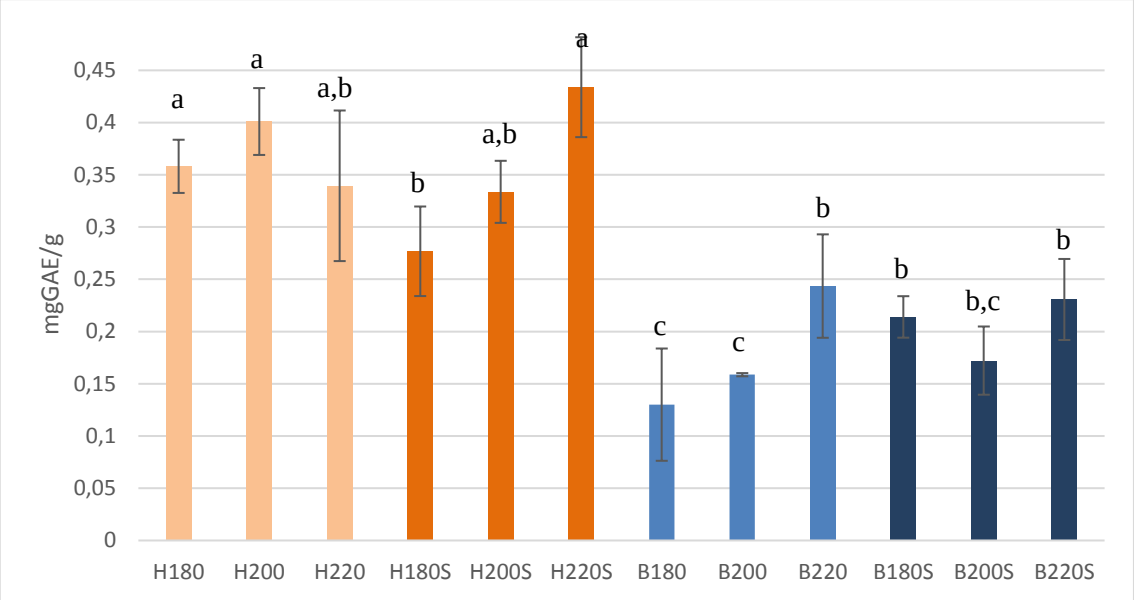


Figure 4.4. Total phenolics of the water extracts, expressed in gallic acid equivalents, GAE. Different letters correspond to significant differences for $p < 0.05$.

In order to compare and quantify the total silica amount, silica was extracted from the rice husks through a conventional sol-gel method described in the previous experimental section (3.2.2.). The silica initially obtained through this method presented a darker colour, due to the presence of organic matter. The silica was then calcinated, to enhance the degree of purity. Figure 4.5. shows the colour and aspect of these silicas.

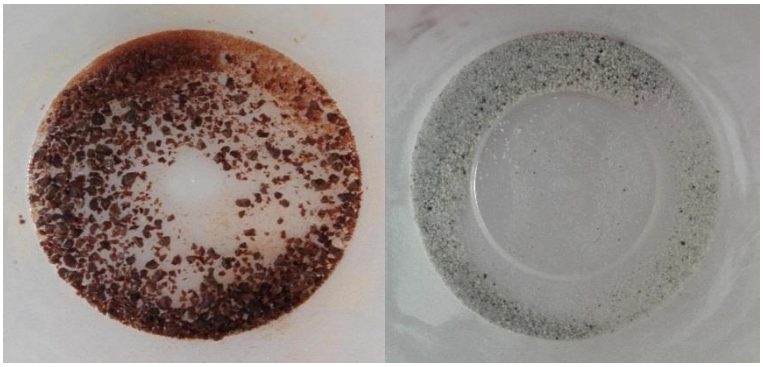


Figure 4.5. Silica obtained from rice husks before (left) and after (right) calcination

The silica before calcination was characterized by TGA (thermogravimetric analysis), and the results are presented in Figure 4.6. The initial mass was reduced in more than 25%. 2% of weight loss was assigned to adsorbed water (lower slope, until 120 ºC), and the remaining corresponds to the degradation of the organic compounds. The remaining mass corresponding to the inorganic fraction is about 70%, which are related with pure SiO_2.

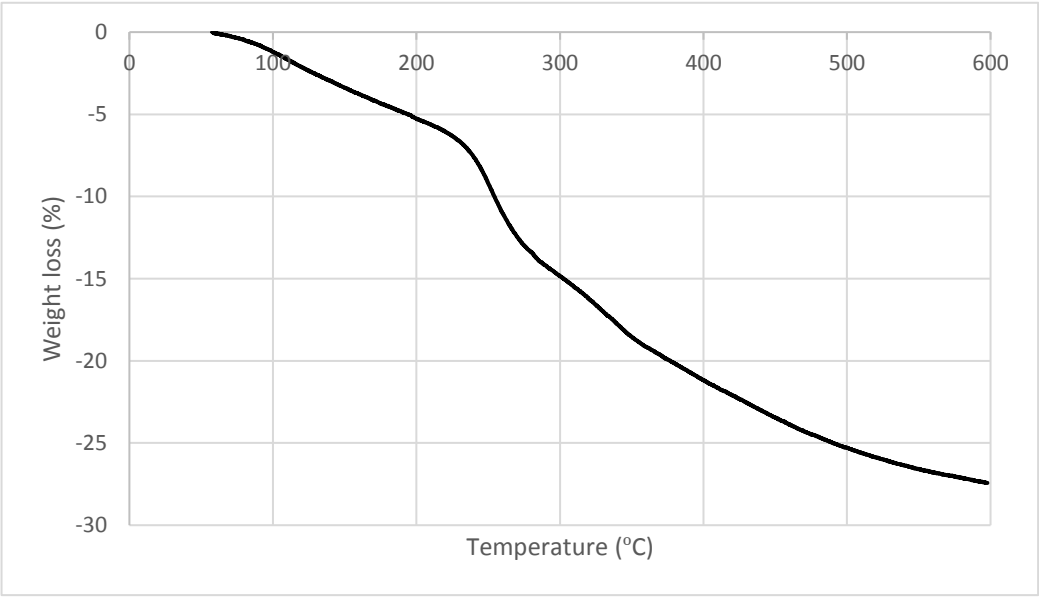


Figure 4.6. Thermogravimetric analysis of the silica obtained from rice husks through sol-gel route.

FTIR analysis was performed for both silicas, and the spectra are presented in Figure 4.7. The calcinated silica seems to be closest to a purified state, displaying clearly the

peaks at 1042 cm^{-1} and 800 cm^{-1} associated to the stretching and bending of the Si-O-Si bonds, being very similar to the spectrum of commercial purified silica (121–123). The silica before calcination, besides these peaks, has a very high peak at 3350 cm^{-1} corresponding to the stretching of the O-H bond, corresponding to water and/or organics, and a smaller peak at 930 cm^{-1} corresponding to the bending of C-H, from organic compounds and O-H bonds, also from organic compounds and/or water, in this case, the sugars present in its composition.

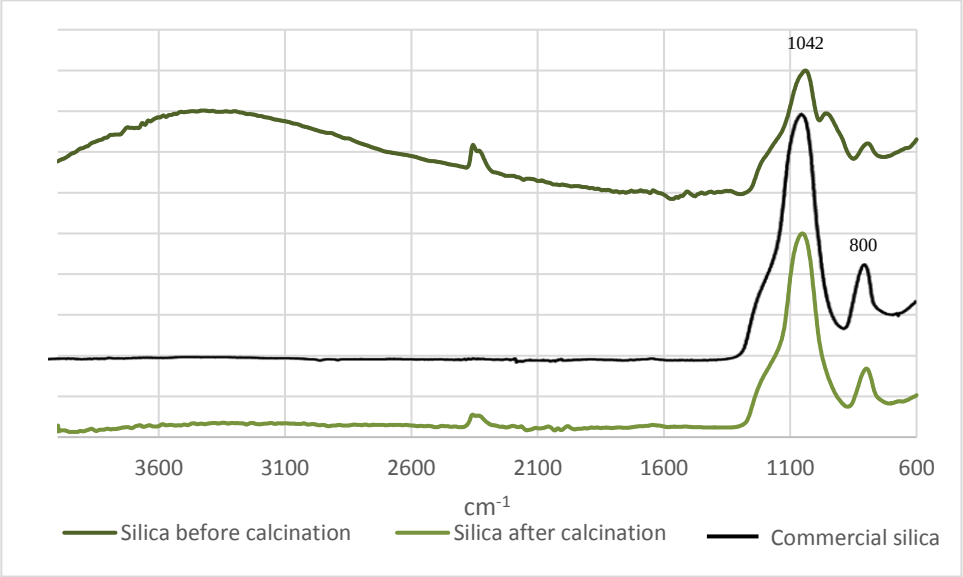


Figure 4.7. FTIR results for rice husks silica before and after calcination

Overall, HT extraction proved to be a good method to extract the polysaccharides, protein, and other soluble compounds from the rice byproducts, ecologically, since the temperatures used only went up to 220 ºC with an holding time of just 1 h and the only solvent used was water, as opposed to other methods to extract these compounds which use very high temperatures or strong solvents (silica extraction uses temperatures up to 800 ºC (79) or strong acids and bases (81,82), and polysaccharide extraction uses strong, concentrated bases such as 1 M KOH or NaOH (127,128)).

Chapter 5 Preparation and characterization of chitosan films

5.1. Preparation of chitosan films with incorporation of rice bran water extract

Two extracts were chosen to use in the chitosan film formulation, H200 and B220. These extracts presented a greater extraction yield among the rest of the water extracts from each group (5% and 37%, respectively). The concentration (relative to initial mass) of protein and carbohydrates was also large in these extracts. Besides, the concentration of total phenolic compounds, and presumably antioxidant activity, was relatively high in both extracts. Control films were made: chitosan only, to compare with the films with both extracts, and chitosan with silica extracted through sol-gel route from rice husks, to compare with the films with rice husk water extracts.

In the first trial, chitosan films were made using B220 in a proportion of 1:0.1 and 1:0.5 (chitosan:extract). Figure 5.1 shows the control film and the films containing the B220 extract for both concentrations. The films with the B220 extract content present a yellow colour (Figures 5.1 b and c) which is stronger for higher amounts of extract (Figure 5.1 c).

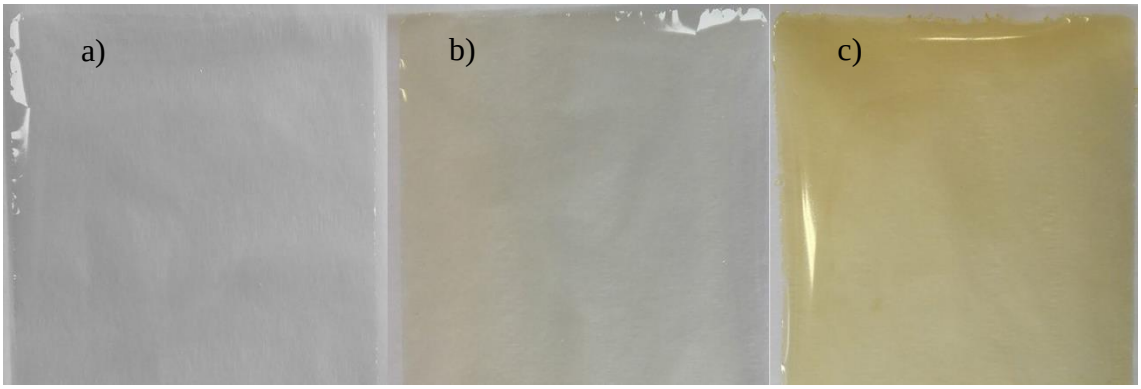


Figure 5.1. Photographs of chitosan-based films: a) control; and films containing chitosan: B220 extract on the ratios of b) 1:0.1; and c) 1:0.5.

The results of the mechanical properties for both films, as well as for control, are presented in Figure 5.2. The tension at rupture was statistically similar ($p \geq 0.05$) for the three films (control, B220 1:0.1, and B220 1:0.5), but the Young's modulus was higher with the increasing concentration of B220 extract ($p \leq 0.05$) with a smaller deformation percentage because of the lower elasticity (129). This could be due to the decreasing of chitosan content with the extract concentration, leading to a weakening of the polymeric

matrix by decreasing elasticity and decreasing flexibility. The deformation percentage of the control chitosan film is in accordance with the literature, with a value of 45% (59), and it is not statistically different from the B220 1:0.1 film, meaning this concentration of extract was not high enough to interfere with the matrix structure stability. Therefore, the greater dilution of chitosan in the films with added water extract, and possibly the interaction of the extract compounds interferes with the chitosan matrix, making it more brittle.

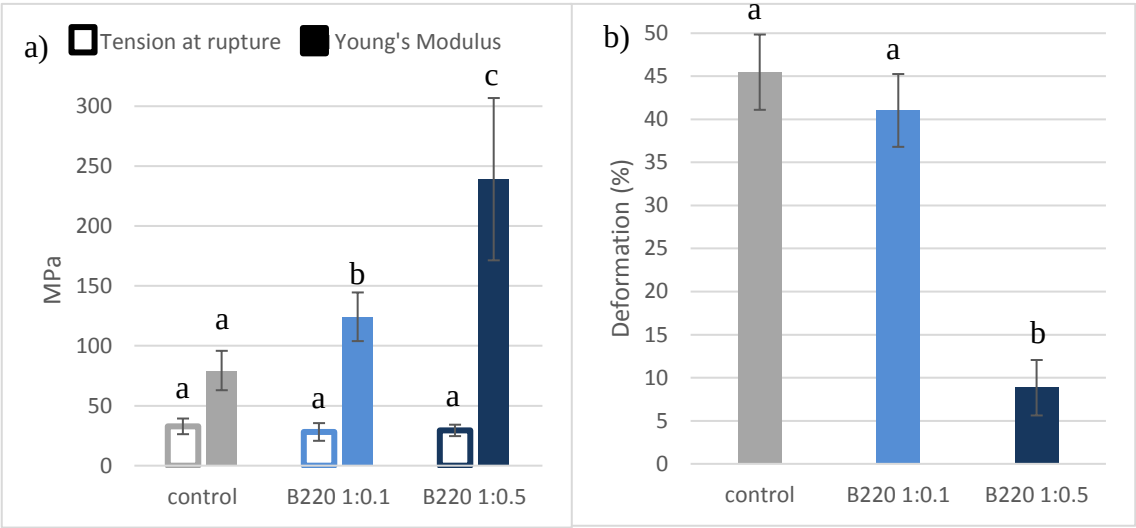


Figure 5.2. Mechanical properties for the control chitosan film, and the two different ratios of chitosan:B220 extract (1:0.1 and 1:0.5): a) tension at rupture and Young's modulus; and b) deformation percentage.

Figure 5.3 shows the humidity degree percentage and the solubility of the films in acidic medium. There are no significant differences between the humidity of B200 films and the control ($p \geq 0.05$), meaning similar water retention capacities among the films. The solubility of the films was evaluated by immersion in acidic water for 7 days. The films with water extract content revealed a lower solubility (40% of mass loss) than the chitosan control (78%), being almost 40% less soluble. The films with extract incorporation are more resistant to acidic medium than a simple chitosan film. This could be due to the smaller quantity of chitosan in the films with extract, because chitosan is easily soluble in acidic medium (10). The control chitosan film presented a greater solubility (78%) than the 25% reported in literature (59). This difference can be justified by the use of acidic conditions (pH 3.5) on the test, instead of slightly acidic (pH 6.5) as typically reported in literature. Chitosan is known to be highly soluble in acidic conditions (10).

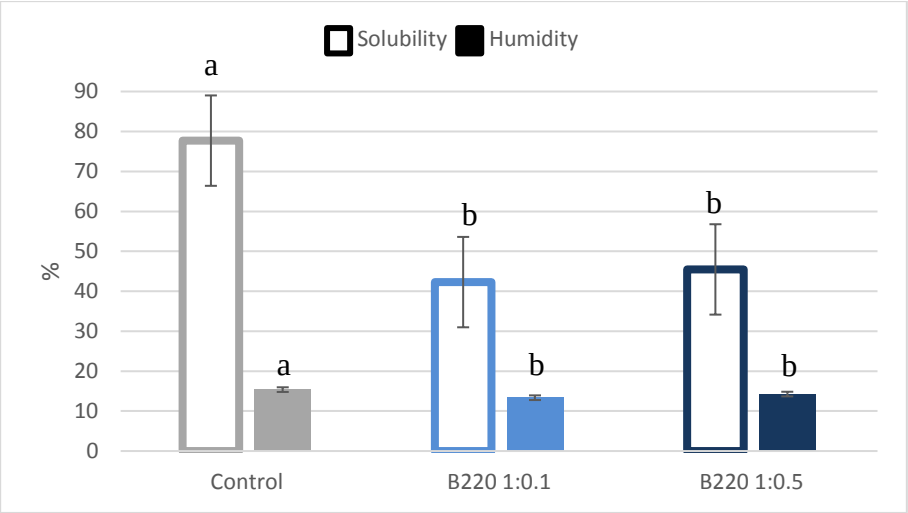


Figure 5.3. Humidity and solubility in acidic medium for the control chitosan film, and the two different ratios of chitosan:B220 extract (1:0.1 and 1:0.5).

As the mechanical properties were highly affected by the low quantity of chitosan, other trial of films were formulated considering the water that had to be added with the extract. Therefore, the chitosan concentration was the same in all the formulations, independently of the quantity of extract added. Using the same selected water extract (B220), two different films were formulated, ratios of chitosan:extract 1:0.1 and 1:0.5. The addition of the rice bran extract gave a yellow tonality to the films, as Figure 5.4 depicts.

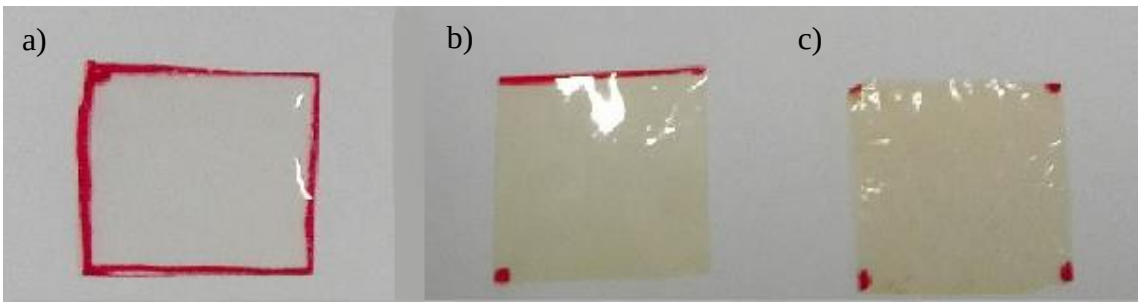


Figure 5.4. Photographs of: a) - Control chitosan film; b) – chitosan film with B220 with a 1:0.1 ratio; c) – chitosan film with B220 with a 1:0.5 ratio.

The results obtained for the mechanical properties are represented in Figure 5.5. Regarding the tension at rupture, there is no considerable difference between the chitosan film and the B220 1:0.1 film ($p \geq 0.05$), meaning that the film maintains its resistance with the incorporation of the extract. The B220 1:0.5 film presents a lower value than the other

films ($p \leq 0.05$), which could be due to the extract interfering with the chitosan matrix interaction between chains, decreasing its overall resistance. On the other hand, this film presents a lower Young's modulus and deformation percentage, meaning it is less brittle and more flexible. There are no significant differences in the mechanical properties between the control and the B220 1:0.1 film ($p \geq 0.05$), leading to conclusions that this concentration of extract is not high enough to weaken the chitosan matrix. A low extract concentration may be the preferable choice if the desired film has to be more resistant; and a high concentration if the film must be more elastic. Similar studies show that a ratio of 1:0.3 of arabinoxylans generally does not cause significant differences in the mechanical properties of chitosan films, which explains the results of the B220 1:0.1 films (61). This study also mentioned the interfering effect of the use of high concentration of compounds within the chitosan matrix (the case of the B220 1:0.5), causing it to be more fragile. The film network microstructure and intermolecular forces play an important role in the mechanical properties of chitosan film. It has been reported that the incorporation different compounds, such as polyphenols, into chitosan films may interrupt the ordered structure formation in the chitosan matrix, causing the intermolecular hydrogen bonding to weaken, disrupting the polymer–polymer chain interactions resulting in decreased mechanical properties (130,131). This allows to infer that with a higher incorporation of the B220 extract, there is a higher interaction with the chitosan matrix, leading to a plasticizer like effect in the mechanical properties of chitosan films by decreasing brittleness and increasing flexibility.

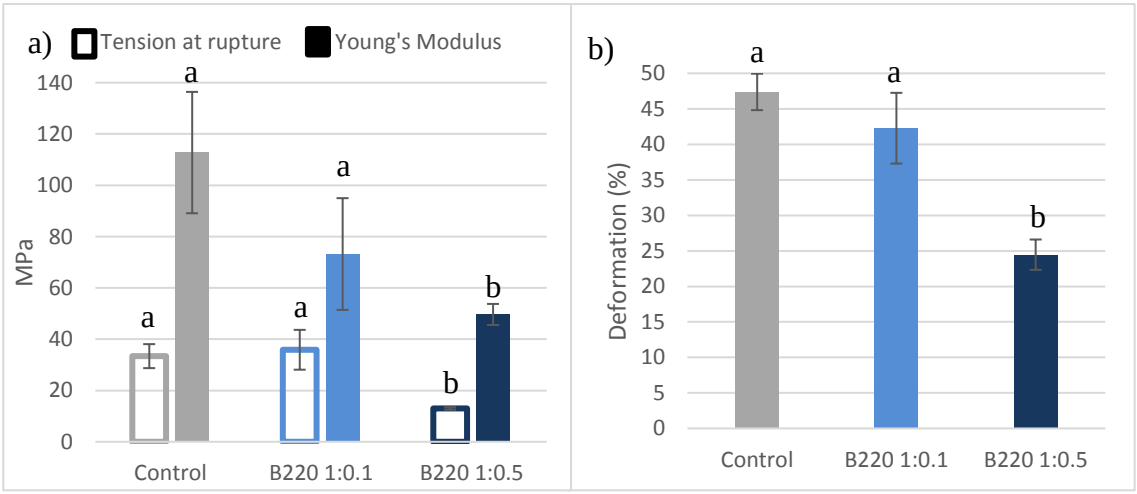


Figure 5.5. Mechanical properties for the control chitosan film, and the two different ratios of chitosan:extract (1:0.1 and 1:0.5): a) tension at rupture and Young's modulus; and b) deformation percentage.

To assess the surface hydrophobicity of the films, the contact angles of the films were measured (Figure 5.6). In the case of the film containing chitosan:B220 in the ratio of 1:0.1, the contact angle was not significantly different from the control film, which had a slightly higher contact angle than the one reported in literature (59), possibly due to the lower content of plasticizer (ratio of 1:4 in the present work, and of 2:5 in the bibliography). The B220 1:0.5 film presents a lower hydrophobicity than the control ($p \leq 0.05$). This can be explained by the fact that a greater quantity of compounds is in the chitosan matrix, originating a less compact structure that allows the water molecules to penetrate and thus reducing hydrophobicity. Some differences between the contact angle of the up and down surfaces are observed. This may be due to the distribution of the components of the film during the casting. Supposedly, denser species go into the bottom surface, while lighter stay up.

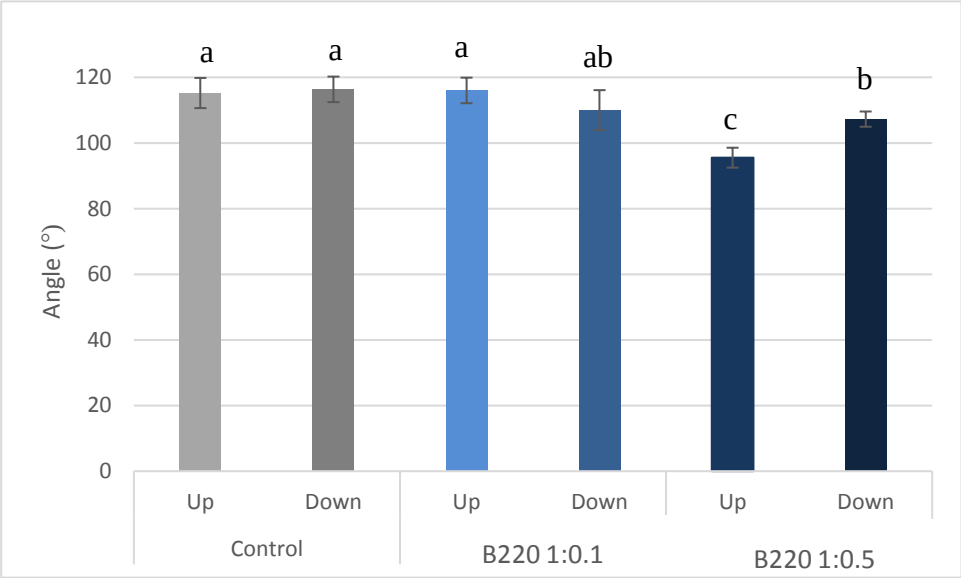


Figure 5.6. Average surface contact angles measured with ultra-pure water on the control and extract containing films.

The humidity and solubility of the films were also tested and the results are shown in Figure 5.7. In terms of humidity, the film with the highest ratio of extract presented a smaller humidity percentage than the one with the lowest ratio and the control ($p \leq 0.05$). This is probably due to the interference of compounds present in the extract, which at a higher concentration bind with chitosan, preventing it from bounding with the water molecules and thus having a lower water retention ability (132). Regarding solubility, the film with B220 1:0.5 showed a greater solubility than the B220 1:0.1 ($p \leq 0.05$). This could be due to the interference of the extracted compounds with the chitosan matrix, making

it more unstable and thus more easily soluble, which is in accordance with the mechanical properties and hydrophobicity (130,131). There were no significant differences between the B220 1:0.1 and the control film ($p \geq 0.05$), meaning the already high solubility of chitosan in acidic media, was not increased by this concentration of extract.

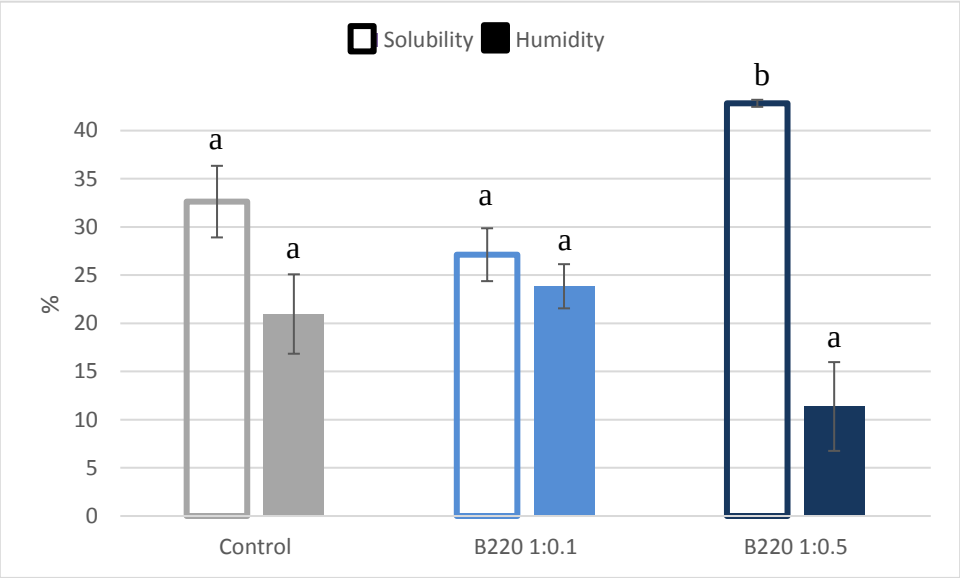


Figure 5.7. Humidity (%) and solubility (% weight loss) of each film in an acidic solution over 7 days.

5.2. Films produced using rice husks water extract

The selected water extract from rice husks was the one obtained with 200 ºC (H200), since it had a relatively high extraction yield (5%), carbohydrate content (24.5%), including arabinoxylans (46.4 mol%) and phenolic compound content (0.43 mg GAE/g). One film was formulated, using a chitosan:extract ratio of 1:0.1. Two controls of chitosan with added silica from the sol-gel extraction from rice husks were also made for comparison. The addition of rice husk extract gave a pale-yellow color to the films, whereas upon silica addition no visible color changes were visible, as can be seen in Figure 5.8.

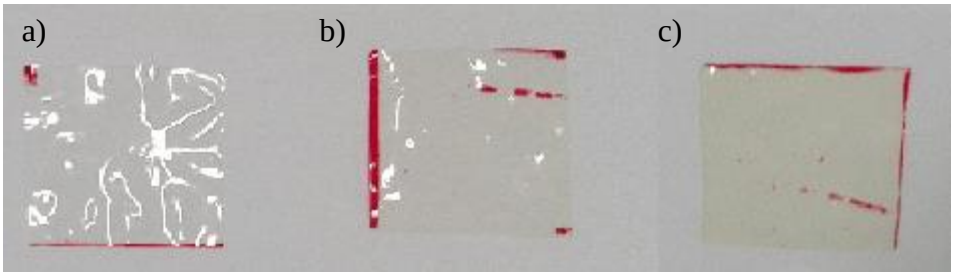


Figure 5.8. a) – Chitosan film with silica with a 1:0.1 ratio; b) – Chitosan film with silica with a a 1:0.5 ratio; c) - chitosan film with H200 with a 1:0.1 ratio.

The results for the mechanical properties are represented in Figure 5.9. Regarding tension at rupture and Young's modulus, there are no significant differences between the control chitosan film and the H200 1:0.1 film ($p \geq 0.05$). Although, the H200 1:0.1 film revealed to have a significantly lower deformation percentage at break ($p \leq 0.05$). This means that the elasticity was not affected by the extract, but the silica and polysaccharides presence in this extract caused the chitosan matrix to be less flexible. It is also possible to see that the addition of silica to the films decrease their resistance and the films become less elastic. A higher quantity of SiO_2 did present a higher tension at rupture than the lower quantity of silica, but not significantly different than the control film ($p \geq 0.05$). Different results have been described in the bibliography, with silica causing the films to be less brittle and more flexible, but the silica was dissolved in acidic medium, and in this case it was dissolved in water, and the same study shows that acidic conditions in the film (pH 3) were a key factor for the increased mechanical properties. At this pH there was a higher ionization of the biopolymer, increasing the interchain repulsion (55). When compared with the B220 films, the H200 1:0.1 film generally presented weaker mechanical characteristics. Regarding elasticity and deformation percentage, both B220 1:01 and B220 1:05 were more flexible than the H200 1:0.1 film, meaning the compounds present in the husks extract water have a greater impact in the elastic properties of the chitosan film. The main difference is that the rice husk extract has a greater quantity of arabinoxylans, and less starch, which has been proven to improve the tensile strength and elongation at break when incorporated in chitosan films. The rice husk extract also has a

larger quantity of total phenolics, compounds with lower molecular weight, which can also affect the chitosan matrix.

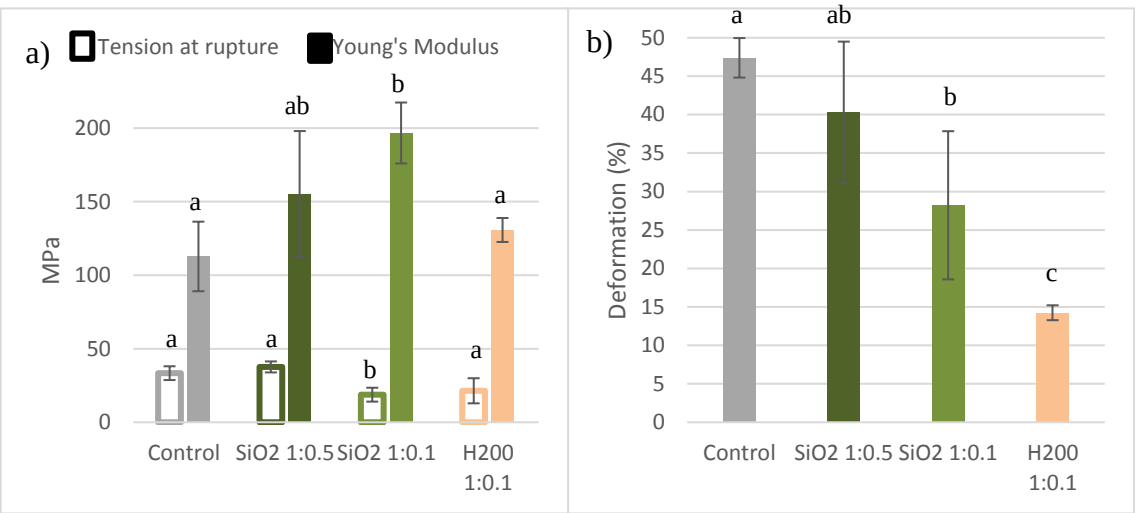


Figure 5.9. Mechanical properties for the control chitosan film, two different ratios of chitosan:silica (1:0.1 and 1:0.5) and at a 1:0.1 ratio of chitosan:H200 extract: a) tension at rupture and Young's modulus; and b) deformation percentage.

To assess the hydrophobicity of the films, the contact angle was tested. These results are presented in Figure 5.10. All films presented contact angles of around 120º. These films also showed no significant difference in hydrophobicity in relation to the control chitosan film. These results were also similar to those obtained for rice bran extract films. Overall, the surface hydrophobicity was not significantly affected by the addition of rice byproducts extracts.

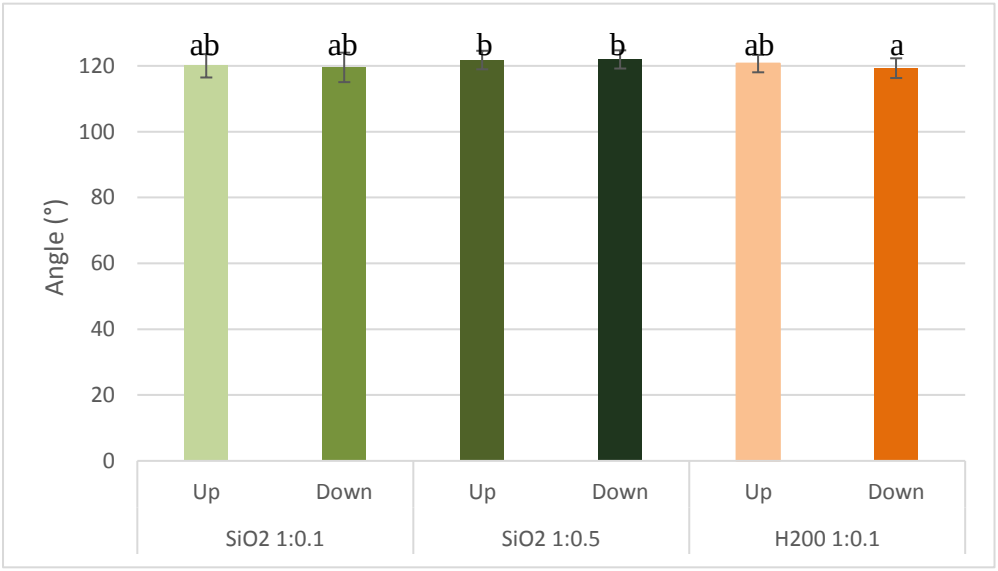


Figure 5.10. Average contact angles of the films with ultra-pure water.

The results for the humidity and solubility of the films with silica and H220 extract are showed in Figure 5.11. The results revealed that there are no significant differences in humidity between the different films, meaning that they have similar water retention capabilities. Similar tests in the literature reported similar results, since the addition of arabinoxylans, present in high quantity in this extract (46.4 mol% - Table 4.3), generally reveal no significant differences in humidity between the chitosan films and the ones with incorporation of arabinoxylans (61). Although, regarding solubility in acidic medium, the film with H200 shows a lower value than the other films ($p \leq 0.05$), which is desirable for food packaging.

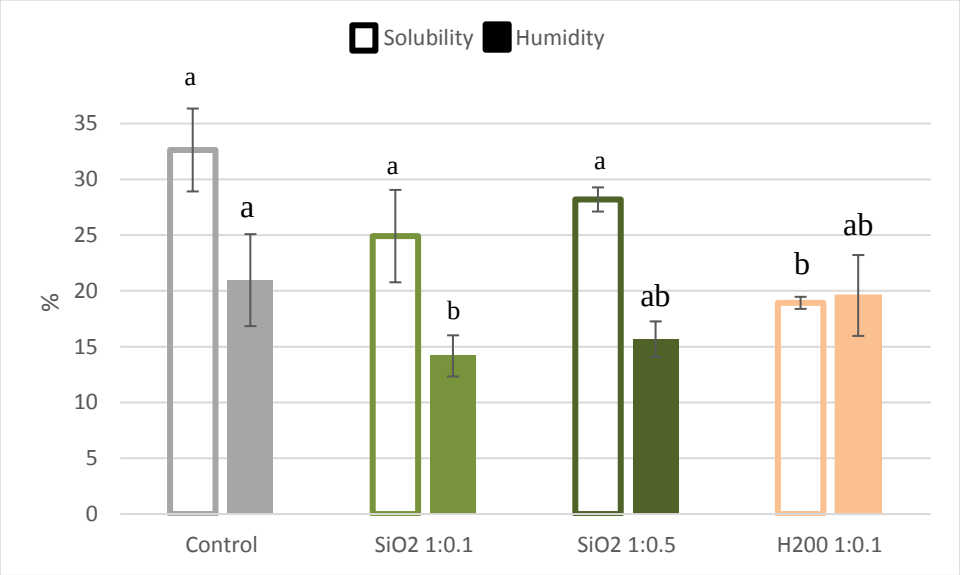


Figure 5.11. Humidity (%) and solubility in acidic water for 7 days (% weight loss) of each film.

5.3. Films antioxidant ability

The antioxidant ability of all the films was determined, to understand if the total phenolic compounds present in the water extracts, as seen in section 4.2 (Figure 4.5), give to chitosan films antioxidant ability when added to the formulation.

The results regarding the antioxidant activity of the films are represented in Figure 5.12.

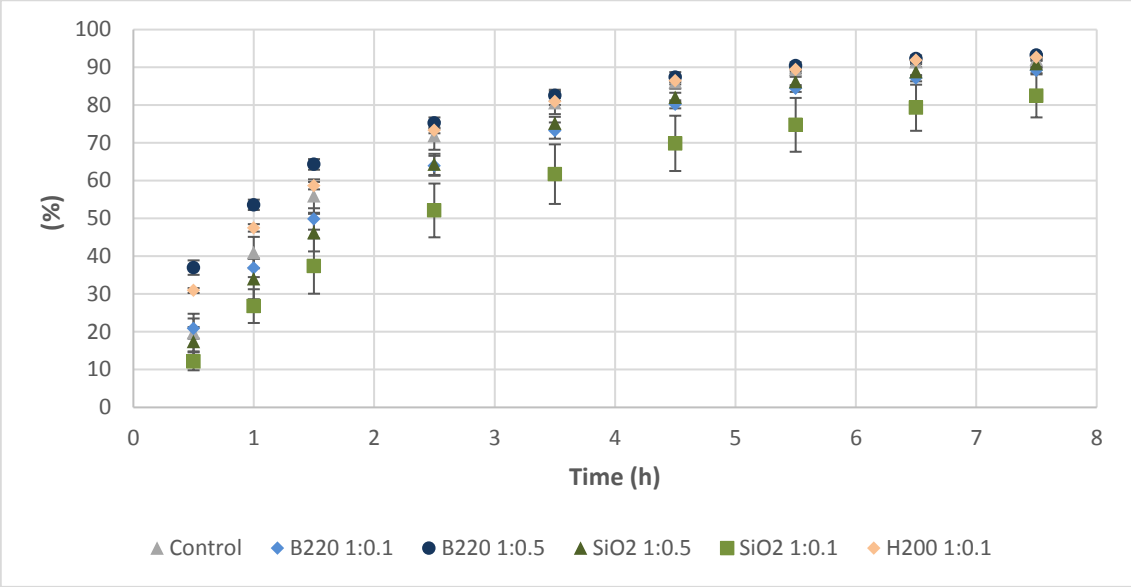


Figure 5.12. ABTS inhibition percentage for all films during 8 h.

All films reached an inhibition percentage around 90% after 6-7 hours. It is possible to see that at 1 h of reaction there is linearity. Therefore, the inhibition percentage after 1 h was the point chosen to compare the films antioxidant activity and it is represented in Figure 5.13. The films with both extracts (B220 1:0.5 and H200 1:0.1) showcased a greater ABTS inhibition and thus antioxidant activity than the remaining ($p \leq 0.05$). This is due to the concentration of phenolic compounds in these extracts, as seen in chapter 4.2 (0.24 mg GAE/mL in B220 and 0.4 mg GAE/mL in H200). This means that the already antioxidant activity of the chitosan film can be further increased by the addition of the byproducts extract waters.

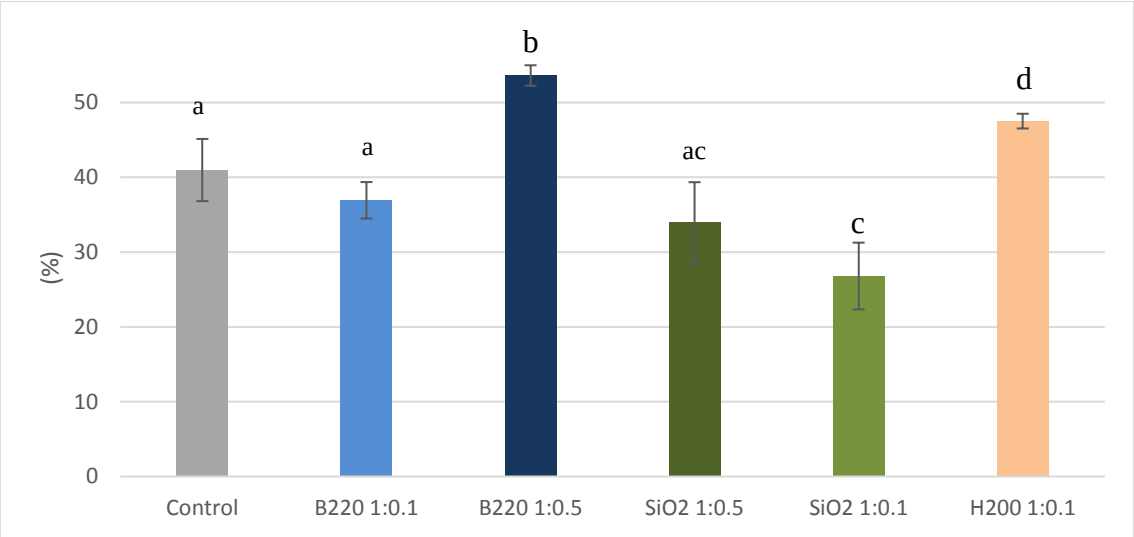


Figure 5.13. ABTS inhibition percentage for each film after 1h of reaction

An important trait to make the films active is the antioxidant activity. With this characteristic, the packages could help preserve food products for longer periods of time, by impeding their oxidation by reactive oxygen species (ROS). The addition of the extracts seems to improve the antioxidant property of chitosan. Films made with these extracts could theoretically protect food products from oxidation for longer periods of time, which is highly desirable.

Chapter 6 Conclusion and future work

6.1. Conclusion

In the first part of this work, a new environmental friendly method for the extraction of valuable active compounds from rice sub-products was tested, using temperature and pressure. Extracts rich in sugars, possibly with the presence of silica in the case of rice husks and protein in the case of rice bran were obtained using temperatures no higher than 220 ºC, just with water as solvent, having an overall inferior ecological impact when compared with the conventional extraction methods for these compounds, while adding value to these byproducts. Sequential extractions did not show great differences in terms of sugar, protein and phenolic compound extraction to the non-sequential extractions, so it seems to be better to perform this method non-sequentially. There was however a direct correlation between temperature and compound extraction, with higher temperatures generally extracting greater quantities of sugars, protein and phenolic compounds.

The second part of this Thesis was devoted to incorporate these extracts in chitosan films. The addition of these extracts managed to decrease solubility and increase antioxidant ability. The mechanical properties were affected, especially in the films with greater concentration of bran extracts (1:0.5 ratio) and in the films with husk extract, mainly the elongation percentage and the tensile strength, due to the interference of the extracts with the chitosan matrix. The hydrophobicity of the films was not severely affected by the addition of extracts, except for the case of the films with the greatest concentration of bran extract. The solubility in acidic medium was lowered by the addition of the husks extract. The films containing B220 had overall higher antioxidant ability than those with H200, as well as a lower solubility. Mechanical properties-wise, the films with bran extracts had better results, so depending on the application, both films present valuable characteristics.

Concluding, the addition of ecologically obtained extracts could originate improved chitosan films, able to preserve acidic foods for longer periods due to enhanced antioxidant activity. The addition of bran extracts can additionally improve mechanical properties.

6.2. Future work

- Testing different conditions for the hydrothermal treatment of rice bran and rice husks, such as temperature and holding time.
- Further characterize the extracts, to know if some important and valuable components are present, such as γ-oryzanol in rice bran water extracts.
- Test different concentrations of extracts in the films.
- Introduce new compounds in the films, such as anthocyanins, to test their use as visual indicators of shelf life.
- Characterize the solid extracts (biochar) and possibly apply them in the films formulation.

References

1. Kamm B, Gruber PR, Kamm M. Biorefineries-Industrial Processes and Products. In: Ullmann's Encyclopedia of Industrial Chemistry. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2016. p. 1–38.

2. Gandini A. Polymers from Renewable Resources: A Challenge for the Future of Macromolecular Materials. Macromolecules. 2008;41(24):9491–504.

3. Fernandes SC, Freire CS, Silvestre AJ, Pascoal Neto C, Gandini A. Novel materials based on chitosan and cellulose. Polym Int. 2011;60(6):875–82.

4. Shahidi F, Arachchi JKV, Jeon Y-J. Food applications of chitin and chitosans. Trends Food Sci Technol. 1999;10(2):37–51.

5. Caner C, Vergano PJ, Wiles JL. Chitosan Film Mechanical and Permeation Properties as Affected by Acid, Plasticizer, and Storage. J Food Sci. 2006;63(6):1049–53.

6. Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, Galed G, Heras A. Functional Characterization of Chitin and Chitosan. Curr Chem Biol. 2009;3(2):203–30.

7. Robert D, Enick M, Eric D, Beckman J, Johnson DJK, Hamilton DA. Chitin Chemistry, The. 1992;

8. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Prog Polym Sci. 2009;34(7):641–78.

9. Bobbink FD, Zhang J, Pierson Y, Chen X, Yan N. Conversion of chitin derived N-acetyl- d -glucosamine (NAG) into polyols over transition metal catalysts and hydrogen in water. Green Chem. 2015 Feb 9;17(2):1024–31.

10. Peniche C, Argüelles-Monal W, Goycoolea FM. Chitin and Chitosan: Major Sources, Properties and Applications. In: Monomers, Polymers and Composites from Renewable Resources. Elsevier; 2008. p. 517–42.

11. Quiroz-Castillo JM, Rodríguez-Félix DE, Grijalva-Monteverde H, Lizárraga-Laborín LL, Castillo-Ortega MM, Del Castillo-Castro T, Rodríguez-Félix F,

Herrera-Franco P. Preparation and Characterization of Films Extruded of Polyethylene/Chitosan Modified with Poly(lactic acid). Mater (Basel, Switzerland). 2014 Dec 30;8(1):137–48.

12. Campos CA, Gerschenson LN, Flores SK. Development of Edible Films and Coatings with Antimicrobial Activity.

13. Fei Liu X, Lin Guan Y, Zhi Yang D, Li Z, De Yao K. Antibacterial action of chitosan and carboxymethylated chitosan. J Appl Polym Sci. 2001;79(7):1324–35.

14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231–7.

15. Park P-J, Je J-Y, Kim S-K. Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. Carbohydr Polym. 2004;55(1):17–22.

16. Sousa F, Guebitz GM, Kokol V. Antimicrobial and antioxidant properties of chitosan enzymatically functionalized with flavonoids. Process Biochem. 2009;44(7):749–56.

17. Vinsova J, Vavrikova E. Recent Advances in Drugs and Prodrugs Design of Chitosan. Curr Pharm Des. 2008;14(13):1311–26.

18. Chien P-J, Sheu F, Huang W-T, Su M-S. Effect of molecular weight of chitosans on their antioxidative activities in apple juice. Food Chem. 2007;102(4):1192–8.

19. Tomida H, Yasufuku T, Fujii T, Kondo Y, Kai T, Anraku M. Polysaccharides as potential antioxidative compounds for extended-release matrix tablets. Carbohydr Res. 2010;345(1):82–6.

20. Kumar Dutta P, Dutta J, Tripathi VS. Chitin and chitosan: Chemistry, properties and applications. J Sci Ind Res. 2004;63:20–31.

21. Crini G. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. Prog Polym Sci. 2005;30(1):38–70.

22. Guibal E. Interactions of metal ions with chitosan-based sorbents: a review. Sep

Purif Technol. 2004;38(1):43–74.

23. Aberg CM, Chen T, Olumide A, Raghavan SR, Payne GF. Enzymatic Grafting of Peptides from Casein Hydrolysate to Chitosan. Potential for Value-Added Byproducts from Food-Processing Wastes. J Agric Food Chem. 2004;52(4):788–93.

24. Martín-Diana AB, Rico D, Barat JM, Barry-Ryan C. Orange juices enriched with chitosan: Optimisation for extending the shelf-life. Innov Food Sci Emerg Technol. 2009;10(4):590–600.

25. Spagna G, Barbagallo RN, Pifferi PG. Fining Treatments of White Wines by Means of Polymeric Adjuvants for Their Stabilization against Browning. J Agric Food Chem. 2000;48(10):4619–27.

26. Yingyuad S, Ruamsin S, Reekprkhon D, Douglas S, Pongamphai S, Siripatrawan U. Effect of chitosan coating and vacuum packaging on the quality of refrigerated grilled pork. Packag Technol Sci. 2006;19(3):149–57.

27. Soultos N, Tzikas Z, Abrahim A, Georgantelis D, Ambrosiadis I. Chitosan effects on quality properties of Greek style fresh pork sausages. Meat Sci. 2008;80(4):1150–6.

28. Cerqueira MA, Sousa-Gallagher MJ, Macedo I, Rodriguez-Aguilera R, Souza BWS, Teixeira JA, Vicente AA. Use of galactomannan edible coating application and storage temperature for prolonging shelf-life of "Regional" cheese. J Food Eng. 2010;97(1):87–94.

29. Galed G, Fernández-Valle M., Martínez A, Heras A. Application of MRI to monitor the process of ripening and decay in citrus treated with chitosan solutions. Magn Reson Imaging. 2004;22(1):127–37.

30. No HK, Meyers SP, Prinyawiwatkul W, Xu Z. Applications of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review. J Food Sci. 2007;72(5):R87–100.

31. Ribeiro C, Vicente AA, Teixeira JA, Miranda C. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biol Technol.

2007;44(1):63–70.

32. Devlieghere F, Vermeulen A, Debevere J. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 2004;21(6):703–14.

33. Caner C, Cansiz O. Effectiveness of chitosan-based coating in improving shelf-life of eggs. J Sci Food Agric. 2007;87(2):227–32.

34. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur J Pharm Biopharm. 2004;57(1):19–34.

35. Ravi Kumar MN. A review of chitin and chitosan applications. React Funct Polym. 2000;46(1):1–27.

36. Sung H-W, Chang Y, Chiu C-T, Chen C-N, Liang H-C. Crosslinking characteristics and mechanical properties of a bovine pericardium fixed with a naturally occurring crosslinking agent. J Biomed Mater Res. 1999;47(2):116–26.

37. Tsai C-C, Chang Y, Sung H-W, Hsu J-C, Chen C-N. Effects of heparin immobilization on the surface characteristics of a biological tissue fixed with a naturally occurring crosslinking agent (genipin): an in vitro study. Biomaterials. 2001;22(6):523–33.

38. Butler MF, Ng Y-F, Pudney PDA. Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. J Polym Sci Part A Polym Chem. 2003;41(24):3941–53.

39. Jelly R, Patton ELT, Lennard C, Lewis SW, Lim () KF. The detection of latent fingermarks on porous surfaces using amino acid sensitive reagents: A review. Anal Chim Acta. 2009;652(1-2):128–42.

40. Lee J-H, Lee D-U, Jeong C-S. Gardenia jasminoides Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. Food Chem Toxicol. 2009;47(6):1127–31.

41. Mi F-L, Sung H-W, Shyu S-S. Synthesis and characterization of a novel chitosan-

based network prepared using naturally occurring crosslinker. J Polym Sci Part A Polym Chem. 2000;38(15):2804–14.

42. Tharanathan RN, Kittur FS, Mcclements DJ. Critical Reviews in Food Science and Nutrition Chitin — The Undisputed Biomolecule of Great Potential Chitin — The Undisputed Biomolecule of Great Potential. Crit Rev Food Sci Nutr. 2003;43(431):61–8761.

43. Santosa FXB, Padua GW. Tensile Properties and Water Absorption of Zein Sheets Plasticized with Oleic and Linoleic Acids. 1999;

44. Ghasemlou M, Khodaiyan F, Oromiehie A. Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran. Carbohydr Polym. 2011;84(1):477–83.

45. Nugraha E. Suyatma, Lan Tighzert * and, Copinet A, Coma V. Effects of Hydrophilic Plasticizers on Mechanical, Thermal, and Surface Properties of Chitosan Films. 2005;

46. Srinivasa PC, Ramesh MN, Tharanathan RN. Effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. Food Hydrocoll. 2007;21(7):1113–22.

47. Debeaufort F, Quezada-Gallo J-A, Voilley A. Edible Films and Coatings: Tomorrow's Packagings: A Review. Crit Rev Food Sci Nutr. 1998;38(4):299–313.

48. Rhim J-W, Ng PKW. Natural Biopolymer-Based Nanocomposite Films for Packaging Applications. Crit Rev Food Sci Nutr. 2007;47(4):411–33.

49. Giannelis EP. Polymer Layered Silicate Nanocomposites. Adv Mater. 1996;8(1):29–35.

50. Radhakrishnan B, Ranjan R, Brittain WJ. Surface initiated polymerizations from silica nanoparticles. Soft Matter. 2006;2(5):386.

51. Rane A V, Abitha VK. Study of Mechanical, Thermal and Micro structural Properties of EPDM/Polypropylene/Nano clay Composites with Variable Compatibilizer Dosage. Environ Sci. 2015;6(1):60–9.

52. Vona ML Di, Marani D, D'Ottavi C, Trombetta M, Traversa E, Beurroies I, Knauth P, Licoccia S. A Simple New Route to Covalent Organic/Inorganic Hybrid Proton Exchange Polymeric Membranes. 2005;

53. Darder M, Colilla M, Ruiz-Hitzky E. Biopolymer−Clay Nanocomposites Based on Chitosan Intercalated in Montmorillonite. 2003;

54. Wang SF, Shen L, Tong YJ, Chen L, Phang IY, Lim PQ, Liu TX. Biopolymer chitosan/montmorillonite nanocomposites: Preparation and characterization. Polym Degrad Stab. 2005;90(1):123–31.

55. Oliveira FC, Barros-Timmons A, Lopes-da-Silva JA. Preparation and Characterization of Chitosan/SiO2 Composite Films. J Nanosci Nanotechnol. 2010;10(4):2816–25.

56. GRAS Notice 000298: Silicon dioxide.

57. Chen H, Zhang Y, Zhong Q. Physical and antimicrobial properties of spray-dried zein–casein nanocapsules with co-encapsulated eugenol and thymol. J Food Eng. 2015 Jan 1;144:93–102.

58. Sun L, Sun J, Chen L, Niu P, Yang X, Guo Y. Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material. Carbohydr Polym. 2017 May 1;163:81–91.

59. Ferreira AS, Nunes C, Castro A, Ferreira P, Coimbra MA. Influence of grape pomace extract incorporation on chitosan films properties. Carbohydr Polym. 2014 Nov 26;113:490–9.

60. Nunes C, Maricato É, Gonçalves FJ, Lopes-da-Silva JA, Rocha SM, Coimbra MA. Properties of Chitosan-Genipin Films Grafted with Phenolic Compounds from Red Wine. Trends Carbohydr Res. 2015;7(1):25–32.

61. Costa MJ, Cerqueira MA, Ruiz HA, Fougnies C, Richel A, Vicente AA, Teixeira JA, Aguedo M. Use of wheat bran arabinoxylans in chitosan-based films: Effect on physicochemical properties. Ind Crops Prod. 2015 Apr 1;66:305–11.

62. FAO Rice market monitor. FAO Rice Mark Monit. 2012;XV(4).

63. Rice by-products [Internet]. Available from: http://www.knowledgebank.irri.org/step-by-step-production/postharvest/rice-by-products#rice-husk

64. Prasad R, Pandey M. Rice Husk Ash as a Renewable Source for the Production of Value Added Silica Gel and its Application: An Overview. Bull Chem React Eng Catal. 2012;7(1):1–25.

65. Kim SP, Kang MY, Park JC, Nam SH, Friedman M. Rice Hull Smoke Extract Inactivates Salmonella Typhimurium in Laboratory Media and Protects Infected Mice against Mortality. J Food Sci. 2012;77(1):M80–5.

66. Kim SP, Yang JY, Kang MY, Park JC, Nam SH, Friedman M. Composition of Liquid Rice Hull Smoke and Anti-Inflammatory Effects in Mice. J Agric Food Chem. 2011;59(9):4570–81.

67. Yang JY, Kang MY, Nam SH, Friedman M. Antidiabetic Effects of Rice Hull Smoke Extract in Alloxan-Induced Diabetic Mice. J Agric Food Chem. 2012;60(1):87–94.

68. Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Sci Nutr. 2014 Mar;2(2):75–104.

69. Imamoglu E, Sukan FV. Scale-up and kinetic modeling for bioethanol production. Bioresour Technol. 2013;144:311–20.

70. Imamoglu E, Dalay MC, Sukan FV. Regional Differences in Rice Hulls Supply for Bioethanol Production. Appl Biochem Biotechnol. 2013;171(8):2065–74.

71. Lee S-C, Kim J-H, Nam KC, Ahn DU. Antioxidant Properties of Far Infrared-treated Rice Hull Extract in Irradiated Raw and Cooked Turkey Breast. J Food Sci. 2003;68(6):1904–9.

72. Singh A, Tuteja S, Singh N, Bishnoi NR. Enhanced saccharification of rice straw and hull by microwave–alkali pretreatment and lignocellulolytic enzyme production. Bioresour Technol. 2011;102(2):1773–82.

73. Mishra SP, Prasad SK, Dubey RS, Mishra M, Tiwari D, Lee S-M. Biosorptive behaviour of rice hulls for Cs-134 from aqueous solutions: A radiotracer study. Appl Radiat Isot. 2007;65(3):280–6.

74. Tsai WT, Lee MK, Chang YM. Fast pyrolysis of rice husk: Product yields and compositions. Bioresour Technol. 2007;98(1):22–8.

75. Patel M, Karera A, Prasanna P. Effect of thermal and chemical treatments on carbon and silica contents in rice husk. J Mater Sci. 1987;22(7):2457–64.

76. Chakraverty A, Mishra P, Banerjee HD. Investigation of combustion of raw and acid-leached rice husk for production of pure amorphous white silica. J Mater Sci. 1988;23(1):21–4.

77. Kamiya K, Oka A, Nasu H, Hashimoto T. Comparative Study of Structure of Silica Gels from Different Sources. J Sol-Gel Sci Technol. 2000;19(1/3):495–9.

78. Mansaray KG, Ghaly AE, Al-Taweel AM, Hamdullahpur F, Ugursal VI. Air gasification of rice husk in a dual distributor type fluidized bed gasifier. Biomass and Bioenergy. 1999;17(4):315–32.

79. Real C, Alcala MD, Criado JM. Preparation of Silica from Rice Husks. J Am Ceram Soc. 1996;79(8):2012–6.

80. Della V., Kühn I, Hotza D. Rice husk ash as an alternate source for active silica production. Mater Lett. 2002;57(4):818–21.

81. Kalapathy U, Proctor A, Shultz J. An improved method for production of silica from rice hull ash. Bioresour Technol. 2002;85(3):285–9.

82. Kamath SR, Proctor A. Silica Gel from Rice Hull Ash: Preparation and Characterization. Cereal Chem J. 1998;75(4):484–7.

83. Chiou T-Y, Ogino A, Kobayashi T, Adachi S. Characteristics and antioxidative ability of defatted rice bran extracts obtained using several extractants under subcritical conditions. J Oleo Sci. 2013;62(1):1–8.

84. Abdus M, Miah S, Satter MA, Ara H, Jabin S, Abedin N, Azad AK, Hossain A, Ara U. Nutritional Composition and Stabilization of Local Variety Rice Bran

BRRI-28 PhD and Ms thesis View project Cereal-based Functional Food for Diarrhea Affected Children View project Nutritional Composition and Stabilization of Local Variety Rice Bran BRRI-. Int J Sci Technol. 2014;3(5).

85. American Association of Cereal Chemists. GB, CRUZ LJ, ESPIRITU SG, SANTIAGO RG, JULIANO BO. Cereal chemistry. Vol. 43, Cereal Chemistry. American Association of Cereal Chemists; 1966. 145-155 p.

86. Saunders RM. Rice bran: Composition and potential food uses. Food Rev Int. 1985;1(3):465–95.

87. Champagne ET, editor. RICE: Chemistry and Technology. 3340 Pilot Knob Road, St. Paul, Minnesota 55121-2097, U.S.A.: American Association of Cereal Chemists, Inc.; 2004.

88. Champagne ET, Wood DF, Juliano BO, Bechtel DB. CHAPTER 4: THE RICE GRAIN AND ITS GROSS COMPOSITION. In: RICE: Chemistry and Technology. 3340 Pilot Knob Road, St. Paul, Minnesota 55121-2097, U.S.A.: American Association of Cereal Chemists, Inc.; 2004. p. 77–107.

89. Sharma R, Srivastava T, Saxena DC. Studies on Rice Bran and its benefits-A Review 1*. J Eng Res Appl www.ijera.com ISSN. 2015;5(52).

90. Rogers EJ, Rice SM, Nicolosi RJ, Carpenter DR, McClelland CA, Romanczyk LJ. Identification and quantitation of γ-oryzanol components and simultaneous assessment of tocols in rice bran oil. J Am Oil Chem Soc. 1993;70(3):301–7.

91. Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF, Mendonça CRB, Ramis-Ramos G. Composition, industrial processing and applications of rice bran γ-oryzanol. Food Chem. 2009 Jul 15;115(2):389–404.

92. Mellen PB, Walsh TF, Herrington DM. Whole grain intake and cardiovascular disease: A meta-analysis. Nutr Metab Cardiovasc Dis. 2008;18(4):283–90.

93. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, Fruit, and Cereal Fiber Intake and Risk of Coronary Heart Disease Among Men. JAMA J Am Med Assoc. 1996;275(6):447.

94. Truswell A. Cereal grains and coronary heart disease. Eur J Clin Nutr. 2002;56(1):1–14.

95. Whelton SP, Hyre AD, Pedersen B, Yi Y, Whelton PK, He J. Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. J Hypertens. 2005;23(3):475–81.

96. Qureshi AA, Sami SA, Khan FA. Effects of stabilized rice bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus Types I and II. J Nutr Biochem. 2002;13(3):175–87.

97. Premakumari S, Balasasirekha R, Gomathi K, Supriya S, Mohan J, Alagusundram K. Development and Acceptability of Fibre Enriched Ready Mixes. Int J Pure Appl Sci Technol Int J Pure Appl Sci Technol. 2012;9(92):74–83.

98. Sekhon KS, Dhillon SS, Singh N, Singh B. Functional suitability of commercially milled rice bran in India for use in different food products. Plant Foods Hum Nutr. 1997;50(2):127–40.

99. Younas A, Bhatti M, Ahmed A, Sci MR-PJA, 2011 U. Effect of rice bran supplementation on cookie baking quality. researchgate.net.

100. Al-Okbi SY, Hussein AMS, Hamed IM, Mohamed DA, Helal AM. Chemical, Rheological, Sensorial and Functional Properties of Gelatinized Corn- Rice Bran Flour Composite Corn Flakes and Tortilla Chips. J Food Process Preserv. 2014;38(1):83–9.

101. Charunuch C, Limsangouan N, Prasert, Wongkrajang K. Optimization of extrusion conditions for ready-to-eat breakfast cereal enhanced with defatted rice bran. Int Food Res J. 2014;21(2):713–22.

102. Pacheco de Delahaye E, Jiménez P, Pérez E. Effect of enrichment with high content dietary fiber stabilized rice bran flour on chemical and functional properties of storage frozen pizzas. J Food Eng. 2005;68(1):1–7.

103. Liu Z, Quek A, Kent Hoekman S, Balasubramanian R. Production of solid biochar fuel from waste biomass by hydrothermal carbonization. Fuel. 2013;103:943–9.

104. Reza MT, Lynam JG, Uddin MH, Coronella CJ. Hydrothermal carbonization: Fate of inorganics. Biomass and Bioenergy. 2013;49:86–94.

105. Parshetti GK, Kent Hoekman S, Balasubramanian R. Chemical, structural and combustion characteristics of carbonaceous products obtained by hydrothermal carbonization of palm empty fruit bunches. Bioresour Technol. 2013;135:683–9.

106. Funke A, Ziegler F. Hydrothermal carbonization of biomass: A summary and discussion of chemical mechanisms for process engineering. Biofuels, Bioprod Biorefining. 2010;4(2):160–77.

107. Busch D, Stark A, Kammann CI, Glaser B. Genotoxic and phytotoxic risk assessment of fresh and treated hydrochar from hydrothermal carbonization compared to biochar from pyrolysis. Ecotoxicol Environ Saf. 2013;97:59–66.

108. Kambo HS, Dutta A. Strength, storage, and combustion characteristics of densified lignocellulosic biomass produced via torrefaction and hydrothermal carbonization. Appl Energy. 2014;135:182–91.

109. Poerschmann J, Weiner B, Wedwitschka H, Zehnsdorf A, Koehler R, Kopinke F-D. Characterization of biochars and dissolved organic matter phases obtained upon hydrothermal carbonization of Elodea nuttallii. Bioresour Technol. 2015;189:145–53.

110. Poerschmann J, Weiner B, Wedwitschka H, Zehnsdorf A, Koehler R, Kopinke F-D. Characterization of biochars and dissolved organic matter phases obtained upon hydrothermal carbonization of Elodea nuttallii. Bioresour Technol. 2015;189:145–53.

111. Poerschmann J, Weiner B, Wedwitschka H, Baskyr I, Koehler R, Kopinke F-D. Characterization of biocoals and dissolved organic matter phases obtained upon hydrothermal carbonization of brewer's spent grain. Bioresour Technol. 2014;164:162–9.

112. Wu Q, Huang L, Yu S, Liu S, Xie C, Ragauskas AJ. Structural elucidation of hydro-products from hydrothermal carbonization of loblolly pine at different temperatures using NMR techniques. Energy. 2017;133:171–8.

113. Kalderis D, Kotti MS, Méndez A, Gascó G. Characterization of hydrochars produced by hydrothermal carbonization of rice husk. Solid Earth. 2014;5(1):477–83.

114. PetroviĿ J, PerišiĿ N, MaksimoviĿ JD, MaksimoviĿ V, KragoviĿ M, StojanoviĿ M, LauševiĿ M, MihajloviĿ M. Hydrothermal conversion of grape pomace: Detailed characterization of obtained hydrochar and liquid phase. J Anal Appl Pyrolysis. 2016 Mar 1;118:267–77.

115. Kalapathy U. A simple method for production of pure silica from rice hull ash. Bioresour Technol. 2000;73(3):257–62.

116. Fujihara S, Sasaki H, Aoyagi Y, Sugahara T. Nitrogen-to-Protein Conversion Factors for Some Cereal Products in Japan. J Food Sci. 2008;73(3):C204–9.

117. Selvendran RR, March JF, Ring SG. Determination of aldoses and uronic acid content of vegetable fiber. Anal Biochem. 1979 Jul 15;96(2):282–92.

118. Coimbra MA, Waldron KW, Selvendran RR. Isolation and characterisation of cell wall polymers from olive pulp (Olea europaea L.). Carbohydr Res. 1994 Jan 15;252:245–62.

119. American Society of Enologists. VL, Rossi JA. American journal of enology and viticulture. Vol. 16, American Journal of Enology and Viticulture. American Society of Enologists; 1965. 144-158 p.

120. Nabarlatz D, Ebringerová A, Montané D. Autohydrolysis of agricultural by-products for the production of xylo-oligosaccharides. Carbohydr Polym. 2007 May 1;69(1):20–8.

121. Galeener FL. Band limits and the vibrational spectra of tetrahedral glasses. Phys Rev B. 1979 Apr 15;19(8):4292–7.

122. Sen PN, Thorpe MF. Phonons in A X 2 glasses: From molecular to band-like modes. Phys Rev B. 1977 Apr 15;15(8):4030–8.

123. Boyd IW. Deconvolution of the infrared absorption peak of the vibrational stretching mode of silicon dioxide: Evidence for structural order? Appl Phys Lett.

1987 Aug 10;51(6):418–20.

124. Anker M, Stading M, Hermansson AM. Aging of whey protein films and the effect on mechanical and barrier properties. J Agric Food Chem. 2001 Feb;49(2):989–95.

125. Liu Y. Recent Progress in Fourier Transform Infrared (FTIR) Spectroscopy Study of Compositional, Structural and Physical Attributes of Developmental Cotton Fibers. Materials (Basel). 2013 Jan 22;6(1):299–313.

126. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997 Apr 1;2(4):152–9.

127. Chanliaud E, Saulnier L, Thibault J-F. Alkaline extraction and characterisation of heteroxylans from maize bran. J Cereal Sci. 1995 Jan 1;21(2):195–203.

128. Fidelis G, Queiroz M, Costa M, Santos P, Rocha H, Costa L. Proteolysis, NaOH and Ultrasound-Enhanced Extraction of Anticoagulant and Antioxidant Sulfated Polysaccharides from the Edible Seaweed, Gracilaria birdiae. Molecules. 2014 Nov 13;19(11):18511–26.

129. Hiebbeler RC. Resistência dos materiais. In: Trimer R, editor. 5th ed. São Paulo: Pearson Education Inc.; 2004. p. 62–72.

130. Wan Y, Creber KA., Peppley B, Bui VT. Ionic conductivity of chitosan membranes. Polymer (Guildf). 2003 Feb 1;44(4):1057–65.

131. Ahmed J, Mulla MZ, Arfat YA. Thermo-mechanical, structural characterization and antibacterial performance of solvent casted polylactide/cinnamon oil composite films. Food Control. 2016 Nov 1;69:196–204.

132. Siripatrawan U, Harte BR. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocoll. 2010 Nov;24(8):770–5.