Este trabajo se ha realizado en la Universidad de Valladolid y ha sido financiado por el Ministerio de Economía y Competitividad y el Fondo Europeo de Desarrollo Regional (FEDER) (Proyecto AGL2015-63849-C2-2-R), el Ministerio de Ciencia e Innovación (Proyecto PID2019-110809RB-I00/AEI/10.1303/501100011033) y la Junta de Castilla y León/FEDER (Proyecto VA195P20). El autor de esta Tesis Doctoral ha sido beneficiario de un contrato predoctoral de la Junta de Castilla y León y de una ayuda de la Fundación IMFAHE para la realización de una Estancia Breve en Purdue University (West Lafayette, IN, Estados Unidos de América).

Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN CIENCIA E INGENIERÍA AGROALIMENTARIA Y DE BIOSISTEMAS

TESIS DOCTORAL:

Physical modification of gluten-free flours by ultrasound treatments. Application to the development of new products suitable for the celiac population

Presentada por Antonio José Vela Corona para optar al grado de Doctor por la Universidad de Valladolid

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“Being fearless isn't being 100% not fearful, it's being terrified but you jump anyway...”

Taylor Swift

AGRADECIMIENTOS

Llega a su fin una meta que en algún momento me plantee, que requirió mucho trabajo y tiempo dedicado a ella, pero que también me permitió vivir muchas experiencias profesionales y personales que me hicieron crecer en todos los sentidos. Siempre me ha gustado la expresión "It takes a village to raise a child", porque me parece muy acertada al hacer referencia que el trabajo que conlleva criar a un hijo no es solo de los padres sino de todo un círculo de personas que oscilan alrededor. Por eso yo creo que también es válido decir "It takes a village to get a PhD", porque es algo que no he hecho solo, y quiero agradecer a todas las personas que me acompañaron durante este proceso.

Gracias a Feli por haber apostado por mí en 2017 cuando me animó a pedir la ayuda predoctoral de la Junta de Castilla y León, y por haber aportado tanto a mi formación profesional durante los últimos 4 años. La oportunidad para hacer la tesis no podía haber llegado en un mejor momento, te voy a estar por siempre agradecido. Espero haber cumplido con lo que esperabas de mí.

Gracias a mis papás, Mario y Mirna, por haberme apoyado en todo lo que he hecho desde niño, por siempre asegurarse de que no me falte nada sin importar en donde me encuentre, y por haberme enseñado a soñar y esforzarme por alcanzar las cosas que quiero. A Andrea, Mario, Dani, y mis sobrinos Mario José y Anna Letizia, y a Bruno. Por su inagotable cariño y constante apoyo, y especialmente durante estos años del doctorado por estar siempre junto a mí a pesar de la distancia. Son lo más valioso que tengo, nada de lo que he logrado sería posible sin ustedes.

Gracias a mis abuelitas Nicolasa Gramajo y Zoila Vela, por estar siempre pendientes de mí, por todo su cariño, y por darme ánimos, consentirme y guiarme desde pequeño. A mis tíos Nolo, Cefalia, Migue, Vero, Dorita, Viole, Estelita y Walter. A mis primos Mirni, Juanma, Mavi, Mike, Javi, Alice, Fabi, William, Isa, Jose, Walter Samuel y Ana Lucia. A mis sobrinos Xime, Pablo, Isabella, Mariana, Elenita, Natalia, Nicolle y Manuel Adrián. Gracias por su cariño y sus ánimos constantes.

Gracias a mis amigos en Guate, en especial a Rodrigo Escobar, Paula Eva, Anne Tongo, Dana Mejia, Sofi García Salas, Yazmin Ortíz, Celeste Gonzalez y Max Hoegg. Porque siguen estando presentes desde nuestros años en la UVG, por todas las experiencias compartidas y por darme ánimos desde que les dije que iba a comenzar el doctorado.

Gracias a Marina Villanueva por haber sido mi amiga y mentora desde el día que llegué a Valladolid. Aprendí mucho de ti, gracias por hacer del sitio de trabajo un ambiente de amistad. Mi experiencia en el doctorando y mi tiempo en Valladolid no habría sido lo mismo sin ti. Gracias por todo.

Gracias a Sandra Pérez Quirce y a Pedro Caballero, miembros de mi grupo de investigación que conocí años atrás cuando cursaba el Master. Por haber sido mis tutores durante el TFM, que me inició en el mundo de la investigación, y por tantos años compartidos desde entonces.

Gracias a mis amigos de PROCEREALtech que me dieron la bienvenida al grupo, Ángela García Solaesa, Ángel Gutiérrez, Laura Murillo y Teresa Sigüenza, por las risas y tantos buenos momentos compartidos. Me sentí parte del grupo desde el principio y fue gracias a ustedes que no me costó nada llegar y adaptarme a una ciudad nueva. Que la vida nos regale muchos encuentros más en el futuro.

Gracias a los miembros de PROCEREALtech que llegaron después de mí, Ainhoa Vicente, Aloisa Deriu, Rito Mendoza, Caleb Calix, Grazielle Náthia, Workineh Abebe, Cristina Gil y Raúl Mauro. Fue un gusto compartir estos años con ustedes, todos han aportado en muchas maneras a mi experiencia durante la tesis.

Gracias a los chicos a quienes dirigí sus Trabajos Fin de Master: Tania Villegas, Oscar Fernández Oraa, Fernando Pérez Yágüez e Isa Revuelta. Disfruté compartiendo mis conocimientos con ustedes a la vez que aprendía con ustedes.

Gracias a mis queridos amigos Mafer González, Iván Navarro y Gabi Porsani. Haberlos tenido en España durante estos años hizo que me disfrutara aún más de esta etapa. Gracias por su amistad durante todos estos años y por tantos buenos momentos compartidos.

Thanks to Dr. Bruce Hamaker and everyone I met at Purdue University, Oluwatoyin Sangokunle, Yony Román Ochoa, Jongbin Lim, Oguz Ozturk, Thaisa Cantu Jungles, Marwa El Hindawy, Veda Krishnan, Nuseybe Bulut, Pablo Torres-Aguilar, Anton Terekhov, and Angie Gutterman, for their warmness and for welcoming me into the group during my time in West Lafayette.

Thanks to the researchers who welcomed me for the short stays I was able to do as a predoc: Joanna Harasym, Agnieszka Orkusz, and Urszula Kaim from the Wroclaw University of Economics and Business, and Costas Biliaderis, Athina Lazaridou, Kostas Mouzakitis, Vasso Sereti, and Evangelia Papagianni from the Aristotle University of Thessaloniki. You were all so open to me even if my time visiting was extremely short. I really appreciated getting to know you, as well as the learning and cultural enrichment that your universities and cities led me experience.

ABSTRACT

Gluten-free market has gained a greater presence in the food industry in recent years due to increasing population being diagnosed with celiac disease or gluten intolerance, and consumers who decide to follow a gluten-free diet for considering it as a healthier alternative, who demand a wider range of good quality products. Gluten removal, however, represents a technical challenge in the development of products, due to the structural role of gluten in most of these products. The natural characteristics of gluten-free flours (and their starch) do not always meet specific industry requirements due to processing limitations, such as low shear resistance, thermal resistance, and high tendency towards retrogradation. Different modification techniques (chemical, enzymatic, genetic, mechanical, and physical) are applied to starches and flours with the aim of increasing their range of industrial applicability, being physical modifications the best perceived for involving more environmentally friendly methods, and for requiring, in general, shorter and simpler processing. Ultrasound treatments are physical modification methods that have demonstrated significant improvements in the modification of starches from different botanical origins. However, the modification of gluten-free flours with ultrasounds has not been greatly studied despite being an important ingredient in the food industry.

The objective of this doctoral thesis was to get deeper knowledge about the physical modification of gluten-free flours by ultrasound treatments, to stablish the impact of key treatment parameters on the degree of modification achieved, and to use ultrasonicated flour in the development of gluten-free breads to determine the effect that the modification has on the quality of the final products. Ultrasound treatments were performed at a frequency of 24 kHz, a maximum power of 180 W, and a on/off cycle of 80 %, and were applied to flours of different nature: rice, tef, corn, and quinoa. Ultrasound treatments are always performed in excess water so that the acoustic waves generate a homogeneous modification in the studied sample.

The study included an initial phase to determine the effect that three important parameters of ultrasound treatments had on the modification generated to rice flour. These parameters were treatment time (between 2 and 60 min), flour concentration in the treated dispersion [between

5 % and 30 % (w/w)], and treatment temperature (between 20 °C and 60 °C). The research began with rice flour since it is the most commonly used ingredient in gluten-free formulations. The results obtained indicated that the modifications were significant starting from short times, and that they could be observed even at high concentrations. Treatment temperature was the parameter that influenced the most the modification caused to the treated flours. Because of this, in the next phase of the study two varieties of tef were investigated, brown and white, applying treatments of 10 min, at a concentration of 25 % (w/w), and temperatures between 20 °C and 55 °C. The modifications caused to rice and tef flours were quantified in their morphological, techno-functional, hydration, structural, thermal, and pasting properties, as well as in the rheological properties of the gels made with them. The results obtained showed that ultrasound treatments led to an overall particle size reduction of the flours, which increased their interaction with water, demonstrated by a marked swelling power increase in ultrasonicated flours. Size exclusion chromatography indicated that ultrasound treatments generated a higher proportion of intermediate length amylose chains (degree of polymerization 300 – 1600), as result of starch chains fragmentation, which took place mostly on α-(1,4) bonds rather than α-(1,6) bonds, as demonstrated by proton nuclear magnetic resonance. Thermal properties indicated a reduction of gelatinization enthalpy, as well as a reduction of the gelatinization temperature range, mainly when treatments were performed at higher temperatures. The pasting temperatures were increased in ultrasonicated flours, while the height of their viscometric profiles was rediced. The ultrasound-modified flours led to gels with higher consistency and a higher elastic component under all studied conditions.

The method applied to remove the water from the dispersion proved to have a very significant effect on the properties of the flours obtained. Freeze-drying, which is the most conservative and preferred method in laboratory trials, was compared with another more easily transferable to the industry method, such as centrifugation followed by low-temperatrue drying. The study was applied to four gluten-free flours of different nature and content of soluble substances, or substances solubilized by ultrasonication (rice, tef, corn, and quinoa) which were treated at optimized conditions [treatments of 10 min, concentration of 25 % (w/w), and temperature of 20 °C]. Results showed marked differences between pairs of treated samples, attributed to the loss of soluble and solubilized compounds during centrifugation, which led to differences in the

composition of ultrasound-treated flours depending on the drying method applied. The influence of water removal method was greater in whole-grain flours (tef and quinoa).

Finally, the ultrasound-treated rice flour was used in the formulation of rice breads replacing 30 % of the rice flour used in the formula. The thermal, pasting, fermentation and rheological properties of the breads' doughs were stablished, as well as the physical quality of the breads obtained. Results showed that the flour particle fragmentation, and the partial starch depolymerization caused by ultrasonication facilitated the accessibility of yeasts to simpler sugars, accelerating the generation of carbon dioxide and a greater development of the dough during proofing, which resulted in breads with greater volume and a softer crumb, showing a maximum effect in the 10 min treatment.

Ultrasound treatments proved to be a feasible technology to modify the physicochemical properties of gluten-free flours. Results indicated that the treatment conditions applied influence the final characteristics of the modified flour. Treatment temperature was the most influential variable in modulating flour properties, due to a combined effect of ultrasonication and annealing, resulting in an amplified effect of the treatments when higher temperatures were applied. The use of ultrasonicated flours in the formulation of gluten-free breads improved the baking performance of rice flour.

RESUMEN

El mercado de los productos sin gluten ha adquirido una mayor presencia en la industria alimentaria en los últimos años debido al aumento de la población diagnosticada con celiaquía o intolerancia al gluten, y a los consumidores que deciden llevar una dieta libre de gluten por considerarla una opción más saludable, que demandan una mayor gama de productos de buena calidad. La eliminación del gluten, sin embargo, representa un reto técnico en el desarrollo de productos, debido al papel estructural que tiene el gluten en la mayoría de estos productos. Las características naturales de las harinas sin gluten (y de su almidón) no siempre se ajustan a los requisitos específicos de la industria debido a limitaciones en su procesamiento, como la baja resistencia al cizallamiento, la resistencia térmica, y la alta tendencia a la retrogradación. Diferentes técnicas de modificación (químicas, enzimáticas, genéticas, mecánicas y físicas) se aplican a almidones y harinas con el objetivo de aumentar su rango de aplicabilidad industrial, siendo las modificaciones físicas las mejor percibidas por utilizar métodos más respetuosos con el medio ambiente, y por requerir, en general, procesados más cortos y sencillos. Los tratamientos con ultrasonidos son métodos de modificación física que han demostrado importantes mejoras en la modificación de almidones de diferentes orígenes botánicos. Sin embargo, la modificación de harinas sin gluten con ultrasonidos no ha sido estudiada de forma generalizada a pesar de ser un ingrediente importante en la industria alimentaria.

El objetivo de esta tesis doctoral fue profundizar en el conocimiento de la modificación física de las harinas sin gluten mediante tratamientos con ultrasonidos, para establecer el impacto que tienen los parámetros clave del tratamiento en el grado de modificación alcanzado, y utilizar la harina ultrasonicada en la elaboración de pan sin gluten para determinar el efecto que la modificación tiene en la calidad del producto final. Los tratamientos con ultrasonidos se realizaron a una frecuencia de 24 kHz, una potencia máxima de 180 W y un ciclo de encendido y apagado del 80 %, y se aplicaron a harinas de diferente naturaleza: de arroz, tef, maíz y quinoa. Los tratamientos con ultrasonidos se realizan siempre en exceso de agua para que las ondas acústicas generen una modificación homogénea de la materia estudiada.

El estudio incluyó una fase inicial de determinación del efecto que tres parámetros importantes del tratamiento de ultrasonidos ejercían sobre la modificación generada sobre harina de arroz. Estos fueron el tiempo de tratamiento (entre 2 y 60 min), la concentración de harina en la dispersión tratada [entre 5 % y 30 % (p/p)], y la temperatura del tratamiento (entre 20 °C y 60 °C). Se inició la investigación con harina de arroz por tratarse del ingrediente más utilizado en las formulaciones sin gluten. Los resultados obtenidos indicaron que las modificaciones fueron significativas desde tiempos cortos, y que se podían observar incluso a concentraciones elevadas. La temperatura fue la variable que más influyó en la modificación generada en las harinas tratadas. Debido a esto, en la siguiente fase del estudio se trabajó con harina de tef de dos variedades, marrón y blanca, aplicando tratamientos de 10 min, una concentración del 25 % (p/p) y temperaturas entre 20 °C y 55 °C. Las modificaciones provocadas en las harinas de arroz y de tef se cuantificaron en sus propiedades morfológicas, tecno-funcionales, de hidratación, estructurales, térmicas y de empastado, así como en las propiedades reológicas de los geles elaborados con las mismas. Los resultados obtenidos mostraron que los tratamientos con ultrasonidos llevaron a una reducción general del tamaño de partícula de las harinas, lo que incrementó su interacción con el agua, demostrado por un marcado incremento de la capacidad de hinchamiento en las harinas ultrasonicadas. La cromatografía por exclusión de tamaño indicó que los tratamientos con ultrasonidos generaban una mayor proporción de cadenas de amilosa de longitud intermedia (grado de polimerización 300 – 1600), como resultado de la fragmentación de las cadenas del almidón, que tuvieron lugar mayoritariamente sobre los enlaces α-(1,4) frente a los α-(1,6), como se demostró mediante resonancia magnética nuclear de protón. Las propiedades térmicas indicaron una reducción de la entalpía de gelatinización, así como una reducción del rango de temperatura de gelatinización, principalmente en los tratamientos realizados a temperaturas más altas. La temperatura de empastado aumentó en las harinas sonicadas al tiempo que se redujo la altura de sus perfiles viscométricos. Las harinas modificadas con ultrasonidos dieron lugar a geles con una mayor consistencia y una mayor componente elástica en todas las condiciones estudiadas.

El método de eliminación de agua de la dispersión demostró tener un efecto muy significativo sobre las propiedades de las harinas obtenidas. La liofilización, que es el método mas conservador y de preferencia en ensayos de laboratorio, se decidió comparar con otro método más fácilmente transferible a la industria, como es la centrifugación seguida de secado a baja

temperatura. El estudio se aplicó a cuatro harinas sin gluten de diferente naturaleza y contenido en sustancias solubles o solubilizables por sonicación (arroz, tef, maíz y quinoa) que fueron tratadas siguiendo condiciones ya optimizadas [10 min tratamiento, 25 % (p/p) concentracion y 20 ºC de temperatura]. Los resultados mostraron marcadas diferencias entre pares de muestras tratadas, atribuibles a la eliminación de componentes solubles y solubilizados perdidos en la centrifugación, lo que llevó a diferencias en la composición de las harinas tratadas con ultrasonidos dependiendo del método de secado aplicado. La influencia del método de eliminación de agua fue mayor en harinas integrales (tef y quinoa).

La harina de arroz tratada con ultrasonidos se utilizó finalmente en la formulación de panes de arroz en sustitución del 30 % de la harina de arroz utilizada en la fórmula. Se establecieron las propiedades térmicas, de empastado, de fermentación y reológicas de las masas de pan elaboradas, y la calidad física de los panes obtenidos. Los resultados obtenidos mostraron que la fragmentación de partículas de harina y la depolimerización parcial de almidón causada por la ultrasonicación facilitó la accesibilidad de levaduras a azúcares más simples, acelerando la generación de dióxido de carbono y un mayor desarrollo de la masa durante la fermentación, lo que dio lugar a panes con mayor volumen y una miga más suave, mostrando un efecto máximo en el tratamiento de 10 min.

Los tratamientos con ultrasonidos demostraron ser una tecnología viable para modificar las propiedades fisicoquímicas de las harinas sin gluten. Los resultados indicaron que las condiciones de tratamiento aplicadas influyen en las características finales de la harina modificada. La temperatura de tratamiento fue la variable más influyente en la modulación de las propiedades de las harinas, debido a un efecto combinado de la ultrasonicación y annealing, resultando en un efecto magnificado de los tratamientos al aplicar las temperaturas más altas. El uso de harinas ultrasonicadas en la formulación de panes sin gluten permitió mejorar el desempeño en panificación de la harina de arroz.

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I INTRODUCTION

Starch is the energy reserve polysaccharide of various plants, and one of the most abundant carbohydrates found in nature. It is found in many different plant organs including seeds, fruits, tubers, and roots, where it is used as a source of energy (Herceg et al., 2010). Starches are found in the form of partly crystalline granules insoluble in water, having different size, morphology, composition, molecular weight, and physicochemical properties depending on its botanical origin (Bai et al., 2017; Sujka, 2017). Starch granules consist of a mixture of mainly two polysaccharides: amylose and amylopectin. Amylose is essentially a linear macromolecule consisting of a α-D-glucan chain linked through α-(1→4) linkages with a degree of polymerization (DP) between 1000 and 10,000 glucose units (Iida et al., 2008; Kang et al., 2016). Only a small portion of amylose (0.1 %) are branched via α-(1→6) linkages. The length of these branch chains varies from 4 to 100 DP (Kang et al., 2016). Amylopectin, on the other hand, is a highly branched macromolecule composed mainly by α-(1→4) linked D-glucopyranose (as in amylose) linked through non-random α-(1→6) linkages at a greater proportion than amylose (Iida et al., 2008; Kang et al., 2016). Both components differ on molecular weight, degree of ramification and chemical properties. Starches show concentric rings of alternating layers of amorphous and crystalline structures of amylose and amylopectin. The amorphous regions consist of amylose and amylopectin chains in a disordered conformation, while the crystalline rings are formed by the double helices in clusters of amylopectin branches (Bel Haaj et al., 2013). The ratio and proportion of these components go from 20 % to 25 % for amylose and from 75 % to 80 % for amylopectin, depending on the starch (Jambrak et al., 2010). Starches also contain small amounts of non-carbohydrate constituents like lipids, phosphates, and proteins (Luo et al., 2008). The interaction of these minor components with amylose and amylopectin can influence the properties and functional behavior of the starch (Chan et al., 2010).

Starches have numerous benefits such as being widespread, abundantly available, cheap, biodegradable, pollution-free and renewable (Zheng et al., 2013). Starches are the main source

of carbohydrate in human diet, supplying almost two thirds of the required daily calories (Bai et al., 2017), representing a valuable ingredient in the food industry, in a wide variety of applications such as thickening, gelling agent, bulking agent, water retention agent and adhesive (Iida et al., 2008; Zheng et al., 2013). Starch is the main component in flours and grains (whose percentage varies depending on each source), with other primary nutrients like proteins, fat, and other carbohydrates (Harasym et al., 2020). Starch intake in human diet derives from consumption of food products containing flours as an ingredient (widely used for food formulations) and cooked grains (e.g., rice, maize, oat, quinoa, buckwheat), a basic food in different cultures all over the world.

However, despite the several industrial use, the proprieties of native starch usually do not fulfill the industry's specific requirements because of limitations such as low shear resistance, thermal resistance, thermal decomposition, and high tendency towards retrogradation (Singh et al., 2007). As a solution to this matter the native starch granules can be modified to obtain improved properties. Starch physicochemical properties can be changed through genetic, mechanical, chemical, enzymatic modification, or physical treatment (Zheng et al., 2013; F. Zhu, 2015). In recent years there has been an increasing attraction towards modifications by physical treatments, especially in food applications. Physical treatments have the advantage of allowing to reach similar properties to those obtained in chemically modified starches but without the need of using chemical agents, hence being considered cleaner and environment-friendly procedures. Another advantage with these treatments is that there is not a legal obligation requiring physically modified starch to be labeled as such, which does not happen in the case of chemically modified starches. Some known methods of physical modification of starches and flours include extrusion, hydrothermal treatments, microwave, radiation, and ultrasound (Zheng et al., 2013).

1. Physical modification of starch by means of ultrasound waves

Among the techniques of physical modification of starches, ultrasound (US) treatment is an approach that has shown many advantages in terms of higher selectivity, efficiency, and quality, requires shorter processing time, represents reduced physical and chemical risks, and reduces the waste generation and energy consumption (Amini et al., 2015; Chemat et al., 2011; Sujka, 2017; Zhu, 2015; Zuo et al., 2009). In recent years several investigations have been carried out

regarding the modification of starches by US treatments, where significant effects have been achieved in their physicochemical properties (Li et al., 2018).

The term ultrasound refers to mechanical waves with a frequency above the threshold of the human hearing (18 kHz) that originate from either a piezoelectric or magnetostrictive transducer within high frequency electrical fields that create high-energy vibrations (Amini et al., 2015; Herceg et al., 2010). These vibrations are later amplified and transferred to a probe/sonotrode or a bath in direct contact with the fluid to be treated (Jambrak et al., 2010). Ultrasound waves need an elastic medium to spread over, that is why starches are always treated in suspensions (Chemat et al., 2011). Water is the most commonly used solvent in these types of modifications, given that it is the safest solvent for food applications. US can be divided in three regions of frequency: Low frequency ultrasound (also known as power ultrasound) in the region of 16 to 100 kHz, high-frequency ultrasound in the range from 100 kHz to 1 MHz, and diagnostic ultrasound from 1 to 10 MHz (Jambrak et al., 2010).

In US treatments the acoustic energy cannot be absorbed by the molecules of the medium, but it is transformed to a usable form by the cavitation phenomenon. The sinusoidal ultrasound waves move through the aqueous medium inducing a longitudinal displacement of particles, resulting in a rapid and successive cyclic motion of compression and rarefaction phases into the medium, which generates pressure that causes mixing within the medium (Chemat et al., 2011; Sit et al., 2014). Multiple tiny bubbles are formed and collapse in the medium during the cycles of compression and rarefaction, which comprises the cavitation phenomenon (see Figure 1), the most important event of the US treatments (Czechowska-Biskup et al., 2005; Patist & Bates, 2008; Chatel et al., 2016). When bubbles collapse, high energy is released and converted to high pressure (up to 20 MPa) and hot spots (high temperatures up to 5000 °C) that can generate both physical and chemical effects in the modification of starches (Amini et al., 2015; Li et al., 2018). The physical effects of cavitation involves intense micro-jets streaming with high velocity (hundreds of m/s) towards the surface of the treated particles in a very short time, shear forces and shock waves produced by the bubble collapse (Li et al., 2018). During the collapse, the acoustic bubble becomes asymmetric and the bubble wall accelerates more on the side opposite to the solid surface, resulting in the formation of a strong micro-jet of water directed towards the particle's surface (Zuo et al., 2012), which brings material fatigue followed by a gradual tearing off of microscopic particles capable of breaking polymeric chains (Czechowska-Biskup

et al., 2005; Degrois et al., 1974). Furthermore, the turbulent flow generated by sonication can also induce granule-granule collision, and the collision between the starch granules and the wall of the container where they are hosted during sonication, which can result in starch granule damage (Zuo et al., 2012). On the other hand, the collapsing bubbles can also have a chemical effect on the modification of starches by the generation of free radicals, such as hydroxide (-OH) and hydrogen (-H), as a result of the dissociation of the solvent molecules by the action of cavitation (Li et al., 2018; Sujka, 2017), which may contribute to the chemical modification of the system (Chemat et al., 2011; Zhu, 2015). It has been observed in general that low-frequency high-power ultrasounds lead to a greater mechanical effect over the granules, while high-frequency low-power ultrasounds are widely used in food applications where the generation of free-radicals is needed (Ashokkumar, 2015; Chatel et al., 2016; Zuo et al., 2009).

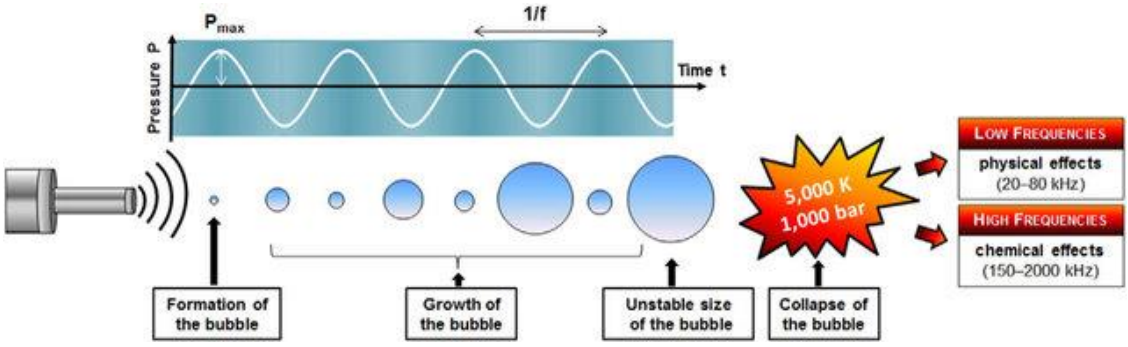


Figure 1. Schematic representation of the acoustic cavitation phenomenon (Chatel et al., 2016).

The total amount of energy released by cavitation depends on the kinetics of the bubble growth and their collapse. The sizes of the bubbles vary depending on the applied frequency (Sujka, 2017). The theoretical resonance size at 20 kHz is around 150 μm, while at a frequency of 1 MHz the resonance size is considered to be about 3 μm (Bai et al., 2017). Hence, it is estimated that low frequency ultrasounds produce relatively large bubbles, compared to the starch granule size (5 – 80 μm), which releases more energy, inducing greater shear damage on the granule surface after the bubbles collapse (Bai et al., 2017). Also, at lower frequencies there is more time to form the bubbles, and the energy of the shock waves is augmented (Gallant et al., 1972). The solvent used for sonication also influences the effect of cavitation over the treated particles. Cavitation intensity increases with surface tension at the bubble interface and decreases with increasing vapor pressure of the medium, thus liquids having small surface tension require lower

energy to produce cavitation bubbles, resulting in cavitation occurring more readily (Hemwimol et al., 2006).

Ultrasound cavitation can significantly increase the temperature of the starch suspension, having a strong thermal effect on the modified starch. Yu et al. (2013) determined that higher temperatures are reached when sonicating at higher power (see Figure 2). If the temperature reached by treatment is higher than the onset temperature of starch gelatinization, the starch will swell and gelatinize in the water during treatment, making temperature a critical variable to be controlled in US modification of starches (Amini et al., 2015). The temperature at which US treatment is performed is also said to influence the effect of cavitation. Temperature affects the vapor pressure of water in which elevated temperatures decrease the transmitted energy, resulting in reduced cavitation intensity (Amini et al., 2015).

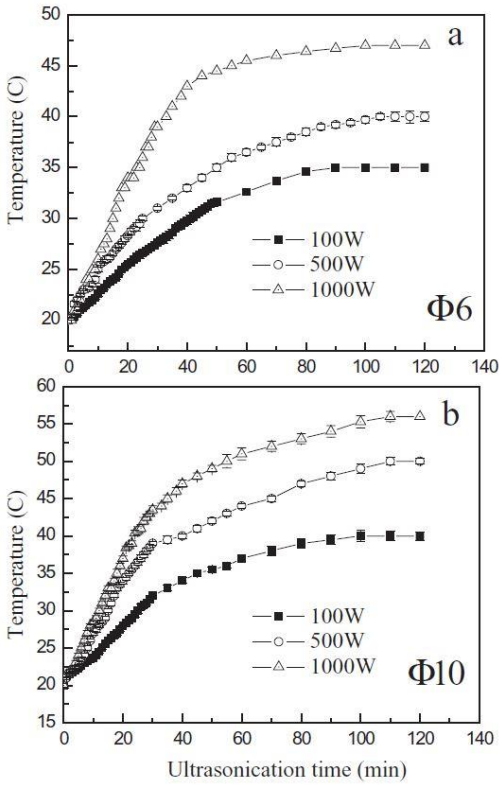


Figure 2. Relationship of ultrasonication time and temperature of rice starch suspension under different ultrasound power and intensity, using a probe of (a) 6 mm and (b) 10 mm (Yu et al., 2013).

The US treatments can affect the starch dispersions in at least three ways: (a) causing physical degradation of the particles, displayed as pitting and cracks in the surface; (b) leading to reduction of the molecular weight of amylose and amylopectin resulting from breakage of C-C bonds; and (c) Solubilizing swollen starch granules, including "ghost" granules that remain even after the complete gelatinization of the starch dispersion (Zuo et al., 2009). These effects depend strongly on treatment conditions, such as applied frequency, power, amplitude, time and temperature, as well as conditions of the treated suspension, such as biological origin of the starch, solvent used, suspension concentration and treated amount (Luo et al., 2008; Zhu, 2015; Zuo et al., 2009). Published results regarding the modification of starches by US are strongly dependent on these complex experimental conditions arrays, making it complicated to reach a consensus regarding the effect caused by ultrasonication on their properties. Several studies have investigated the properties of ultrasound-modified starches from different botanical origins, and confirmed different effects on the morphological, molecular, physicochemical, functional, rheological and digestion properties depending on the applied treatment conditions (refer to Table 1). The most important findings are presented below.

2. Effect of ultrasound modifications in the morphological properties of starches

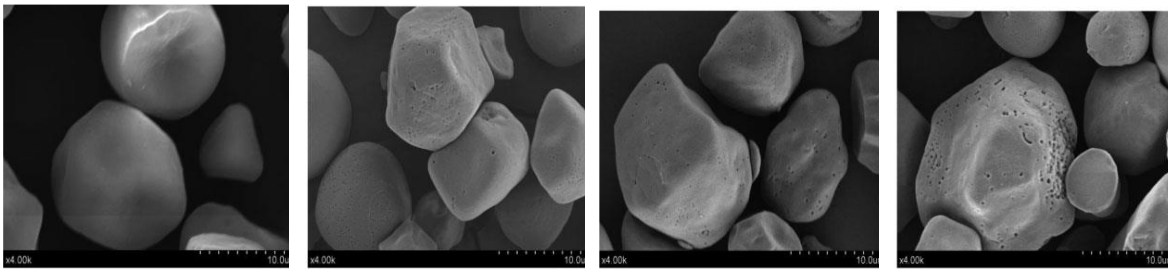
The morphology of starch granules after being ultrasonicated has been studied by different techniques, including light microscopy (LM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), optical microscopy, field-emission scanning electron microscopy (FE-SEM) and confocal laser scanning microscopy (CLSM) to evaluate surface modification, and laser diffraction analysis to determine the granule size distribution.

2.1 Surface damage

It has been widely found that ultrasounds lead to granular damage, which in the starch granule surface has been describes as cracks (Amini et al., 2015; Babu et al., 2019; Gallant et al., 1972), holes (Falsafi et al., 2019; Hu et al., 2019), pits (Bai et al., 2017; Czechowska-Biskup et al., 2005; L. Wang et al., 2022), pores (Flores-Silva et al., 2017; Hu et al., 2019; Li et al., 2019; Yang, Kong et al., 2019), scratches (Li et al., 2018; Sujka & Jamroz, 2013), roughness (Ding et al., 2019; Rahaman et al., 2021), grooves (Yang, Lu et al., 2019; Zhu et al., 2012), fissures (Chan et al., 2010; Jin et al., 2020; Luo et al., 2008), fractures (Karwasra et al., 2020) and channels (Kaur &

Gill, 2019; Monroy et al., 2018). This surface damage derives from the cavitation phenomenon, attributed to high pressure induced in starch granules vicinity, causing shear forces on starch granules surface (Falsafi et al., 2019; Luo et al., 2008), which might even induce the destruction of starch granules (Wang et al., 2022). Some studies did not report any effect of ultrasonication on starch granule surface, which might be due to low sonication power during treatment or the application of US by bath, given that more marked effects of sonication have been reported when applying by probe (Jambrak et al., 2010; Zuo et al., 2009).

Porosity of the granules influences the starch's chemical reactivity, since pores, channels and cavities increase the surface area of the starch and may allow reagents and enzymes to more easily penetrate into the bulk of the granule which may potentially speed up chemical and enzymatic reactions (Huang et al., 2007; Sujka, 2017). It is generally accepted that greater power (lower frequencies), longer times, and higher temperatures lead to a more noticeable impact over starch surface. The damage appreciated on granule surface would greatly depend on the applied frequency given that it limits the size at which bubbles collapse (Bai et al., 2017). The number of pits generated per granule would increase with increasing sonication frequencies. Furthermore, Zheng et al. (2013) determined a higher number of dents and holes in sweet potato starch after treatment by dual frequency (25+80 kHz) than what was observed in single frequency treatments (25 or 80 kHz) (see Figure 3). Dual frequency ultrasound, meaning two beams of US propagating together at the same time in the treated solution, could cause greater damage to starches because the cavitation yield of dual-frequency ultrasound is higher and the bubbles collapse faster than in single-frequency treatments (Hu et al., 2015, 2019; Zheng et al., 2013).



a. Native starch b. 25 kHz US c. 80 kHz US d. 25 kHz + 80 kHz US

Figure 3. SEM photos of sweet potato starch samples sonicated for 60 min at different frequencies (4000×) (Zheng et al., 2013).

Table 1. Summary of the available literature about modifications of starches, flours, and grains by ultrasound treatments.

Botanical origin/Reference	Treatment parameters / Method of analysis	Effect of treatment
STARCHES		
Potato starch (Gallant et al., 1972)	• US probe (10 cm tip Φ); Freq.: 960 kHz and 280 kHz; Int.: 15 W/cm²; Time: 3, 6, 16 and 32 min; Temp.: 5 °C; Atm.: Air, O_2, H_2, CO_2, vacuum; Conc.: 3, 11 mg/mL; Vol.: 1, 2 mL • Light microscopy (LM); Scanning electron microscopy (SEM)	In atmosphere of H_2, many deep pits were produced. In air or CO_2, deep pitting was less pronounced, but injury to other parts of the surface was greater. Virtually no effect was produced in vacuum and under CO_2. Damage increased with time and decreased with increasing concentration of starch.
Potato starch (Degrois et al., 1974)	• Freq.: 960 and 280 kHz; Int.: 1-15 W/cm²; Time: 3-32 min; Temp.: 5 °C; Atm.: Air, O_2, H_2, CO_2, vacuum; Conc.: 3, 11 mg/mL; Vol.: 1, 3 mL • SEM, Optical microscopy	The type of gas affected the nature and degree of granule erosion. The damage caused to starch was greater at lower concentrations and volumes.
Potato starch Sweet potato powder (Azhar & Hamdy, 1979)	• US probe (1.9 cm tip Φ); Freq.:20 kHz; Power: 300 W; Amp.: 98 %; Time: 15, 30 and 60 min; Temp.: 1 – 2 °C; Conc.: 1, 0.75 and 0.5 % • Presence of reducing sugars; Hydrolysis of starch by β-amylase; Polyacrylamide-gel electrophoresis	A rapid decrease in relative viscosity of sonicated starch was determined, particularly during the first 5 min exposure. Maximum degradation of potato starch was observed after 30 min sonication, evidenced by the decrease in relative viscosity.
Waxy rice starch (Isono et al., 1994)	• US probe (36 mm tip Φ); Freq.:20 kHz; Power: 600 W • Gel permeation chromatography (Number average molecular weight and molecular weight distribution)	US decreased average molecular weight, accelerated by higher power. At long sonication, the number average molecular weight tended to a constant value, and the molecular weight distribution tended to be fairly narrow.
Wheat starch (Seguchi et al., 1994)	• US probe (1/4 in tip Φ); Time: 300, 600,900, 1200, 1500, 1800, 2100, 2400 and 2700 s; Temp.: 40 °C; Solv.: 90 % DMSO; Sample: 250 mg • Gel filtration chromatography (molecular weight); Light scattering microscopy; Transmission electron microscopy (TEM)	With increasing US time, the solution turned more transparent, its viscosity decreased, and the linear increase of starch reducing power was observed. Wheat starch agglomerates were gradually reduced in size by shear force of sonication
Mung bean starch Potato starch Rice starch (Chung et al., 2002)	• US probe (3.2 mm tip Φ); Power: 30 W; Time: 1, 3 and 5 min; Temp.: pre-heating at 95 °C; Conc.: 5 % (w/w). Sample: 28 g • Apparent viscosity; Inherent viscosity; Paste clarity; Degree of polymerization (DP); Swelling power (SP)	Decrease of the apparent and inherent viscosities of starches after ultrasonication. Average degree of polymerization did not change by US. Starch paste became more transparent.
Corn starch (Czechowska-Biskup et al., 2005)	• US bath; Freq.: 360 kHz; Power: 170 W/kg; Temp.: 22±2 °C; Atm: Ar, He and O_2; Sample: 10 mL • Viscosity measurements (viscosity-average molecular weight.)	Starch molecular weight was reduced by US treatment. The yield of this degradation process depended on polymer concentration, ultrasound power and gas used to saturate the solution.
Corn starch (Huang et al., 2007)	• US probe (6 mm tip Φ); Power: 500 W; Time: 3 - 15 min; Pulse: 15 s on / 5 s off; Conc.: 30 % (w/v); Sample: 200 g • Degree of hydrolysis; Polarized light microscopy (PLM); SEM; X-ray diffraction (XRD); Differential scanning calorimetry (DSC); Reaction with propylene oxide; Pasting properties	The crystalline structure of treated corn starch did not change, but the amorphous area was slightly destroyed. Pores and channels were detected. Gelatinization enthalpy and temperature first increased and then slightly dropped with increasing degree of hydrolysis.
Starches: waxy maize, potato, tapioca, sweet potato, and corn *(Sonication applied to gelatinized starches)* (Iida et al., 2008)	• US [probe (12.7 mm tip Φ); Power: 120 W; Sample: 100 g] [bath; Power: 100 W; Sample: 500 g]; Time: 30 min; Temp.: 60 °C; Conc.: 5, 10 % • Degree of granule disintegration; Degree of solubilization; High performance gel permeation chromatography (HP-GPC); ^1H and ^{13}C Nuclear magnetic resonance (NMR) spectroscopy	Sonication drastically depressed the solution viscosity without altering the chemical structure of the polymer chains. The molecular weight sharply decreased in the initial period of 10-30 min and thereafter the depolymerization proceed slowly.

Starches: normal maize, waxy maize, and amylomaize V (Luo et al., 2008)	• US bath; Power: 100 W; Time: 30 min; Temp.: 30 °C; Conc.: 30 %; Sample: 100 g • SEM; Laser light scattering (particle size); SP and solubility (S); XRD; DSC; Freeze-thaw stability; Pasting properties	Porous and a fissure were observed on the surface of treated starches. X-ray pattern was not changed by US, while swelling power, solubility and gelatinization transition temperatures were increased. A viscosity drop was observed. US degraded preferentially the amorphous regions of starches.
Waxy rice starch (Zuo et al., 2009)	• US bath; Freq.: 211 kHz; Power: 2.5, 4.1 W; Int.: 0.11, 0.18 W/cm^2; Time: ≤ 60 min; Temp.: 25–70 °C; Conc.: 5 % (w/w); Sample: 60 g • Pasting properties; Particle size distribution (PSD); SEM; High-performance size exclusion chromatography-multiple laser light scattering (SEC-MALLS)	A viscosity reduction was found in samples sonicated at high temperature. Particle size was reduced by US, while no surface damage was seen by SEM, and no reduction of starch molecules was found with SEC-MALS.
Starches: corn, potato, mung bean and sago (Chan et al., 2010)	• US bath; Time: 10 min; Solv.: water and sodium dodecyl sulphate (2 % w/v); Conc.: 40 % (w/v); Sample: 250 g • Amylose content by spectrophotometric method; SEM; SP and S; Pasting properties [Rapid Visco Analyzer (RVA)]	Sonication appeared to induce a rough surface and fine fissures on starch granules. The combination of SDS and sonication increased amylose content, swelling and solubility of starches. Peak viscosity was increased while pasting temperature was reduced.
Corn starch (Herceg et al., 2010)	• US [probe (7 mm tip Φ); Freq.: 24 kHz; Power: 100, 300, 400 W; Int.: 34, 55, 73 W/cm^2; Amp.: 100 %] [bath; Freq.: 24 kHz; Power: 300 W; Int.: 2 W/cm^2]; Time: 15 and 30 min; Conc.: 10 % (w/w); Vol.: 500 mL • Pasting properties; S; Gel textural properties; Micrograph pictures; DSC	Sonication with probes caused lowering of the starting gelatinization temperature. US treatment caused disruption of starch granules and made them more permeable to water, resulting in increased solubility. More mechanical damage was observed when applying more powerful ultrasound.
Corn starch (Jambrak et al., 2010)	• US [probe (7 mm tip Φ); Freq.: 24 kHz; Power: 100, 300, 400 W; Int.: 34, 55, 73 W/cm^2; Amp.: 100 %] [bath; Freq.: 24 kHz; Power: 300 W; Int.: 2 W/cm^2]; Time: 15, 30 min; Conc.: 10 % (w/w); Vol.: 500 mL • DSC; Rheological properties; Turbidity; SP; Micrography	Ultrasonication distorted the crystalline region in starch granules, caused a decreased in gelatinization enthalpy, a significant decrease in consistency coefficient (k), and increase in swelling power. Micrography showed impact on the granules structure and size.
High-amylose maize starch (Lima & Andrade, 2010)	• Melt-processing + Ultrasound; US probe (13 mm tip Φ); Freq.: 20 kHz; Power: 750 W; Amp.: 40 %; Time: 30 min; Temp.: 10 °C; Conc.: 5 g/L • ^{1}H NMR; Viscosity measurements; XRD	US caused a significant reduction in intrinsic viscosity for the sample previously processed with the highest glycerol content, revealing a significant reduction in amylose molar mass.
Tapioca starch (Manchun et al., 2012)	• US probe; Freq.: 24 kHz; Power: 400 W; Amp.: 50 and 100 %; Time: 10, 20 and 30 min; Conc.: 3 % (w/w) • SEM; SP and S; XRD	US treatment distorted the crystalline region in starch granules, especially at higher amplitude or longer time. Swelling power and solubility increased after treatments, associated with improved water absorption capacity.
Potato starch (Zhu et al., 2012)	• US probe (13 mm tip Φ); Freq.: 20 kHz; Power: 60, 105, and 155 W; Time: 30 min; Pulse: 2 s on / 2 s off; Temp.: Controlled; Conc.: 10 % (w/w); Vol.: 30 mL • SEM; PLM; XRD; Small angle X-ray scattering (SAXS)	US induced notch and groove on starch granule surface. B-type crystal structure was scarcely affected. Ultrasonication affected cluster structure, especially the crystalline region, with a reduction in the molecular order in crystalline lamellae. No particle size reduction was determined.
Potato starch (Zuo et al., 2012)	• US probe (13 mm tip Φ); Freq.: 20 kHz; Power: 0 - 60 W; Time: 30 min; Temp.: 5 °C; Conc.: 0.1 % (w/w); Vol.: 5 mL • LM; Starch granule damage; Rheological measurements	US caused starch surface damage. The number of defects first increased linearly with an increase in US power up to a threshold level. There was a linear dependence of the number of defects on the US power.
Waxy maize starch Standard maize starch (Bel Haaj et al., 2013)	• US probe (13 mm tip Φ); Freq.: 24 kHz; Power: 170 W; Time: > 75 min; Temp.: 8 °C; Conc.: 1.5 % (w/w); Vol.: 100 mL • Wide-angle-X-ray diffraction (WAXD); Raman spectroscopy; Transmittance; PSD; Dynamic light scattering (DLS) (Z-average size); Field effect scanning electron microscopy (FE-SEM)	Particle size of starch granules decreased continuously with ultrasonication time, generating nanoparticles between 30 and 100 nm. Sonication seriously disrupted the crystalline structure of clustered amylopectin.

Starches: potato, wheat, corn, and rice (Sujka & Jamroz, 2013)	• US probe; Freq.: 20 kHz; Power: 170 W; Time: 30 min; Temp.: 20 °C; Solv.: water and ethanol; Conc.: 30 % (w/v) • Blue value and iodine absorption spectra; Fat and water absorption; Transmittance; Paste viscosity; Least gelling concentration; SP and S; SEM; TEM	Depolymerization of starch was higher when it was sonicated in water than in ethanol. Cracks and depressions were found on the surface of granules, especially in potato and wheat starches. Ultrasonication increased fat and water absorption, least gelling concentration, solubility and swelling power, and decreased starch paste viscosity.
Sweet potato starch (Zheng et al., 2013)	• Freq.: single (25 and 80 kHz) and dual (25+80 kHz); Power: 720 W; Time: 10, 20, 30, 45 and 60 min; Temp.: 30±2 °C; Conc.: 5 % (w/w) • SEM; Starch-iodine complex absorption; Fourier transform infrared spectroscopy (FTIR); Pasting properties; S; Transparency	Starch-iodine complex analysis showed that US destroyed amylopectin and starch chains. Damage to the crystalline structure was detected by FTIR. Peak viscosity was reduced, while solubility and transmittance were increased. Dual frequency US caused more changes than single frequency.
Non-waxy rice starch (Yu et al., 2013)	• US probe (6 and 10 mm tip Φ); Freq.: 24 kHz; Power: 100, 500 and 1000 W; Time: 0 – 120 min; Conc.: 5 % (w/v); Sample: 15 g; Vol.: 300 mL • DSC	Higher temperatures were reached in starch suspensions when sonicating at higher power. High ultrasound power and strong intensity can effectively change the gelatinization and retrogradation properties or rice starch.
Corn starch (Hu et al., 2014)	• US bath; Freq.: single (20 and 25 kHz) and dual (20+25 kHz); Time: 5, 10, 15, 20, 30, 40 min; Temp.: 30 °C; Solv.: ethanol solution (20 % v/v); Conc.: 5 % (w/v); Sample: 50 g. • Transmittance; Gel texture properties; DSC; Freeze-thaw stability; XRD	Transparency of starch paste was improved by US, but hardness, brittleness, elasticity, adhesiveness, conglutination degree, chewiness and recoverability decreased, as well as starch crystallinity and enthalpy values. Dual frequency treatment was found to be more effective than single frequency treatment.
Taro starch (Sit et al., 2014)	• US probe (7 mm tip Φ); Freq.: 30 kHz; Power: 100 W; Amp.: 50 and 100 %; Time: 5, 10 min; Cycle: 0.5, 1; Temp.: 20 °C; Sample: 100 g • SP and S; Clarity of starch paste; Color; Pasting properties; Texture analysis; Freeze-thaw stability	A significant increase in swelling, solubility, pasting and texture properties of the ultrasonically extracted starch was observed. The freeze-thaw stability was slightly better after ultrasonication. The whiteness of the starch powders was lower after ultrasonication.
Corn starch (Amini et al., 2015)	• US probe (3 mm tip Φ); Freq.: 24 kHz; Power: 150 W; Amp.: 0, 50, 100 %; Cycle: 80 %; Time: 5, 10, 15 min; Temp.: 25–65 °C; Conc.: 10, 15, 20 % (w/w); Vol.: 50mL • LM; SEM; SP and S; Transmittance; DP; DSC; XRD; Rheological properties	The influence of sonication strongly depended on temperature and treatment time, while concentration and amplitude had little influence on functional and rheological properties. The most effective parameter was sonication temperature followed by exposure time and starch concentration.
Corn starch (Hu et al., 2015)	• US bath; Freq.: single (20 and 25 kHz) and dual (20+25 kHz); Time: 40 min; Temp.: 30 °C; Conc.: 5 % (w/w) • Pasting properties; SEM; Determination of cavitation yield by iodine release method	After US the structure of starch granules presented many dents and holes. Gel properties were decreased, while thermal stability and retrogradation was enhanced. Peak viscosity decreased as US frequency increased. Dual-frequency treatment caused more obvious damage than single-frequency.
Pinhão starch (Pinto et al., 2015)	• US probe (5 mm tip Φ); Single US treatment, dual ANN+US, HMT+US, US+ANN and US+HMT treatments; Freq.: 20 kHz; Amp.: 50 %; Time: 90 min; Pulse: 30 s on / 5 s off; Temp.: controlled; Conc.: 25 % (w/v); Sample: 100 g; Vol.: 400 mL • HP-GPC; WAXD; SEM; SP and S; RVA; DSC	Relative crystallinity decreased in sonicated starch, where no visible cracks were detected. Sonication provided high swelling power, with no effect on solubility. When US was applied as second treatment, an peak viscosity increase was determined. US-treated starch presented the highest breakdown viscosity. Gelatinization enthalpy decreased after US.
Plantain starch Taro starch (Carmona-García et al., 2016)	• US probe (7 mm tip Φ); Freq.: 25 kHz; Power: 80 W; Amp.: 20 %; Time: 20 and 50 min; Temp.: 4 °C; Conc.: 5 % (w/v); Vol.: 100 mL • Optical microscopy; SEM; Laser diffraction analysis; SP and S; XRD; DSC; Pasting properties; Rheological properties	Ultrasounds caused profound cavities and fractures, without causing a reduction of granule size. Peak viscosity, swelling power and solubility increased after treatments. Sonication of high granule size starch resulted in more pronounced decrease in storage modulus (G').

Corn starch *(Sonication applied to gelatinized starches)* (Kang et al., 2016)	• US probe (13 mm tip **Φ**); Freq.: 20 kHz; Power: 13.5 and 29.9 W; Time: ≤ 20 min; Conc.: 5 and 10 % (w/w); Vol.: 20 mL • LM; FTIR; Rheological properties; Particle size measurements (dynamic light scattering)	Viscosity and hydrodynamic radius decreased with increasing US time. FTIR showed molecular scission at C-O-C bond of α-1,6 glycosidic linkage, with the extent of breakage being inversely correlated to amylose content. High-amylose starch pastes were more resistant to US due to aggregation.
Normal potato starch Waxy potato starch (Bai et al., 2017)	• US bath; Freq.: 1 MHz, 850 kHz and 500 kHz; Power: 0.2, 2 and 3.7 W; Time: 2.5, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120 min; Temp.: 2 °C; Conc.: 1 (w/w); Sample: 10 g • LM; SEM; FTIR; PSD	The number of pits per starch granule was independent of the amylose content, but strongly depended on granule size. Small granules were more pitted than the large ones. High frequency ultrasound was more favorable to produce ultrasonic pitting.
Corn starch (Flores-Silva et al., 2017)	• US probe (7 mm tip **Φ**); Freq.: 24 kHz; Int.: 300 W/cm²; Amp.: 80 %; Time: 1, 2, 4, 8 and 16 min; Temp.: 20 °C; Conc.: 30 % (w/v) • Laser diffraction analysis; SEM; XRD; FTIR ; DSC; Apparent viscosity; *In vitro* starch digestibility	SEM images showed disruption of granules. XRD indicated increased relative crystallinity content. Resistant starch content increased after 16 min sonication, attributed to morphological and crystallinity changes that reduced the structure of the starch granule channels.
Starches: Potato, wheat, corn, and rice (Sujka, 2017)	• US probe; Freq.: 20 kHz; Power: 170 W; Time: 30 min; Temp.: 20 °C; Solv.: water and ethanol; Conc.: 30 % (w/v) • Low temperature nitrogen adsorption (to investigate porosity of starch by specific surface area, average pore size and pore size distribution)	Modification of starch with US resulted in the formation of new pores in the studied range of diameter. Results varied due to botanical origin of starch and solvent used.
Yam starch (Bernardo et al., 2018)	• US probe; Freq.: 25 kHz; Power: 450 W; Amp.: 12, 40, 68 and 70 %; Time: 3, 6, 9 and 15 min; Conc.: 50 % (w/w) • Color; SEM; LM; PSD; XRD; SP and S; Paste clarity; RVA; DSC; Absolut density	Starch extraction yield increased with ultrasonication. Starch surface became damaged, and amorphous region was reduced, but no change was observed in the crystalline pattern. Except for starches treated at 70 % amplitude for 15 min, all others showed no change or slight increase of peak viscosity.
Maize starch (Flores-Silva et al., 2018)	• US probe (7 mm tip **Φ**); Dual US-HMT and HMT-US treatments; Freq.: 50 kHz; Int.: 300 W/cm²; Amp.: 80 %; Time: 1, 2, 4, 8 and 16 min; Temp.: 20 °C; Conc.: 30 % (w/v) • FTIR; DSC; *In vitro* starch digestibility	Dual treatments rearranged starch structure, increasing RS content. HMT-US produced thermos-stable SDS and RS. FTIR indicated that changes in SDS and RS fractions may be associated with variations in the crystallinity and with the packing of double helices within the crystalline lamella.
Corn starch (Li et al., 2018)	• US bath; Freq.: 40 kHz; Power: 420, 480 and 540 W; Time: 20, 30 and 40 min; Temp.:40, 50 and 60 °C; Conc.: 30 % (w/v) • Hydrolysis degree; RVA; DSC; SEM; PLM; FTIR; XRD; HP-GPC	US reduced the time in liquefaction process and resulted in increased hydrolysis degree. The appearance of notch and groove on granules surface was detected, and the polarized cross became smaller or even disappear.
Cassava starch (Monroy et al., 2018)	• US probe (13 mm tip **Φ**); Power: 750 W; Amp.: 40 and 60 %; Time: 5, 10, 20 min; Temp.: with and without control; Conc.: 5 % (w/v) • SEM; Confocal laser scanning microscopy (CLSM); Granule size distribution; Attenuated total reflection FTIR (ATR-FTIR); XRD; DSC; SP; Rheological characterization	US produced morphological and crystallinity changes, reduction of particles size and increase of swelling power. Gelatinization enthalpy was decreased, while transition temperatures were not affected. Higher *G'* values were observed for the treatment performed for 20 min at 60 % amplitude.
Foxtail millet starch (Babu et al., 2019)	• US bath; Single US treatment and dual ANN+US and US+ANN treatments; Freq.: 33 kHz; Time: 30 min; Temp.: 50 °C; Conc.: 50 % (w/v); Sample: 200 g; Vol.: 400 mL • Amylose content; Water absorption capacity (WAC); SP; Acid resistance; Shear and Freeze-thaw stability; *In vitro* starch digestibility; RVA; Color; Gel texture properties; Gel filtration chromatography and number average molecular weight; XRD; FTIR; SEM	Modified starches contained higher amylose. US had a predominant effect on RS level. Pasting properties were increased by sonication, while color was not affected. Hardness of gels was decreased by treatments. Ultrasonication led to increased proportion of very short and short chains after depolymerization of long chain amylose and amylopectin molecules. FTIR indicated reduced values of the 1047/1022 cm^{-1} ratio. SEM displayed deep pitting and cracks on granular surface after ultrasonication.

Retrograded starch (RS3) (Ding et al., 2019)	• US probe (6 mm tip Φ); Freq.: 20 kHz; Power: 100, 200; 300, 400, 500 and 600 W; Time: 30 min; Pulse: 2 s on / 2 s off; Temp.: controlled; Conc.: 10 % (w/v) • SEM; PSD; Zeta potential; Apparent amylose content (AAC); XRD; FTIR; DSC; *In vitro* digestibility; SP and S	High power resulted in more compact block-shape structure. US decreased the long-range orders but increased the median particle size, short-range orders, and V-type polymorph of RS3. Some RDS fractions were converted into SDS and/or RS. The molecular structures and starch digestibility were changed to different degrees depending on the US power.
Oat starch (Falsafi et al., 2019)	• US [probe (12 mm tip Φ); Freq.: 20 kHz; Int.: 39, 48, 63 W/cm²; Pulse: 5 s on / 5 s off] [bath; Power: 350 W; Int.: 5 W/cm²]; Time: 10 and 20 min; Temp.: 25 °C; Conc.: 5 %; Vol.: 100 mL • SEM; Granule size; AAC; DSC; XRD; Paste viscosity; SP and S; Transmittance; Oil and water absorption; Gel hardness; Syneresis	Fissures and pores were detected on the surface of granules treated with the probe. Size reduction was observed at the highest intensity. US increased amylose content, swelling power, solubility, transmittance, water and lipid holding capacity, and gel hardness was diminished. Gelatinization temperatures increased and crystallinity degree decreased.
Potato starch Millet starch (Hu et al., 2019)	• US bath; Freq.: single (40 kHz) and dual (40+80 kHz); Power: ≤ 720 W; Temp.: 25 and 60 °C; Conc.: 5 % (w/w) • Pasting properties; PSD; SP; FTIR; DSC; XRD; SEM	US caused the partial collapse of potato starch. Reduction of particle size, peak viscosity and relative crystallinity was greater in potato starch. Dual frequency US caused more changes than single frequency.
Starches: Wheat, barley, rice, and maize (Kaur & Gill, 2019)	• US probe (2 mm tip Φ); Freq.: 24 kHz; Power: 100 W; Amp.: 100 %; Time: 15 and 30 min; Temp.: controlled; Vol.: 50 mL • SP and S; PSD; SEM; XRD; FTIR; Gel rheological properties; *In vitro* starch digestibility	US increased swelling power and solubility. Depressions and pores were seen on granules' surface. Sonicated starches presented increased RDS and RS content, while SDS content decreased. Increase G' and G'' were determined after treatments of 15 min, and decreased values after 30 min.
Millet starch (Li et al., 2019)	• US bath; Freq.: 25 + 40 + 80 kHz; Power: 700 W; Time: 15, 30, 45, 60 min; Conc.: 10, 15, 20, 25 and 30 % (w/w); Sample: 20 g • Pasting properties; SP; Transparency; ATR-FTIR; DSC; XRD; *In vitro* enzymatic digestibility; SEM	US increased swelling power (at short sonication time) and transparency. The 1047/1022 cm^{-1} ratio was gradually increased with the increase of concentration and treatment time. US increased ΔH and *in vitro* enzymatic digestibility. SEM showed cracks and pores, even some broken granules.
Cassava starch Corn starch Yam starch (Minakawa et al., 2019)	• US probe (1.27 mm tip Φ); Freq.: 20 kHz; Time: 30 min; Temp.: 25 °C; Conc.: 10 % (w/w); Vol. 100 mL • SEM; Atomic force microscopy (AFM); Blue value (DP of amylose and amylopectin); XRD; FTRI; Thermogravimetric analysis (TGA)	Starch microparticles and nanoparticles were obtained after US. Yam starch, exhibiting higher amylose content, was more susceptible to ultrasonication, generating smaller starch nanoparticles and microparticles than those obtained from corn and cassava starches.
Waxy corn starch (Yang, Lu et al., 2019)	• US probe (38 mm tip Φ); Freq.: 15 kHz; Power: 100 and 400 W; Time: 40 min; Temp.: 25 °C; Conc.: 30 % (w/w) • High-Performance Anion-Exchange chromatography (HPAEC-PAD); HPSEC-MALLS; ^{1}H NMR; ^{13}C CP/MAS NMR; XRD; SEM; PSD; Pasting properties; DSC	Lower proportion of linear chains and higher proportion of external chain were observed in US starch. Lower degree of branching was observed in US samples, and α-1,4 glycosidic linkages were more stable than α-1,6 glycosidic linkages. ^{13}C NMR indicated that the content of double helices was decreased, and single helix and amorphous components were increased.
Rice starch (Yang, Kong et al., 2019)	• US probe (10 mm tip Φ); Freq.: 22 kHz; Power: 150, 300, 450 and 600 W; Time: 20 min; Pulse: 5 s on / 5 s off; Temp.: 25 °C; Conc.: 30 % (w/w); Vol.: 50 mL • XRD; FTIR; Raman spectroscopy; HPAEC; SEM; PSD; DSC; RVA	US slightly destroyed the amorphous region of granules, while the crystalline pattern remained unchanged. US induced fissures and pores on granules surface. Peak and breakdown viscosities increased, while gelatinization enthalpy decreased.
Potato starch (Cao & Gao, 2020)	• US bath; Single US treatment and dual US+electric field and electric field+US treatments; Freq.: 20 kHz; Power: 600 W; Time: 30 min; Temp.: 30 °C; Conc.: 20 % (w/w) • SEM; XRD; FTIR; AAC; Granule size; Pasting behavior; Transmittance; WAC; SP; *In vitro* starch digestibility; Texture properties; DSC	Deep pitting was observed in treated granules. US treatment increased relative crystallinity, granule size and amylose content. Peak viscosity was increased, and setback reduced. RS and RDS contents were increased after US, while SDS was reduced. Hardness of US-gels was improved. T_o, T_p and T_c values were higher after ultrasonication.

Sweet potato starch (Jin et al., 2020)	• Freq.: 20 kHz; Int.: 2, 4, 8, 12, 16 W/mL; Time: 10, 15, 20, 25, 30 min; Pulse: 3 s on / 5 s off; Temp.: 20, 30, 40, 50 and 60 °C; Conc.: 60, 80, 100, 125, 150 and 200 g/L • RVA; DSC; FTIR; XRD; SEM	US decreased peak and setback viscosities, and the gelatinization range and enthalpy. FTIR indicated damage to the ordered structures and crystallization zone. Relative crystallinity was reduced by 15 %. US destroyed the surfaces and the linkages between starch granules.
Wheat starches (Karwasra et al., 2020)	• US probe (2 mm tip Φ); Freq.: 30 kHz; Int.: 600 W/cm^2; Amp.: 100 %; Time: 15 and 30 min; Cycle: 80%; Conc.: 10 % (w/v); Vol.: 25 mL • SP and S; Oil absorption capacity (OAC); Amylose content; SEM; XRD; FTIR; PSD	Scraps and deformities were clearly observed on granule surfaces after US, which were more prominent in large size granules. Relative crystallinity increased after US. FTIR spectra showed no new or broken bonds after US, whereas the absorbance ratio 1022/995 cm^{-1} decreased significantly.
Taro starch (Thomaz et al., 2020)	• US probe; Dual HMT and US treatments; Freq.: 20 kHz; Amp.: 60 %; Time: 30 min; Conc.: 10 % (w/v) • DSC, XRD; SEM; RVA	Morphology of granules did not show major changes. Relative crystallinity increased, and RVA profiles decreased after ultrasonication. Dual treatment resulted in lower gelatinization temperature range and decreased enthalpy.
Chestnut starch (Wang, Wu et al., 2020)	• US probe (6 mm tip Φ); Treatments: Single US and dual US+MW (UM) and MW+US (MU); Freq.: 20 kHz; Power: 500 W; Time: 60 min; Pulse: 2 s on / 2 s off; Temp.: < 25 °C; Conc.: 10 % (w/v); Sample: 2 g • SEM; PLM; XRD; FTIR; DSC; RVA; SP; Freeze-thaw stability; WAC and OAC	UM and MU dually modified samples exhibited more severe surface damage, weaker birefringence, and lower relative crystallinity and gelatinization enthalpy than the native and single-treated starches. Swelling power, peak, trough, final and breakdown viscosities, and pasting temperature all decreased regardless of single or dual modification.
Sweet potato starch (Wang, Xu et al., 2020)	• US probe (13 mm tip Φ); Freq.: 20 kHz; Power: 300 W; Time: 15, 20, 25 and 30 min; Pulse: 3 s on / 5 s off; Temp.: 30 °C; Conc.: 6 % (w/w) • Amylose content; SEM; PSD; XRD; FTIR; Raman spectroscopy; DSC; SP and S; RVA; Dynamic rheological properties	Pores and cracks were observed in treated granules. Structural disorganizations were more evident with increasing time, especially in crystallinity, short-range molecular orders and ordered molecular structures. US increased SP and S, decreased pasting properties, and strengthened gels.
Corn starch Cassava starch (Rahaman et al., 2021)	• US bath; Freq.: 40 kHz; Amp.: 99 %; Time: 10 and 20 min; Temp.: 26 ± 2 °C; Conc.: 20 % • XRD; FTIR; DSC; SEM	Groove and notch appeared on the surface of the starch granules after US. Gelatinization temperature did not change with ultrasonication, but enthalpy decreased. XRD showed slight decrease in the crystallinity degree after US.
Corn starch Potato starch Pea starch (Zhang et al., 2021)	• US probe (6 mm tip Φ); Freq.: 25 kHz; Power: 100, 200, 300, 400, 500 and 600 W; Time: 5, 10, 15, 20, 25, 30 min; Pulse: 5 s on / 5 s off; Temp.: 25 °C; Conc.: 30 % (w/w) • AAC; FTIR; DSC; *In vitro* starch digestibility; Rheological properties	Power and time decreased AAC in corn and pea starches, while increased it in potato starch. US enhanced 1047/1022 values of corn starch, whereas those of potato and pea starches were decreased. Us decreased the RS content of pea and potato starches, but increased that corn starch.
Cowpea starch (Acevedo et al., 2022)	• US probe (13 mm tip Φ); Dual HMT+US treatment; Freq.: 20 kHz; Amp.: 80 %; Time: 30 min; Pulse: 2 s on / 2 s off; Temp.: 25 – 30 °C; Conc.: 10 % (w/v); Sample: 100 mL • SEM; ^{1}H NMR; XRD; DSC; *In vitro* starch digestibility; Pasting properties	Granule shape and XRD pattern were not modified by US. ^{1}H NMR revealed that US treatment caused a decrease of amylopectin branching degree. Gelatinization enthalpy was not affected by treatment. Slow digestible starch was increased up to 30 % by HMT-US treatments. Pasting viscosity of starch was decreased by US.
Normal maize starch Potato starch (Wang et al., 2022)	• US probe (6 mm tip Φ); Single US treatment and dual US+MW and MW+US treatments; Freq.: 20 kHz; Power: 12.4 W; Int.: 43.9 W/cm^2; Time: 60 min; Pulse: 2 s on / 2 s off; Temp.: controlled; Conc.: 10 % (w/v); Sample: 2.0 g • Total starch; Damaged starch; SEM; PLM; PSD; XRD; DSC	Ultrasonication loosened the internal space and destroyed the structure of starch granules, increased starch damage, decreased relative crystallinity and increased the median size values. The type of starch influenced the effect of US treatment.

Freq. = Frequency. Int. = Intensity. Temp. = Temperature. Atm. = Atmosphere. Solv. = Solvent. Conc. = Concentration. Vol. = Volume. US = Ultrasound. ANN = Annealing. HMT = Heat moisture treatment. MW = Microwave. RS = Resistant starch. SDS = Slowly digestible starch. RDS = Rapidly digestible starch.

The damage caused by US on starch surface is aggravated as power increases (since it is inversely correlated to frequency) (Zhu et al., 2012; Zuo et al., 2012). Yang, Kong et al. (2019) determined that the outer layer of rice starch granules was gradually peeled off from the periphery as the ultrasonic power was increased from 150 W to 600 W (see Figure 4). In agreement to these finding, Ding et al. (2019) demonstrated that roughness and erosion of starch granules was increased with increasing power from 100 W up to 400 W, where the granules almost lost their integrity and formed a discontinuous compact fibrous structure with many surface cracks. When sonicating at 500 W and 600 W, agglomerated granules were observed, resulting from interactions among the trimmed amylose chains and partial surface gelatinization, where the original shape had completely disappeared (Ding et al., 2019).

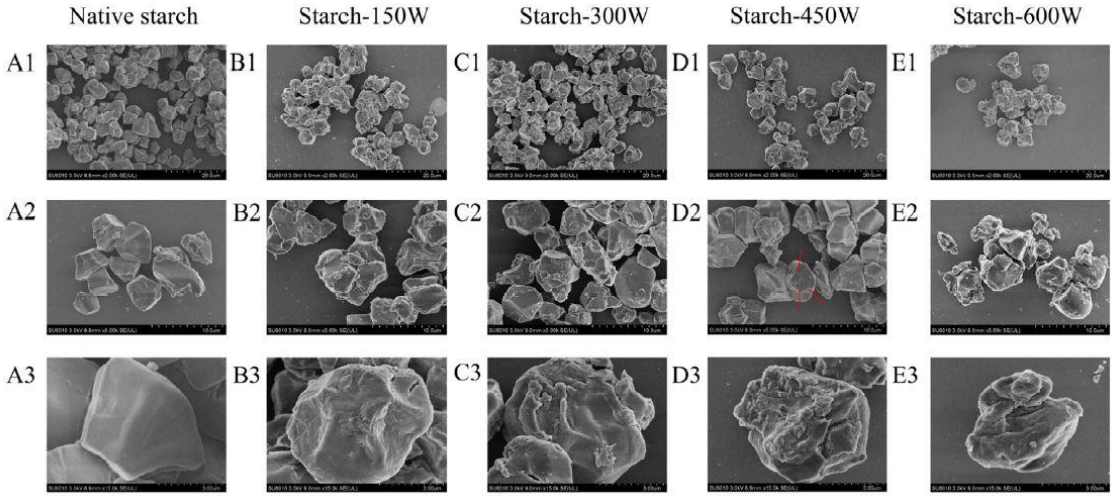


Figure 4. Scanning electron micrographs of native rice starch (A1-3) and rice starch treated by 150 W (B1-3), 300 W (C1-3), 450 W (D1-3) and 600 W (E1-3) ultrasound. The magnification of image from top to bottom in the same line was 2, 5 and 15 K, respectively (Yang, Kong et al., 2019).

Longer sonication exposure (Bai et al., 2017; Gallant et al., 1972; Manchun et al., 2012; Monroy et al., 2018; Wang, Xu et al., 2020) and higher treatment temperature (Hu et al., 2019; Rahaman et al., 2021) have widely been determined to intensify the damage induced by ultrasonication on granule surface. Flores-Silva et al. (2017) determined higher damage with increasing treatment time. While 4 min ultrasonication showed fissures and cracks, 8 min led to severe disruption of granule surface, and at 16 min there were signs of granule fragmentation and disintegration. It was indicated by Bel Haaj et al. (2013) that with increasing sonication time, the surface of waxy maize starch granules appeared to be progressively broken down and eroded, with the release of

nano-particles about 20-200 nm in size. Treatment temperature also increases the damage caused by ultrasounds, due to melting that temperature can provoke (Zuo et al., 2009), being able to partially gelatinize the surface of US-treated starch (Rahaman et al., 2021) and disintegrate some starch granules (Amini et al., 2015; Hu et al., 2019) during treatments. It has been said that even when temperature of the treated suspension is controlled during treatment, US can lead to local heating that damages the outer regions of the starch granules, magnifying the disruption effects induced by cavitation (Carmona-García et al., 2016).

The susceptibility of starch to ultrasonication treatments is greatly related to its botanical origin and granule size. Hu et al. (2019) demonstrated that the damage of US on larger size starch granules (potato) was more serious than on small granules (millet), in agreement with Carmona-García et al. (2016), since larger starch granules have greater probability of trapping kinetic energy than smaller granules do. This found different susceptibilities originate from the type and structure of the starch, affecting, among other factors, the amylose/amylopectin ratio, and the molecular characteristics of the native starches (Luo et al., 2008; Zhu, 2015). Luo et al. (2008) noted that under the same treatment conditions, normal maize and waxy maize starches showed a porous surface after sonication, while in the surface of amylomaize V starch a fissure was clearly observed. In agreement, Chan et al. (2010) determined that when sonicating different types of starches (sago, potato, corn, and mung bean) at the same conditions, fissures were only found on corn starch granules, possibly attributed to a relatively weaker granular structure, more prone to be disrupted by cavitation effect.

The nature and degree of erosion caused by cavitation also depends on the concentration of starch in the suspension (Amini et al., 2015; Gallant et al., 1972), the solvent used for the treatment (Sujka, 2017), and the gas present in the medium during treatment (Degrois et al., 1974). The damage caused to starch has been said to be greater at lower concentrations because of increased acoustic energy due to reduced impedance of the medium (Amini et al., 2015). The lower damage at higher concentrations can be explained by a diminution of the density of the acoustic energy due to the diffusion of the waves by the particles (Gallant et al., 1972). Regarding the medium, cavities are more readily formed in solvents with high vapor pressure, low viscosity, and low surface tension (Sujka, 2017). Water has high surface tension and low viscosity and vapor pressure, making it a good medium for cavitation and degradation of starch (Sujka, 2017). The solubility of the surrounding gas in water has been found to be inversely proportional to

the size of generated pits generated (Gallant et al., 1972). It was determined by Gallant et al. (1972) that in the presence of air, the surface of potato starch granules becomes rugged and pitted, whereas in hydrogen atmosphere the surface remains smooth with large and deep pits, while in the presence of oxygen there is less detectable damage, carbon dioxide led to a much weaker effect, and virtually no effect is produced in vacuum.

2.2 Particle size

There is no consensus on the effect of US treatment on the size of starches after treatment, and the available literature seems to indicate that it greatly depends on treatment factors. Some authors have reported that ultrasounds can significantly rupture starch granules by the collapse of cavitation bubbles, reducing the size of particles (Hu et al., 2019; Jambrak et al., 2010; Kang et al., 2016; Yang, Lu et al., 2019; Yang, Kong et al., 2019), while others indicate finding slight changes (Herceg et al., 2010; Karwasra et al., 2020; Kaur & Gill, 2019; Zuo et al., 2009), no effect at all (Bernardo et al., 2018; Carmona-García et al., 2016; Falsafi et al., 2019; Sujka & Jamroz, 2013), or even an increase in granular size (Cao & Gao, 2020; Ding et al., 2019; Herceg et al., 2010; Wang, Xu et al., 2020; Wang et al., 2022) after ultrasonication.

It seems like the devise used for applying the treatments greatly determines the effect on particles' size. Fewer effects (even no significant changes) have been reported when treatments were performed using ultrasound bath (Herceg et al., 2010), but the size of granules was greatly reduced when using probes (Jambrak et al., 2010). When treating with US bath, the granules tend to agglomerate due to superficial adhesiveness among granules and the liberated bonds, providing the opportunity of connecting linkages between the polymers which results in increased sizes after ultrasonication (Cao & Gao, 2020; Harasym et al., 2020; Herceg et al., 2010), a phenomenon that seems to be linked to high US powers. When sonicating using a probe, the generation of small-sized particles has been commonly reported, although there are also some authors that reported the agglomeration of granules in treatments performed at high powers (Ding et al., 2019; Yang, Lu et al., 2019; Wang, Wu et al., 2020). The particle reduction has been indicated to be more likely due to disintegration of large starch agglomerates rather than fragmentation of individual granules (Falsafi et al., 2019; Yang, Kong et al., 2019). The particle size modification do not entirely depend on whether sonication was applied by US bath or probe,

or the sonication power, and the composition and type of the starch and its susceptibility to be modified may also have an influence.

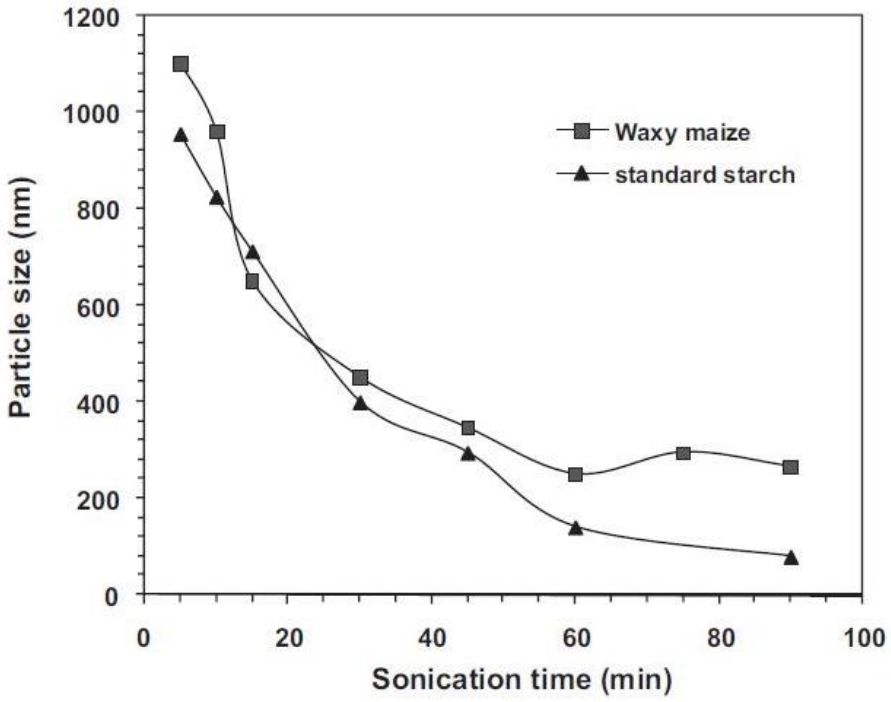


Figure 5. Change in mean particle size vs. ultrasonication time for waxy maize and standard maize starch (only particles larger than 100 nm were taken into consideration) (Bel Haaj et al., 2013).

Degrois et al. (1974) and Gallant et al. (1972) were the first authors to report the physical degradation of starch granules after ultrasound treatments. When a significant size reduction is determined, it is believed that the progressive erosion caused by mechanical collision and shear forces from cavitation leads to granule fragmentation (Bel Haaj et al., 2013; Yang, Kong et al., 2019). Bel Haaj et al. (2013) said that particle reduction was mainly caused by violent collision of starch particles due to high-speed streams resulting from implosion of the cavitation bubbles, rather than the effect of direct impact of the micro-jets towards the starch particles. The concentration of the suspension, the temperature and time of ultrasonic processing, and the nature of the treated sample (e.g., amylose content) are factors that also influence the extent of granule fragmentation achieved (Minakawa et al., 2019; Yang, Lu et al., 2019). Greater particle fragmentation has been reported in starches with increasing sonication time, until a limiting size is reached (see Figure 5) (Bel Haaj et al., 2013). Minakawa et al. (2019) determined that amylose content also influences particle reduction. These authors showed that yam starch, exhibiting

higher amylose content, presented smaller starch microparticles (1-3 μm) and nanoparticles (8-32 nm) after ultrasound treatment than those obtained from ultrasonication of starches with lower amylose content (corn and cassava) (Minakawa et al., 2019). The size of the starch, on the other hand, was not found to influence the fragmentation of particles. It was indicated by Carmona-García et al. (2016) that even if a big size starch (plantain, D_{50} = 22.4 μm) was more susceptible to surface damage than a small size starch (taro, D_{50} = 2.3 μm), no significant size reduction was determined in any of them, indicative that treatment conditions were more determinative factors than the size of the granules in their study.

3. Effect of ultrasounds on the physico-chemical properties of starches

3.1 Molecular structure

Many of the physical and functional properties of starch depend on its molecular conformation, like molecular weight, chain length distributions and amylose to amylopectin ratio, which has been proved to be modified by ultrasonication (Chemat et al., 2011). Depolymerization as consequence of ultrasonication can involve two mechanisms: i) mechanical polymeric degradation due to cavitation, and ii) chemical degradation resulting from reactions between the polymer and high energy molecules such as hydroxide (-OH) and hydrogen (-H) radicals generated from dissociation of water molecules due to cavitation (Chemat et al., 2011; Jambrak et al., 2010). The free radicals may induce the scission of starch molecular chains, which disrupts the fine molecular structure and thus destructs the integrity and rigidity of starch granules (Wang, Wu et al., 2020). Polymeric degradation by chemical reactions stands out in high-frequency ranges (>500 kHz) since the amount of free radicals generated at low frequencies is very low. In low-frequency treatments the main effect is due to mechanical degradation (Sujka, 2017). In food industry, depolymerization by ultrasound is generally performed using immersion probes working at 20 kHz (Chemat et al., 2011).

3.2 Molecular weight and chains length distribution

Starch depolymerization has been extensively reported after ultrasound treatments (Amini et al., 2015; Babu et al., 2019; Czechowska-Biskup et al., 2005; Jambrak et al., 2010; Zheng et al., 2013). The experiment conditions determine the yield of polymer degradation caused by treatments. Temperature has been reported to have a direct correlation with the diminishment of degree of

polymerization (DP), while an inverse correlation has been reported for starch concentration in the sonicated suspension (Amini et al., 2015). The solvent used, and more precisely on its vapor pressure, viscosity, and surface tension, has also been reported to influence (Sujka & Jamroz, 2013). Depolymerization was reported to be higher when sonicated in water than in ethanol (Sujka & Jamroz, 2013). The molecular weight of starches has been indicated to be reduced by ultrasonication (Iida et al., 2008; Li et al., 2018; Seguchi et al., 1994; Yang, Lu et al., 2019), where the reduction has been reported to be sharp at the beginning of treatment, to slow down as degradation progresses, and to ultimately tend towards a constant value (Isono et al., 1994). A characteristic feature of polymer degradation by US is that it proceeds in a non-random manner, and that there is a minimum chain length limiting the degradation process, that once is reached no further chain degradation occurs (Czechowska-Biskup et al., 2005).

The amylose to amylopectin ratio in starches differ depending on the nature of each starch. The crystalline regions in granules appear in branched glucose units of amylopectin molecules, while amylose, the linear polysaccharide of starch, is largely amorphous and randomly distributed between the amylopectin clusters (Zhu, 2015). It is believed that ultrasound treatments preferentially degrade (but not exclusively) the easily attacked linear amylose of the amorphous regions with low structural integrity (Amini et al., 2015; Flores-Silva et al., 2017; Kaur & Gill, 2019; Luo et al., 2008). Linear polymeric conformations seem to be easier to break, since they may accumulate the applied forces of the same spatial orientation on much longer distances along the chain (Czechowska-Biskup et al., 2005), while the destruction of crystalline regions and the unwinding of double helices would require more energy (Huang et al., 2007; Yang, Lu et al., 2019; Yang, Kong et al., 2019). The distortion of the crystalline regions has been reported after high intense ultrasonication (>420 W) of corn starch (Li et al., 2018) and rice starch (Yang, Kong et al., 2019). High power US starch depolymerization occurs mainly on the C-O-C bond of the α-(1→6)-glycosidic bond (the branching points of amylopectin) resulting in amylopectin gradually converted to amylose and low molecular weight segments (Falsafi et al., 2019; Li et al., 2018). The degradation of the side chains of amylopectin molecules would increase the apparent amylose content in US-treated starches, given that the released long-branch chains would be recorded as amylose (Ding et al., 2019; Li et al., 2018). Higher amylose contents have been reported with increasing sonication time (Wang, Wu et al., 2020) and increasing US power (Isono et al., 1994; Zhang et al., 2021). Zheng et al. (2013) reported increased absorbance of starch-

iodine complex in sweet potato starch with increasing frequency, denoting an increase of apparent amylose content.

3.3 Crystallinity and degree of branching (DB)

The crystallinity structure of starches and their degree of branching have been studied by various characterization techniques [Polarized light microscopy (PLM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy and nuclear magnetic resonance (NMR) spectroscopy], which seem to agree with a higher susceptibility of the amorphous zone to ultrasound treatments, compared to the crystalline region (Yang, Lu et al., 2019).

3.3.1 Polarized light microscopy (PLM)

The density and refractive index difference in crystalline and amorphous structures generates the Maltese cross, clearly seen when granules are exposed in polarized light (Li et al., 2018; Zhu et al., 2012). These Maltese crosses are characteristic of intact granules and reflect their crystalline organization (Huang et al., 2007; Wang, Wu et al., 2020). Ultrasonication has either been reported to cause no change or to decrease the luminance of the Maltese cross. When no effect has been found, it has been concluded that ultrasonication cannot change the whole structure of the starch granules but may change parts of them (likely the amorphous parts), barely affecting the crystalline structure (Huang et al., 2007; Zhu et al., 2012). On the contrary, whenever a decrease in the brightness of the native starch Maltese cross is detected, it has been stated that it is due to disruption of the crystal layer of granules by ultrasonication, leaving a more fragile structure after treatment (Carmona-García et al., 2016; Gallant et al., 1972; Li et al., 2018). This reduced brightness indicates that the double-helix structure of amylopectin is damaged by ultrasonication, and that the order of the molecular chain is reduced (Wang et al., 2022).

3.3.2 X-ray diffraction (XRD)

X-ray diffraction (XRD) allows the evaluation of X-ray pattern and degree of long-range order crystallinity of starches. Not many effects have been reported over the XRD patterns of ultrasonicated starches. There seems to be a general consensus in the literature indicating that US treatments do not modify the position of the characteristic peaks, while the diffraction intensities of the modified starches have been reported to remain unchanged (Acevedo et al.,

2022; Babu et al., 2019; Falsafi et al., 2019; Flores-Silva et al., 2017; Huang et al., 2007; Li et al., 2019; Luo et al., 2008; Monroy et al., 2018; Wang et al., 2022; Zhu et al., 2012) or to be slightly reduced (Amini et al., 2015; Carmona-García et al., 2016; Ding et al., 2019; Karwasra et al., 2020; Kaur & Gill, 2019; Li et al., 2018; Manchun et al., 2012; Rahaman et al., 2021; Thomaz et al., 2020; Wang, Wu et al., 2020; Yang, Lu et al., 2019). The susceptibility of starches to ultrasonication is influenced by their packing of the crystalline and amorphous regions in the granules (Hu et al., 2019; Kaur & Gill, 2019). As Figure 6 illustrates, it has been indicated that increasing US times lead to greater reduction of the characteristic peaks' intensities (Carmona-García et al., 2016; Karwasra et al., 2020; Wang, Xu et al., 2020). Increasing temperatures have also been reported to lead to greater diffraction intensity reduction (Amini et al., 2015).

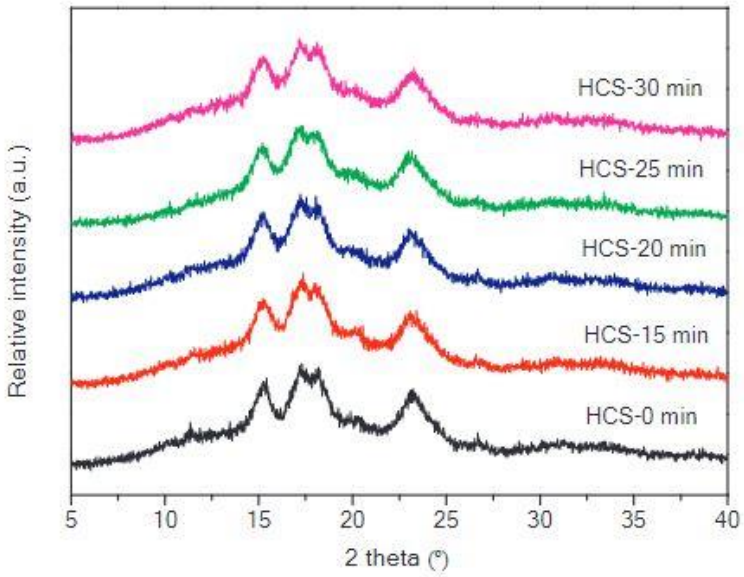


Figure 6. X-ray diffraction patterns of sweet potato starch treated by ultrasonication (Wang, Xu et al., 2020)

In starches XRD patterns, peak diffraction characteristics and dispersion diffraction characteristics correspond to the crystalline and the amorphous regions, respectively (Hu et al., 2014). The long-range order crystallinity of starch granules, also called relative crystallinity (RC), can be determined as the ratio between the crystalline and total region (Bernardo et al., 2018). In agreement with the lower XRD pattern intensities reported after US treatments, starch RC has been commonly indicated to be reduced by ultrasonication, in A-type [rice (Yang, Kong et al., 2019), corn (Hu et al., 2014; Li et al., 2018; Rahaman et al., 2021), waxy corn (Yang, Lu et al., 2019), normal maize (Bel Haaj et al., 2013; Wang et al., 2022), oat (Falsafi et al., 2019), tapioca

(Manchun et al., 2012), millet (Hu et al., 2019) and sweet potato (Jin et al., 2020; Wang, Wu et al., 2020)], B-type [potato (Zhu et al., 2012), and retrograded starch RS3 (Ding et al., 2019)] and C-type [cassava (Monroy et al., 2018) and pinhão (Pinto et al., 2015)] starches. The decreased RC after US has been attributed to damage caused to the amorphous regions rather than the crystalline regions, because of their higher susceptibility to ultrasonication (Yang, Kong et al., 2019). It may be attributed to the breaking of hydrogen bonds and starch chain structures resulting from cavitation and mechanical oscillation pressure during US treatments, causing the destruction of amorphous regions, the loose packing of lattices and the transformations of double-helix orientation, eventually leading to a decrease of starch crystallinity (Wang, Wu et al., 2020). However, the inner lamellae (presumably more crystalline and richer in amylopectin chains) are also susceptible to the attack of ultrasonic waves (Flores-Silva et al., 2017; Monroy et al., 2018). It has been said that greater US conditions (both power and time) (Falsafi et al., 2019; Yang, Kong et al., 2019; Zhu et al., 2012) decline crystallinity content because they cause the fragmentation of the lamellar array of starch granules. More intense treatment conditions would result in a more unstable starch arrangement after ultrasonication, causing disruption of the double-helix structure of the crystalline regions (Wang et al., 2022; Wang, Wu et al., 2020). Bel Haaj et al. (2013) determined that prolonged ultrasonication of starch under high treatment conditions resulted in serious disruption of the crystalline structure of clustered amylopectin, leading to nanoparticles with low crystallinity. Small crystallites may not produce sufficiently detectable reflection intensities, presenting an amorphous character, and resulting in lower crystallinity values (Manchun et al., 2012). Even if ultrasonication can damage the starch crystalline structure, their breakdown strength is not enough to induce a crystal type change (Hu et al., 2014).

Some studies have reported no change of RC after ultrasonication, while few authors even report a long-range crystallinity increase following treatments. These results tend to be attributed to preferential degradation of the amylose-rich amorphous regions by ultrasonication, leaving the crystalline structure rather unaffected (Huang et al., 2007; Luo et al., 2008). It may be assumed that in these studies, the treatment conditions were less prone to lead to an effect over the crystalline regions, rather than lower susceptibility of the starch structure to the ultrasonication (Kaur & Gill, 2019). Flores-Silva et al. (2017) reported an increase of crystallinity from about 25 % in the native corn starch to about 33 % after 4 min sonication (see Figure 7). These authors

concluded that ultrasounds did not lead to a modification in the composition of the starch molecules but only their relative organization within the granule microstructure. Cleavage of starch chains in the amorphous regions allows the formation of new crystallites, and some reordering of the fragmented chains that produce a more crystalline structure (Acevedo et al., 2022; Cao & Gao, 2020; Flores-Silva et al., 2017).

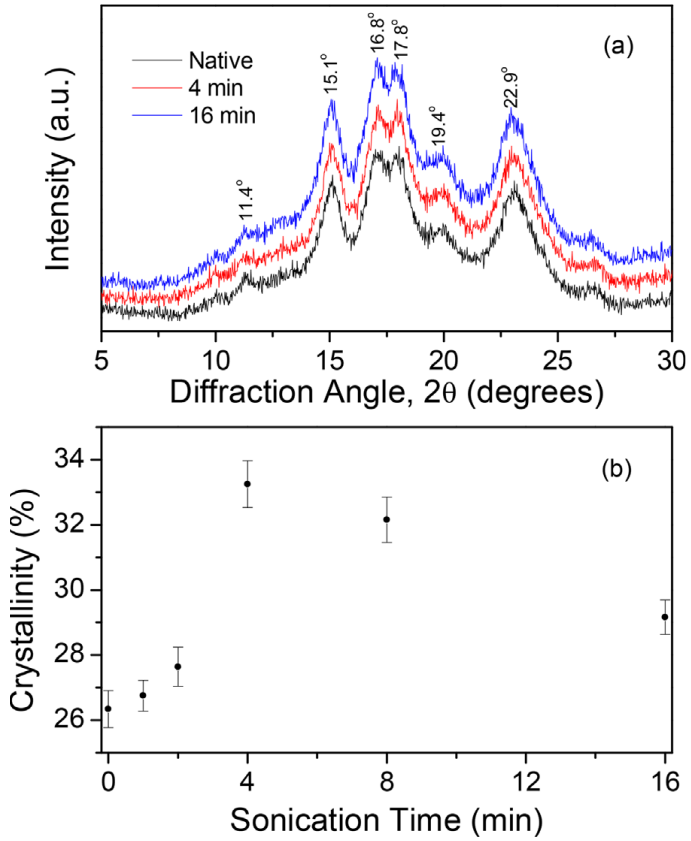


Figure 7. (a) XDR pattern for native starch and two sonicated samples; (b) Crystallinity content determined for different treatment times (Flores-Silva et al., 2017).

3.3.3 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is used to verify changes in the chemical structures of starch molecules (Kang et al., 2016). The specific bands associated with vibrations, stretching, flexion, and deformation of bonds corresponding to the main functional groups characteristics of starch are in the region of 800 - 1200 cm^{-1} (Kaur & Gill, 2019; Rahaman et al., 2021). Ultrasound treatments have been indicated to modify the shape, width, and intensity of FTIR spectra, but the positions of the characteristic absorption peaks are not obviously changed by ultrasonication (Jin et al., 2020; Karwasra et al., 2020; Monroy et al., 2018). Changes of the

peak intensities are due to damage caused to the starch conformation such as the structure of the double helices and crystalline regions (Li et al., 2019). Neither the loss of absorption peaks nor the generation of new ones has been reported after treatments, suggesting that US do not alter chemical bonds and functional groups, indicating that the modification was purely physical (Bai et al., 2017; Hu et al., 2019; Li et al., 2018).

The absorbance bands at 1047 cm^{-1}, 1022cm^{-1} and 995 cm^{-1} are particularly sensitive to modifications caused to starches and are associated to the crystalline structure, the amorphous structures, and bonding in hydrated carbohydrate helices, respectively (Flores-Silva et al., 2017; Monroy et al., 2018). The short-range order crystallinity can be determined from the absorbance ratio 1047/1022, calculated as the intensity of 1047 cm^{-1} divided by the intensity of 1022 cm^{-1}, used to quantify changes in starch structure because of its positive correlation with the helical structure packing enclosed by the interior microstructure (Kaur & Gill, 2019; Wang, Wu et al., 2020; Yang, Kong et al., 2019). It has been generally indicated that US treatments lead to a reduction of the 1047/1022 values, indicative of disruption of short-range molecular order (Babu et al., 2019). It is presumed that this decrease occurs because ultrasonication destroys the amorphous and crystalline regions of starch granules, resulting in irregular packing with a double helical reorientation within crystalline domains, and/or disruption of some hydrogen bonds linking adjacent double helices (Jin et al., 2020; Li et al., 2018; Wang, Xu et al., 2020). This ratio has presented a decreasing tendency with increasing power, suggesting that ultrasonication might weaken starch short-range crystallinity (Yang, Kong et al., 2019). Sonication time, on the other hand, does not seem to have a direct correlation with the decrease of 1047/1022 values. Flores-Silva et al. (2017) determined a significant decrease of the 1047/1022 ratio in US treatments of 1 min up to 4 min (when the lowest value is reached), which remained constant until 16 min sonication, indicating that the limit of short-range crystallinity crack down is achieved even by short treatments. These findings agree with the statement presented by Czechowska-Biskup et al. (2005), that depolymerization by US happens in a non-random manner and is limited by a minimum chain length. It was determined by Zhang et al. (2021) that different starches presented different trends in the evolution of 1047/1022 values after being subjected to the same US treatments. While sonicated pea and potato starches showed a remarkable decrease on these values, sonicated corn starch presented much higher values than the control sample, with a constant increase with increasing time, suggesting the strengthen of short-range order (see

Figure 8). Whenever an increase of 1047/1022 values has been determined, it has been explained as more serious damage caused to the amorphous regions, and that ultrasounds enhanced the associations between starch chains and favored the formation of relatively ordered structure (single- and double- helices) due to the recrystallisation of short chains (Ding et al., 2019; Li et al., 2019; Zhang et al., 2021). Increasing 1047/1022 values suggest that US reduced the chain lengths of starch molecules, since these short chains could form double helical order structures in the amorphous region, resulting in an ordered short-range structure that increase 1047/1022 (Zhang et al., 2021). Another evaluated ratio, although to a much lower degree, is 1022/995, assumed to represent the organization state of the double helices located inside the crystallites (Monroy et al., 2018). In general, a decrease of 1047/1022 is joined by an increase of 1022/995, indicative of higher proportion of amorphous to ordered structure zones in the sonicated starches (Monroy et al., 2018; Rahaman et al., 2021), and confirms the weakening of short-range order (Hu et al., 2019; Yang, Kong et al., 2019).

3.3.4 Raman Spectroscopy

Starch short-range ordered structure has also been studied by Raman spectroscopy, which has been informed to be more sensitive than FTIR to local changes in polymer microstructure (Bel Haaj et al., 2013). The Raman spectra bands of starches have been closely associated with the vibration modes of the C-O-C bond in the glucose ring and the α-1,4-glycosidic linkage (Yang, Kong et al., 2019). The bands due to the skeletal mode vibrations of the glucose pyranose ring of starches have been reported to undergo a shift towards higher wavenumber, which has been mentioned in starch nanoparticles and resulted from the disruption of crystalline structure by high power ultrasonication (Bel Haaj et al., 2013; Yang, Kong et al., 2019). Vibrations related to the C-O-C of α-1,4 and α-1,6 glycosidic linkages are characterized by strong bands in 900-960 cm^{-1} and a weak band at 1155 cm^{-1}. A significant modification of these bands was reported by Bel Haaj et al. (2013), where the band at 905 cm^{-1} seemed to vanish, the band at 940 cm^{-1} was shifted towards lower wavenumber, and the band at 1155 cm^{-1} decreased in intensity. These authors inferred that these results indicated that the branching points in the amylopectin were mostly affected by high power ultrasonication since the band at 905 cm^{-1} is associated with α-1,6 glycosidic linkages (Bel Haaj et al., 2013). The 480 cm^{-1} Raman band has been reported to have a strong correlation with starch short-range order structure. Wang, Xu et al. (2020) indicated that the full width at half height (FWHH) at 480 cm^{-1} was used to characterize this crystallinity value,

while Yang, Kong et al. (2019) indicated that it was the height of the band that was used in the determination. Lower degree of short-range molecular structure was suggested by both studies, reaching the conclusion that US weaken the ordered packing of double helix structure in starch granules. This conclusion agrees with the commonly reduced 1047/1022 values reported by FTIR analyses (see section 3.3.3). Furthermore, the intensity of the band at 2900 cm^{-1} has been reported to be decreased as the US treatment intensifies, which might be related to distortion of ordered molecular structure, closely associated with changes in the amylose/amylopectin ratio (Wang, Xu et al., 2020; Yang, Kong et al., 2019).

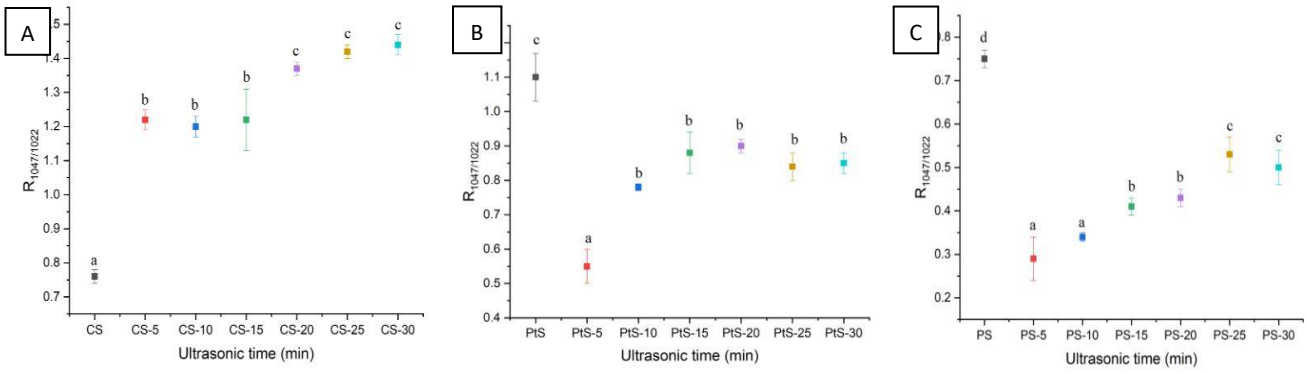


Figure 8. 1047/1022 cm^{-1} values of native and sonicates (A) corn (CS), (B) potato (PtS) and (C) pea (PS) starches under different ultrasonic time. Means followed by different letters indicate statistical difference ($p < 0.05$) (Zhang et al., 2021).

3.3.5 Nuclear magnetic resonance spectroscopy (NMR)

Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) allows the determination of the degree of branching (DB) in starches to quantify changes caused to starch molecules. The high sensitivity of ^{1}H NMR allows the resolution of the anomeric proton resonance of starch, distinguished between the α-1,4 and α-1,6 glycosidic linkages, to characterize the structural features of native and ultrasonicated starches (Acevedo et al., 2022; Yang, Lu et al., 2019). Signals at 5.12 ppm and 4.80 ppm are assigned to α-(1,4) and α-(1,6) glycosidic bonds in starches, respectively (Acevedo et al., 2022). Ultrasounds have been reported to weaken the intensity of both peaks (5.12 and 4.80 ppm), which suggests higher damage to the amorphous regions in starches. After ultrasonication, Acevedo et al. (2022) reported the decrease of the 4.80 ppm signal, while Yang, Lu et al. (2019) indicated a relative decrease of both peaks (see Figure 9). Consequently, the DB determined in the sonicated starches were decreased. In the case of waxy corn starch, it has been said that the steric hindrance of α-1,4 glycosidic linkages are more stable

than that of α-1,6 glycosidic linkages, hence being more resistant to ultrasonication (Yang, Lu et al., 2019). Iida et al. (2008) indicated that the integrated intensity of the peaks increased with sonication, indicative that the fraction of the mobile starch molecules increased after treatment. These results suggest that ultrasounds break both α-1,4 and α-1,6 glycosidic linkages, with greater rupture to one or the other depending on the US power applied and the amylose content in the starch.

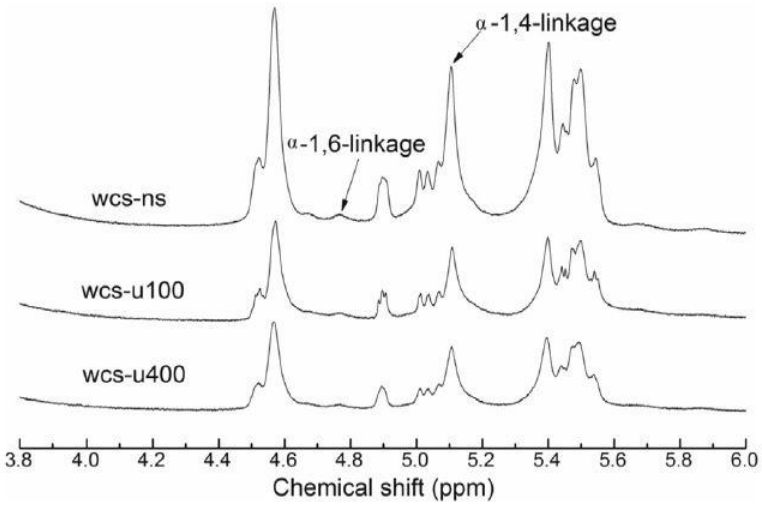


Figure 9. ^{1}H NMR spectra of native and ultrasonicated waxy corn starches. wcs-ns, wcs-u100 and wcs-400 refer to the waxy corn starch that was not sonicated, sonicated at 100 W and sonicated at 400 W, respectively (Yang, Lu et al., 2019).

In carbon nuclear magnetic resonance spectroscopy (^{13}C NMR) the two broad shoulder peaks near 103 and 82 ppm provide information about the amorphous components in starch, while the triplet peaks in the C-1 region (90-110 ppm) provide information about the crystalline state (Yang, Lu et al., 2019). It was determined by Yang, Lu et al. (2019) that as ultrasound power increased, the signals representing the amorphous state were gradually intensified, while the intensity of the peaks in the crystalline state were gradually decreased (see Figure 10). Iida et al. (2008) found that the ^{13}C NMR spectral intensity was largely increased by the sonication, supporting the argument that the mobile fraction of starch was largely increased by sonication. Both results indicate that the crystalline state decreased, and the amorphous state increased with US treatment. The increase of single helix might be because crystalline region was destroyed and double helices were unwound into many short single helices and amorphous state (Yang, Lu et al., 2019).

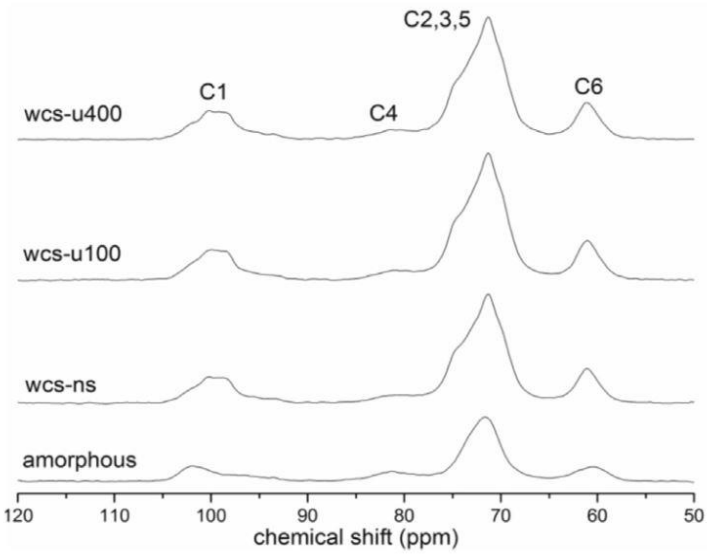


Figure 10. ^{13}C NMR spectra of waxy corn starches. wcs-ns, wcs-u100 and wcs-400 refer to the waxy corn starch that was not sonicated, sonicated at 100 W and sonicated at 400 W, respectively. "Amorphous" corresponds to the amorphous subspectra obtained from the native starch (Yang, Lu et al., 2019).

4. Effect of ultrasounds on the techno-functional properties of starches

Techno-functional properties of starches are completely related to the interaction they have with water, which deeply depends on starch granule morphology and composition (Chan et al., 2010). As a result of the morphological impact of US on starches (see section 3), the surface area of starch granules is increased, facilitating interaction with water, resulting in the alteration of their techno-functional properties. The sonication process provides heat to the medium during treatment that results in temperature increase, unless this heat is removed by a cooling system. When starch is heated in the presence of excess water, the amorphous regions of the granules absorb water and swell, which causes stress between the amorphous and crystalline regions. This process continues in a reversible way until the gelatinization temperature is reached, when the granules collapse because the tension between both regions is too high. At this point the process becomes irreversible, the viscosity of the solution increases and the original starch structure is lost (Miles et al., 1985). As it has been indicated, temperature influences in a great extent the modification caused by the ultrasonication, so it is necessary to be controlled during treatment. It was indicated by Monroy et al. (2018) that the complete starch sample gelatinized happened during US treatment when an ice bath was not used, given that the slurry reached 65 °C in 20 min sonication.

4.1 Swelling power and solubility

Swelling power (SP) and solubility (S) of starches and flours are parameters closely related to the starch granule fine structure, the gelatinization temperature, the amylose to amylopectin ratio, degree of branching and branch length, conformation and molecular weight, the degree of association between their chains and the aggregation structure of the granules (Chan et al., 2010; Kaur & Gill, 2019; Sujka & Jamroz, 2013; Wang, Xu et al., 2020). These two properties are linked together because when the granule swells, more amylose can be released out to the aqueous medium, so high swelling power contributes to high solubility (Chan et al., 2010).

Parallel to the generalized impact reported starch granules surface, an increase in both SP and S is commonly reported after US treatments (Chan et al., 2010; Falsafi et al., 2019; Hu et al., 2019; Karwasra et al., 2020). Structural weakening and depolymerization of starch have been pointed out as possible explanations to the increase in these properties (Carmona-García et al., 2016; Herceg et al., 2010; Sujka & Jamroz, 2013). As consequence of the increased starch surface area after ultrasonication, water diffuses easier into the granules, so US-treated samples present higher water uptake and retention, increasing their SP (Amini et al., 2015; Falsafi et al., 2019; Jambrak et al., 2010; Luo et al., 2008). The generation of nano-particles also favors starch interaction with water, since small-sized granule fragments have higher specific surface area than large native granules, increasing water absorption capacity (Falsafi et al., 2019). The increase in SP and S can also be consequence of starch depolymerization due to treatment, and the disassociation of ordered molecular structure. Higher SP and S indicate improved binding between water molecules and free hydroxyl groups of starch polymer chains through hydrogen bridge, suggesting that the molecular disruption of chains by ultrasounds contributed to the increase in linear chains (Luo et al., 2008; Manchun et al., 2012; Sujka & Jamroz, 2013; Wang, Xu et al., 2020). Ultrasonication enhanced the mobility of amylose chains and reduced their molecular weight after the release of side chains of amylopectin or cleavage of amylose chains, thus improving their hydration performance and promoting the leaching of amylose outside of the swollen granules to the aqueous medium (increasing solubility) (Chan et al., 2010; Herceg et al., 2010; Jambrak et al., 2010; Wang, Xu et al., 2020). Within the crystalline structures, the disintegration of intermolecular bonds leads to a less compact granular arrangement and generates free hydroxyl groups of amylose and amylopectin, where water molecules can bind by hydrogen bonds, increasing the SP in the granules (Kaur & Gill, 2019).

The final effect of US treatments over these properties depends on the treatment parameters array, where time and temperature have been demonstrated to have a high influence. Shot sonication times (below 30 min) have been said to increase SP (Karwasra et al., 2020; Monroy et al., 2018; Sit et al., 2014), while longer times lead to decreased values (Li et al., 2019). This behavior is believed to happen because with the increase in starch damage at long exposure, there is a reduction in the starch structural stability, which detriments its SP (Li et al., 2019). Solubility (S), on the contrary, has been reported to be increased with increasing ultrasonication time, favored by the disruption of amorphous regions (Manchun et al., 2012; Zheng et al., 2013). It was mentioned by Karwasra et al. (2020) that S increased at short times (15 min) but decreased by sonication at 30 min in US-treated wheat starches. This tendency switch is believed to be related to the temperature reached during treatment, since these authors did not report to have controlled the treatment temperature, which would have resulted in a significant increase after 30 minutes ultrasonication. Amini et al. (2015) concluded that treatment temperature was in fact more determinative in modifying starch solubility than ultrasonication time. These authors determined that S was almost independent of exposure time at sonication temperatures below 45 °C, but at higher temperatures a significant direct dependence was observed (Amini et al., 2015). One possible explanation is that high temperatures cause more serious physical destruction within the granules, and unraveling of double helices of the crystalline region, which reduces the stability of starch (Hu et al., 2019; Vela, Villanueva, & Ronda, 2021).

Swelling power and solubility of starches are properties whose susceptibility to be modified by US seem to also depend on its type and composition, and not just on the sonication parameters, particularly by the structural arrangement of amylose and amylopectin. Ultrasonication breaks the intermolecular bonds of starches generating a less compact granular arrangement and results in an increase of apparent amylose content (i.e., higher amount of short light chains), which allows a greater possibility to interact with water (increase in SP) and eases the release of amylose out of the granule to the aqueous medium (increase in S) (Kaur & Gill, 2019; Luo et al., 2008). Carmona-García et al. (2016) concluded that granule size also positively influences the modification of SP and S, since larger granules have larger surface area prone to be affected by sonication.

4.2 Pasting properties

These properties are among the main physicochemical properties of starches and flours, given that their functionality is greatly determined by pasting behavior, which transforms starches into a usable form (i.e., paste, gel) and therefore influences their application in starch-based food products (Wang, Xu et al., 2020). Changes in pasting profiles after ultrasonication are attributed to modifications caused to starch structure, and to the minor components. The structural changes that happen during gelatinization include the melting of crystalline regions and unwinding of double-helices, absorption of water in the amorphous regions, displacement of amylopectin units and leaching of amylose from the granules (Iida et al., 2008). Ultrasounds present different effects in the modification caused to pasting properties depending on the source of starch and the parameters employed. For instance, amylose content appears to be directly correlated with a higher susceptibility to modification, probably related to easier mobility of amylose chains compared to amylopectin chains (Thomaz et al., 2020).

Pasting temperature (PT) is the temperature at which the viscosity begins to increase during heating process, which indicates the structural resistance of starches to heat-induced swelling and rupture in water (Harasym et al., 2020; Wang, Xu et al., 2020). There is no consensus regarding the effect of US on PT. Some authors have reported an increase of PT after ultrasonication, which indicates better resistance to high temperature and strong mechanical shearing force due to US treatments (Hu et al., 2019; Sit et al., 2014; Yang, Lu et al., 2019; Zheng et al., 2013). Increase in PT after US supports the argument that the modification tends to increase crystalline perfection, resulting from reorientation of starch granules molecules or chains. The strengthening of intragranular bonded forces makes starch granules to require more heat and longer time before structural disintegration and paste formation occurs (Babu et al., 2019; Thomaz et al., 2020). When a significant lowering of PT has been reported, it has been indicated that ultrasonication caused granule disruption and partial degradation of amylopectin, which made them more permeable to water and less resistant to swelling (Herceg et al., 2010; Li et al., 2018; Wang, Xu et al., 2020).

Ultrasounds have not been reported to change pasting profile shapes (Hu et al., 2019; Wang, Xu et al., 2020), however they have been generally reported to decrease the viscosity achieved during pasting event (Acevedo et al., 2022; Hu et al., 2019; Luo et al., 2008; Thomaz et al., 2020). The

reduction of pasting viscosity profiles is attributed to both the physical damage caused to starch granules, and to changes in starch molecular structure (Falsafi et al., 2019; Zuo et al., 2009). Starch granules breakdown promotes the penetration of water, while the degradation of the starch macromolecular chains by sonication causes chains to become shorter, which would result in lower viscosity during gelatinization. This reduction of the molecular chain length and molecular weight of starches by cavitation results in weaker interaction force of starch granules and a partially degraded starch gel network that is less resistant to shear, resulting in lower viscosity profiles (Hu et al., 2019; Huang et al., 2007; Sujka & Jamroz, 2013; Yang, Kong et al., 2019).

Because of continuous swell of starch granules, peak viscosity (PV) can be acquired from a complete rupture of its inherent hierarchical structures (Wang, Xu et al., 2020). The reduction of PV after sonication indicates that US could weaken the granule structure and integrity and rigidity due to glycosidic bond cleavage (Chan et al., 2010; Li et al., 2018). The lessened amounts of crystallites, amorphization of short-range ordered structure and disruption of helical structures caused by violent physical forces (e.g., micro jets, shear forces, shock waves) and -OH free radicals during ultrasonication might reduce PV, as the disordered aggregation structures of starch often show weak resistance when subjected to shearing and heating (Wang, Xu et al., 2020; Yang, Lu et al., 2019). Breakdown viscosity (BV) reflects the degree of granule disruption after reaching maximum viscosity value. Lower BV values after ultrasonication suggest stronger resistance of the starch granules to shear-thinning during cooking and strengthen stability in the hot paste (Li et al., 2018; Wang, Xu et al., 2020; Wang, Wu et al., 2020). Final viscosity (FV) is a critical quality parameter that indicates the ability of the sample to form a viscous paste or gel after heating and cooling (Harasym et al., 2020), while setback viscosity (SV) reflects the retrogradation capacity of starchy foods, positively correlated with amylose content (Singh et al., 2007; Wang, Wu et al., 2020). The lower FV and SV values after US are attributed to the decrease in the degree of polymerization of treated starches, due to degradation and depolymerization of the leached amylose and long-chain amylopectin (Jin et al., 2020; Yang, Kong et al., 2019).

It is worth mentioning that pasting properties have not always been reported to be reduced by ultrasonication, since the modification depends on the treatment conditions matrix. Some authors have indicated no significant changes (Li et al., 2019) or higher values after ultrasonication (Bernardo et al., 2018; Cao & Gao, 2020; Pinto et al., 2015; Yang, Kong et al.,

2019). Increased pasting values have been attributed to (a) a possible loosening of the interaction between amylose and amylopectin chains after ultrasonication (Bernardo et al., 2018), (b) micro-crystallization and reorientation of starch molecules or chains (Cao & Gao, 2020), and (c) a softer starch matrix induced by sonication resulting in easier pasting and higher viscosity (Park & Han, 2016). It was said by Herceg et al. (2010) that increasing US power (300 and 400 W) caused a greater disruption of starch granules and weakening of the crystalline region, causing more water being entrapped within the starch molecule, which leads to higher viscosity. Increase of SV could be attributed to higher amount of linear chains after degradation and depolymerization of long-amylose chains, facilitating the re-association or re-arrangement of starch molecular chains during cooling (Sit et al., 2014; Wang, Xu et al., 2020).

4.3 Paste clarity

Starch paste clarity is important quality parameter in food applications, as it influences the optical properties of final product (Falsafi et al., 2019). The transmittance of starch pastes is associated with the particle size distribution of starch, and the proportion of amylose and amylopectin, larger expanded particle size and greater amylopectin content tend to increase starch paste transparency (Hu et al., 2014; Zheng et al., 2013). Ultrasounds can break the chains of amylopectin by disrupting covalent bonds, which results in a decrease in association between starch molecules and causes a paste clarity increase (Hu et al., 2014; Zheng et al., 2013). US treatments led to an increase in paste clarity of potato (Sujka & Jamroz, 2013), corn (Hu et al., 2014; Jambrak et al., 2010), sweet potato (Zheng et al., 2013), maize (Bel Haaj et al., 2013), mung bean (Chung et al., 2002), millet (Li et al., 2019) and rice (Sujka & Jamroz, 2013) starches, which seems to be improved by longer sonication times (Bel Haaj et al., 2013). The extent of the effect has been reported to depend on the sonication device used. At the same US frequency, Jambrak et al. (2010) and Falsafi et al. (2019) reported a greater increase of transmittance when US were applied using a probe than when a bath was used. It has also been demonstrated that the solvent used for preparing the suspension influences the effect of US over paste clarity, due to the medium's surface tension and the dynamics of bubble formation and implosion. Sujka & Jamroz (2013) determined that potato starch's paste clarity was significantly increased after sonication in water, but it was not modified when sonicated in ethanol. Increase paste clarity probably results from damage caused by US to the starch particle surface and the crystalline regions, leading to increased association between starch and water (thereby resulting in decreased

association between starch molecules) making starch particles to expand easily and increase the starch paste transparency (Hu et al., 2014). However, some authors have indicated no significant change (Sit et al., 2014) and even a paste clarity reduction (Bernardo et al., 2018) after ultrasonication at high US conditions (15 min at 70 % amplitude). A clarity decrease has been attributed to starch granules disintegration, allowing them to swell more, making the starch paste more viscous and decreasing the transmittance (Sit et al., 2014). It could also be attributed to the degradation of amylose and amylopectin, and the fissures formed on starch granules, favoring the essential linear amylose molecule to leave the starch granule, hence reducing paste clarity (Bernardo et al., 2018).

5. Effect of ultrasonication on the thermal properties of starches

The onset (T_O) and conclusion (T_C) temperatures reflect the melting temperatures of the weakest crystallites and high-perfection crystallites in the starch granules, respectively (Wang, Wu et al., 2020), while the gelatinization temperature range ($\Delta T = T_C - T_O$) represents the extent of homogeneity of crystallites within the granules (Falsafi et al., 2019). It has been said that power, time and temperature highly influence the modification caused to gelatinization temperatures (Amini et al., 2015; Li et al., 2019; Yang, Lu et al., 2019; Yu et al., 2013). Some authors have indicated that US do not lead to significant changes of gelatinization temperatures (Carmona-García et al., 2016; Jambrak et al., 2010; Monroy et al., 2018; Rahaman et al., 2021; Yang, Kong et al., 2019), while most of the literature has reported significant modifications.

A significant shift towards higher temperatures has been reported in a wide variety of starches such as maize (Luo et al., 2008), corn (Amini et al., 2015; Flores-Silva et al., 2017), rice (Park & Han, 2016), yam (Bernardo et al., 2018), oat (Falsafi et al., 2019), waxy corn (Yang, Lu et al., 2019), potato (Cao & Gao, 2020; Zhang et al., 2021) and cowpea (Acevedo et al., 2022). The less ordered double-helical structures within the granules are more prone to disruption during sonication, reflected by a delay of T_O in sonicated samples, requiring higher temperatures to dissociate the more stable and stronger crystalline structure after treatments (Bernardo et al., 2018; Luo et al., 2008; Falsafi et al., 2019; Yang, Lu et al., 2019; Zhang et al., 2021). On the contrary, a significant reduction of gelatinization temperatures has been reported after ultrasonication of rice (Yu et al., 2013), maize (Flores-Silva et al., 2018), corn (Li et al., 2018), millet and potato (Hu et al., 2019), chestnut (Wang, Wu et al., 2020) and sweet potato (Wang,

Xu et al., 2020) starches. It was found by Yu et al. (2013) that T_O and T_P values showed an inverse correlation with the applied US power, which could explain the different results reported in the literature. Lower gelatinization temperatures could be caused by a change in starch matrix due to greater mobility of starch polymers after ultrasonication, promoting water entering the interior of the granules and accelerating the irreversible hydration of the starch (Hu et al., 2019). The bonds formed between freely moving amylose molecules and amylopectin chains present in crystalline regions would require less energy to be destroyed (Li et al., 2018). Despite the different results reported for T_O, T_P and T_C after US treatments, there seems to be a general agreement that ΔT is significantly reduced after ultrasonication, particularly when applying high US intensity (Bernardo et al., 2018; Falsafi et al., 2019) and high temperature (Amini et al., 2015). The narrowing of ΔT may happen because ultrasound treatments disrupt the ordered double-helical structures containing flaws and lead to the breakage of crystallites of different stabilities, decreasing the degree of diversity in the crystals and lowering the dissociation temperature range (Ding et al., 2019; Luo et al., 2008; Wang, Xu et al., 2020). Said narrowing has also been explained as distortion of the amorphous and non-organized parts of the starch granule by ultrasonication, which might enhance the homogeneity of starch granular structure towards a well-ordered crystalline remnants with narrower ΔT (Amini et al., 2015).

The gelatinization enthalpy (ΔH) values reflect the loss of double-helical order in the crystalline and non-crystalline regions of the granules that unravel and melt during starch gelatinization (Huang et al., 2007; Jin et al., 2020; Li et al., 2019). The effect of ultrasonication on ΔH depends greatly on treatment conditions and sample's botanical origin (amylose/amylopectin composition). Yang, Kong et al. (2019) and Yu et al. (2013) determined that the change is influenced by the applied US power, where low powers (≤300 W) leads to reduced ΔH values, while an increase of ΔH was obtained when applying higher powers (≥450 W). Higher ΔH denotes greater number of double helices with more compact packing (indicating that the amorphous regions are degraded by US prior to the crystalline regions) (Huang et al., 2007; Li et al., 2019), a rearrangement of the molecular packing within the granule microstructure, and that ultrasonicated starches presented a higher amylopectin content resulting from leaching of amylose in the liquid medium where treatment was performed (Carmona-García et al., 2016; Flores-Silva et al., 2017). The amylose/amylopectin composition of the treated matter and the degree of damage that its amorphous and crystalline regions suffer due to US treatment also

determine the final effect over Δ*H*. Hu et al. (2019) reported that by applying the same treatment conditions, Δ*H* was decreased in potato starch, while it was increased in millet starch. With this complex array of variables, it is easy to understand why there is not a consensus in the literature regarding the effect of US treatments on Δ*H*. It is worth mentioning, however, that in general more intense US conditions modify the crystallinity of the starch and reduce the gelatinization enthalpy (Thomaz et al., 2020).

A decrease of Δ*H* after ultrasonication has been attributed to: i) surface damage cause to starch granules, increasing the diffusion of water molecules into the starch granules and hence their access to the crystalline regions (Yang, Kong et al., 2019); ii) disintegration of the double helices present in the crystalline and non-crystalline regions of the granule by cavitation (Huang et al., 2007; Luo et al., 2008; Zuo et al., 2009); iii) distorting the crystalline regions by H^+ ions released during the sonochemical ionization of water molecules (Falsafi et al., 2019). Δ*H* has been positively correlated to branch-chain length of amylopectin. A decrease in Δ*H* indicates that some of the external chains of amylopectin are destroyed after ultrasonication, so the US-treated starch would require less energy for gelatinization (Yang, Lu et al., 2019; Yang, Kong et al., 2019).

6. Effect of ultrasonication on gels made with treated starches

Starch finds many industrial applications as thickener, colloidal stabilizer, gelling agent, bulking agent, water retention agent, and adhesive, mainly due to its gelling capacity when heated in presence of water (Herceg et al., 2010). In the gelatinization process, linear and short-chain amylose and amylopectin chains are leached out from the granules, forming a highly viscous continuous matrix, while amylopectin-rich insoluble remnants are dispersed in the continuous matrix, forming a microstructure with complex rheological response (Flores-Silva et al., 2017). The rheological behavior and textural properties of the gels depend strongly on starch molecular structure, such as chain length distributions and ramification degree of leached starch chains (Flores-Silva et al., 2017), so the fragmentation of amylose chains and debranching of amylopectin molecules caused by ultrasound waves also modify the structure of the gels formed with the US-treated starches and flours.

6.1 Rheological properties

Rheology reflects the flow and deformation behaviors of fluid foods, which is typically determined by processing conditions such as high shearing, stirring, mixing and pumping. The rheological behavior of starch paste or gel is considered as one of the most important physical properties for determining the eating quality of starch or starch-based foods and the acceptance by consumers (Wang, Xu et al., 2020). The rheological properties of starch gels are used as indicators of processing suitability and gelling properties of food products, and are strongly dependent on the accessibility and entanglement of amylose and amylopectin, chain length distributions and ramification degree of leached starch chains, the interactions and tightness between polymer chains, and the dissolution ability of amylose, which determine the stability of the formed three-dimensional network structure (Flores-Silva et al., 2017; Wang, Xu et al., 2020).

6.1.1 Steady shear flow behavior

The flow behavior of starch gels is analyzed by measuring its apparent viscosity in a determined shear rate range (at constant temperature), usually upwards and downwards, to later apply least-squares fitting to a power-law model (Herschel-Bulkley model, Ostwald de Waele model) that describes the state flow behaviors of the gels. The flow behavior index (n), the consistency coefficient (k), and the time-dependence behavior are determined with these models. Rotational tests allow to analyze the rheological behavior of the gels under large shearing deformations (Monroy et al., 2018).

It has been indicated that the apparent viscosity of starch gels is decreased by ultrasonication due to granular structure destruction and depolymerization of the main components (fragmentation of amylose chains and debranching of amylopectin molecules) caused by the high shear forces of ultrasound waves in C-O-C linkages (Amini et al., 2015; Azhar & Hamdy, 1979; Chung et al., 2002; Falsafi et al., 2019; Kang et al., 2016). Flores-Silva et al. (2017) determined decreasing apparent viscosity values with longer sonication times (see Figure 11), indicative of weaker gels that reflect the fragmentation of long-chain starch molecules. Kang et al. (2016) also reported a sharp apparent viscosity decrease at the early stage of sonication and then a slower reduction reaching a limiting value. This slowdown in the rate of apparent viscosity reduction reflects how starch molecule chains get shorter with increasing ultrasound exposure, and

progressively approach the minimum chain length that limits the US degradation process (Jambrak et al., 2010), which was firstly mentioned by Czechowska-Biskup et al. (2005).

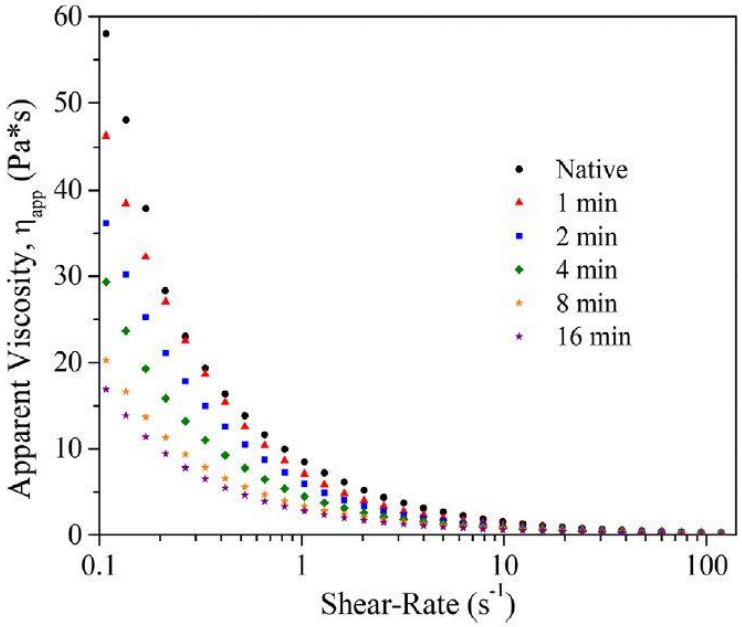


Figure 11. Behavior of the apparent viscosity of native corn starch and corn starch sonicated for different times (Flores-Silva et al., 2017).

The flow behavior index (*n*) and consistency coefficient (*k*) have been reported to be modified by ultrasonication at different extents. Starch concentration and sonication amplitude do not appear to have a significant influence on the value of *n* (Amini et al., 2015), while time and temperature highly influence the effect of US on *n*. Starches usually present values of $n < 1$, displaying a pseudo-plastic behavior (Zhang et al., 2021). Kang et al. (2016) reported an increase of *n* with sonication time, indicating the formation of weaker gels as the starch dispersion was subjected to longer US exposure, diminishing pseudoplasticity to the point where starches showed a Newtonian behavior ($n = 1$). These results could be due solubilization (disaggregation) of the aggregated macromolecules in the starch pastes by ultrasonication, so the shear applied during viscosity measurements will no longer contribute to the break-down of the starch aggregates resulting in a reduction of the thixotropic behavior (Kang et al., 2016). Starch molecular size reduction will also result in the transition from a pseudoplastic behavior to a Newtonian behavior, characteristic of a dilute macromolecular suspension (Kang et al., 2016). Amini et al. (2015) determined that the effect of ultrasonication on *n* depends highly on the

interaction between treatment temperature and time. At temperatures close to 45 °C, sonication time did not affect the value of *n* but at lower temperatures, increasing sonication time decreased the value of *n*. In contrast, increasing exposure time at higher temperatures (> 45 °C) caused a profound increase of *n*. Zhang et al. (2021) agreed on this in the study where the ultrasonication of corn, potato and pea starches was performed at different times and constant temperature (25 °C), where *n* values were reduced (Zhang et al., 2021).

In the case of consistency coefficient (*k*), the available literature suggests that temperature is the main variable determining the modification caused by ultrasonication. Amini et al. (2015) determined that *k* increased in corn starch as the treatment temperature increased up to 45 °C, while increasing the temperature to 65 °C diminished *k* considerably. Also, regarding the ultrasonication of corn starch, Zhang et al. (2021) concluded that the values of *k* were increased by US treatments at different powers (100 – 600 W) and times (5 – 30 min), while Jambrak et al. (2010) in treatments performed in the same power (100 – 400 W) and time (15 and 30 min) ranges indicated that *k* values were reduced after ultrasonication. The different reported results are believed to derive from the treatment temperature. In the treatments performed by Zhang et al. (2021) it is indicated that treatment temperature was 25 °C, which lays in the temperature range where Amini et al. (2015) said that ultrasonication causes an increase of *k* (< 45 °C). In the treatments performed by Jambrak et al. (2010), however, the treatment temperature is not indicated, which usually means that it was not controlled, so treatment temperature would have presumably risen beyond 45 °C, which would agree with the explanation of Amini et al. (2015) and would lead to a reduction of *k*. In the cases where Jambrak et al. (2010) obtained an increase of *k* (treatments applied using US bath, and the one performed using a probe at 100 W for 15 min) it is believed that ultrasonication conditions were rather soft (low power and/or short time), so treatment temperature may not have been significantly increased, possibly laying in the < 45 °C range. Higher *k* values suggest gels with high structural strength and resistance to flow, which might result from the loose internal crystalline structure of sonicated corn starch (Zhang et al., 2021). The alteration of rheological parameters may be attributed to a combined effect of starch granule disruption and breakdown of the linear amylose molecules (Amini et al., 2015).

6.1.2 Dynamic oscillatory assays

6.1.2.1 Strain/Stress sweeps

Strain (or stress) sweeps at constant angular frequency are performed to determine the limit of linear viscoelasticity of the gels (Wang, Xu et al., 2020). These assays indicate two different regions in gels behavior, the linear viscoelastic region (LVR), where the elastic (G') and viscous (G'') moduli as well as the loss tangent [tan(δ)] are constant, and the non-linear region, where the gels quickly lose their structure's integrity. The maximum deformation that gels are able to resist before disruption of their structure is denoted by τ_{max}, which marks the end of the LVR. These tests have been used by some authors (Amini et al., 2015; Carmona-García et al., 2016; Kaur & Gill, 2019; Monroy et al., 2018; Wang, Xu et al., 2020) when studying US-treated starches to determine the LVR in order to carry out frequency sweeps. However, there have not been results reported regarding the effect of US treatments on τ_{max} or the cross over point ($G' = G''$), despite the valuable information that these parameters give about the gels' resistance.

6.1.2.2 Frequency sweep

Frequency sweeps are performed on gels to determine their frequency dependence (within the LVR) at a specific temperature, applying a constant strain (or stress). The viscoelastic properties of gels as function of frequency are characterized by storage (G') and loss (G'') moduli, and loss tangent [$tan(\delta) = G''/G'$], which are used to quantify the strength of starch gels (Amini et al., 2015). Higher values of G' than G'' show a predominance of the solid/elastic behavior (Kaur & Gill, 2019). The modification that US treatments cause to the frequency sweep parameters is influenced by starch granule disruption and breakdown of amylose chains (Amini et al., 2015). There seems to be a general agreement in the literature that ultrasonication induces starch macromolecules fragmentation, leading to short polymeric chains in US-treated starches. However, there is no consensus regarding the association that the shorter chains may have during gelatinization, and while some sources report increased moduli after treatments, others indicate the contrary. Treatment time seems to be the main factor influencing the extent of modification achieved in frequency sweep parameters.

At short treatment times the moduli have been reported to be increased, believed to be due to weakening of the crystalline regions, causing the molecules to entrap more water which leads to

higher viscosity (Kaur & Gill, 2019). Within the short time range (≤ 20 min), Monroy et al. (2018) concluded that increasing times lead to stronger gels (higher G' values), since starch polymer chains fragmentation occurs, which would facilitate the gelation process and subsequent retrogradation phenomenon by reassociation of short polymeric chains through hydrogen-bonds to form a more elastic three-dimensional network. Zhang et al. (2021) reached a similar conclusion after ultrasonication of corn, potato and pea starches at different powers and times. These authors determined increased G' and G'' values after US treatments attributed to degradation of starch granules by cavitation, making them more permeable to water, and the tendency of starch molecules (especially amylose) to re-form double helices resulting in a harder gel (Zhang et al., 2021). Higher G' values after US could be attributed to the increase of amylose content, the pores and cracks on granules surface, and the disruption of crystalline and ordered molecular structure, which enhanced the entanglement and interactions of amylose and amylopectin and lead to the formation of a strengthened gel network structure (Wang, Xu et al., 2020). However, at longer ultrasonication times [30 min according to Kaur & Gill (2019)], it has been indicated that both moduli are reduced, attributed to the severe damage caused to the starch granules by the shear forces of cavitation, leading to the straightening out of amylose molecules that reduce the shear action within the fluid layers and contribute to viscosity decrease. A sharp decrease of G' was determined by Carmona-García et al. (2016) after 50 min ultrasonication of plantain and taro starches, suggesting that amylopectin was largely affected by treatment, which generated lineal chains that were not able to form a consolidated compact network during gelatinization (Carmona-García et al., 2016).

Loss tangent [*tan(δ)*] gives information about the strength of the gel, which indicates the ratio of energy lost to the energy stored (G''/G'). The lower the value of *tan(δ)*, the higher the strength of the gel (Wang, Xu et al., 2020). Despite the different results reported for G' and G'' depending on the applied time, the available literature seems to agree about the lowering of *tan(δ)* after ultrasonication, suggesting that US could change the state of starch pastes to more solid-like (Zhang et al., 2021). Reduced values of the *tan(δ)* have been reported after ultrasonication of a wide variety of starches (wheat, barley, rice, maize, plantain, taro, corn, potato, pea and sweet potato) (Carmona-García et al., 2016; Kaur & Gill, 2019; Wang, Xu et al., 2020; Zhang et al., 2021). Lower *tan(δ)* values have been indicated for longer treatment times, while at shorter times only slight variations (or even no change at all) have been reported (Amini et al., 2015; Carmona-

García et al., 2016; Kaur & Gill, 2019). Amini et al. (2015) obtained an increase of the value of *tan(δ)* in corn starch ultrasonicated at 65 °C, which is clearly caused by the high treatment temperature and not by ultrasonication, given that treatments at lower temperatures did not show the same effect. In that study, the treatment temperature was beyond T_o of the native starch (64.8 °C), which could have caused the *tan(δ)* increase. When there is a rise in temperature, the molecules absorb translational energy and gradually cease to retain their hydration, which causes the lowering of viscosity (Kaur & Gill, 2019). The effect of ultrasonication in reducing *tan(δ)* could be attributed to structural rearrangement, straightening out of amylose, and disruption of starch granules in sonicated samples (Zhang et al., 2021). It has been said that ultrasonicated starches could be utilized as strong gelling agents in food industry given the higher strength of gel showed by *tan(δ)* (Wang, Xu et al., 2020).

6.2 Textural properties

Gel's texture profile is usually characterized by hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness (Babu et al., 2019). Gel hardness is mainly caused by retrograding starch, associated with water syneresis and amylopectin crystallization loss (Herceg et al., 2010). The available literature has demonstrated that ultrasonication led to lower hardness in gels prepared from corn, (Hu et al., 2014), oat (Falsafi et al., 2019), and foxtail millet starches (Babu et al., 2019). Cohesiveness, springiness (Babu et al., 2019), elasticity, conglutination degree, chewiness, and recoverability (Hu et al., 2014) have also been reported to been reduced by ultrasonication. The intramolecular hydrogen bonding of starches can be broken due to mechanical vibration, thermal and ultrasonic effects, thus the molecular structures become loose and molecular winding nodes are reduced (Hu et al., 2014). Larger texture properties reductions are reported for higher frequency and longer sonication times, since starch granules become further disrupted and depolymerized by greater sonication exposure (Babu et al., 2019). The liberation of low molecular weight short glucan fractions from amylose and amylopectin following cavitation reduces the ability of starch to form homogenous gel structures. Chains with a polymerization degree below 12 negatively affect the gel formation ability of starches (Falsafi et al., 2019). The study carried out by Herceg et al. (2010), on the other hand, reported higher values of hardness, adhesiveness, cohesiveness, springiness, and gumminess on gels made with US-treated corn starch. In this case, these apparently contrary results are believed to be explained by the temperature reached by the starch dispersions during treatments. As it has been

mentioned before, temperature is an important treatment parameter determining the modification caused to starches and the gels made with them. In the treatments performed by Herceg et al. (2010) temperature was not controlled and, given the US powers (100 – 400 W) and times (15 and 30 min) that these authors studied, there was surely a temperature rise during ultrasonication which must have probably caused an annealing effect. This combined annealing-ultrasound treatment would lead to significantly different results than what is obtained only by US (Amini et al., 2015; Hu et al., 2019). In the study presented by Babu et al. (2019) comparing the texture properties obtained in gels made with foxtail millet starch modified by US and annealing treatments separately and by a combination of both (annealing+ultrasound and ultrasound+annealing), they determined that by applying ultrasound alone the hardness of the gel decreased, but when applying annealing after ultrasonication the hardness was increased. Sonication prompted high pressure gradients and high local velocities that contributed to damaging granules and cutting long chains into appropriate length ones, which further get re-associated during annealing and retrogradation process, forming harder gels (Babu et al., 2019). If there was in fact a temperature rise during the ultrasound treatments performed by Herceg et al. (2010), resulting in simultaneous ultrasounds-annealing treatments, the explanation provided by Babu et al. (2019) could justify the determined hardness increase, opposite to the generally reported tendency of reduced gel hardness after ultrasonication.

7. Effect of ultrasonication on starch digestibility

Starch digestion properties are important because of their extensive use in food, brewing fermentation, and pharmaceutical and chemical industries (Ding et al., 2019). Starch *in vitro* digestion properties are commonly evaluated following the procedure of Englyst et al. (1999), that simulates human gastrointestinal tract digestion, where starch samples are incubated with pancreatin from porcine pancreas and amyloglucosidase enzymes at 37 °C for 120 min. For nutritional applications, starch comprises three portions: rapidly digestible starch (RDS) is the fraction digested within 20 min, slowly digestible starch (SDS) is the fraction digested between 20 and 120 min, and resistant starch (RS), the remaining fraction after 120 min (Zhang et al., 2021). RDS is digested in the mouth and small intestine, resulting in a rapid increase of postprandial blood sugar level, whereas SDS is digested slowly in the small intestine, leading to stable postprandial blood sugar levels (Zhang et al., 2021). RS is not digested in the small intestine and does not contribute to blood glucose level, becoming a substrate for the intestinal

microbiota, thus providing health benefits and reducing risk factors for diet-related diseases (risk of developing type II diabetes, obesity, and cardiovascular disease) (Ding et al., 2019; Zhang et al., 2021). Starch digestion properties can be evaluated in raw and in gelatinized starches, where gelatinized samples present lower values than their raw counterparts due to cooking (Kaur & Gill, 2019).

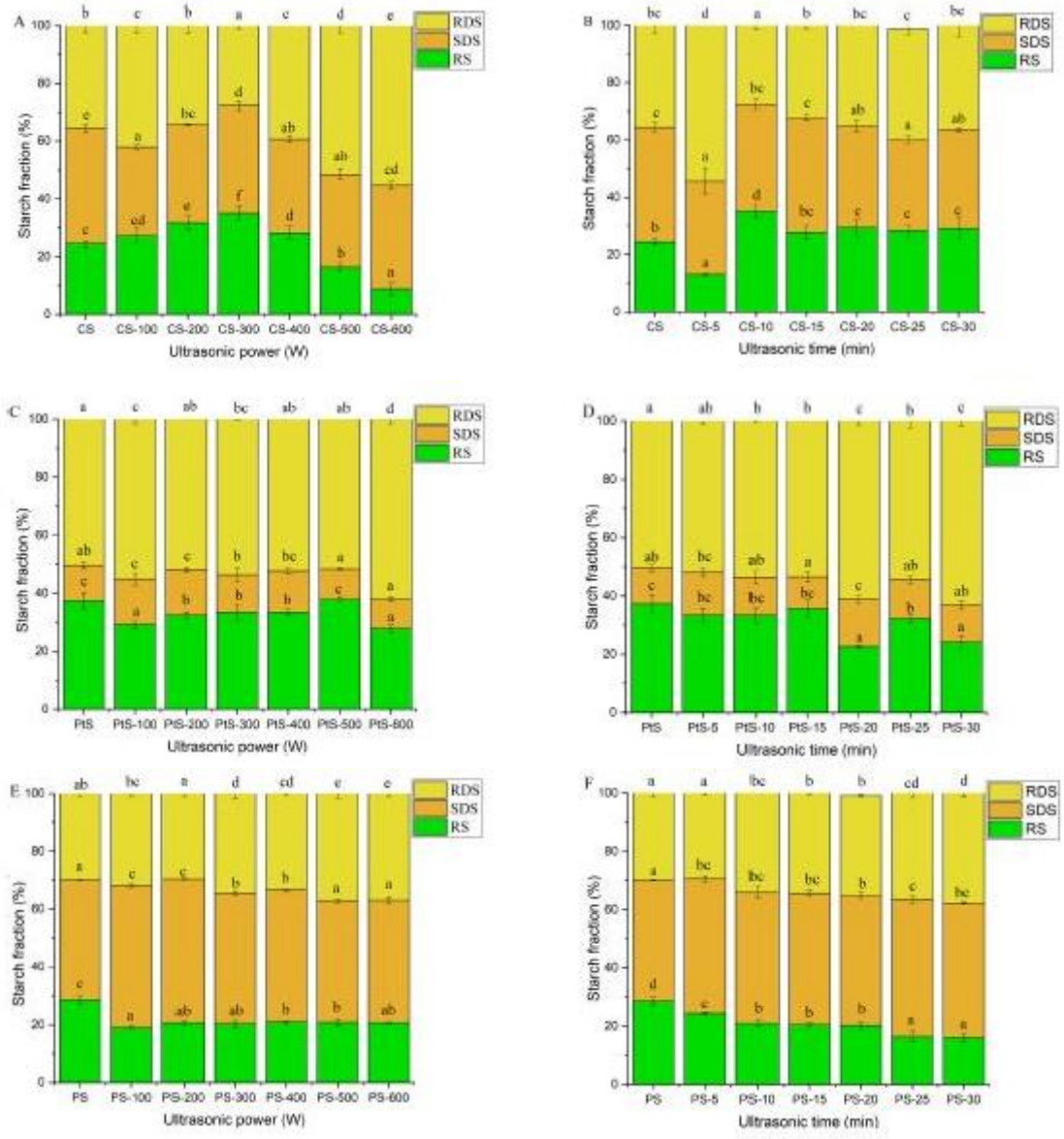


Figure 12. Digestion profile of native and sonicated corn, potato and pea starches under different ultrasonic powers and times; A-B, native and sonicated corn starch (CS); C-D. native and sonicated potato starch (PtS); E-F, native and sonicated pea starch (PS). Different letters upon the pillars of the same color indicate statistical differences ($p < 0.05$) (Zhang et al., 2021).

Some authors have not reported a significant difference of RDS after ultrasonication (Acevedo et al., 2022; Flores-Silva et al., 2017), while others reported an RDS increase (Cao & Gao, 2020; Flores-Silva et al., 2017; Kaur & Gill, 2019). RDS content of different US-treated starches (wheat, barley, rice, and maize) increased with increasing sonication time, both in raw and gelatinized starches (Kaur & Gill, 2019). This direct correlation of increasing RDS values with sonication time could be explained by the holes and cracks formed on granules due to ultrasonication, making the porous surface more susceptible to enzymatic attack. Crystalline regions are more compactly arranged than amorphous regions and thus less susceptible of attack by digestive enzymes, but after sonication the double-helix structure of starches might be disrupted, allowing easier access of enzymes to the inside of starch granules (Flores-Silva et al., 2017; Kaur & Gill, 2019). The RDS increase in gelatinized starch might also be explained by the increased availability of short-length chains obtained from the fragmentation of amylose chains and debranching of amylopectin molecules, which are more amenable for enzymatic degradation (Flores-Silva et al., 2017). In the case of SDS, there is not a definite effect of US treatments. While some studies have reported a significant decrease (Cao & Gao, 2020; Kaur & Gill, 2019), others indicated an increase after ultrasonication (Acevedo et al., 2022). SDS is associated with positive health effects including improved glycemic control, reduction of postprandial circulated free fatty acids and reduction of oxidative stress (Acevedo et al., 2022). US treatments could increase the digestion of starch (RDS and SDS) due to the partial loss of starch crystallinity caused by the disruption of double helices, and promotion of polymer degradation by cavitation (Acevedo et al., 2022; Zhang et al., 2021).

RS content has said to increase after US treatments (Babu et al., 2019; Cao & Gao, 2020; Flores-Silva et al., 2017; Kaur & Gill, 2019), possibly due to interactions between different chains of starch or different microconstituents (such as amylose and amylopectin, amylose and protein, amylopectin and lipid, etcetera), and the rearrangement and formation of hydrogen bonds within starch inner structure (Cao & Gao, 2020). It was determined by Zhang et al. (2021), however, that such increase would only happen in a specific US power range. In ultrasonication of corn starch, Zhang et al. (2021) determined that RS content increased significantly at low powers (100 – 300 W), while with further power increase (400 – 600 W) the RS content was reduced. It is believed that sonicating starch at low power led to a rearrangement of starch molecules that resulted in increased RS content (Zhang et al., 2021). However, excess power resulted in a

decrease of RS content, possibly because it caused more serious physical destruction within the starch granules and broke the crystalline molecular structure to a state that was easily accessible to enzymes. These results revealed that the rearrangement and destruction of starch structures occur simultaneously during the US treatment, and that the destruction plays the dominant role at high power ultrasonication (Zhang et al., 2021).

The effect of ultrasonication on starch digestion properties are complex and depend on the applied treatment parameters, such as time (Flores-Silva et al., 2017), power (Zhang et al., 2021), starch botanical origin (Zhang et al., 2021) and even granule size (Kaur & Gill, 2019) (see Figure 12). Granule size may also affect starch digestibility since smaller size starches exhibit higher digestibility rate, due to their increased surface area for specific volume, subsequently, the chance of enzymatic attack on substrate increases (Kaur & Gill, 2019). Further research is needed to reach solid conclusions regarding the effect of US on starch digestion properties.

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II OBJECTIVES

The use of ultrasonication in the modification of starches has been greatly covered, however flours have not been deeply covered by the literature. Flours are more complex matrixes than starches given the presence of other components (proteins, lipids, and other carbohydrates) that interact with starch. The main objective of this research was to gain deeper understanding of the effect of ultrasound treatments on the modification of gluten-free flours, and to stablish the impact that key treatment parameters (time, concentration, temperature, and botanical origin of the flour) have on the extent of the modification achieved. To reach the main objective, the following specific objectives and working plan were proposed:

- Study the influence that sonication time and flour concentration have on the extent of modification achieved by US treatments.

  With that purpose rice flour was studied for being the most suitable ingredient in gluten-free formulations due to its natural lack of gluten, hypoallergenic properties, bland taste, and white color. Ultrasound treatments were performed on at a constant frequency of 24 kHz, maximum power of 180 W, 80 % on-off cycle, and temperature of 20 °C. Time was varied from 2 to 60 minutes, while flour concentration was studied in the range from 5 to 30 % (w/w). The modifications achieved were quantified in morphological, techno-functional, hydration, structural, thermal, and pasting properties of the flours, and rheological properties of gels made with the flours. This study is presented in Chapter 1.

- Determine the influence that temperature has on treatments, and differentiate the modification caused exclusively by the treatment temperature (ANN) from the combination of US and ANN.

  To accomplish this aim, rice flour was modified at a constant frequency of 24 kHz, maximum power of 180 W, 80 % on-off cycle, concentration of 10 % (w/w) and time of

60 min. The studied temperatures were 20, 40, 50 and 60 °C. A set of samples were modified exclusively by ANN, by keeping the rice flour at 90 % (w/w) water during 60 min at the designated temperature, while the other set was also sonicated, resulting in combined US+ANN treatments. The results of both samples sets were compared, as well as with the native rice flour. The modifications were quantified in morphological, techno-functional, hydration, structural, thermal, and pasting properties of the flours, and the rheological properties of the gels made with the flours. This study is presented in Chapter 2.

- Evaluate the influence of temperature in the modification of nutritionally more complex flours by US treatment.

  To achieve this objective, the modification of two varieties of tef flours (white and brown) was performed by ultrasonication. In this study the sonication parameters were kept constant at 24 kHz, maximum power of 180 W, 80 % on-off cycle, time of 10 min and concentration at 25 % (w/w), while temperature was varied from 20 to 55 °C. The modification caused to the flours was quantified in their morphological, techno-functional, hydration, and pasting properties of the flours, and the rheological properties of the gels made with the flours. Part of this research was carried out at Purdue University (U.S.A.) under the supervision of Dr. Hamaker. This study is presented in Chapter 3.

- Analyze the impact of ultrasonication on the microstructure of two varieties of tef flours (white and brown), modified at different temperatures.

  For that purpose, the modification of the two previously studied varieties of tef flours (white and brown) was characterized by changes in their microstructure, using X-ray diffraction, size exclusion chromatography, Fourier transform infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, and thermal properties. Part of this research was carried out at Purdue University (U.S.A.) under the supervision of Dr. Hamaker. This study is presented in Chapter 4.

- Study the influence of drying method on the modification achieved by ultrasound treatments in gluten-free flours from different botanical origin.

  To achieve this objective, 4 gluten-free flours (rice, tef, corn and quinoa) were modified by the same ultrasonication conditions [frequency of 24 kHz, concentration of 25 % (w/w), time of 10 min and temperature of 20 °C]. After treatment, a set of flours were dried by freeze-drying and another set was dried by sedimentation by centrifugation followed by air-drying at 40 °C. The modification achieved in the flours was quantified by the morphological, techno-functional, thermal, and pasting properties of the flours, and the rheological properties of the gels made with the flours. This study is presented in Chapter 5.

- Study the effect of the incorporation of US-treated flours in the development of gluten-free breads.

  To reach this goal, rice breads were elaborated, in which 30 % of the native flour was replaced with ultrasonicated flour at different times. Based on the previously determined results, flours were modified at a constant frequency of 24 kHz, maximum power of 180 W, 80 % on-off cycle, concentration of 25 % (w/w), temperature of 20 °C and times of 2, 5, 10 and 20 minutes. The modification achieved by the incorporation of US-treated flours was determined by changes in the doughs' rheological properties and the physical parameters of the elaborated breads. This study is presented in Chapter 6.

All the chapters presented in the present study correspond to scientific publications. A schematic representation of the properties studied in each chapter is presented in Table 13.

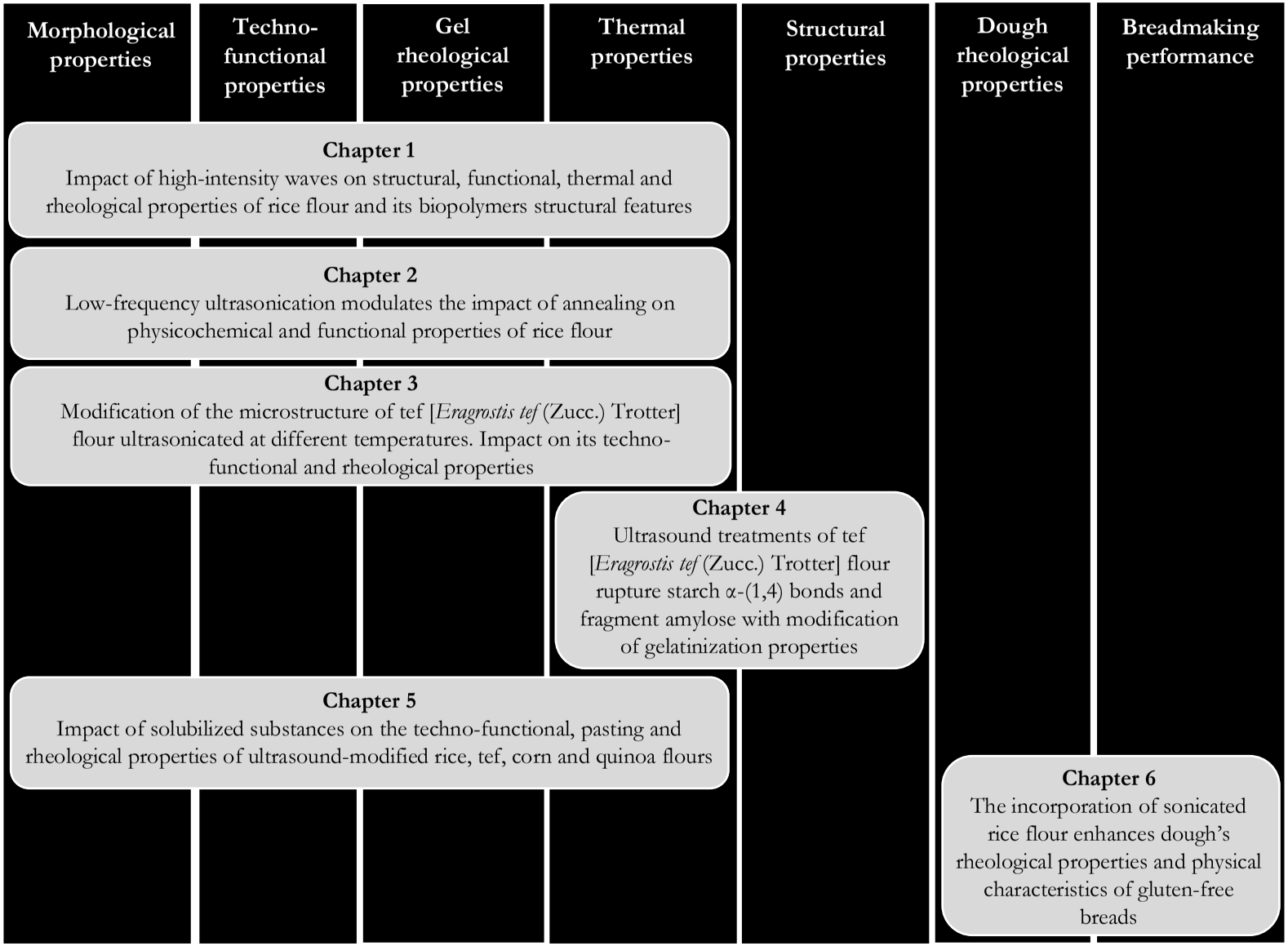

Figure 13. Schematic representation of the information covered in each chapter.

III RESULTS

CHAPTER 1

Impact of high-intensity ultrasound waves on structural, functional, thermal and rheological properties of rice flour and its biopolymers structural features

Food Hydrocolloids (2021), 113, 106480

Impact of high-intensity ultrasound waves on structural, functional, thermal and rheological properties of rice flour and its biopolymers structural features

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http://doi.org/10.1016/j.foodhyd.2020.106480

CHAPTER 2

Low-frequency ultrasonication modulates the impact of annealing on physicochemical and functional properties of rice flour

Food Hydrocolloids (2021), 120, 106933

Low-frequency ultrasonication modulates the impact of annealing on physicochemical and functional properties of rice flour

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http://doi.org/10.1016/j.foodhyd.2021.106933

CHAPTER 3

Modification of the microstructure of tef [*Eragrostis tef* (Zucc.) Trotter] flour ultrasonicated at different temperatures. Impact on its techno-functional and rheological properties

Current Research in Food Science (2023), 6, 100456

Modification of the microstructure of tef [*Eragrostis tef* (Zucc.) Trotter] flour ultrasonicated at different temperatures. Impact on its techno-functional and rheological properties

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https://doi.org/10.1016/j.crfs.2023.100456

CHAPTER 4

Ultrasound treatments of tef [*Eragrostis tef* (Zucc.) Trotter] flour rupture starch α-(1,4) bonds and fragment amylose with modification of gelatinization properties

LWT – Food Science and Technology (2023), 174, 114463

Ultrasound treatments of tef [*Eragrostis tef* (Zucc.) Trotter] flour rupture starch α-(1,4) bonds and fragment amylose with modification of gelatinization properties

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http://doi.org/10.1016/j.lwt.2023.114463

CHAPTER 5

Impact of solubilized substances on the techno-functional, pasting and rheological properties of ultrasound-modified rice, tef, corn and quinoa flours

Foods (2023), 12(3), 484

Impact of solubilized substances on the techno-functional, pasting and rheological properties of ultrasound-modified rice, tef, corn and quinoa flours

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http://doi.org/10.3390/foods12030484

CHAPTER 6

The incorporation of sonicated rice flour enhances dough's rheological properties and physical characteristics of gluten-free breads

(IN PRESS)

The incorporation of sonicated rice flour enhances dough's rheological properties and physical characteristics of gluten-free breads

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Abstract

Ultrasound (US) energy is an environment-friendly technology used in the present study to physically modify rice flour and improve its poor bread-making performance. The treatment was performed at different times (2, 5, 10 and 20 min) on an aqueous flour dispersion (25 g rice flour/100 g). The sonicated dispersion was used to substitute 30 % native (untreated) rice flour in the gluten-free bread control formulation. The breadmaking performance was characterized by doughs' pasting, thermal, and rheological properties, and physical quality of the resulting gluten-free breads. Ultrasonication time presented a direct correlation with particle fragmentation that led to a significant reduction of the onset and conclusion gelatinization temperatures, reflecting starch molecular rearrangement. Doughs' rheology presented reduced $tan(\delta)_1$ values and improved recovery after the application of a stress, denoting an enhanced elastic behavior in doughs containing sonicated rice flour. The partial depolymerization of starch by US facilitated yeast accessibility to simpler sugars, accelerating the generation of CO_2 and a higher dough development during proofing. The incorporation of US-treated flours led to breads with higher specific volumes (up to 24 %) and softer crumbs, showing a maximum effect at treatment of 10 min. The use of sonicated flour also delayed the hardening of bread during storage. In conclusion, short ultrasonication times can lead to significant improvement of the viscoelastic behavior and breadmaking performance of rice flour doughs.

Keywords: Dough rheology, Gluten-free bread; Rice flour; Thermal properties; Ultrasound treatment

6.1 Introduction

The gluten free (GF) market has been increasingly growing in recent years due to an increasing population diagnosed with celiac disease, and a popular trend of reducing gluten ingesta for considering it as a healthy improvement (Witczak et al., 2016). The development of GF products with a comparable quality to the traditional wheat-based products represents a difficult challenge due to structural problems derived from the limited baking capacity and natural characteristics of the raw GF ingredients. The lack of gluten leads to liquid batters rather than doughs, and result in baked breads with a crumbling texture, poor color, and inability to sufficiently retain gas bubbles during fermentation (Villanueva et al., 2019). Breads without gluten can only retain the gas generated during fermentation using additives in the formulation. The GF sources greatly depend on starch to provide structure and texture to the products made with them. The proprieties of native starch usually do not fulfill the industry's specific requirements because of limitations such as low shear and thermal resistance, and high tendency towards retrogradation (Singh et al., 2007). A strategy adopted by the food industry is the application of modifications by different means (i.e., genetic, mechanical, physical, chemical, or enzymatic) to alter the natural physicochemical properties of GF sources (starches and flours) so they can adapt better to the requirements of the industry (Yang, Kong et al., 2019; Zhu, 2015). It has been demonstrated that the characteristics of starch granules have a strong influence on processing and quality of the final products, derived from the interaction that starch has with other compounds present in the bread system (Witczak et al., 2016). Processing of the raw GF ingredients prior the dough making is important to improve the quality of the bread.

Physical modifications are well perceived in the food industry for being environmentally respectful, regarded as "clean label". These modifications impact water absorption and rheological properties of the doughs, degree of starch gelatinization, texture of the bread, and allow obtaining recipes with well controlled properties (Villanueva et al., 2019; Witczak et al., 2016). Among the physical modification methods of starches and flours, ultrasound (US) treatments have been recognized as a promising approach, attributed to its higher selectivity and efficiency, and reduced processing time (Amini et al., 2015; Yang, Kong et al., 2019). Ultrasounds can be classified into two categories: low intensity (1 W/cm^2) with a frequency of 5-10 MHz, and high intensity (10-1000 W/cm^2) with a frequency of 20-100 kHz (Vera et al., 2019). The energy from US acoustic waves is transformed into a chemically feasible form by the cavitation effect, in which multiple

collapsing bubbles induce high pressure gradients and high local velocities of liquid layers, resulting in shear forces and microjets that cause granular damage and alter the molecular structure of starches and proteins (Amini et al., 2015; Jambrak et al., 2010; Vera et al., 2019; Yang, Kong et al., 2019). The extent of modification caused by ultrasonication depends on treatment parameters (frequency, power, time, temperature, and starch/flour concentration in the suspension) and the nature of the treated matter (composition and type of starch). The structural damage after ultrasonication modifies the interaction that flours have with water as consequence of increased surface area after particle fragmentation, resulting in modification of the water-dependent properties, such as swelling power, solubility, thermal, pasting, and rheological properties (Amini et al., 2015; Kaur & Gill, 2019; Yang, Kong et al., 2019).

Rice (*Oryza sativa* L) flour is one of the leading crops in the world, and the most suitable ingredient in GF bakery formulation given its natural lack of gluten, hypoallergenic properties, bland taste, white color, low protein and sodium content, and the presence of easily digested carbohydrates (Kim & Shin, 2014; Villanueva et al., 2019). Previous studies have shown the suitability of US technology to modify the physicochemical properties of rice flour, resulting in significant differences in the thermal, pasting, and rheological properties (Vela, Villanueva, & Ronda, 2021; Vela, Villanueva, Solaesa, et al., 2021). Modifications in rice starch and proteins after US treatments led to the formation of stronger gels able to resist higher stress before the disruption of their structure (Vela, Villanueva, Solaesa, et al., 2021). It is important to investigate the influence that this physical modification technique has in the performance that this raw ingredient will have when developing new food products. According to our knowledge, the incorporation of ultrasonicated flours in bread recipes is unexplored. The aim of this research was to incorporate US-modified rice flour treated at a high concentration (25 % w/w) at different times (2, 5, 10, and 20 min) in the formulation of GF breads, to determine the influence of high intensity ultrasonication on the doughs' rheological properties, and the physical quality of the breads obtained, as function of ultrasonication time.

6.2 Materials and methods

6.2.1 Rice flour

Indica rice flour used in this study was kindly supplied by Herba Ricemills S.L.U. (Herba Ricemills S.L.U., Valencia, Spain). It presented the following composition: moisture content: 12.62 %, proteins: 6.5 %, fat: < 1 %, ash: < 0.9 %, provided by the manufacturer. The flour was kept at 4 °C until use.

6.2.2 Ultrasound treatment

An ultrasonicator Hielscher UP400St (Hielscher Ultrasonics, Teltow, Germany) coupled with a S24d22D titanium tip was used for flour modification. Treatment conditions were a frequency of 24 kHz, on-off pulse of 80 %, and maximum output power of 180 W. Rice flour dispersions (400 g) at a concentration of 25 % (w/w) were US-treated in a glass jacket containing recirculating water from a RA12 LAUDA water bath (LAUDA, Lauda-Königshofen, Germany) to keep the treatment temperature constant (20 °C). Ultrasound treatments were performed for 2, 5, 10, and 20 minutes. Dispersions were stirred during treatments to avoid flour sedimentation, and to ensure a homogenous temperature and ultrasonication effect. The whole sonicated aqueous dispersion (flour + water) was used in dough formation. For the particle size distribution analysis (section 6.2.3), the sonicates dispersion were freeze-dried using a Telstar Lyoquest equipment (Telstar, Terrassa, Spain) to retrieve the ultrasonicated flour, which was subsequently sieved to < 250 μm. Untreated rice flour was used as control in the study.

6.2.3 Particle size distribution

Granulometry of the flours was determined using a Mastersizer 2000 coupled with a dry dispersion unit (Malvern Panalytical, Malvern, U.K.). The median diameter (D_{50}) and span values [$(D_{90}-D_{10})/D_{50}$] are reported as indicators of particles' dispersion, according to Abebe et al. (2015). Samples were measured in triplicate.

6.2.4 Formulation and elaboration of doughs and breads

For dough and bread formulation, the following formula on a 100 g rice flour (13 % moisture) basis was used: 5 % sugar, 1.5 % salt, 2 % HPMC, 6 % sunflower oil, 90 % water and 3 % dry yeast (all amounts are expressed as % flour). Yeast was only included in the preparation of doughs destined to the elaboration of breads and for the proofing test with rheofermentometer; not in

those used to study the properties of the dough. In doughs and breads containing US-treated rice flour, the whole aqueous flour dispersion was incorporated in the formulation after treatment, which substituted 30 % of the native rice flour, and 86.2 % of the required water.

Yeast was rehydrated in water and mixed with the previously homogenized dry ingredients (flour, sugar, salt and HPMC) for 2 min using a KitchenAid Professional mixer (KitchenAid, Benton Harbor, MI, U.S.A.) with a K45DH dough hook at speed 2. Sunflower was added to the dough and the mixing continued for 8 min at speed 4. To study doughs rheological and fermentative properties, the doughs were evaluated right after being prepared, while for the study of the thermal and pasting properties, doughs were freeze-dried using a Telstar Lyoquest equipment (Telstar, Terrassa, Spain) and ground prior measurements.

To prepare the breads, 160 g of the dough was placed into an aluminum pan, followed by fermentation at 32 °C and 80 % RH for 50 min in a HPP260eco Memmert constant climate chamber (Memmert GmbH, Buechenbachm, Germany), and baking at 170 °C for 20 min in a S400 Sveba Dahlen oven (Sveba Dahlen AB, Fristad, Sweden). Breads were left to cool down for 60 min at room temperature before the measurement of their physical characteristics. All bread elaborations were studied in duplicate.

6.2.5 Dough measurements

6.2.5.1 Pasting properties

Pasting properties of the doughs were determined using a Rapid Visco Analyzer 4500 equipment (PerkinElmer Inc., Waltham, MA, U.S.A.) following the AACC International Method 76-21.02 Standard 1 (AACC International Approved Methods, 2017). The freeze-dried dough sample (3.50 g on a moisture basis of 14 %) was placed in the RVA canister with 25 mL of distilled water. The paddle speed was set to rotate at 960 rpm for the first 10 s to disperse the sample, and continued at 160 rpm the rest of the essay. The parameters determined were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV), using TCW3 software (PerkinElmer Inc., Waltham, U.S.A.). Samples were measured in duplicate.

6.2.5.2 Thermal properties

Thermal properties of the doughs were studied using a DSC3 equipment (Mettler Toledo, Barcelona, Spain). Freeze-dried doughs samples (~ 6 mg) were placed in 40 μL aluminum pans

with the corresponding amount of deionized water to reach the original moisture content of the doughs (50.4 %). The sealed pans were kept at room temperature for 60 min to allow moisture homogenization before measurements. The scan was performed from 0 to 120 °C at 5 °C/min using an empty pan as reference. A first scan was performed to determine the gelatinization enthalpy (ΔH, J/g of dry matter, dm) and temperatures [Onset (T_O), peak (T_P), and conclusion (T_C) temperatures (°C)] of the doughs. A second run was performed after 7 days of sample storage at 4 °C to study the retrogradation parameters. The dissociation of the amylose-lipid complex was determined in both runs. Each dough was measured in duplicate.

6.2.5.3 Rheological characterization of doughs

Oscillatory measurements were performed using a Kinexus Pro+ rheometer (Malvern Panalytical, Malvern, UK) with a parallel serrated plate geometry (40 mm diameter, 1 mm gap), coupled with a Peltier KNX2002 C25P plate temperature control at 25 °C.

a. Frequency and oscillation sweeps

Before measurement, the doughs were placed in the plate and left for 5 min to allow relaxation. Strain sweeps were performed from 0.01 to 200 % strain at constant frequency of 1 Hz, while frequency tests were performed at a range of 10 – 1 Hz and 0.05 % strain, within the linear viscoelastic region (LVR). Frequency sweeps data were fitted to the potential equations indicated by Ronda et al. (2014). The coefficients G'_1, G''_1 and $tan(\delta)_1$ of power law obtained from fitting represent the elastic and viscous moduli and the loss tangent, respectively, at a frequency of 1 Hz, while the exponents a, b and c quantify the dependence of these parameters to the oscillation frequency (ω). All doughs were evaluated in duplicate.

b. Creep-recovery assays

Creep-recovery measurements were performed applying a constant stress of 10 Pa (outside of the LVR) to the dough for 60 s, followed by removal of the stress and a strain recovery phase for 180 s. The obtained data were analyzed and fitted to the 4-parameter (creep) and 3-parameter (recovery) Burgers models, as described by Villanueva et al. (2019), using Statgraphics Centurion XVIII software (Statgraphics Technologies Inc., The Plains, VA, U.S.A.). The recovery (%) was calculated as the ratio J_{steady}/J_{max} (elastic recovery). Where J_{max} is the maximum creep compliance obtained at the end of the creep step and J_{steady} is the steady-state compliance in recovery step, that

was calculated by substracting the compliance value at the terminal region of curve (where dough recovery reached equilibrium) from the J_{max}. All measurements were performed in triplicate.

6.2.5.4 Rheofermentometer

Development and gas production of the doughs was measured using a Chopin rheofermentometer F3 (Chopin Technologies, Villenueve-La-Garenne, France). In contrast to the traditional method, the weight of dough was reduced to 160 g and the four weights of 0.5 kg were removed adapted to dough softness. Fermentation was performed at 32 °C for 180 min. The determined parameters were: H_m, height at the maximum development of the dough (mm); T_1, time required for the maximum development of the dough (min); Dough tolerance, time in which the dough is stable at a volume beyond 90 % of H_m (min); H'_m, maximum heigh of CO_2 production (mm); T'_1: time corresponding to H'_m (min); V_T, total volume of CO_2 produced (mL); V_R, total volume of CO_2 retained by the dough (mL); V_{NR}, total volume of CO_2 not retained by the dough (mL); R_C, CO_2 retention coefficient V_R/V_T (%); T_x, time of appearance of dough's porosity (min), in which all the CO_2 generated is no longer retained by the dough (Villanueva et al., 2015). All samples were measured in duplicate.

6.2.6 Evaluation of bread quality

6.2.6.1 Bread appearance

A PowerShot SX410 IS camera (Canon, Tokyo, Japan) was used to photograph the breads elaborated and the slices obtained from them.

6.2.6.2 Bake loss

Breads were weighted immediately after removal from the pan using a COBOS precision scale (COBOS, Barcelona, Spain). The baking loss was stablished as the percentual weight difference between the weight of the bread and the weight of the dough placed in the pans before proofing and baking (160 g).

6.2.6.3 Volume

The volume of breads was determined using a Volscan profiler 300 (Stable Microsystems, Godalming, U.K.) analyzer. The specific volume was calculated as the ratio between the bread volume and its weight (mL/g). Two different breads were measured in each elaboration (four measurements in total for each formulation).

6.2.6.4 Color

Color parameters of crust and crumb were measured using a PCE-CSM 2 colorimeter (PCE Instruments, Spain), controlled with the 3nh Color Quality Controller System (CQCS3) (Shenzhen ThreeNH Technology Co. Ltd, Shenzhen, China) software. Results were obtained in the CIE L^* a^* b^* and CIE L^* C^* h coordinates using the D65 standard illuminant and the 10 ° standard observer. Five measurements were made for the evaluation of the crust, while the crumb was measured in quadruplicate. The color difference (ΔE) of the crust and the crumb of each bread containing US-modified rice flour with respect to the control bread was calculated using the equation: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

6.2.6.5 Texture

Crumb texture was determined with a "Texture Profile Analysis" (TPA) double compression test using a TA-XT2 texture analyzer (Stable Microsystems, Godalming, U.K.) provided with the Texture Expert software (Villanueva et al., 2019). Analyses were carried out on bread slices of 20 mm thickness taken from the center of the loaf. A 20 mm diameter cylindrical aluminum probe was used to penetrate 50 % depth, at a speed of 1 mm/s, with 30 s delay between compressions. Hardness (N), springiness, cohesiveness, chewiness (N), and resilience were determined from the TPA graphs. The hardness was also evaluated after 7 days of bread storage at 4 °C in hermetic bags. Samples were evaluated in quadruplicate.

6.2.7 Statistical analysis

Statgraphics Centurion XVIII software (Statgraphics Technologies Inc., The Plains, VA, U.S.A.) was used to model the creep-recovery data into the 3- and 4-parameter Burger models, and to perform analysis of variance (ANOVA) by Least Significant Differences (LSD) test at p-value ≤ 0.05 to evaluate statistical differences between samples.

6.3 Results and discussion

6.3.1 Particle size of sonicated rice flour

The determined values of median particle diameter (D_{50}) and size dispersion [(D_{90}-D_{10})/ D_{50}] of the control and ultrasonicated flours are presented in Table 6.1, and their size distributions are shown in Figure 6.1. Results showed a direct correlation of median size reduction with longer US exposure, increasing the proportion of particles with a diameter < 50 µm, leading to greater size dispersion values. Longer ultrasound treatments have been indicated to intensify the damage induced to the treated particles (Cui & Zhu, 2020). Particle fragmentation has been reported to happen due to mechanical collision and high shear forces caused by the cavitation phenomenon, that progressively erodes the particles' surface until fragmentation (Bel Haaj et al., 2013). Besides particle fragmentation, another effect of cavitation's mechanical degradation has been said to be the partial depolymerization of starch due to the fast formation and collapse of the cavitation bubbles (Chemat et al., 2011), modifying the molecular structure of starch and the chain length distributions after ultrasonication (Yang, Lu et al., 2019). This fine structure modification would consequently lead to changes of the techno-functional properties of the US-treated flours and their performance in starch-based food product development.

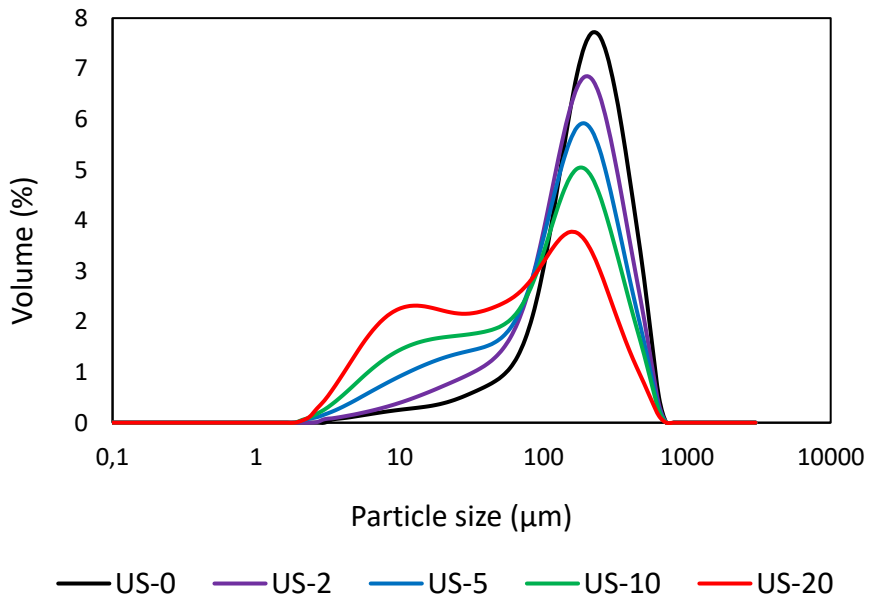

Figure 6.1. Particle size distribution of the native rice flour and flours sonicated at different times.

Table 6.1. Median diameter (D_{50}) and size dispersion [(D_{90}-D_{10})/ D_{50}] of native and sonicated flours.

US time (min)	D_{50} (μm)	(D_{90}-D_{10})/ D_{50}
0 (Control)	190e	1.70a
2	160d	1.99b
5	133c	2.35c
10	107b	2.79d
20	56a	4.30e
SE	1	0.02
Analysis of variance and significance (p-values)		
	***	***

SE: Pooled standard error from ANOVA. Different letters in the same column indicate statistically significant differences between means at $p < 0.05$.
Analysis of variance and significance: *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

6.3.2 Effect of incorporating US-treated flours in dough properties

6.3.2.1 Pasting properties

The pasting properties of the studied freeze-dried doughs are presented on Table 6.2, and the pasting profiles are shown in Supplementary Figure 6.1. The structure changes that take place during starch gelatinization in RVA analysis include a crystallite melting and double-helix unwinding, water absorption in the amorphous regions, displacement of amylopectin units and leaching of amylose from the grains (Iida et al., 2008). Results obtained showed that pasting temperature (PT), the temperature at which gel formation begins and paste viscosity development starts, was significantly increased by US treatments, where doughs containing flour treated at 5, 10 and 20 min presented equal values, about 2 °C higher than the control. It has been reported that ultrasonication causes a delay in PT in rice flour, with the same result independently of exposure time in the range from 5 to 60 min (Vela, Villanueva, Solaesa, et al., 2021), which could explain the uniform effect observed in the freeze-dried doughs. The obtained results showed that the delay reported for flours can also be determined in dough, even in the studied formulations where the US-modified flour only represented 30 % of the total flour used, meaning most of the flour was in the native state exactly as in the control dough. The higher PT values indicate reorganization of starch structures that require more energy for structural disintegration and paste formation, suggesting a denser cross-linking within the starch granules after US treatments (Villanueva et al., 2019).

The profiles obtained after the incorporation of US-treated flour presented significantly lower peak (PV) and trough (TV) viscosities, while the final (FV), breakdown (BV) and setback (SV) viscosities were not significantly different to the values obtained in the control dough. Ultrasound can rupture the macromolecular chains of starch, and destroy its crystalline structure, which would decrease the viscous resistance of starch paste, leading to reduced values of PV (Li et al., 2018). The unaffected BV values indicate that doughs containing US-treated flours still presented the same stability to heating and stirring as the control dough containing exclusively native rice flour. During cooling, the starch molecules begin to reassociate into an ordered structure and undergo retrogradation of amylose. FV results showed that the samples containing US-treated flours were able to form an equally viscous structure after amylose retrogradation as the native dough. These results might surge from partial depolymerization of starch components caused by US treatment, as other studies have demonstrated (Amini et al., 2015; Falsafi et al., 2019; Vela et al., 2023), diminishing the starch ability to develop viscosity during gelling, but allowing a significantly equal retrogradation capacity due to higher availability of linear chains from the release of side chains of amylopectin or cleavage of chains of amylose.

6.3.2.2 Thermal properties

The thermal properties of the studied freeze-dried doughs are presented in Table 6.2. Two scans were performed on the studied doughs to characterize their gelatinization (first) and retrogradation (second) properties. The thermograms obtained from the first scan presented two endothermic peaks, a bigger first one due to starch gelatinization, and a smaller peak at higher temperatures corresponding to amylose-lipid complex dissociation (Vela, Villanueva, & Ronda, 2021). Starch gelatinization enthalpy (ΔH_{gel}) was not significantly affected by the incorporation of ultrasonicated rice flour. ΔH_{gel} values reflect the disruption of hydrogen bonds or double helices in the crystalline regions of the granules (Li et al., 2019). The fact that no significant modification of ΔH_{gel} was determined after the addition of ultrasonicated flour indicates that US treatment did not have an important impact on the number of those ordered structures. It is believed that ultrasonication has a higher impact on linear amylose chains of the amorphous regions, while the highly branched amylopectin molecules are more resistant and require greater energy to be affected, either high US power or longer sonication exposure (Amini et al., 2015; Kaur & Gill, 2019; Li et al., 2019).

The onset gelatinization temperature ($T_{O\text{-}gel}$) represents the melting temperature of the weakest crystallites in the starch granules (Wang et al., 2020). Results showed a significant decrease of $T_{O\text{-}gel}$ when US-treated flour was used. It was demonstrated by Yu et al. (2013) that $T_{O\text{-}gel}$ in rice starch was constantly reduced by increasing sonication times in treatments below 60 min, attributed to breakage of polymeric chains and damage caused to the starch granule. In the present study, it is possible that the incorporation of US-treated flours at 30 % in the doughs had a dilution effect, hiding the effect of longer exposure on leading to greater modification of $T_{O\text{-}gel}$. Lower $T_{O\text{-}gel}$ values could result from a change in starch matrix, allowing greater mobility of starch polymers after US treatments, promoting water entering the interior of the starch granules and accelerating the gelatinization (Hu et al., 2019). The conclusion temperature ($T_{C\text{-}gel}$) was not significantly modified by US treatments.

The enthalpy determined for the second peak obtained in the first scan ($\Delta H_{am\text{-}lip}$), corresponding to the amylose-lipid complex dissociation, was found to be significantly reduced by the incorporation of US-treated flour in the doughs. These results indicate that ultrasonication led to the formation of less stable amylose-lipid complexes, and probably also lower amounts, as has been determined in chemically modified starches (Eliasson, 1994). The amylose-lipid complex is described as a helical inclusion complex with amylose forming a helix around the hydrophobic chain of the ligand (Eliasson, 1994). It was indicated by Eliasson (1994) that each monoacyl chain would require a helix composed of at least 18 glucosyl residues to form the amylose-lipid complex. In the present study, it could be possible that the fragmentation of linear chains by cavitation would lead to short chains that were unable to form a complex, resulting in lower total amount of complexes, hence reducing the $\Delta H_{am\text{-}lip}$ values in doughs incorporating sonicated flour.

The second scan performed after 7 days of sample storage at 4 °C allowed the analysis of the extent of amylopectin recrystallization. The melting enthalpy of retrograded amylopectin (ΔH_{ret}) was not modified by US treatments, suggesting that the crystalline regions of starch were not significantly affected by ultrasonication, in agreement with the results obtained in ΔH_{gel}. However, the onset melting temperature of retrograded samples ($T_{O\text{-}ret}$) was significantly increased in US-10 and US-20, in agreement with the results reported by Yu et al. (2013) after ultrasonication of rice starch. The conclusion melting temperature of retrograded samples ($T_{C\text{-}ret}$) was slightly reduced when applying US-treatments. Both $T_{O\text{-}ret}$ and $T_{C\text{-}ret}$ results illustrate a lower degree of heterogeneity in the retrograded structure when US-treated flour was incorporated in the doughs.

Table 6.2. Pasting and thermal properties of the studied doughs.

Freeze dried dough properties	US-0	US-2	US-5	US-10	US-20	SE	Analysis of variance and significance
PT (°C)	84.0a	85.4b	86.2b	86.2b	86.0b	0.4	*
PV (cP)	2271b	2126a	2125a	2167a	2172a	23	*
TV (cP)	1998b	1863a	1876a	1920ab	1911a	23	*
BV (cP)	274a	264a	249a	265a	261a	12	ns
FV (cP)	3252ab	3213ab	3144a	3251ab	3279b	36	ns
SV (cP)	1255a	1350a	1268a	1295a	1369a	35	ns
ΔH_{gel} (J/g)	8.6a	8.3a	8.2a	8.4a	8.4a	0.2	ns
$T_{O\text{-}gel}$ (°C)	67.4c	66.8ab	66.7a	66.9ab	67.0b	0.1	**
$T_{P\text{-}gel}$ (°C)	83.1b	83.1b	82.4a	83.7c	83.1b	0.2	**
$T_{C\text{-}gel}$ (°C)	99.1b	98.8ab	98.6ab	98.7ab	98.4a	0.2	ns
$\Delta H_{am\text{-}lip}$ (J/g)	1.03d	1.02cd	0.83ab	0.80a	0.93bc	0.04	**
$T_{P\text{-}am\text{-}lip}$ (°C)	113.0c	110.7a	112abc	112.4bc	111.3ab	0.5	ns
ΔH_{ret} (J/g)	6.7ab	6.7ab	6.7ab	6.4a	6.9b	0.1	ns
$T_{O\text{-}ret}$ (°C)	35.2ab	35.1a	36.1bc	36.5cd	36.9d	0.3	**
$T_{P\text{-}ret}$ (°C)	49.6ab	49.4ab	48.8a	49.1ab	50.2b	0.4	ns
$T_{C\text{-}ret}$ (°C)	69.8b	69.2a	69.5ab	69.5ab	69.1a	0.2	*
$\Delta H_{am\text{-}lip\text{-}ret}$ (J/g)	0.6a	0.6a	0.8a	0.8a	0.8a	0.2	ns
$T_{P\text{-}am\text{-}lip\text{-}ret}$ (°C)	108.0b	106.4a	106.2a	105.9a	106.4a	0.4	**

PT = Pasting Temperature. PV = Peak Viscosity. TV = Trough Viscosity. BV = Breakdown Viscosity. FV = Final Viscosity. SV = Setback Viscosity. ΔH_{gel} = Enthalpy of dough gelatinization. $T_{O\text{-}gel}$, $T_{P\text{-}gel}$, $T_{C\text{-}gel}$ = Onset, peak and conclusion temperatures of gelatinization. $\Delta H_{am\text{-}lip}$ = Enthalpy of the amylose-lipid dissociation. $T_{P\text{-}am\text{-}lip}$ = Peak temperature of the amylose-lipid complex dissociation. ΔH_{ret} = Enthalpy of melting of retrograded dough. $T_{O\text{-}ret}$, $T_{P\text{-}ret}$, $T_{C\text{-}ret}$ = Onset, peak and conclusion temperatures of melting of retrograded amylopectin. $\Delta H_{am\text{-}lip\text{-}ret}$ = Enthalpy of the amylose-lipid dissociation at the second scan. $T_{P\text{-}am\text{-}lip\text{-}ret}$ = Peak temperature of the amylose-lipid complex dissociation at the second scan. ΔH_{gel}, $\Delta H_{am\text{-}lip}$, ΔH_{ret}, and $\Delta H_{am\text{-}lip\text{-}ret}$ are referred to dry matter.

SE: Pooled standard error from ANOVA. Different letters in the same column indicate statistically significant differences between means at $p < 0.05$.

Analysis of variance and significance: *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

Table 6.3. Oscillatory tests, creep-recovery parameters and rheofermentometer data obtained for the studied doughs.

Dough properties	US-0	US-2	US-5	US-10	US-20	SE	Analysis of variance and significance
τ_{max} (Pa)	1.4a	1.5a	1.7a	1.8a	1.9a	0.2	ns
Cross over (Pa)	7a	6a	8a	7a	8a	1	ns
G'_1 (Pa)	908a	767a	903a	787a	919a	48	ns
a	0.341c	0.336bc	0.313a	0.318ab	0.317ab	0.006	*
G''_1 (Pa)	564c	436ab	514bc	406a	497abc	28	*
b	0.450b	0.431ab	0.442ab	0.437ab	0.426a	0.006	ns
$tan(\delta)_1$	0.647b	0.638b	0.586a	0.581a	0.574a	0.007	**
c	0.124ab	0.115ab	0.129ab	0.139b	0.114a	0.007	ns
J_{0c} (10^{-4} Pa^{-1})	31.8d	28.5c	25.5b	26.3bc	22.4a	0.7	**
J_{1c} (10^{-3} Pa^{-1})	34b	33b	26a	22a	24a	1	**
λ_c	5.3a	6.5b	6.1b	6.0b	6.1b	0.2	*
μ_0 (10^3 Pa·s)	2.1a	2.0a	2.6b	2.8b	2.8b	0.1	*
$(J_{0c} + J_{1c}) / J_{max}$	51a	56b	57b	56b	57b	1	**
J_{0r} (10^{-4} Pa^{-1})	42b	40b	34a	34a	33a	1	**
J_{1r} (10^{-3} Pa^{-1})	5.66d	5.25c	4.92b	4.80b	4.38a	0.05	***
λ_r (s)	18.4a	19.9b	21.5c	22.2cd	23.3d	0.3	***
Recovery (%)	15.1a	15.5ab	16.6ab	17.2b	17.0b	0.6	ns
H_m (mm)	62bc	58ab	58a	61abc	64c	1	*
T_1 (min)	113b	110ab	105a	110ab	112b	2	ns
Dough tolerance (min)	50.0a	53.3ab	54.8b	55.5b	60.8c	0.9	**
H'_m (mm)	72.8a	74.5ab	75.6bc	74.5ab	78.1c	0.8	*
T'_1 (min)	70c	65b	63ab	64b	59a	1	**
V_T (mL)	1282a	1279a	1284a	1278a	1303a	17	ns
V_R (mL)	1170a	1199b	1203b	1201b	1198b	6	*
V_{NR} (mL)	101.5d	94.5c	95.0c	87.5b	85.0a	0.4	***
R_C (%)	92.15a	92.55b	92.60b	93.15c	93.45c	0.09	***
T_x (min)	71b	56ab	51a	62ab	51a	5	ns

τ_{max} and the cross over were obtained from strain sweeps. The power law model was fitted to experimental results from frequency sweeps. $G'=G'_1 \cdot \omega^a$; $G''=G''_1 \cdot \omega^b$; $tan(\delta)=tan(\delta)_1 \cdot \omega^c$. $tan(\delta)_1$ was calculated as G''_1/G'_1, and *c* as *b-a*. J_0 and J_1 indicate the instantaneous and retarded elastic compliances. λ is the retardation time and μ_0 the steady state viscosity. Subscript "c" refers to parameters in the creep phase; Subscript "r" refers to parameters in the recovery phase. Recovery is the elastic recovery obtained in the recovery phase expressed as percentage of the maximum compliance. H_m: Height at the maximum development of the dough. T_1: time corresponding to H_m. H'_m: Maximum height of CO_2 production. T'_1: time corresponding to H'_m. V_T: total volume of CO_2 produced. V_R: Total volume of CO_2 retained by the dough. V_{NR}: Total volume of CO_2 not retained by the dough. R_C: CO_2 retention coefficient. T_x: time of appearance of dough's porosity.

SE: Pooled standard error from ANOVA. Different letters in the same column indicate statistically significant differences between means at $p < 0.05$.

Analysis of variance and significance: *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

6.3.2.3 Doughs' rheological properties

a. Dynamic oscillatory rheology

The rheological properties derived from the dynamic oscillatory tests determined for the studied doughs are presented in Table 6.3. Strain sweep tests allow the determination of the maximum stress that the doughs were able to resist before the collapse of the structure (τ_{max}), which marks the end of the linear viscoelastic region (LVR), and the cross over point, where the elastic modulus matches the viscous modulus ($G' = G''$). Results showed that τ_{max} and the cross over point of the doughs were not significantly changed by the ultrasonicated flour, indicative that the structural damage caused to starch molecular structure by cavitation was faded due to the high amount of native flour (70 %) in the formulations, resulting in an equal resistance to deformation as the one presented by the control dough.

Frequency sweep tests were performed to determine the frequency dependence of the viscoelastic moduli and the loss tangent of studied doughs within the LVR. The determined parameters from fitting the frequency sweep data to the power-law model were: the coefficients G'_1, G''_1, and $tan(\delta)_1 = G''_1 / G'_1$, that represent the elastic and viscous moduli and the loss tangent at 1 Hz, and are used to quantify the strength of the doughs (Amini et al., 2015), and the exponents *a*, *b*, and *c* that quantify the variation of G', G'', and $tan(\delta)$ to oscillation frequency, respectively, and inform about the stability of dough structure versus the rate of deformation. For all the studied samples the elastic modulus was above the viscous one, resulting in $tan(\delta)_1 < 1$, denoting the predominant solid/elastic behavior in the doughs (Villanueva et al., 2019). While there were not clear differences found in G'_1 and G''_1 with the incorporation of US-treated flours, $tan(\delta)_1$ was constantly reduced with extended ultrasonication exposure, suggesting an improved structuring effect with increasing treatment times. The $tan(\delta)_1$ value determined in the dough containing rice flour sonicated by 20 min (0.574) was 11 % lower than that determined for the control (0.647), which indicates an important effect of ultrasonication, given that the substitution in the dough was just 30 %. In the rheological study of gels entirely made with US-treated rice flours, it was reported by Vela, Villanueva, Solaesa, et al. (2021) that ultrasonication led to lower values of $tan(\delta)_1$ for longer treatment times. In both matrices (doughs and gels), this behavior could be related to starch macromolecules fragmentation leading to short polymeric chains capable to reassociate to form a more elastic network (Monroy et al., 2018). In the case of the studied doughs, however, the changes caused to the molecular structure of starch in rice flours by ultrasonication would also

alter its interactions with the rest of the components present in the matrix (i.e., proteins, sugar, oil, and HPMC), so the lower values of $tan(\delta)_1$ cannot be exclusively attributed to the starch macromolecules fragmentation. The exponents *a*, *b* and *c* indicated that the dependance of the moduli and the loss tangent on angular frequency was not particularly affected by ultrasonicated flour.

b. Creep-recovery tests

The creep-recovery tests were performed at 10 Pa, outside of the LVR, where the stress applied overpasses the maximum stress the doughs could stand without breaking their structure. During baking process (i.e., mixing, molding, fermentation, baking) the doughs are subjected to stress outside the LVR, so these conditions are useful to predict the deformation that the doughs will experience during processing (Villanueva et al., 2018). The parameters determined after adjusting the creep-recovery tests data to the Burgers models are presented in Table 6.3. The studied doughs showed a typical viscoelastic behavior (data not shown), as was previously reported for rice flour doughs (Perez-Quirce et al., 2018; Villanueva et al., 2019). The instantaneous compliance (J_0) is related to the energy of elastic stretching of the bonds, when stress is applied, and vanishes immediately after its removal, while the retarded elastic compliance (J_1) is related to the disruption and conversion of the bonds (Witczak et al., 2012). Results showed that the incorporation of ultrasonicated flours in the doughs led to a significant decrease of J_0 and J_1 values, both in creep and recovery phases, associated to stiffer doughs with lower dough deformation when submitted to a constant stress, and a higher recovery when stress was removed (Perez-Quirce et al., 2018; Ronda et al., 2014). This effect of sonication on dough elastic compliances increased with increasing sonication exposure, where J_{0c}, J_{1c}, J_{0r} and J_{1r} determined for US-20 showed a reduction of 30 %, 29 %, 21 % and 23 %, respectively, compared to the values presented by the control dough. The $[(J_{0c} + J_{1c}) / J_{max}]$ values represent the elastic (instantaneous + retarded) to total (elastic + viscous) compliance ratio (Villanueva et al., 2018). A significant increase of the $(J_{0c} + J_{1c}) / J_{max}$ values was determined with the incorporation of US-treated flours. These results indicate a higher elastic deformation with respect to the total (elastic + viscous) deformation, and confirm the higher elasticity of the doughs made with 30 % sonicated rice flour in comparison with that of the control dough (100 % untreated rice flour). This is of relevance given the gas retention capacity of a dough needs a well-structured matrix with enough elastic behavior (Lazaridou et al., 2007).

Higher retardation times were determined in both creep (λ_c) and recovery (λ_r) phases of tests performed on doughs made with sonicated flour. Higher λ values mean more time needed to obtain the viscoelastic deformation of the dough (Villanueva et al., 2019). Retardation times in the creep tests (λ_c) were equally improved by the incorporation of ultrasonicated flours, regardless of the treatment time. The increased retardation times were probably due to the increased swelling ability derived from particle fragmentation which allows a higher interaction with water (see section 6.3.1) (Vela, Villanueva, Solaesa, et al., 2021; Witczak et al., 2012). A major factor in the growth of doughs' elasticity is water capacity, which depends on the flour particle size and number of damaged starch granules (Witczak et al., 2012), which according to previous research is greatly modified by the US treatments via the cavitation effect (Vela, Villanueva, & Ronda, 2021; Vela, Villanueva, Solaesa, et al., 2021). Changes in water binding ability lead to changes in the structure of the dough because hydration of starch determines its shape and interconnectivity (Witczak et al., 2012).

The highest values of the steady state viscosity (μ_0) were determined for US-10 and US-20, statistically equal to that presented by US-5, while the value determined for US-2 was not significantly different to the control. A proper consistency in gluten-free doughs, with high enough μ_0, helps to hold the carbon dioxide produced during fermentation (Ronda et al., 2014). Dough viscosity increase might be attributed to higher water retention capacity (Perez-Quirce et al., 2018), associated to particle rupture and disintegration of starch intermolecular bonds by ultrasonication, which allows water molecules to bind with the free hydroxyl groups of amylose and amylopectin (Kaur & Gill, 2019).

The recovery capacity of the doughs after applying the stress is related to the contribution of the elastic deformation with respect to the total deformation (Villanueva et al., 2019). Results indicated that recovery capacity was slightly improved using US-treated flour, where US-10 and US-20 were significantly different than the control. The increased recovery values reflect improved bonding between the structural elements of the dough, resulting from higher elastic behavior, in agreement with the reduced $tan(\delta)_1$ values determined.

6.3.2.4 Fermentative properties of the doughs

The fermentative properties of the studied doughs were evaluated using a rheofermentometer, the obtained results are presented in Table 6.3. During essays, the volume increase was measured

(related to the development of the dough) as well as the gas produced and retained by the dough (see Figure 6.2 and 6.3, respectively). H_m and T_1 indicate the height at the maximum development of the dough, and the time at which it is achieved, respectively. Results showed that both properties were not particularly affected by the incorporation of ultrasonicated flours. The dough tolerance, however, showed a positive correlation with sonication time in the treated flours, reaching a value of 60.8 min in US-20, which represents an increase of 21.6 % with respect to the value determined for the control dough (50.0 min). This property indicates the time that dough remains stable at a volume beyond 90 % of H_m. Dough stability during fermentation depends on the amount of CO_2 produced and the rheological properties of the dough, which both seemed to be improved by the incorporation of US-treated flours.

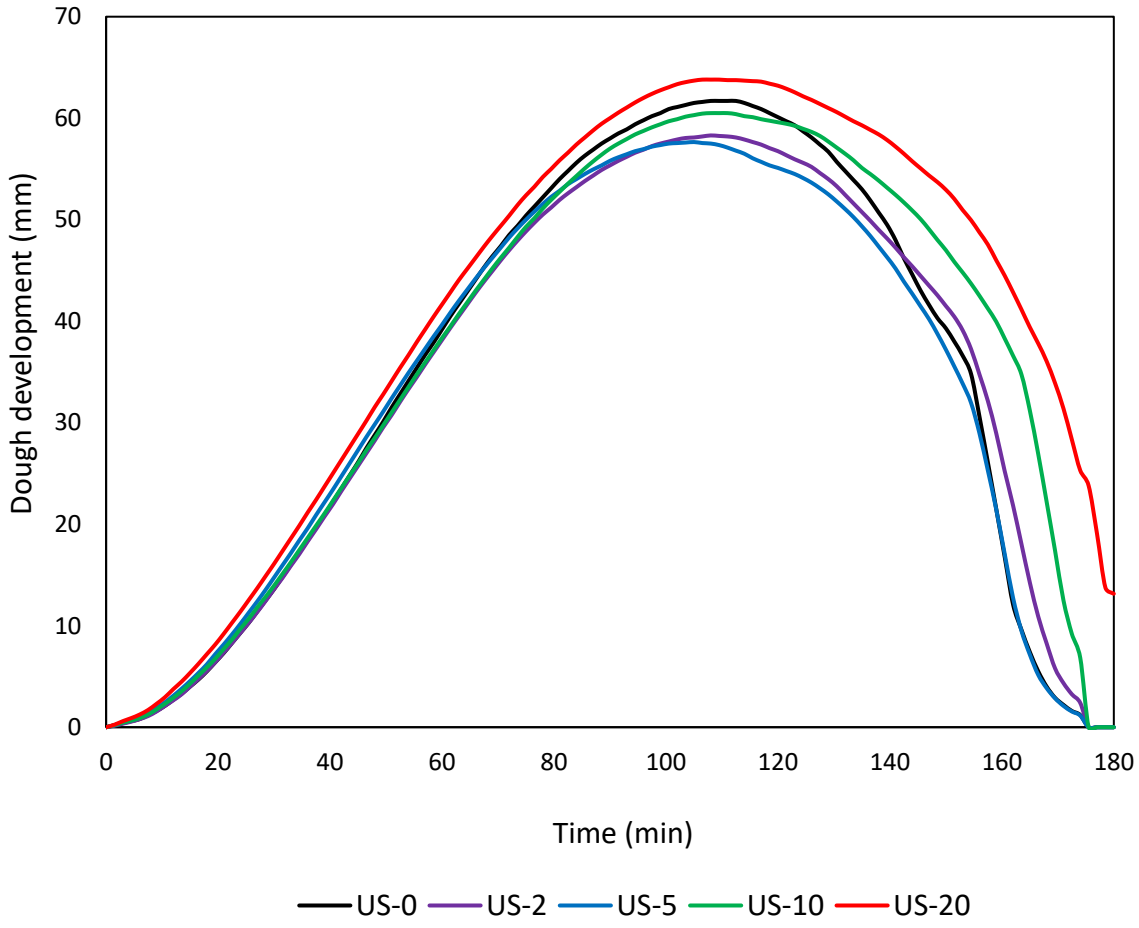

Figure 6.2. Development of the studied doughs during fermentation.

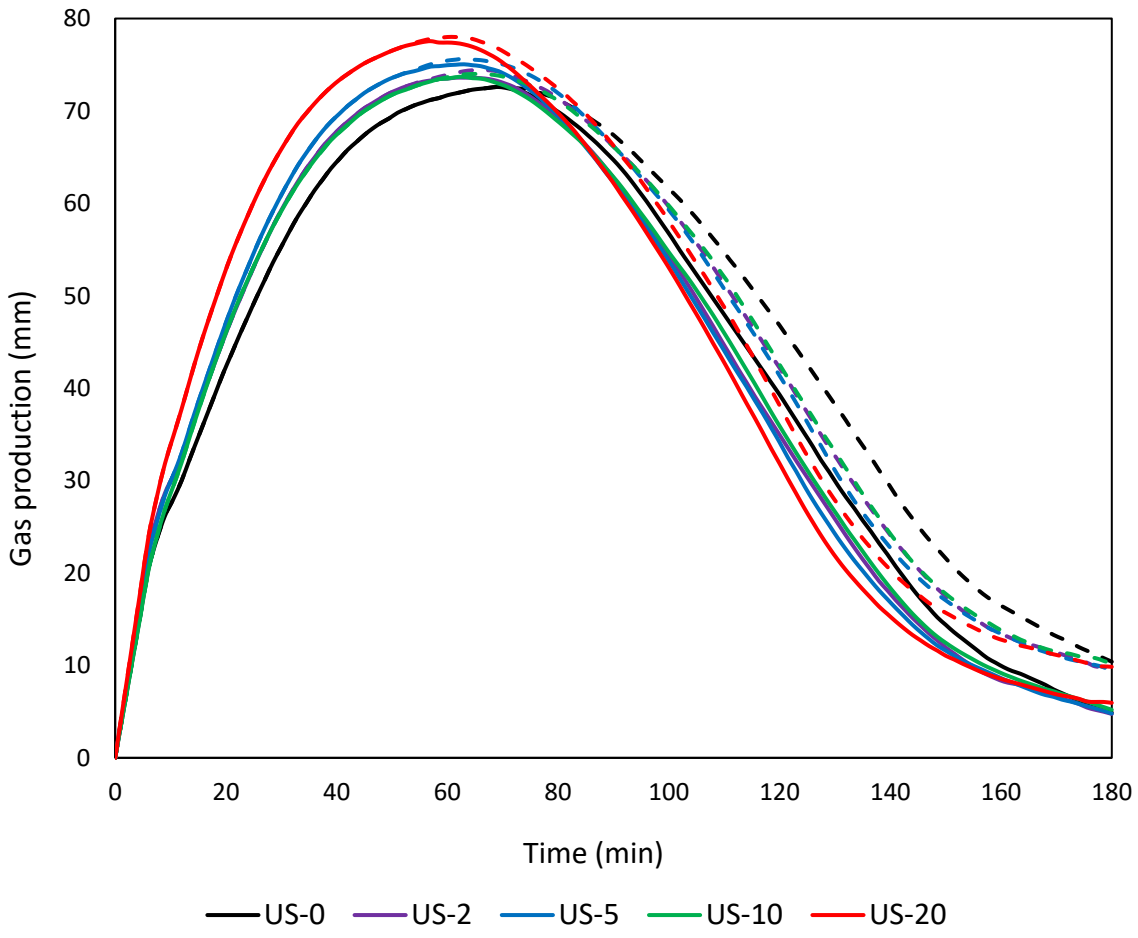

Figure 6.3. Gas production (discontinuous line) and gas retention (continuous line) in the studied doughs during fermentation.

Regarding the gas generated, results showed that the use of US-treated flours in the doughs increased the maximum heights of CO_2 produced during fermentation (H'_m), and that they were achieved at shorter times (T'_1) compared to the control dough. It is believed that this improved CO_2 production derives from starch molecule fragmentation caused by cavitation (Czechowska-Biskup et al., 2005; Vela et al., 2023). The fragmentation of linear chains would result in the generation of simpler sugar in the US-modified rice flours, facilitating their accessibility to yeast during proofing, accelerating the generation of CO_2 and achieving a higher amount of CO_2 produced. The total volume of CO_2 produced (V_T), on the other hand, was not altered by the use of ultrasonicated flours, while the total volume of CO_2 retained (V_R) was significantly increase, resulting in increased values of the retention coefficient ($R_C = V_R/V_T$). R_C measures the fraction of CO_2 generated and retained by the dough; therefore, is related to the porosity of the dough (Villanueva et al., 2015). These greater values obtained in doughs containing US-treated flours

result from the faster generation of CO_2 and the improved elastic behavior and increased viscosity in those doughs, allowing them to better retain the gas generated during proofing. The time of appearance of dough porosity (T_x) was shorted in doughs containing US-treated flours, also related to the improved generation of CO_2 in these flours, giving an accelerated profile when compared to the control dough.

6.3.3 Effect of incorporating US-treated flours in bread physical properties

6.3.3.1 Bake loss and specific volume of the breads

The determined values of bake loss and specific volume of the studied breads are presented in Table 6.4. Results showed that the incorporation of US-treated flour in the doughs led to significantly higher values of bake loss and specific volumes in the obtained breads, for all the evaluated ultrasonication times. The higher volumes achieved by doughs containing US-treated flours (4.23 – 4.63 mL/g vs. 3.71 mL/g) could be related to the partial depolymerization due to cavitation, leading to improved fermentation and higher CO_2 produced, retained in these doughs (see section 6.3.2.4) thanks to its enhanced viscoelastic properties. It was demonstrated by Aoki et al. (2020) that amylose plays an important role in achieving a high loaf volume in rice breads, after comparing the volumes obtained in breads using rice flours with amylose contents ranging from 9 to 22 %. These authors determined that the amylose content was positively correlated with the specific volume of the rice breads. The linear chain fragmentation induced by ultrasonication (Czechowska-Biskup et al., 2005; Vela et al., 2023) would result in the generation of amylose-like structures that could have shown a similar behavior as that of increasing amylose content indicated by Aoki et al. (2020). Results seem to indicate that when the US doughs containing a higher amount of gas in their structure and a higher development were baked, an easier evaporation of water was obtained probably due to a higher surface exposed to dryness in the oven (Villanueva et al., 2019). The upper and side photographs of these breads, as well as a photograph of a slice, are presented in Figure 6.4; significantly bigger pieces are clearly observed after the incorporation of ultrasonicated flours. The highest specific volume was determined for US-10, which represents a volume increase of 24 % with respect to the control bread. The pasting properties of the dough are also related to the bread volume and baking loss. The higher pasting temperatures (see section 6.3.2.1) in doughs with US-treated flours in their formulation would allow a greater development of the doughs during baking before the fixation of the crumb structure upon baking, allowing as well longer time for water to evaporate during proofing (Villanueva et al., 2019).

6.3.3.2 Color

The color parameters determined for the crust and crumb of the studied breads are presented in Table 6.4. Results showed that lightness (L^*_{crust}), redness (a^*_{crust}) and yellowness (b^*_{crust}) of the crust were reduced by the incorporation of ultrasonicated flours, where significant differences were found at treatment times ≥ 5 min with respect to the control values. The color of the crust results from the Maillard reaction and caramelization of sugars during baking, which depends directly on the available water and the concentration of carbonyl groups from reducing sugars (Villanueva et al., 2019). The lower L^*_{crust} values in breads containing US-treated flours indicate a darker crust as consequence of Maillard reaction to a greater extent. Particle fragmentation and partial starch depolymerization caused by cavitation may be the precursor of increased Maillard reaction due to higher availability of simpler sugars that increased the reaction potential. Lower values of a^*_{crust} and b^*_{crust} and the concomitant reduction in C^* indicate less vivid colors in the crust of the breads containing ultrasonicated rice flours. Park et al. (2014) reported a decrease of a^*_{crust} and b^*_{crust} values in breads made using fine rice flour fractions, in agreement with the results of the present study, suggesting that the change of these color properties could be related to the reduced particle sizes after US treatments (see section 6.3.1). The values determined for L^*_{crust}, a^*_{crust} and b^*_{crust} were very similar among US breads, despite the applied sonication time, which could be due to presence of 70 % of native flour in all breads containing US-treated flour, hiding the possible effect of increasing ultrasonication time, resulting in very uniform values. ΔE_{crust}, which combines these three parameters, showed an increasing trend with increasing sonication time. However, none of the determined differences represented a significant difference from a sensory point of view, since a difference of at least 5 would be required to be sensory noticeable (Gutiérrez et al., 2022). This parameter demonstrated that there was not a linear trend with treatment time, but rather an increase that was faster at short times (+0.714 when going from US-2 to US-5) and slowed down at longer times (+0.190 when going from US-10 to US-20). The hue (h_{crust}) of the crust was increased by the incorporation of ultrasonicated flours which indicates a slight increase towards more yellow tones.

The color parameters determined for the crumb were also significantly modified by the use of ultrasonicated flours, mainly by longer treatment times. The color of the crumb is mainly related to the color of the ingredients (Pérez-Quirce et al., 2014), so the determined differences could be related to alterations of US treatment to the rice flour. L^*_{crumb} values were not particularly affected

(only in US-20), while in C^*_{crumb} a significant decrease was found at US-10 and US-20, resulting from the reduction of a^*_{crumb} and b^*_{crumb} values, indicative of less vivid colors for the crumb of breads containing flours exposed to longer US times. The values of h_{crumb} were significantly increased starting at 5 min of ultrasonication, indicative of more yellowish hues. The highest ΔE_{crumb} was determined for US-20, as well as in ΔE_{crust}, which was also not relevant at a sensory point of view.

Table 6.4. Effect of incorporation of 30 % US-treated rice flour at different times on rice flour bread quality properties.

Bread properties	US-0	US-2	US-5	US-10	US-20	SE	Analysis of variance and significance
Bake loss (%)	19a	21.4d	20.8cd	20.3bc	19.7ab	0.3	***
Specific volume (mL/g)	3.71a	4.23b	4.52c	4.63c	4.49c	0.05	***
L^*_{crust}	62.3c	61.3ab	60.9a	61.5ab	61.3ab	0.4	*
a^*_{crust}	15.6c	15.3bc	14.8ab	14.6a	14.7a	0.2	***
b^*_{crust}	31.7d	31.4cd	30.9bc	30.4ab	30.2a	0.3	***
C^*_{crust}	35.5c	34.9bc	34.4b	33.5a	33.6a	0.4	***
h_{crust}	63.7a	63.8ab	64.3cd	64.4d	64.1bc	0.2	***
ΔE_{crust}	---	1.1	1.8	1.8	2.0	---	---
L^*_{crumb}	68.9b	69.4b	69.4b	68.9b	67.8a	0.3	**
a^*_{crumb}	0.408c	0.345bc	0.258ab	0.201a	0.304b	0.04	***
b^*_{crumb}	5.56c	5.55c	5.40c	5.17b	4.93a	0.09	***
C^*_{crumb}	5.57c	5.55c	5.40c	5.18b	4.95a	0.09	***
h_{crumb}	85.8a	86.0a	87.4b	88b	86.9b	0.4	***
ΔE_{crumb}	---	0.50	0.55	0.44	1.27	---	---
Hardness (N)	1.00c	0.69a	0.75ab	0.79b	0.74ab	0.79	***
Springiness	0.91ab	0.89a	0.91ab	0.93b	0.92ab	0.01	ns
Cohesiveness	0.536a	0.599c	0.581b	0.589bc	0.619d	0.005	***
Chewiness (N)	0.51c	0.37a	0.40ab	0.43b	0.43b	0.02	***
Resilience	0.228a	0.275cd	0.261b	0.268bc	0.285d	0.004	***
Hardness-7 d (N)	3.21c	3.19c	2.38a	2.85b	2.47a	0.06	***

L^*, a^*, b^*: CIELAB color coordinates, C^*: chroma; h: hue; ΔE: Difference of color between each sample and the control.
SE: Pooled standard error from ANOVA. Different letters in the same column indicate statistically significant differences between means at $p < 0.05$.
Analysis of variance and significance: *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

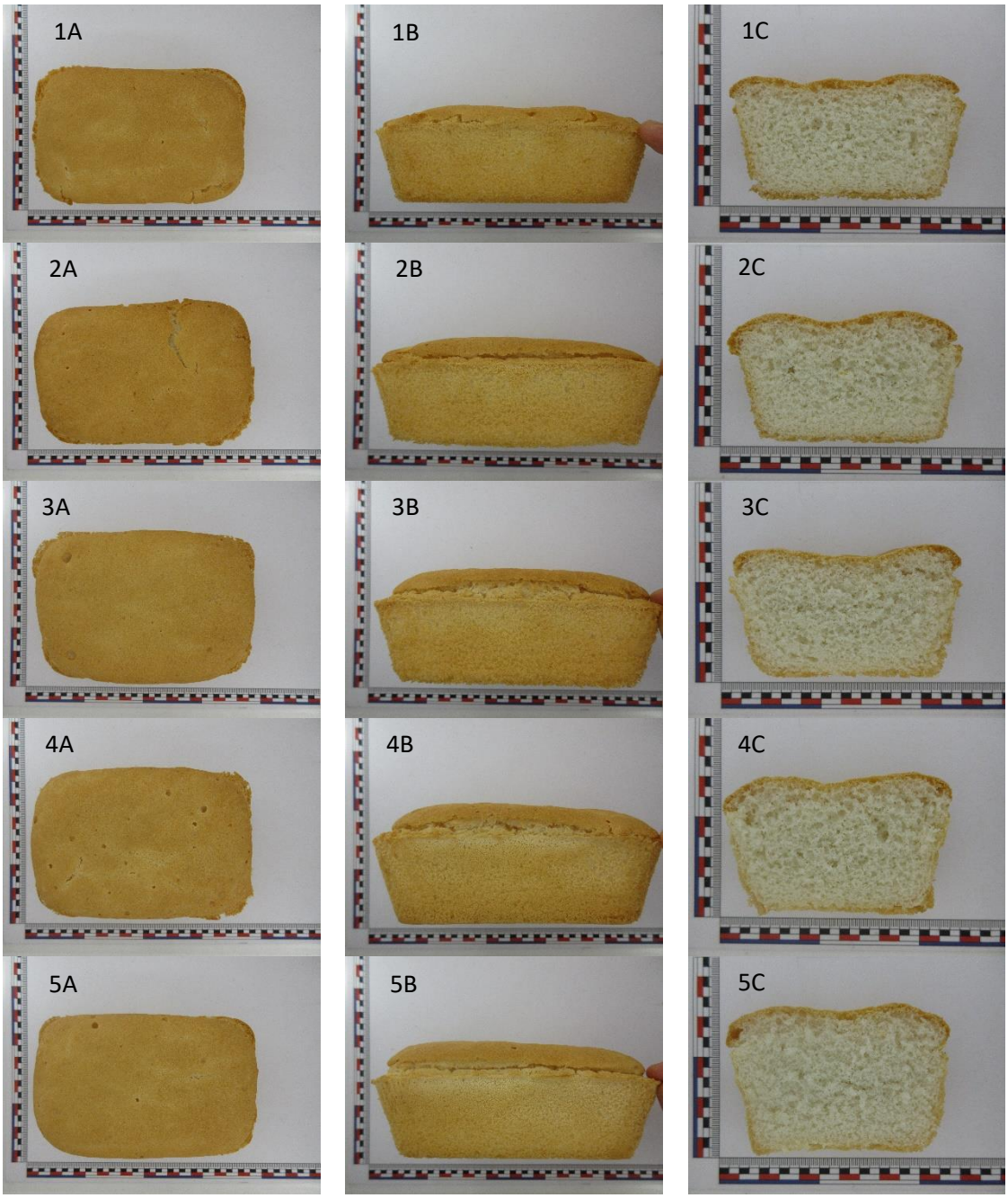

Figure 6.4. Effect of US treated-rice flour addition on the external appearance and internal structure of gluten-free breads depending on sonication time. 1) US-0, 2) US-2, 3) US-5, 4) US-10, 5) US-20. A) Upper surface, B) Side surface, C) Bread crumb structure.

6.3.3.3 Texture

The use of US-treated rice flour led to breads with a softer crumb (see Table 6.4). The lack of gluten in rice breads increases the role of starch in providing structure and texture (Witczak et al., 2016). Crumb hardness decreased from 1.00 N in the control to values ranging between 0.69 N and 0.79 N in breads incorporating ultrasonicated flours. The effect of sonication time could not be observed in this property, since all crumb hardness of breads containing US-treated samples were equal. Lower values of hardness were related to the higher bread volume, due to higher amount of air retained in the dough structure during proofing and baking (Pérez-Quirce et al., 2014; Villanueva et al., 2019). The same trend was observed for chewiness, probably because this parameter is mainly affected by hardness. Flour granulation and uniformity of particle size are also important factors that also affect the processing performance of the flours by determining their hydration and pasting properties (Abebe et al., 2015). As it was mentioned before (section 6.3.1), ultrasonication led to particle fragmentation, so doughs made with 30 % US-treated flour and 70 % native flour contained a mixture of normal-size and small-size particles. It is believed that the presence of particles of smaller size (both starch and proteins) had a Pickering stabilization effect that helped the doughs to better retain the gas generated during proofing, hence reducing the bread's hardness. Nanoscale particles have been indicated to perform good stabilizing droplets and gas bubbles in food applications (Dickinson, 2012). Resilience and cohesiveness increased significantly in the breads made with sonicated flour which demonstrate the higher recovery capacity (instantaneous or retarded, respectively) after a compression of their bread crumbs with respect to the control bread, indicative of freshness and a higher elastic behavior (Ronda, Quilez, et al., 2014). Springiness was not found to be affected by ultrasonication.

Hardness determined after 7 days of bread storage showed that the use of sonicated flour delayed the hardening of bread during storage (see Table 6.4). The hardening of the crumb is a complex phenomenon in which multiple mechanisms operate, involving starch recrystallization and moisture loss (Pérez-Quirce et al., 2014; Villanueva et al., 2015). Considering the hardness stablished for the fresh crumbs, the values determined after storage represented an increase of 2.21 N, 2.50 N, 1.63 N, 2.06 N and 1.73 N, for the control, US-2, US-5, US-10, and US-20, respectively. This retarded hardening could derive from particle fragmentation caused by US treatments, enhancing interaction with water and favoring crumb moist over time.

6.4 Conclusion

Ultrasound treatments demonstrated to be a technology capable of modifying the physicochemical properties of rice flour and influence its breadmaking performance, even at treatments performed at short times, of 5 – 10 min, and high flour concentration, of 25 %. US treatments led to particle fragmentation and molecular reorganization of starch by the action of cavitation, that caused a significant increase of pasting temperature, a reduction of $T_{O\text{-}gel}$ and improved elasticity when 30 % of the US-treated flours were incorporated to dough formulations. It is believed that the linear chain fragmentation allowed an improved fermentation by easier accessibility of yeast to simpler sugars, accelerating the generation of CO_2 and achieving a higher amount of CO_2 produced. The partial depolymerization and its consecutive increased generation of CO_2 were also confirmed in the breads obtained, where the incorporation of ultrasonicated flours led to higher volumes and softer crumbs, and lower L^* values in the crust, all indicative of higher availability of simpler sugars in the US-treated flours. The breads containing ultrasonicated flours presented a crumb with a lower tendency towards staling, possibly due to improved interaction of smaller particles with water, better preserving their moist.

Acknowledgement: Authors thank the financial support of Ministerio de Ciencia e Innovación (PID2019-110809RB-I00) and the Junta de Castilla y León/FEDER VA195P20. A. Vela thanks the financial support of Junta de Castilla y León for the doctoral grant.

Author statement

Antonio Vela and Felicidad Ronda conceived and designed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper. Antonio Vela performed the experiments and wrote the original draft. Marina Villanueva analyzed and interpreted the data - review & editing. Felicidad Ronda: Funding acquisition, conceptualization, methodology, resources, investigation, visualization, supervision, writing - review & editing, project administration.

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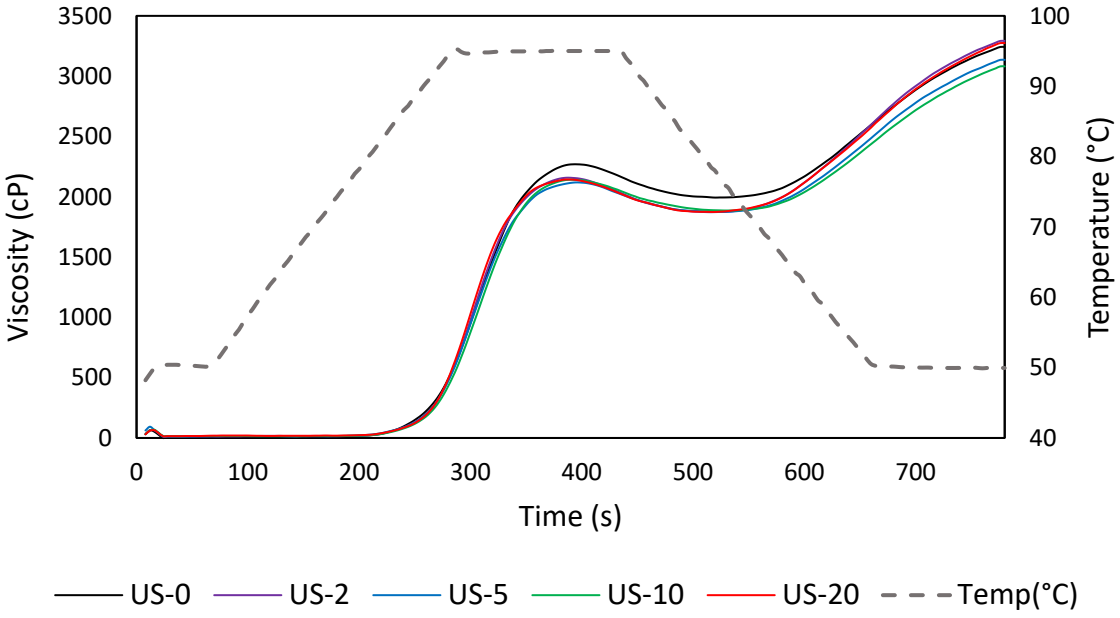
Supplementary Figures



Supplementary Figure 6.1. Pasting profiles of the studied doughs.

IV CONCLUSIONS

The research carried out about the physical modification of gluten-free flours by ultrasound treatments indicates clear changes in the physicochemical properties, where the treatment parameters and the botanical origin of the flour have shown to be decisive in the extent of modification achieved. The extent of modification achieved depends on the effect of ultrasound cavitation on the treated particles and their molecular configuration, which could be controlled by adjusting the treatment conditions.

The following conclusions can be highlighted:

- There is not a constant increasing impact of ultrasonication with increasing sonication exposure. After reaching a summit, few effects are determined with longer treatment times. In the case of rice flour, said limit was seen to be 10 min of ultrasonication.
- Flour concentration during US treatments did not show to be highly influential in the final properties obtained for the ultrasonicated flours, while treatment temperature was a parameter that greatly determined the impact of the ultrasound treatment.
- Ultrasound cavitation leads to particle rupture and molecular fragmentation, which alter their interaction with water and, consequently, the properties that derive from said interaction (hydration, thermal, pasting, and rheological properties).
- The closer the treatment temperature to the onset gelatinization temperature of the native flour, the more marked changes obtained by the modification.
- Ultrasound treatments performed at higher temperatures led to a narrowing of the gelatinization temperature range, indicative of a more homogeneous starch structure after the molecular rearrangement due to higher mobility induced by annealing, and increased interactions of the amorphous regions due to chain fragmentation.
- Cavitation led to molecular depolymerization in both amylose and amylopectin, with preferential chain fragmentation on the α-(1,4) glycosidic bonds leading to increased proportions of intermediate length amylose chains (DP 300 – 1600).

- The method employed to remove water after ultrasound treatment greatly defines the physicochemical properties of the modified flour, mainly due to the differences in the final composition of the flour after the elimination of the soluble compounds when freeze-drying is not applied.
- The rheological properties of gels made with ultrasonicated flours indicated higher consistency after treatments, with improved ability to withstand stress (τ_{max}) and lower values of $tan(\delta)_1$ reflecting a higher solid-like behavior and higher strength of the gel.
- The particle fragmentation and partial depolymerization induced by ultrasonication allowed an improved fermentation in the elaboration of bread by easier accessibility of yeast to simpler sugars, accelerating the generation of CO_2 and enhancing its retention within the dough. This effect resulted in breads with lower lightness (improved Maillard reaction), higher volumes and softer crumbs. The breads containing ultrasonicated flours presented a crumb with a lower tendency towards hardening.

Ultrasound treatments demonstrated to be a feasible technology to modify the physicochemical properties of gluten-free flours from different botanical origins. Treatment temperature was the most important variable determining the extent of modification achieved, magnifying the effect of ultrasonication due to a combined effect with annealing.

Further chemical analyses to characterize potential changes in other constituents of flours different than starch, and the impact of the treatment on the nutritional value in treated flours would be interesting to widen the scope and perspective of ultrasound modification of flours. Regarding breadmaking, the use of ultrasonicated flours from different botanical origins would be needed to determine if the beneficial effect observed with rice flour is extensive to other complex matrixes, and to gain deeper information about the influence that the different components in the flours could have on the quality of the obtained breads. It would also be interesting to investigate the use of other levels of sustitution of ultrasonicated flour in gluten-free bread formulations, particularly higher amounts, to determine if the beneficial effect seen at a dosification of 30 % can also be obtained at other substitutions. Given the beneficial effect in the volume of breads containing ultrasonicated flours, and their improved capacity to retain CO_2, it would be interesting to further investigate the possibility of using reduced amounts of additives in formulations containing US-treated flours.