University of Sao Paulo
"Luiz de Queiroz" College of Agriculture

Ultrasound assisted hydration of grains with sigmoidal behavior: kinetics of hydration, cooking, germination and nutrient incorporation

Alberto Claudio Miano Pastor

Thesis presented to obtain the degree of Doctor in Science. Area: Food Science and Technology

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Agroindustrial Engineering

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Advisor:
Prof. Dr. **PEDRO ESTEVES DUARTE AUGUSTO**

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Hidratação assistida por ultrassom de grãos com comportamento sigmoidal: cinética de hidratação, cozimento, germinação e incorporação de nutrientes

A presente Tese teve como objetivos estudar o processo de hidratação de grãos com comportamento sigmoidal de hidratação, avaliar a tecnologia de ultrassom para a sua melhora, os possíveis efeitos na germinação, cozimento e possível incorporação de nutrientes. O trabalho foi feito em três etapas. A primeira etapa consistiu em realizar uma prospecção de hidratação de diversos grãos, auxiliando na escolha dos futuros grãos a serem estudados. Ainda, comparou-se as cinéticas de hidratação associando-as com as propriedades intrínsecas dos grãos, avaliando diversas inferências publicadas sobre o processo. Na segunda etapa estudou-se o efeito do ultrassom de alta potência no processo de hidratação de três grãos com comportamento sigmoidal: tremoço andino, feijão moyashi e feijão branco. Também foi avaliado o efeito combinado do ultrassom e a temperatura da água de hidratação. O efeito da tecnologia de ultrassom foi avaliado na cinética de absorção de água, extração de compostos, germinação e cozimento. A terceira etapa consistiu em aproveitar o longo tempo do processo de hidratação para incorporar um nutriente hidrossolúvel dentro do grão. O ultrassom foi também usado para acelerar este processo. Os resultados mais importantes da Tese foram que se corroborou que a hidratação de grãos é um processo complexo e que há muita dificuldade em correlacionar as propriedades intrínsecas dos grãos com as características da hidratação. Ainda, foi demonstrado que o comportamento sigmoidal de hidratação é somente encontrado em grãos da família das leguminosas. Também foi demostrado que o ultrassom acelera a hidratação de grãos com comportamento sigmoidal e que esta tecnologia não afeta o amido dos grãos, melhora ou piora a germinação (dependendo do grão), e não afeta o cozimento. Corroborou-se que quanto maior a temperatura da água, menor é o efeito do ultrassom na cinética de hidratação. Finalmente, demonstrou-se que é possível incorporar nutrientes solúveis dentro dos grãos, aproveitando assim o longo tempo de hidratação. Encontrou-se que a incorporação de nutrientes segue mecanismo similar que a entrada de água, e que pode ser acelerado usando ultrassom. Também, foi demonstrado que o ferro foi incorporado no cotilédone e no tegumento (em maior quantidade), e que este composto melhora o cozimento do feijão, porém prejudica a sua germinação. Em conclusão, o ultrassom pode ser usado para melhorar o processo de hidratação de grãos e para a incorporação de algum nutriente.

Palavras-chave: Hidratação; Ultrassom; Grãos; Leguminosa; Comportamento sigmoidal; Incorporação de nutrientes

ABSTRACT

Ultrasound assisted hydration of grains with sigmoidal behavior: kinetics of hydration, cooking, germination and nutrient incorporation

This Thesis had as objectives to study the hydration process of grains with sigmoidal behavior, evaluating ultrasound technology to improve it, the possible effects on germination, cooking and a possible incorporation of nutrients. The work was developed in three parts. The first part consisted of describing the hydration kinetics of many grains to have a data bank of hydration kinetics, allowing further selections. Furthermore, this part compared the differences among grains hydration kinetics, associating them with the intrinsic properties of the grains and questioning some inferences previously published in the literature. The second part was related to study the effect of ultrasound on the hydration kinetics of grains with sigmoidal behavior, being studied Andean lupin, mung beans and white kidney beans. Further, the effect of ultrasound in combination with soaking water at high temperatures was studied to demonstrate if they have additive, synergic or antagonist effect. The effect of ultrasound technology was evaluated in the water uptake, extraction of components, germination and cooking kinetics. The third part consisted of studying and describing the incorporation of a nutrient inside the grains, taking advantage of the hydration process. Further, ultrasound was used to improve this incorporation. The main results of this work corroborate that the hydration process is a complex phenomenon, and many intrinsic properties of the grains causes the kinetics differences. In addition, the fact that only legume grains have sigmoidal behavior of hydration was proved. Further, the ultrasound accelerated the hydration process of grains with sigmoidal behavior without affecting the grain starch, improving the extraction of undesirable components, enhancing or hindering the germination (depending on the grain), but without affecting the cooking process. Another result was that the higher the soaking water temperature is, the lower the ultrasound effect on hydration is. Finally, regarding nutrient incorporation, a hydrophilic nutrient (iron) was incorporated in a grain during hydration process demonstrating that it is possible. The iron incorporation had the same behavior as the water uptake, suggesting similar mechanisms and phenomena of mass flow. In addition, the incorporated iron was attached not only to the bean cotyledon, but also mostly in the seed coat. Furthermore, ultrasound technology enhanced this process. The cooking process of the studied grains was improved, and its germination was hindered by the incorporated iron. In conclusion, ultrasound technology can be used for improving the hydration process of grains with sigmoidal behavior, being also practical for nutrient incorporation into grains.

Keywords: Hydration; Ultrasound; Grains; Pulses; Sigmoidal behavior; Nutrient incorporation

RESUMEN

Hidratación asistida por ultrasonido de granos con comportamiento sigmoidal: cinética de hidratación, cocción, germinación e incorporación de nutrientes

La presente tesis doctoral tuvo como objetivos estudiar el proceso de hidratación de cereales y leguminosas con cinética sigmoidal, evaluando el uso de ultrasonidos para su mejora, el posible efecto en la germinación, cocimiento e incorporación de nutrientes. Este trabajo fue realizado en tres etapas. La primera etapa consistió en comparar la cinética de hidratación de diversos cereales y leguminosas para verificar que granos presentan una cinética sigmoidal y poder ser escogidos en trabajos futuros. Además, en esta etapa se investigó cuáles de las características intrínsecas de los granos se correlacionan con las características de la cinética de hidratación. La segunda parte estuvo relacionada al uso de ultrasonidos de alta potencia para mejorar el proceso de hidratación. Se aplicó en tarwi, frejol blanco y loctao, demostrando que el ultrasonido acelera el proceso de hidratación. Asimismo, se verificó el efecto combinado de la temperatura y el ultrasonido en la hidratación. El efecto de la tecnología de ultrasonido fue evaluado en la cinética de ganancia de agua, extracción de compuestos, germinación y cocción. La última etapa de investigación tuvo como objetivo describir y acelerar la incorporación de fierro como nutriente hidrosoluble dentro de leguminosas usando ultrasonidos de alta potencia. Los principales resultados de la presente tesis fueron la confirmación de la complejidad del proceso de hidratación por la dificultad de asociar las propiedades intrínsecas de los granos con la cinética de hidratación. Además, se demostró que la cinética sigmoidal de hidratación es sólo presente en leguminosas. Asimismo, se demostró que el ultrasonido acelera el proceso de hidratación sin afectar el almidón, pudiendo o no mejorar la germinación, mejorando la extracción de compuestos indeseados y sin afectar el proceso de cocción. A su vez, fue demostrado que el efecto de los ultrasonidos disminuye al realizar la hidratación con altas temperaturas. Finalmente se demostró que es posible incorporar fierro en los granos durante la hidratación y que este proceso puede ser acelerado usando ultrasonidos de alta potencia. Se observó que la cinética de incorporación de fierro (nutriente hidrosoluble) es similar a la cinética de entrada de agua y que el fierro es mantenido en el cotiledón y el tegumento del grano. La incorporación de fierro en los granos aceleró el proceso de cocción. Sin embargo, la germinación del grano fue reducida. Como conclusión general se tiene que es el uso de ultrasonidos de alta potencia es eficaz en la aceleración del proceso de hidratación de granos y que puede además ser usado para incorporar nutrientes fortificando los granos.

Palabras clave: Hidratación; Ultrasonidos; Granos; Leguminosas; Comportamiento sigmoidal; Incorporación de nutrientes

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1. INTRODUCTION, OBJECTIVES AND THESIS STRUCTURE

The hydration of grains is important prior to many other processes such as germination, cooking, extraction, malting, fermentation and extraction. This process consists of immersing the grains in water to increase their moisture and water activity, bringing many benefits for the mentioned processes. This process is studied by its kinetics recording the gained mass of water through the time.

The hydration kinetics gives information about how water is absorbed, the velocity of the process and the water holding capacity of the grain, which can be mathematically described. In fact, the hydration kinetics has two different behaviors: downward concave shape and sigmoidal shape (Albert Ibarz & Augusto, 2014; Alberto Claudio Miano & Augusto, 2015) (Figure 1). The first is the most common behavior in which the grain presents a fast water uptake at the beginning of the process. The hydration rate is reduced until no more water can be hold by the grain (equilibrium moisture content). On the other hand, the second behavior presents a slow water uptake at the beginning of the process due to the impermeability of the grain's seed coat. After certain time, due to changes on the permeability, the hydration rate is increased, and the grain absorbs water until reaching the equilibrium.

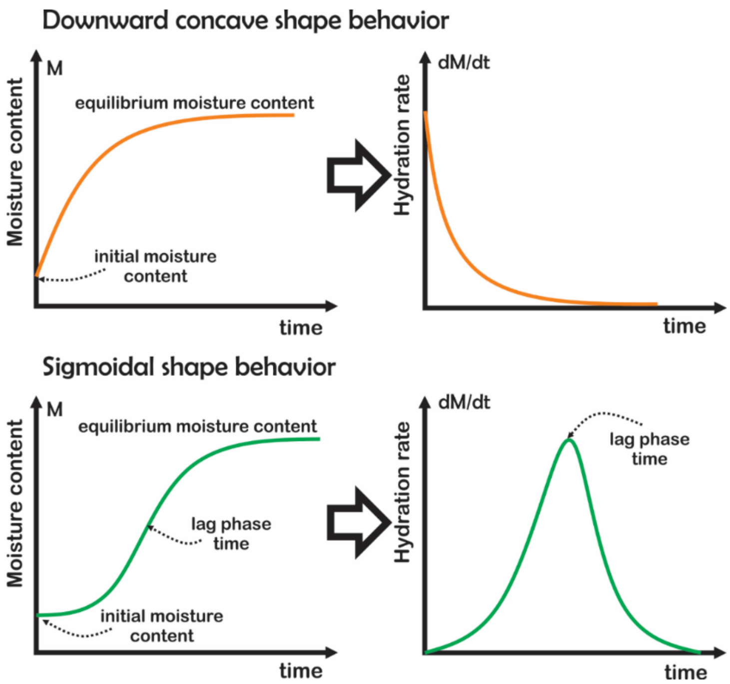

Figure 1. Behaviors of the hydration kinetics of grains.

Apparently, the hydration of grains seems to be a simple process. However, it involves different mechanisms and phenomena, being highly complex. That was one reason why this process was chosen to be studied.

During my Master research, the hydration kinetics of some grains were described and explained (A. Miano, 2015). The reason why the sigmoidal behavior is presented in some grains and how the temperature (Alberto Claudio Miano, García, & Augusto, 2015) and the initial moisture content affected it (Alberto Claudio Miano & Augusto, 2015) was studied. In addition, the water uptake pathways were associated with the structure of grains in order to reinforce the idea that grains must be considered as anisotropic bodies and the hydration takes place not only by diffusion, but also by capillarity. Finally, the use of ultrasound and its mechanisms was studied for model foods (Alberto Claudio Miano, Ibarz, & Augusto, 2016) and corn kernels hydration (Alberto Claudio Miano, Ibarz, & Augusto, 2017), an important grain from the industrial point of view and whose hydration kinetic has downward concave shape (most common behavior).

Nevertheless, there was the need to perform more studies to better understand the hydration process. For instance, looking for which property of the grain causes the sigmoidal behavior of hydration and its intensity (different sigmoidal shapes), as well as how ultrasound enhances this process. In addition, because of an unexpected experience during my Master studies, we observed that particles of cupper, which were eroded by ultrasound from a heat interchanger, were found inside the grains after hydration. Consequently, the idea of incorporating some nutrient in the grains during this process arose. For those reasons, this Thesis was proposed in order to complement my Master studies.

In fact, the hydration process has as main disadvantage that it takes long time to finish and it is conducted in batch. Therefore, many studies were conducted to accelerate the process. The most used approach was the use of water at elevated temperatures, demonstrating that as the water temperature is increased, the hydration process is faster (Montanuci, Jorge, & Jorge, 2015; Oliveira et al., 2013; Sopade & Obekpa, 1990). However, this has the limitation that high temperatures can change the grains components and can bring costs for heating and isolating. Other used technology is ultrasound, which has shown successful results in many mass transfer unit operation, being studying for hydration process (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Alberto Claudio Miano et al., 2017; Patero & Augusto, 2015;

Simal, Benedito, Sánchez, & Rosselló, 1998; Ulloa et al., 2015). For a more detailed information about the hydration process, its description, its enhancement, and its modeling, we performed a critical revision, which was published as part of my Doctorate studies (Appendix A) (Alberto Claudio Miano & Augusto, 2018a).

The present Thesis used the ultrasound technology for improving mass transfer unit operations. This technology uses acoustic waves with frequencies higher than 20 kHz and intensities higher than 1 W/cm^2 to perform physical chemical changes in food (Awad et al., 2012). This technology enhances the mass transfer processes by many mechanisms that depend on the studied process (for more information, a book chapter about theses aspects was published during the Doctorate studies (Alberto C. Miano, Rojas, & Augusto, 2017); please see Appendix B). This technology was used for enhancing the hydration process of grains with downward concave shape behavior (Ghafoor, Misra, Mahadevan, & Tiwari, 2014; Alberto Claudio Miano et al., 2017; Patero & Augusto, 2015; Yildirim, Öner, & Bayram, 2011), but there was not any work with sigmoidal behavior grain before the development of the present Thesis.

The present Thesis is organized in three parts, as shown in Figure 2, as a result of seven articles and one book chapter (Appendix from A to H). In the first part, the complexity of the hydration process of grains was evaluated by trying to associate some intrinsic properties of the grains with the hydration kinetics, especially for explaining the sigmoidal behavior. In addition, grains with sigmoidal behavior of hydration kinetics were investigated to be used in the following studies. The second part was related to the use of ultrasound to enhance the hydration process of grains with sigmoidal behavior, as well as to verify if this technology affects the grain components or the later processes. Although the ultrasound technology improved the hydration process, it still takes significant time to be applied. Therefore, the third part was conducted, where one way to take advantage of the process time was investigated. This part was related to study the incorporation of a nutrient into the grains during the hydration process. This process was described as well as enhanced using ultrasound.

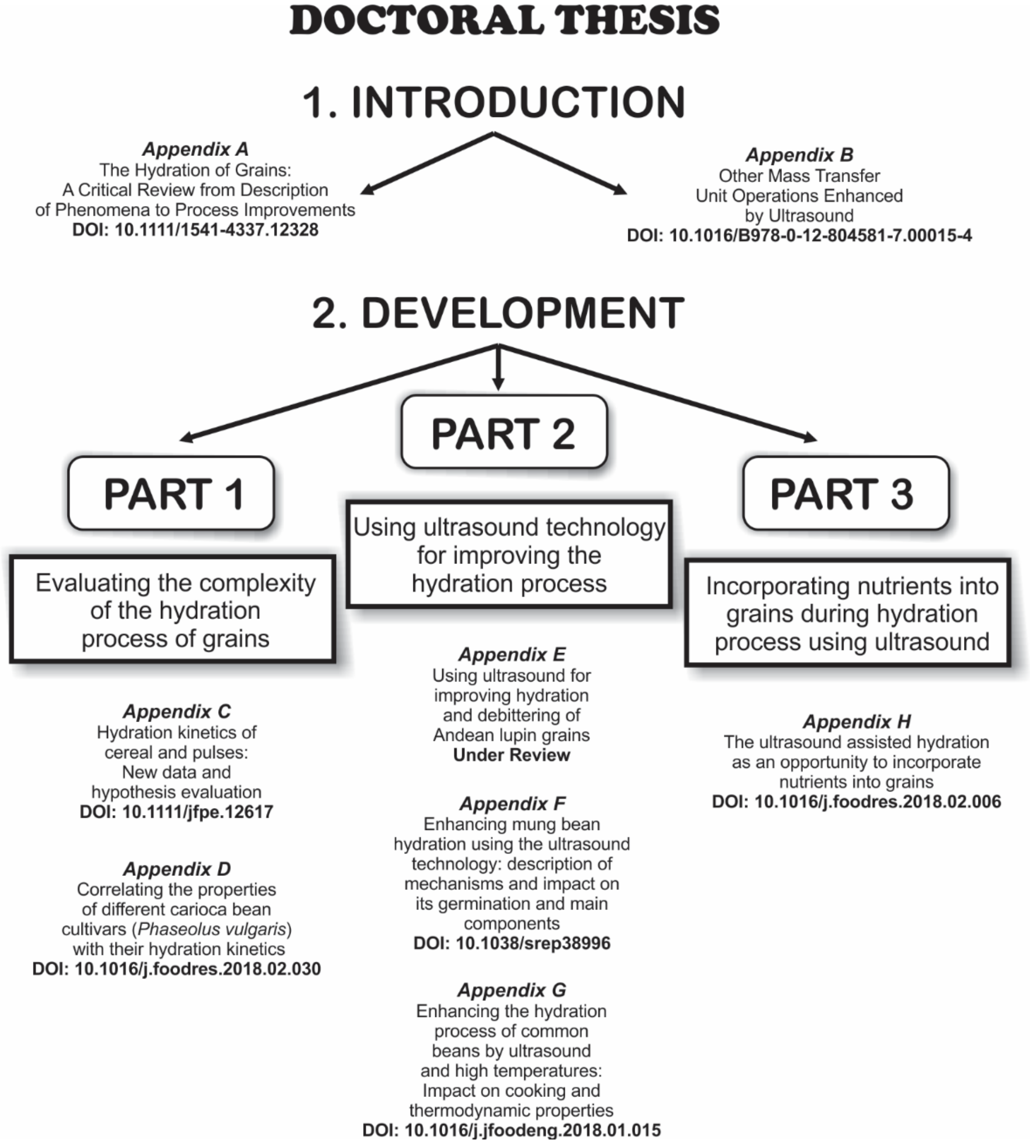

Figure 2. Thesis organization

The general objective of this work was to describe, to model and to enhance the hydration process of grains with sigmoidal behavior, leading to utilize this process for incorporating nutrients into the grains. For that, some specific objectives were intended:

- Obtain a database of hydration kinetics from different grains: cereal and legume grains;
- Evaluate and correlate some intrinsic characteristics of the grain with the hydration kinetics;
- Describe the mechanisms and model the effect of ultrasound technology on the hydration process of grains with sigmoidal behavior;

- Evaluate the combined effect of ultrasound and temperature on the hydration kinetics of grains with sigmoidal behavior.
- Evaluate the effect of ultrasound assisted hydration of grains on some of their main components and on their later processes, such as germination, cooking and extraction process.
- Describe the mechanisms and model the incorporation of a hydrophilic nutrient in a grain during the hydration process: ionic iron in solution;
- Correlate the kinetics of hydration and nutrients incorporation to evaluate the relation between both, in order to obtain an optimized process.

2. THESIS RESULTS

Figure 3 organizes the results of the present Thesis according to the reasoning order.

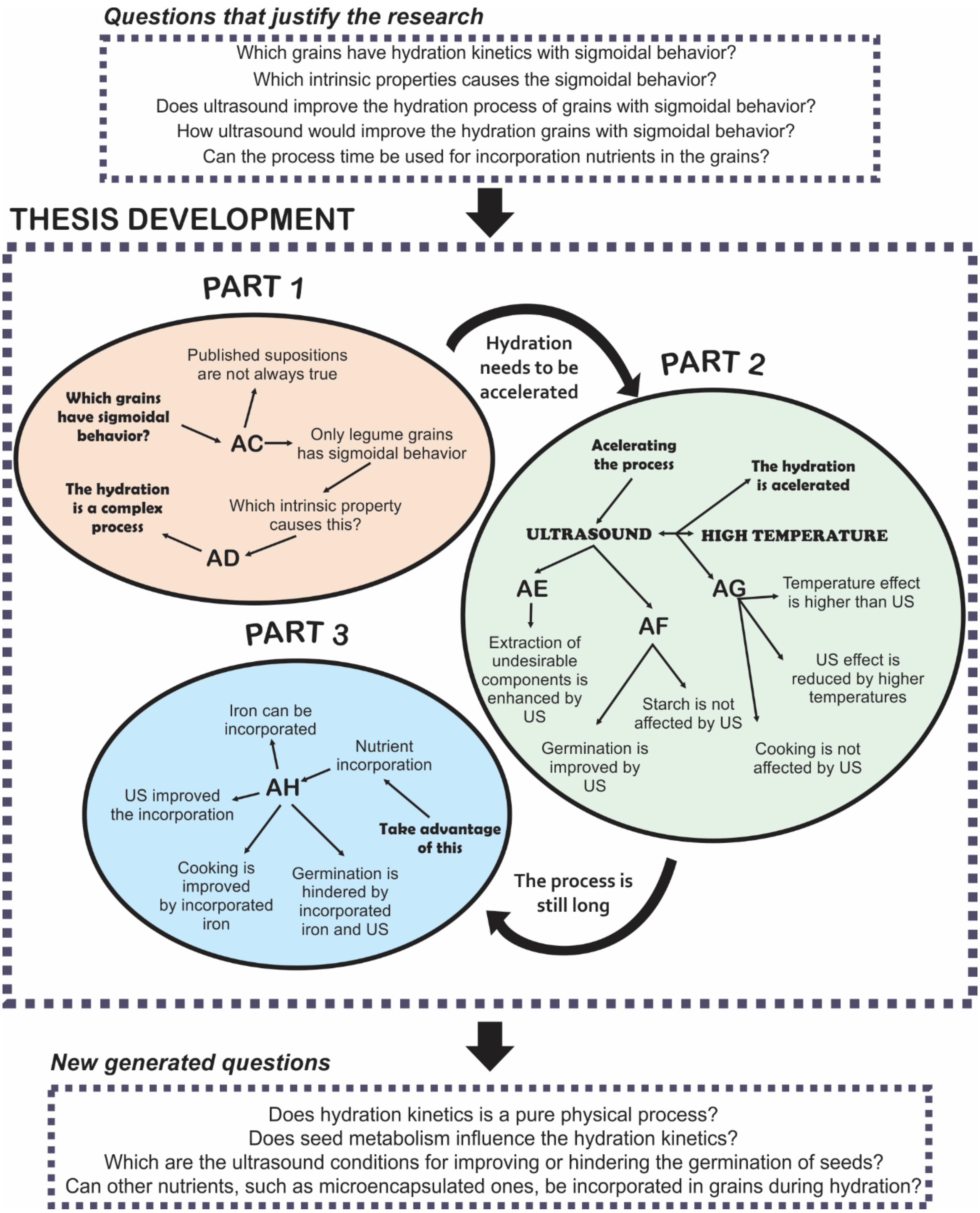

Figure 3. Objective-results scheme of the present Thesis. AC, AD, AE, AF, AG and AH are the published works that are in Appendix (A) section.

Notice that some questions were raised which brought the idea for the Thesis research. After the Thesis development, those questions were answered, and other questions were generated during the discussion of the obtained results, which should be answered by performing future researches (as our suggestions).

As stated, the Thesis development was performed in three parts which are described as follow.

2.1. Evaluating the complexity of the hydration process of grains

In this part, the hydration kinetics of many grains (cereal and legume grains) was studied to verify how this process is affected by the family differences, varieties and even cultivar as well as to have a data bank of grains hydration kinetics to be selected in future studies. The main objectives of this part were to describe grains with sigmoidal behavior (which is very rare in the literature) and select grains for the further studies.

2.1.1. Study of the hydration kinetics of diverse grains: suppositions evaluation.

The first work consisted of hydrating several grains to evaluate the different hydration kinetics of cereal and legume grain, as well as to have a data bank to select future grains to be studied. In fact, 19 species of grains from *Fabaceae* family (legume grains) and 4 from *Poaceae* Family (cereal grains) were studied (Figure 4), where 10 of them had never been studied before. It was demonstrated that there are only two hydration behaviors, the downward concave shape and the sigmoidal shape. Further, the sigmoidal behavior is rare, being exclusive for grains from *Fabaceae* family.

The obtained results also allowed us to question some published suppositions about the hydration kinetics. For instance, it was demonstrated that the ideas about darker colored grains hydrate slowly and that smaller grains hydrate faster are not always fulfilled. Further, it was observed that grains with sigmoidal behavior did not always hydrate slower than grains with downward concave behavior. In addition, by performing a Hierarchical Cluster Analysis, it was observed than the behavior hydration kinetics of grains from the same taxonomic family, genre or specie had similar characteristics of hydration rate, lag phase and equilibrium moisture content. For instance, the hydration kinetics of legume grains with sigmoidal behavior were discriminated in three groups, which were associated with the genre: *Vigna* genre, *Phaseolus* genre and *Lens* genre (Figure 5). In other words, despite legume grains have similar chemical compositions, the morphology and structure affect how the water enter the grains. Therefore, it is recommended to hydrate more varieties of grains to determine if the hydration kinetics behavior changes according to the grain genre.

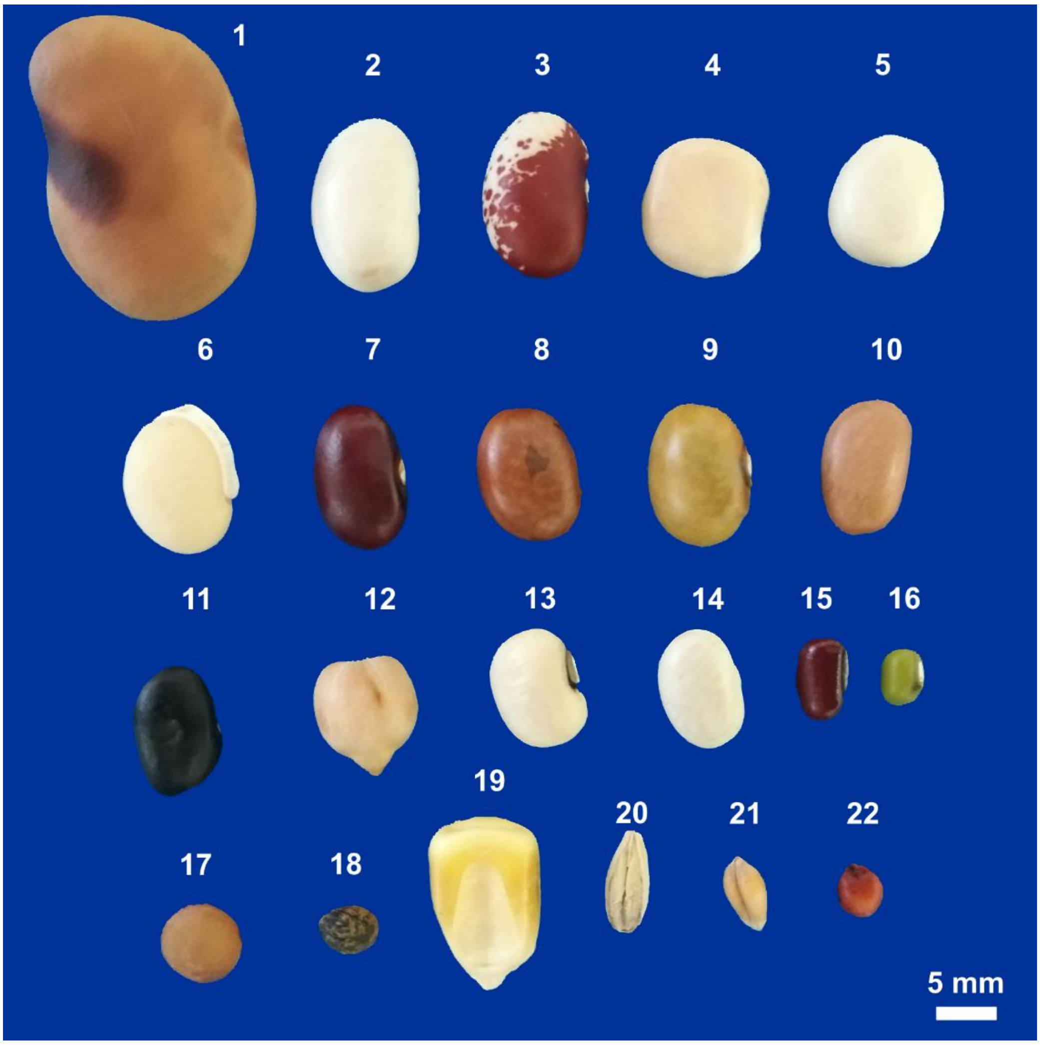

Figure 4. Studied grains for the first part of the Thesis. *Fabaceae* family: 1. Faba bean; 2. White kidney bean; 3. Ñuña bean; 4. White lupin; 5. Caballero bean; 6. Egyptian kidney bean; 7. Dark red kidney bean; 8. Carioca bean; 9. Canary bean; 10. Pink kidney bean; 11. Black kidney bean; 12. Chick pea; 13. Black eye pea; 14. Panamito bean; 15. Adzuki bean; 16. Mung bean; 17. Lentil; 18. Green lentil. *Poaceae* family: 19. Corn kernel; 20. Barley kernel; 21. Wheat kernel; 21. Sorghum kernel.

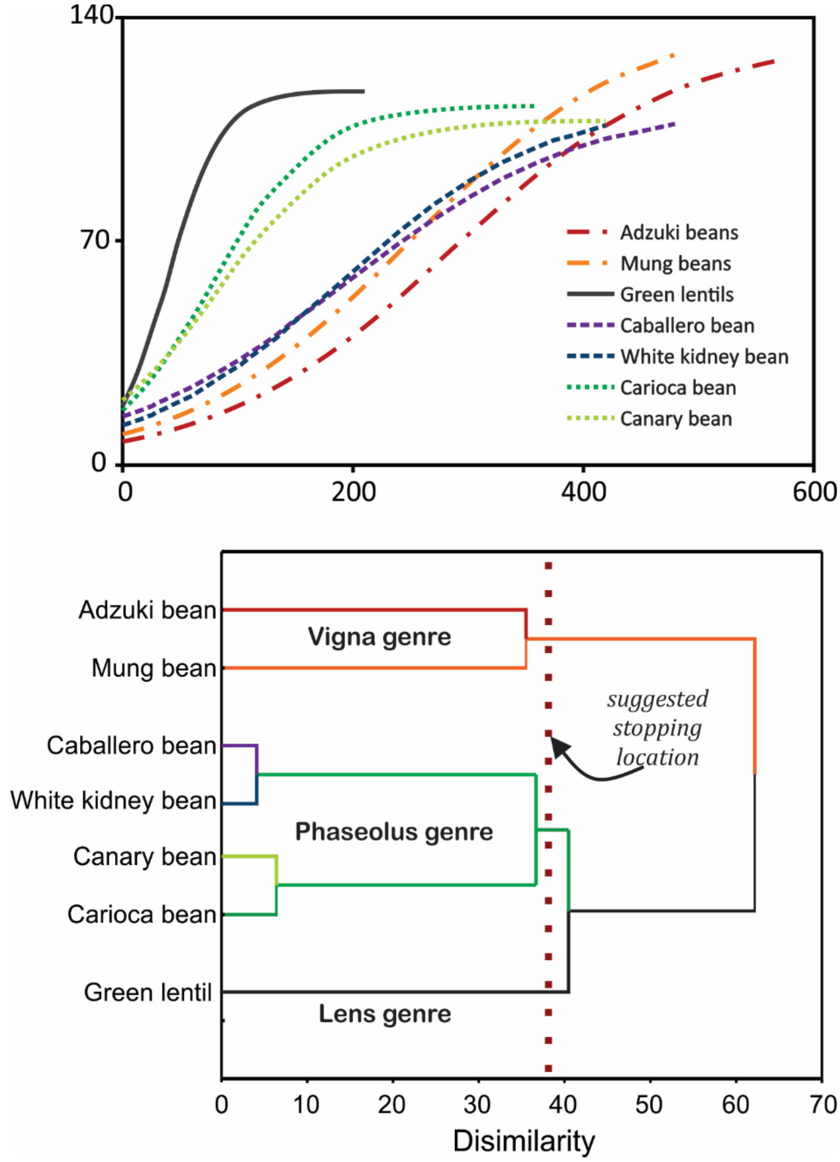

Figure 5. Discrimination of the hydration kinetics of grains with sigmoidal behavior by hierarchical cluster analysis. Note that according to the suggested stopping location, the hydration kinetics of grains from the same taxonomical genre have similar shape.

Overall, these results indicate that there is a big difficulty on associating some isolated characteristics of the grain to the hydration behavior. To do this, it is required to keep any other characteristic constant. However, this is challenging since not only extrinsic properties as growth, storage and hydration conditions, but also intrinsic properties as structure, chemical composition and physical properties of the grain can affect the hydration kinetics – justifying the next study.

This part is described in the article “Hydration kinetics of cereal and pulses: New data and hypothesis evaluation”, published in the journal “Journal of Food

Process Engineering” (Alberto Claudio Miano, Sabadoti, Pereira, & Augusto, 2018) showed in Appendix C.

2.1.2. Correlation of grain’s intrinsic properties with the sigmoidal hydration kinetics.

In this scenario, the second work was performed. By keeping many factors constant, such as how grains were growth and stored and their initial moisture content, six cultivars from the same species (*Phaseolus vulgaris)* and variety (carioca beans) of beans were evaluated. The study performed an association with their intrinsic characteristics with the hydration kinetics. The evaluated intrinsic characteristics were the chemical composition (starch, protein, lipids, functional groups from the seed coat analyzed by FT-IR and specific minerals (Mg, P, S, K, Ca, Mn, Fe, Cu, Zn), physical properties (size, 1000 grain weight, seed coat thickness, energy to penetrate the bean) and microstructure. The hydration kinetics characteristics were the hydration rate, lag phase and equilibrium moisture content. The correlation was performed using the Multiple Factorial Analysis (MFA), which allowed to know which intrinsic property had strong correlation with the characteristics of the hydration kinetics (Figure 6).

As main result, few of the studied factors did strongly correlate with the hydration characteristics. The total fat content, the potassium content and the specific surface correlated positively with the lag phase time, while the protein/lipid relation correlated negatively with this characteristic. In addition, the necessary energy to penetrate the seed coat correlated negatively with the hydration rate. Hypothesis for each factor were proposed.

These results suggested that there are other factors that can affect the sigmoidal behavior, in special the association between composition and structure. Therefore, the development of more studies looking for more factors that can affect the hydration kinetics is still necessary.

This part is described in the article “Correlating the properties of different carioca bean cultivars (*Phaseolus vulgaris*) with their hydration kinetics”, published in the journal “Food Research International” (Alberto Claudio Miano, Saldaña, Campestrini, Chiorato, & Augusto, 2018) showed in Appendix D.

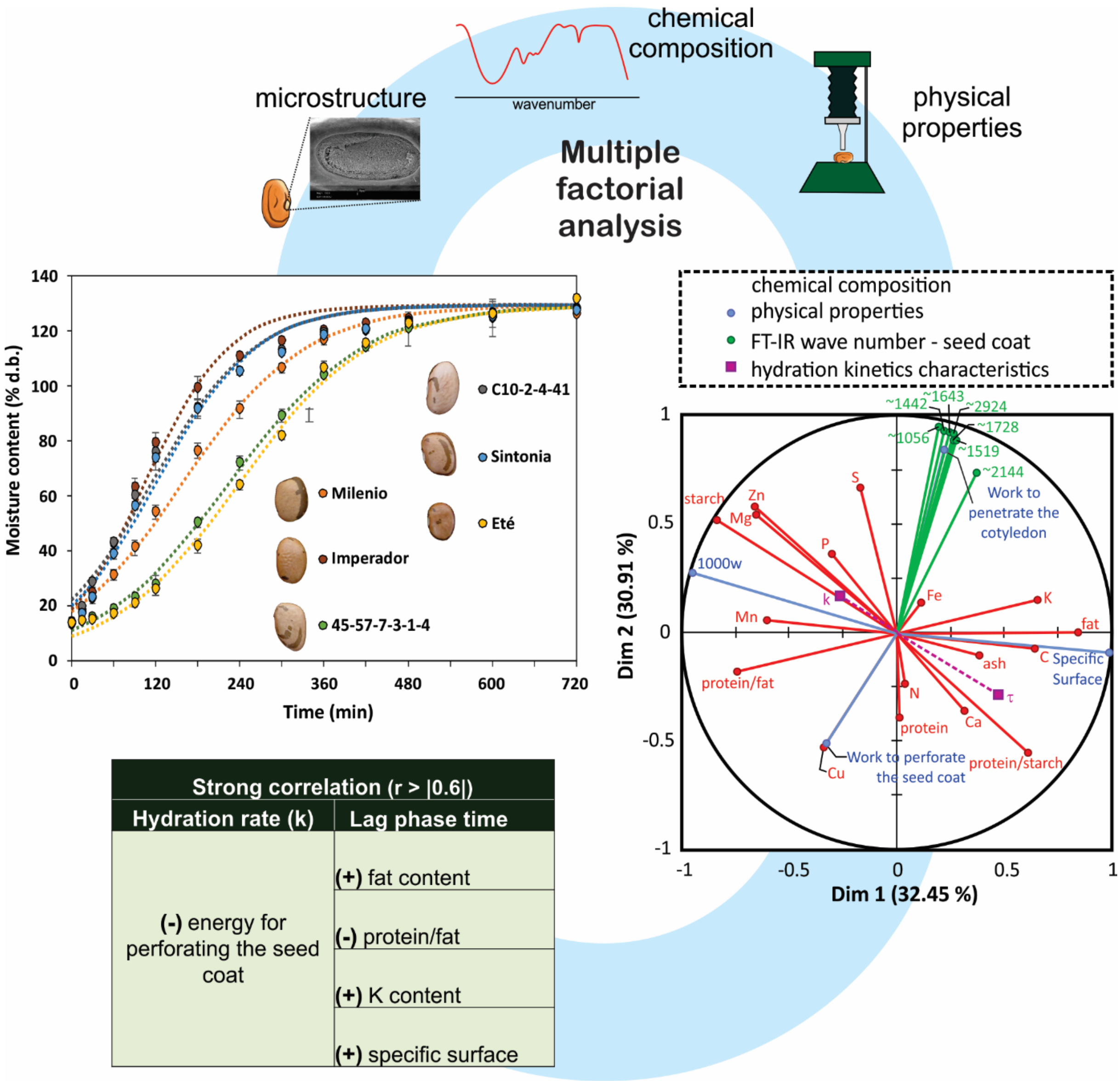

Figure 6. Correlation of different intrinsic properties of 6 cultivars of carioca beans with the hydration characteristics of a sigmoidal behavior.

2.1.3. Conclusions

This part concluded that the hydration of grains is not a simple process as it seems. Despite that, some hypotheses and suppositions were questioned and clarified and a data bank, especially of grains with sigmoidal behavior, was obtained for choosing grains for the following studies. As stated in the introduction, the hydration process of grains has as disadvantage that it takes long time, being its enhancement very desirable since the industrial point of view. Furthermore, as the grains with sigmoidal behavior had been little studied, this behavior was chosen to be enhanced.

Therefore, the use of ultrasound technology on the hydration process of grains with sigmoidal behavior was studied in the second part.

2.2. Using ultrasound technology for improving the hydration process

This part consists of three studies where the effect of ultrasound was evaluated on the hydration kinetics of three grains. The difference among these three studies is the used grain, the other processes or components evaluated (germination, cooking, starch and extraction) and the use of different temperatures in combination with ultrasound.

2.2.1. Ultrasound assisted hydration of Andean lupin (*Lupinus Mutabilis* Sweet)

In the first work, the effect of ultrasound on the hydration kinetics of Andean lupin grains was studied. This grain was the first studied grains during my Master research, and it was chosen as it showed sigmoidal behavior (A. C. Miano et al., 2015). In addition, this pulse is important due to its high amount of proteins (~44 %w.b) (Jacobsen & Mujica, 2006), even more than soybean. However, it has also a great number of toxic alkaloids that must be extracted before being consumed. Therefore, besides evaluating the effect of ultrasound on the hydration process, it was also verified if the alkaloid was extracted.

As result, the ultrasound (bath, 41 W/L of volumetric power, 25 kHz of frequency) accelerated 40% the hydration process, by reducing the lag phase time (13%) and increasing the equilibrium moisture content (14%) (Figure 7a). This improvement was attributed to the ultrasound mechanisms that enhances mass transfer (sponge effect, inertial flow and microchannels formation), whose effect depends on the water activity and porosity of the grains (Alberto Claudio Miano, Ibarz, et al., 2016). Therefore, as the grains absorb water, the ultrasound effect is improved, which was also related to the convergence of acoustic impedance.

Further, after the hydration without and with ultrasound, the alkaloid content of the grains was determined (Figure 7b). The grains hydrated with ultrasound had 21% less alkaloid content than the conventionally hydrated grains, demonstrating that ultrasound has potential to extract undesirable components from grains.

This part is described in the article "Using ultrasound for improving hydration and debittering of Andean lupin grains", which is under review at Journal of Food Process Engineering showed in Appendix E.

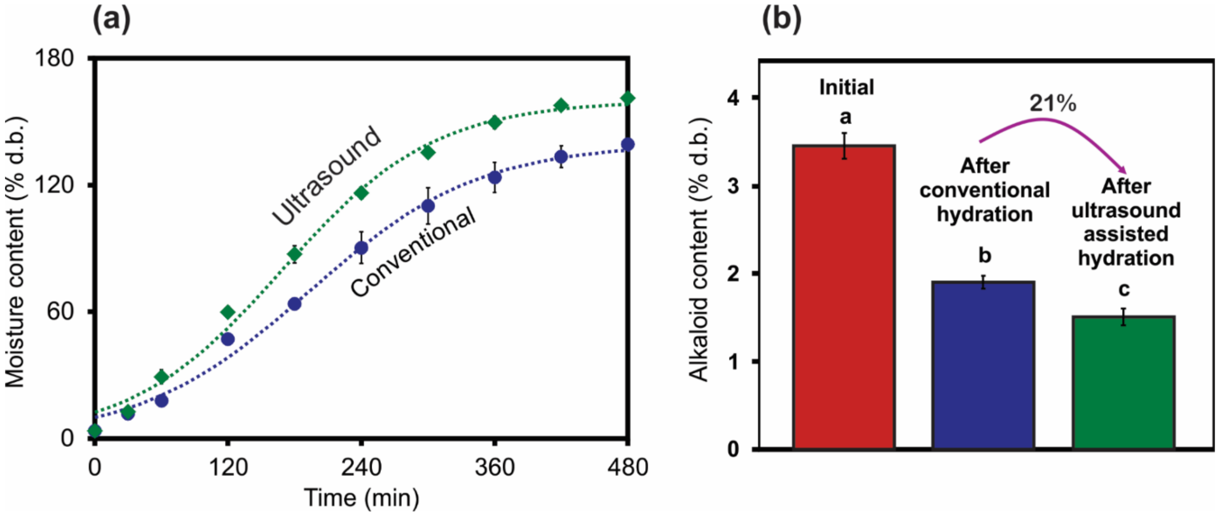

Figure 7. (a) Hydration kinetics of Andean lupin (*Lupinus mutabilis* Sweet) with and without ultrasound (41 W/L of volumetric power and 25 kHz of frequency) at 25 °C. **(b)** Alkaloid content of Andean lupin grains hydrated with and without ultrasound. The dots represent the experimental data, the discontinuous lines represent the Kaptso et al. model (Kaptso et al., 2008), the vertical bars represent the standard deviation and the lower case letters represent the Tukey comparison test (95% of confidence).

2.2.2. Ultrasound assisted hydration of mung bean (*Vigna radiata*): Effect on the hydration kinetics, germination and starch properties.

In the second work of this first part, the ultrasound assisted hydration of mung beans (*Vigna radiata*) was studied. This grain is used for the sprout production, starch extraction and direct consumption (Dahiya et al., 2015).

This work firstly described the hydration behavior of this bean. In fact, it was verified that the hydration kinetics of this grains was affected by its initial moisture content. As the initial moisture content is increased, the hydration behavior changes from sigmoidal to downward concave shape corroborating previous studies with other grains (Alberto Claudio Miano & Augusto, 2015; Ross, Arntfield, Beta, Cenkowski, & Fulcher, 2008). In addition, it was also corroborated the water uptake pathway that was proposed for grains with sigmoidal behavior during my Master studies (A. C. Miano et al., 2015). This was determined by sealing some structures of the grain and by evaluating the microstructure through scanning electronic microscopy (SEM) images.

Due to the impermeability of the seed coat, water enters by the hilum for then filling the space between the cotyledon and the seed coat. The seed coat is hydrated by the inside face, causing physicochemical changes that increases its permeability to water. Finally, water enters not only by the hilum (capillarity), but also by the seed coat (diffusion) to hydrate the cotyledon, until reaching the equilibrium moisture content.

Then, the effect of ultrasound on the hydration kinetics of this bean was studied (Figure 8a). As result, ultrasound (bath, 41 W/L of volumetric power and 25 kHz of frequency) accelerates the hydration process of mung bean, reducing the processing time in 25%, by increasing the hydration rate (44%) and reducing the lag phase (28%). The effect of ultrasound on the equilibrium moisture content could not be stablished due to the fast germination of this pulse. As in the previous works, the mass transfer enhancement was attributed to the sponge effect, inertial flow and microchannels formation by ultrasound technology. Furthermore, any change on the grain microstructure were found (SEM images), suggesting that ultrasound did not cause a significant change on structure.

In addition, ultrasound accelerated the germination process of mung bean, a desirable result for producing sprouts. In fact, seeds imbibition is composed by three stages during their germination (Bewley, Black, & Halmer, 2006): the first stage consists of the hydration process itself (the stage that this Thesis studied), the second stage consist of the nutrient catabolism and molecules synthesis (without water gain) before germination and the third stage consist of the visible germination by the radicle growth. In the present part, ultrasound caused the reduction of the second stage, leading to rapid radicle growth. Therefore, the germination was accelerated. Many possible causes were given as the mass transfer improvement (water, oxygen and metabolites) and enzymes activation.

In fact, as the grains is hydrated, their metabolism activity is increased. Consequently, this study brought the idea that there is a possibility that the hydration process may be enhanced not only by physical phenomena, but also due to metabolic/biological phenomena, since the fact that ultrasound can enhance the germination and vigor of seeds had been proved (A. Miano et al., 2015). However, this could not be proved in this study. Therefore, this possibility must be further evaluated.

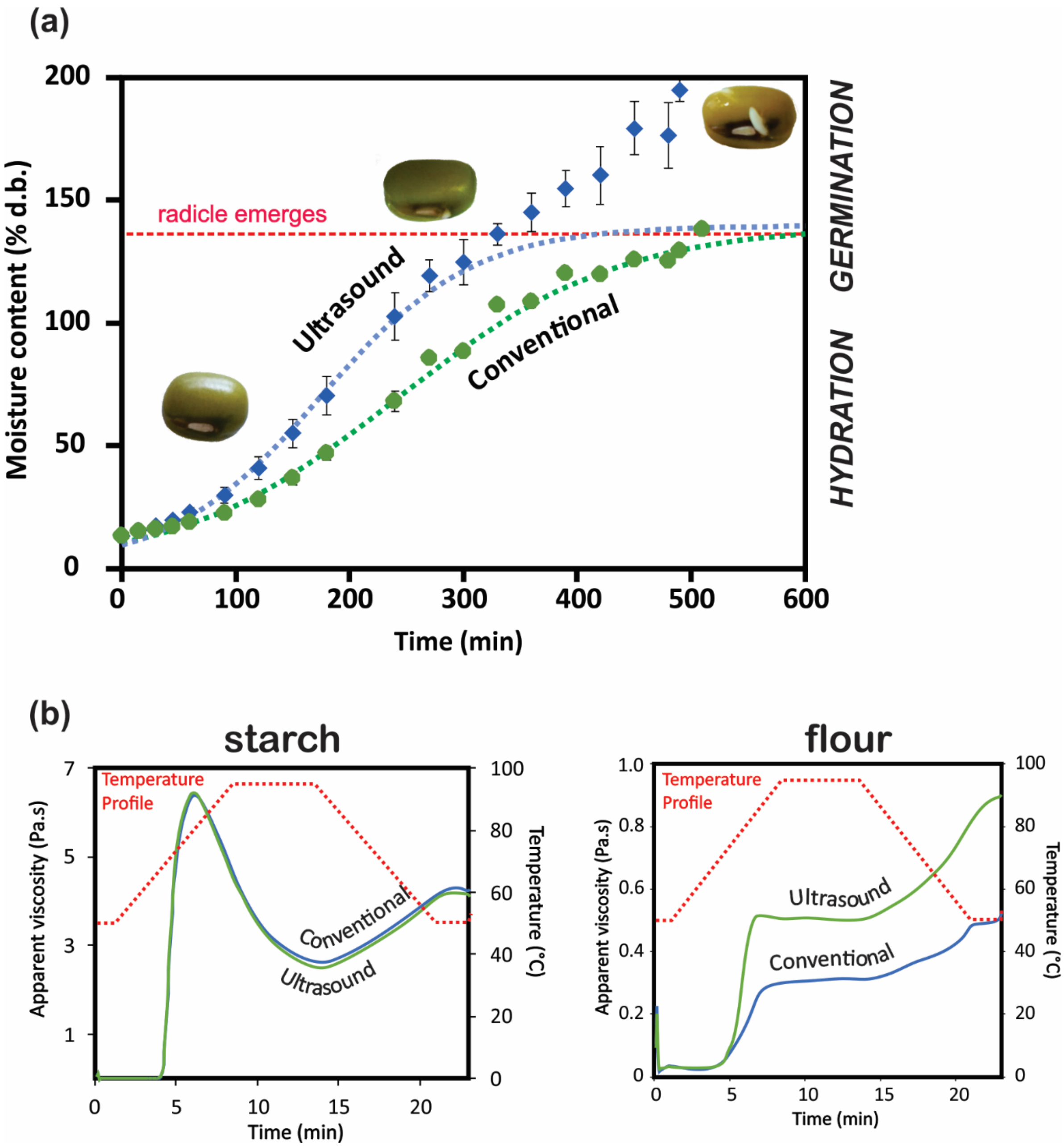

Figure 8. (a) Hydration kinetics of mung bean (*Vigna radiata*) with and without ultrasound (41 W/L of volumetric power and 25 kHz of frequency) at 25 °C. The dots represent the experimental data, the discontinuous lines represent the Kaptso et al. model (Kaptso et al., 2008), the vertical bars represent the standard deviation **(b)** Pasting properties of the starch and flour extracted from mung beans hydrated with and without ultrasound.

Finally, starch from mung beans hydrated with and without ultrasound was extracted to be evaluated through different analysis: granule microstructure, pasting properties, mechanical properties of the gel and molecular mass distribution. All the evaluations suggested any change on starch structure and properties by ultrasound application during hydration process. This corroborated the result obtained in my

Master's degree Dissertation, when corn starch was studied. On the other hand, the mung bean flour was analyzed through its pasting properties. We observed that the apparent viscosity of the flour obtained from grains hydrated with ultrasound was increased. Since starch was not modified, this result suggested the protein modification by ultrasound (Figure 8b).

This part is described in the article "Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components", published in the journal "Scientific Reports" (Alberto Claudio Miano, Pereira, Castanha, Júnior, & Augusto, 2016) showed in Appendix F.

2.2.3. Ultrasound assisted hydration process of white kidney beans (*Phaseolus vulgaris*) at different temperatures: Effect on hydration kinetics, cooking process and thermodynamic properties.

The last work of this part consisted of evaluating the combined effect of ultrasound and soaking water at hot temperatures on the hydration kinetics of grains. Both approaches were demonstrated to be efficient accelerating the hydration process individually in many published studies. Therefore, this work aimed to verified if the using of both approaches in combination would have synergic, additive or antagonist effect on the hydration process.

For that, white kidney beans were hydrated with and without ultrasound (28 W/L of volumetric power and 40 kHz of frequency) at 25 °C, 35 °C, 45 °C and 55 °C. As a result, ultrasound and elevated temperatures accelerated the hydration process when they are used individually (Figure 9). In contrast, as the temperature of the soaking water is increased, the effect of ultrasound technology is hindered. This result was also observed in other mass transfer processes as drying (Clemente, Sanjuán, Cárcel, & Mulet, 2014; Rodrigues, Fernandes, García-Pérez, & Cárcel, 2016; Sabarez, Gallego-Juarez, & Riera, 2012) and on hydration of a grain with downward concave shape behavior (Patero & Augusto, 2015). In fact, the energy provided by water at high temperatures is very high compared to the energy provided by ultrasound. In addition, according to Raso, Mañas, Pagán, and Sala (1999), the ultrasonic effect can be reduced by the cushioning effect of the additional vapor presented in the cavitation bubbles due to the vapor pressure reduction by the high temperatures. Further, reduction on the water viscosity and surface tension could reduce the energy dispersed in the implosion of the cavitation bubble. Therefore, the combination of both technologies may not be useful for mass transfer unit operations.

The thermodynamic properties were determined with and without ultrasound: activation enthalpy, activation Gibbs free energy, activation entropy and activation energy. Regarding the hydration rate and according to the activated complex theory, “dried” molecules from the grains have to form an activated complex with water molecules before getting hydrated. Therefore, it was considered that the hydration of the dry bean followed Equation 1: during the hydration of a dry bean (DB), the activated complex ($[DB - H_2O]$) is formed, before obtaining the hydrated bean (WB). For the activated complex formation, many mechanisms take place, as molecular rearrangement, bond angles, interatomic distances changes, and so on, involving energy and organization changes (enthalpy, entropy and Gibbs free energy of activation) (Atkins & de Paula, 2009).

$$DB + H_2O \leftrightarrow [DB - H_2O] \rightarrow WB \quad (1)$$

The activation enthalpy ($\Delta H^{\#}$) for the hydration rate had positive sign, meaning that the hydration process is endothermic. Consequently, by providing energy, the hydration is accelerated. Further, ultrasound caused a reduction of the activation enthalpy suggesting that this technology provided part of the energy to form the activated complex.

Further, the free energy ($\Delta G^{\#}$) had positive sign and the activation entropy had negative sign ($\Delta S^{\#}$). Therefore, the activation complex formation was considered as a non-spontaneous reaction and a more organized molecule. Ultrasound technology could hinder the organization of the activated complex. For that reason, the value $\Delta S^{\#}$ was more negative, but with a small influence on $\Delta G^{\#}$, suggesting that the chaotic and turbulence movement of the molecules caused by ultrasound avoid the molecules organization during the activated complex.

On the other hand, regarding the lag phase time of the hydration process, Equation 2 was proposed to explain the formed activation complex during the state transition. It is important to notice that the lag phase time can also be interpreted as a kinetic parameter, being related to the glass transition of the seed coat components.

$$G_{SC} + H_2O \leftrightarrow [G_{SC} - H_2O] \rightarrow R_{SC} \quad (2)$$

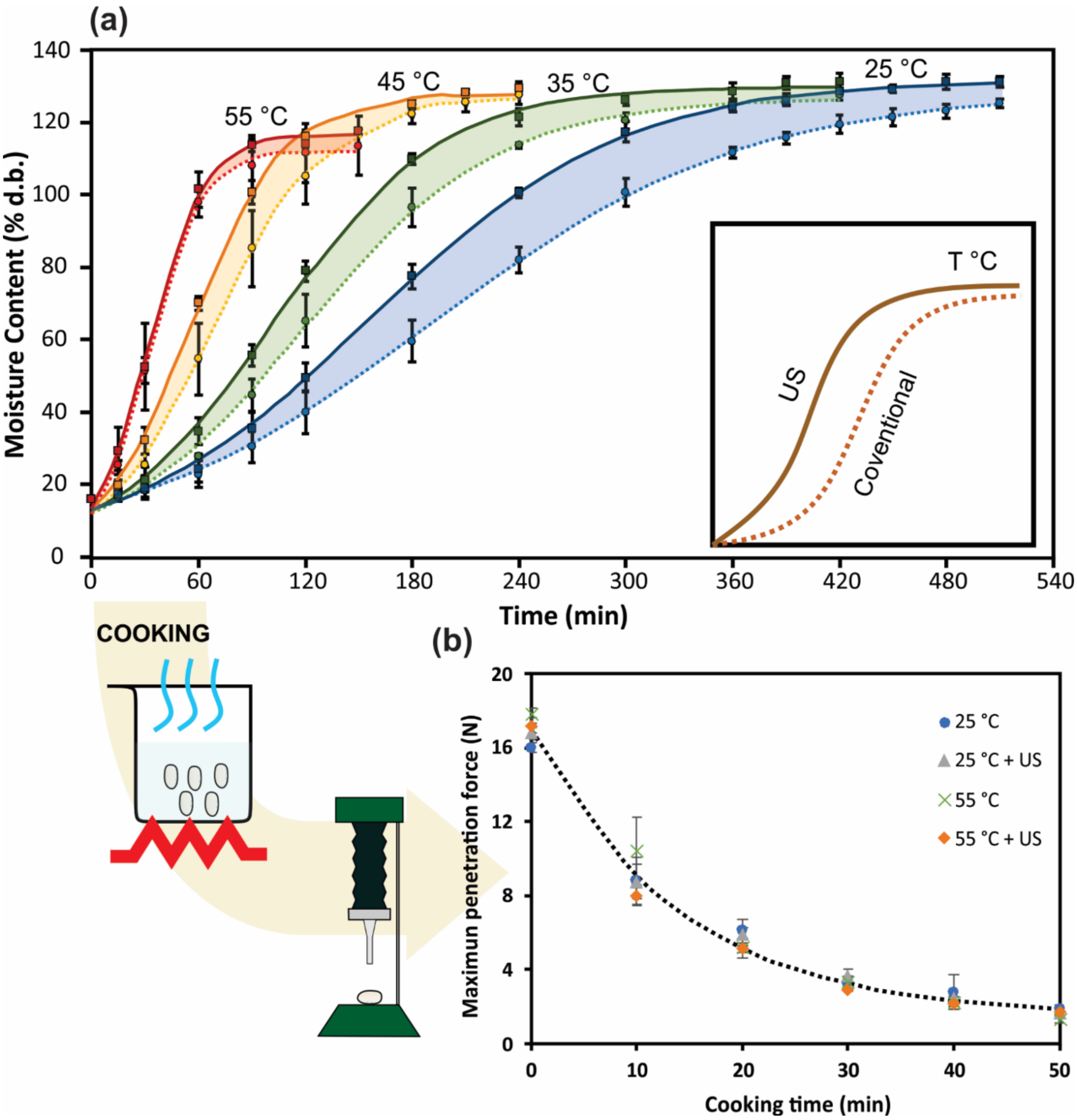

Figure 9. (a) Hydration kinetics of white kidney beans (*Phaseolus vulgaris*) with and without ultrasound (28 W/L of volumetric power and 45 kHz of frequency) at different temperatures. The dots represent the experimental data, the lines represent the Kaptso et al. model (Kaptso et al., 2008) and the vertical bars represent the standard deviation. **(b)** Softening kinetics of white kidney bean during cooking process. The dots represent the experimental data, the line represent the first order kinetics model and the vertical bars represent the standard deviation.

The seed coat components in glassy state (G_{SC}) interacts with water forming the "activated complex" ($[G_{SC} - H_2O]$) in order to change the components to the rubbery state (R_{SC}). The thermodynamic properties revealed that formation of the activated complex is an exothermic and nonspontaneous process. In addition, this complex is a more organized molecule. However, neither temperature, nor ultrasound affected the thermodynamic properties. Although temperature affects the state transition, the results showed that temperature did not affect the organization, spontaneity and involved energies to form the activated complex. In fact, the evidence that the seed coat is a multicomponent structure and that each component has its own state transition temperature must be considered. Therefore, there is not an exact state transition temperature for the seed coat (Ross et al., 2008). Consequently, the thermodynamic properties would represent an average value.

Finally, the cooking process was evaluated studying the softening kinetics of the beans during the cooking time. The fact that neither the use of elevated soaking temperatures nor the use of ultrasound during hydration of the beans affected the softening velocity during cooking process was demonstrated (Figure 7). Despite some published works reported that ultrasound enhances the cooking process due to structural change, it was perceived that those works did not control the initial moisture content of the grains during cooking (equilibrium moisture content of the hydration process). In fact, A. Ibarz, González, and Barbosa-Cánovas (2004) demonstrated that grains cook faster when their moisture content is higher (here is the importance of hydration process). Consequently, this work took this issue into account in order to isolate the treatment effects.

This part is described in the article “Enhancing the hydration process of common beans by ultrasound and high temperatures: Impact on cooking and thermodynamic properties: description of mechanisms and impact on its germination and main components”, published in the journal “Journal of Food Engineering” (Alberto Claudio Miano, Sabadoti, & Augusto, 2018) showed in Appendix G.

2.2.4. Conclusions

In conclusion, ultrasound technology is a good alternative for accelerating the hydration process of grains with sigmoidal behavior, increasing the hydration rate and reducing the lag phase time. Therefore, ultrasound improved the hydration of the seed coat, changing is permeability and accelerating the whole process.

In addition, some subsequent processes were affected as extraction (improved) and germination (sometimes improved, sometimes hindered, depending of the grain), while other processes as cooking was not affected. Further, the starch from the grains hydrated with ultrasound was not affected, but the proteins seem to be affected.

Nevertheless, despite ultrasound reduced from 25% to 35% the hydration time, this process still takes time. Therefore, other alternatives to take advantage of this times were studied. For example, the incorporation of some nutrient.

2.3. Incorporating nutrients into grains during hydration process using ultrasound

This last part had as objective to demonstrate if it is possible to incorporate some nutrient into the grains during the hydration process. Therefore, the incorporation kinetics, its description and improvement using ultrasound, and the effect of this incorporation on the cooking and germination process were studied.

The selected nutrient for this work was iron from ferrous sulfate, since it is soluble in water and since it is the most common iron fortification substance. Carioca beans from *IAC Imperador* cultivar was used, since it has sigmoidal behavior of hydration and it is a very consumed grain in Brazil. The grains were soaked in ferrous sulfate solution (0.271% w/v), evaluating the iron content of the grains during the process time. Ultrasound technology (91 W/L of volumetric power and 25 kHz of frequency) was also used to study not only the water uptake kinetics, but also the iron uptake kinetics (Figure 8a). First, it was verified that the presence of ferrous sulfate did not affect significantly the grain hydration kinetics. Consequently, the mass contribution of ferrous sulfate was insignificant compared to the mass of water.

By soaking the grains in ferrous sulfate solution for 8.5 h, the iron content is increased approximately 15 times. In addition, iron entered the grain following the same behavior of water uptake (sigmoidal), suggesting a similar entrance pathway as water. Further, by covering the hilum and measuring the iron content of the cotyledon after certain time of the process, it was demonstrated that iron can pass through the seed coat. Consequently, this proved that iron enters through hilum mainly by capillarity and through the seed coat mainly by diffusion as the water uptake. Then, it was verified that iron is fixed in the seed coat and the cotyledon (these structures were rinsed with water at least three times before being analyzed, to assure that iron was not superficially held). In fact, iron was fixed in higher quantity in the seed coat than the cotyledon, possibly due to the presence of chelating molecules. Finally, ultrasound improved the iron incorporation, as well as the water uptake, increasing the iron content of the grains approximately 27 times (Figure 8b). It should be mentioned that it was verified that the incorporated quantities were not considered toxic for human consumption according to Ellenham and Barceloux (1988).

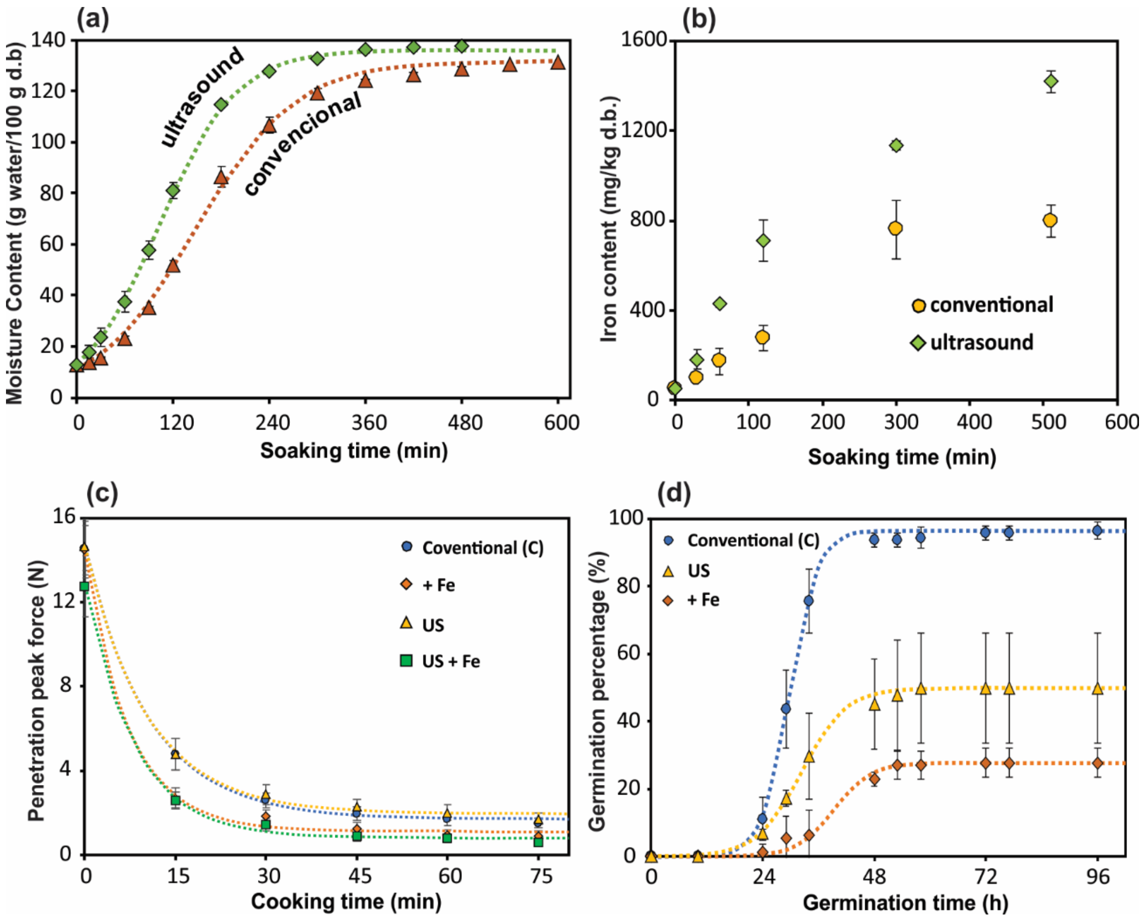

Figure 10. (a) Hydration kinetics of carioca beans (*Phaseolus vulgaris*) with and without ultrasound (91 W/L of volumetric power and 25 kHz of frequency) at 25°C. **(b)** Iron incorporation kinetics in carioca beans with and without ultrasound. **(c)** Softening kinetics during cooking of carioca beans hydrated at different conditions. **(d)** Germination kinetics of carioca beans hydrated at different conditions. For all, the dots represent the experimental data, the lines represent the modeled data and the vertical bars represent the standard deviation.

On the other hand, the cooking and germination processes of the studied beans were studied (Figure 8c). By incorporation iron, the softening kinetics of the beans during cooking were improved. Apparently, the iron weakened the structure of the beans, especially the seed coat structure. It reduces the cooking time, which is a highly desirable result. Iron probably helped to reduce the insoluble calcium salts by interchanging the calcium from phytate and pectate, thus improving the softening of the beans. In addition, the use of ultrasound during the hydration process did not affect the softening kinetics, corroborating the work of Appendix G. However, the combined effect of ultrasound and iron incorporation synergic improved the grains softening during cooking. Although, ultrasound did not affect the softening kinetics, it enhanced the iron incorporation. Therefore, maybe more insoluble salts were reduced by the additional incorporated iron, improving more the cooking process.

Furthermore, the germination process was hindered by both ultrasound and iron incorporation (Figure 8d). The iron presence had a severe toxicity for the grain embryo, reducing the germination capacity and rate (velocity). In addition, the ultrasound technology also reduced the germination capacity and rate, but less than the iron presence. Despite the results from the work of mung beans (Alberto Claudio Miano, Pereira, et al., 2016) (Appendix E) and other published works stating that ultrasound improves the germination process (Chiu & Sung, 2013; Goussous, Samarah, Alqudah, & Othman, 2010; Liu et al., 2016; López-Ribera & Vicient, 2017; A. Miano et al., 2015; Yaldagard, Mortazavi, & Tabatabaie, 2008), this work demonstrated the opposite, maybe due to the grains metabolisms difference, or the process conditions. In fact, depending on the ultrasound system and power, enzymes can be activated or inactivated (Rojas, Hellmeister, & Augusto, 2016). This may be one of the causes of reducing germination. Finally, when iron incorporation was assisted by ultrasound, the grains did not germinate due to the synergic effect of these factors on reducing the germination capacity of the seeds.

This part is described in the article “The ultrasound assisted hydration as an opportunity to incorporate nutrients into grains”, published in the journal “Food Research International” (Alberto Claudio Miano & Augusto, 2018b) showed in Appendix H.

2.3.1. Conclusions

This part demonstrated that taking advantage of the hydration process time by incorporating nutrients inside grains is conceivable. In addition, besides accelerating the hydration of grains, ultrasound technology also can be used to improve the nutrients incorporation. Furthermore, the effect of iron incorporation on further processes as germination and cooking was studied. Therefore, future studies should be conducted using different nutrients evaluating the incorporation kinetics, and how affect other processes after hydration.

3. GENERAL CONCLUSION

The hydration of grains is a complex process which involves many mechanisms, behaviors and water pathways. The hydration kinetics depends not only on extrinsic characteristic as temperature, storage conditions, growth conditions and so on, but also on intrinsic characteristics as chemical composition, structure and physical properties of the grains. In fact, giving conclusive ideas of the process such as saying that darker grains hydrate slower or saying that smaller grains hydrate faster cannot be stated as an absolute truth. In this work, some intrinsic factors that are associated with the sigmoidal behavior of hydration were found. However, finding there is still the need of looking for more intrinsic properties to be correlated.

Furthermore, ultrasound has shown excellent results accelerating the hydration process of beans with sigmoidal behavior. This technology increases the hydration rate, reduces the lag phase and sometimes increases the equilibrium moisture content, without affecting the grain starch, improving the extraction of alkaloids, sometimes improving the germination and without any effect on the following cooking process. In addition, it was proved that by combining the use of ultrasound with hot soaking water, the effect of ultrasound is hindered. However, despite this acceleration of the process, the hydration of grains was still long. For that reason, the incorporation of nutrients during the hydration process was studied and described.

Finally, the incorporation of some water-soluble components in the grains during their hydration process was proved. Therefore, the hydration time can be useful. This Thesis demonstrated that iron, as ferrous sulfate, can be incorporated in common beans during its hydration. The iron incorporation kinetics followed the same behavior as the water uptake and it was kept not only in the seed coat but also in the cotyledon. Furthermore, ultrasound improved the process, increasing the iron incorporation rate. Finally, the iron incorporation improved the cooking process of the beans, but it hindered their germination.

4. FUTURE STUDIES

From the results of the present Thesis, new hypotheses were generated for future studies. For example, despite some intrinsic properties of the beans were associated with their hydration kinetics, more intrinsic properties, as for example seed coat components, needs to be studied and correlated. In fact, the seed coat is the responsible for the water impermeability of legume grains and for their sigmoidal behavior. Therefore, its chemical components and physical properties should be studied to verify their effect on the hydration kinetics.

Further, another hypothesis to be study is the idea if the hydration process is taken place only by physical phenomena or biological phenomena and seed metabolisms also interfere. This is important, since the effect of ultrasound affect the grain metabolism; thereby, this could also affect the hydration kinetics.

In addition, as the incorporation of a nutrient using ultrasound was demonstrated as a possibility to take advantage of the hydration time, other nutrients and process conditions should be studied. For examples, another water-soluble nutrient, at different concentrations, using different grains, temperatures and ultrasound power can be studied. Further, microencapsulated lipophilic nutrient could be incorporated. After that, the bioavailability and bioaccessibility of those nutrients must studied to verify if they can be usable by the body. Finally, it can be verified if the incorporated nutrients are kept in further processes as cooking process.

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APPENDIX

APPENDIX A: THE HYDRATION OF GRAINS: A CRITICAL REVIEW FROM DESCRIPTION OF PHENOMENA TO PROCESS IMPROVEMENTS

Comprehensive REVIEWS in Food Science and Food Safety

The Hydration of Grains: A Critical Review from Description of Phenomena to Process Improvements

Alberto Claudio Miano and Pedro Esteves Duarte Augusto

Abstract: Hydration is a crucial step during grain processing. It is performed prior to many other processes, such as germination, cooking, extraction, malting and fermentation. The number of publications on this topic studying the description of the mechanisms involved and recent technologies for processing enhancement has increased recently. However, due to the complexity of the hydration process, there are still many aspects that are little understood. For that reason, this review provides not only an overview of recent developments in this field, but also a critical discussion of publications from the last 2 decades, as well as suggestions for future innovative studies. This review discusses the importance of hydration in the grain industries, the pathway for water entry into the various grains, the mass transfer and fluid flow mechanisms in the process, the behavior of the hydration kinetics, the mathematical modelling, the technologies used to accelerate the process and other necessary requirements that must be performed to complement and complete our knowledge of this process.

Keywords: cereals, grains, hydration, legumes, steeping

Introduction

Grains are plant seeds that are produced for purposes other than that of plant reproduction. For example, they are used for animal and human consumption, as well as for the extraction of different components (oils, proteins, and more). The most widely-consumed grains are the cereals (from the *Poaceae* family) and legumes (from the *Fabaceae* family), even though there are grains from others, such as the *Cucurbitaceae* (gourd seeds) and *Amaranthaceae* families (Figure 1). Cereal grains, such as corn kernels, barley kernels, sorghum kernels, and wheat kernels, are important mainly for their starch content (more than 60%) (Koehler and Wieser 2013). On the other hand, legumes are important mainly for their protein content (20% to 40%), as well as for some minerals, such as iron and zinc. Common beans, peas, lentils, chickpeas, cowpeas, lupins, and soybeans are examples of legumes (Siddiq and others 2011). Gourd seeds are rich in lipids and proteins, and are consumed in some regions of the world, mainly in African countries and India (Patel and Rauf 2017). Grains from the *Amaranthaceae* family have high a protein content and are rich in lysine (Berghofer and Schoenlechner 2002). These are widely consumed in Africa and Latin America. In fact, grains are part of a "healthy" diet due to their nutritional composition, especially legumes as a protein source (FAO 2016)

CRF3-2017-0194 Submitted 9/27/2017, Accepted 11/30/2017. *Authors are with Dept. of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz Coll. of Agriculture (ESALQ), Univ. of São Paulo (USP), Piracicaba, SP, Brazil. Direct inquiries to authors Miano and Augusto (E-mail: cmiano@usp.br, pedro.ed.augusto@usp.br).*

In general, grains are harvested dry, as dryness is a big advantage to extend shelf-life during storage. Therefore, before being consumed or processed, they need to be hydrated to increase the moisture content. For these reasons, the process of hydrating grains is a crucial step, one which has already been studied for decades (Table 1). Distinct aspects have been studied, such as characterizing different kinetics behavior, describing the process and looking for innovative technologies to accelerate it (Table 1). In addition, many mathematical models have been used to describe hydration kinetics and predict the moisture content as a function of the process time (Table 2). Despite that, there is still the necessity to conduct more studies to better understand and optimize the process. In fact, there has been a recent increase in the number of grain hydration studies, especially those using innovative technologies to improve the process and proposing new mathematical models to describe it. Therefore, a review which organizes the knowledge about this complex process is important to avoid future studies replicating work already done and to point out which areas need to be studied. In addition, the latest review related to hydration was published by Swanson and others (1985) and dealt only with legumes. Consequently, this comprehensive review presents a discussion of the results about the grain hydration process and gives information about aspects that still need to be explored.

Importance of the Hydration Process

The hydration of grains is a process that consists of soaking them in water in order to increase their moisture content. This is a crucial step in industrialized processing and provides several beneficial effects on their physicochemical and nutritional

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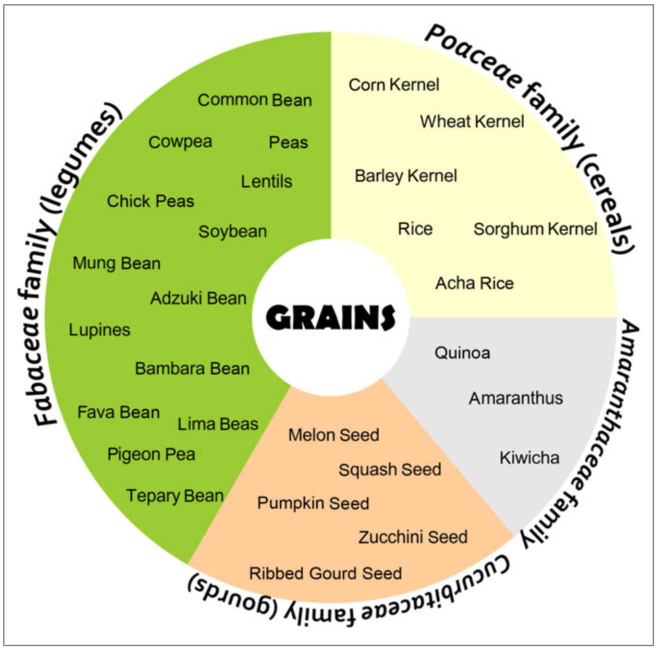

Figure 1–Example of different grains consumed in the world classified by their taxonomic family.

quality (Drumm and others 1990; Carmona-García and others 2007; Huma and others 2008; Yasmin and others 2008; Bordin and others 2010). Soaking is widely used in processing different grains for many reasons, as hydration is necessary for processes like cooking, extraction, fermentation, germination and malting.

The hydration of grains before cooking helps to soften the bean structure and so, reducing the cooking time (Silva and others 1981). This process promotes the activation of cell-wall enzymes, decreases the degree of polymerization of rhamnogalacturonan I and increases the solubility of polygalacturonan and galactan, which results in better polysaccharide solubility and shorter cooking time (Martínez-Manrique and others 2011). In addition, hydration enhances the homogeneous gelatinization of the starch and the homogeneous denaturation of proteins during cooking (Wood 2016). Therefore, a similar texture is obtained in the whole grain. Moreover, the heat transfer through the grain during cooking is enhanced by the absorbed water, thus improving the inactivation of anti-nutritional factors (Sefa-Dedeh and Stanley 1979) such as protease inhibitors, lectins, saponins, vicine, convicine, phytates, alkaloids, and indigestible oligosaccharides (Wang and others 2009)

Hydration also improves component extraction from grains, which in this case is sometimes called the steeping process. The most commonly-extracted component from grains is starch, especially from cereal grains, and is conducted by wet milling. Softening the grains by hydration improves their wet grinding and so facilitates starch purification (Singh and Eckhoff 1996). In addition, the hydration process is used to extract toxic components from beans. For instance, the Andean lupin (*Lupinus mutabilis* Sweet) has a high level of toxic alkaloids (lupanine), which needs to be extracted before being consumed. This extraction is performed in water; thus, the grains need to be hydrated (Carvajal-Larenas and others 2013). In addition, during the hydration process, some anti-nutritional compounds, such as phytic acid, tannins, phenols, α-amylase, and trypsin inhibitors, are extracted (Abd El-Hady and Habiba 2003).

Some grains are fermented before being consumed or to produce sauce products, which need elevated water activity for microbial development. For that reason, the hydration process is important. For example, soy sauce is a popular sauce in whose production the soybean is fermented after a hydration process (Luh 1995). In addition, fermentation has been demonstrated as an effective way to reduce antinutritional factors in common beans (*Phaseolus vulgaris*) (Barampama and Simard 1995; Granito and others 2002).

Germination is the natural process after the hydration of grains. This is mainly used for the development of new plants. The water uptake of the grains is important for the biochemical activation and the increase in breathing rate for embryo growth (Bewley and Black 1978). Besides the reproductive function, germination is important for sprout production and the malting process. Some pulses are germinated to produce sprouts that are consumed as food. The sprouts have the advantage of better nutritional properties than the nongerminated beans (breakdown of complex molecules and a lower level of antinutritional factors), for example in mung beans, lentils, kidney beans, and purple corn (Kylen and McCready 1975; Mbithi and others 2001; Paucar-Menacho and others 2017). Cereal grains are germinated for enzyme activation and the characteristic color, aroma, and flavor formation of malt to produce some alcoholic beverages (Barreiro and others 2003; Montanuci and others 2016), a process known as malting. This process is performed mainly with barley kernels and other grains, such as corn kernels and sorghum kernels.

Consequently, the hydration process is of significant importance in the industrialization of grains. However, this step is a batch process, which can take many hours and uses a substantial quantity of water. For that reason, its study, description and optimization are very desirable.

Description of Mechanisms and Mathematical Modeling

Seeds need to absorb water to activate their metabolism for germination. In fact, they naturally have a hydration kinetics with 3 stages (Figure 2) (Bewley and Black 1978). In stage I, the seeds absorb water mainly through physical mechanisms in order to reach an appropriate moisture content to activate their metabolism. Bewley and Black (1978) stated that this stage is independent of the seeds' metabolic activity, so this occurs equally well in live and dead seeds. However, sometimes, dead seeds can absorb more water than the living seeds as the turgor pressure in living seed counteracts hydration (Bewley and others 2013), and the effect of grain metabolism in this stage still needs investigation. In stage II, the seeds prepare for germination by breaking the reserve molecules into simple ones to be used by the embryo. In this stage, there is no significant gain of water. Germination starts in stage III. The cells start to reproduce and the tissues to grow, increasing the moisture content again. The present review is focused on stage I of hydration, since the development of the new plant is not our target, but rather the hydration of grains for food processing and consumption.

The grain hydration process is mainly a mass transfer unit operation, in which the water activity difference acts as the driving force. In other words, the water is transported from a substance with a high effective water concentration (soaking water) to a substance with a low effective water concentration (grain), a phenomenon called diffusion. In addition, the complex structure and different tissues and cells of the grains form channels of many

Table 1–Grains hydration works of grains with Downward concave shape behavior (DCS) and sigmoidal behavior performed from the last 2 decades.

Grain common name	Grain scientific name	Family	Hydration behavior	Model	Studied effect	Reference
Acha rice	*Digitaria exilis*	*Poaceae*	DCS	Peleg	Temperature	Tunde-Akintunde Toyosi (2010)
Adzuki beans	*Vigna angularis*	*Fabaceae*	Sigmoidal	Kaptso et al.	Temperature	Oliveira and others (2013)
Adzuki beans	*Vigna angularis*	*Fabaceae*	Sigmoidal	Kaptso et al.	Initial moisture content	Miano and Augusto (2015)
Adzuki beans	*Vigna angularis*	*Fabaceae*	Sigmoidal	Weibull	High hydrostatic Pressure	Ueno and others (2015)
Amaranth grain	*Amaranthus cruentus*	*Amaranthaceae*	DCS	Fick	Temperature	Resio and others (2006)
Andean lupins	*Lupinus mutabilis*	*Fabaceae*	Sigmoidal	Kaptso et al.	Temperature	Miano and others (2015b)
Bambara beans	*Vigna subterranea*	*Fabaceae*	DCS	Peleg	Temperature	Jideani and Mpotokwana (2009)
Barley kernels	*Hordeum vulgare*	*Poaceae*	DCS	Peleg, Fick, 1st order, Weibull	Temperature	Montanuci and others (2015)
Barley kernels	*Hordeum vulgare*	*Poaceae*	DCS	Peleg	Any evaluated	Miano and others (2017c)
Black Bambara groundnuts	*Voandzeia subterranea*	*Fabaceae*	Sigmoidal	Kaptso et al.	Temperature	Kaptso and others (2008)
Black kidney beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Any evaluated	Miano and others (2017c)
Caballero beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Kaptso et al.	Nothing	Miano and others (2017c)
Calabash seeds	*Lagenaria siceraria*	*Cucurbitaceae*	DCS	Peleg, Kaptso et al.	Temperature	Edith and others (2016)
Chickpeas	*Cicer arietinum* L.	*Fabaceae*	DCS	Ibarz et al.	High hydrostatic Pressure	Ibarz and others (2004)
Chickpeas	*Cicer arietinum* L.	*Fabaceae*	DCS	Peleg	Temperature	Gowen and others (2007)
Chickpeas	*Cicer arietinum* L.	*Fabaceae*	DCS	Peleg	Ultrasound	Yildirim and others (2010)
Chickpeas	*Cicer arietinum* L.	*Fabaceae*	DCS	Peleg	Ultrasound	Ranjbari and others (2013)
Chickpeas	*Cicer arietinum* L.	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
Chickpeas	*Cicer arietinum* L.	*Fabaceae*	DCS	Peleg, Khazaei	Nothing	Shafaei and Masoumi (2014)
Chickpeas (Split)	*Cicer arietinum* L.	*Fabaceae*	DCS	Peleg	Temperature	Johnny and others (2015)
Chickpeas (Split)	*Cicer arietinum*	*Fabaceae*	DCS	Peleg, Fick, Exponential	Temperature	Prasad and others (2010)
Canary beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Kaptso et al.	Any evaluated	Miano and others (2017c)
Carioca kidney beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Kaptso et al.	Any evaluated	Miano and others (2017c)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Peleg, 1st order, Kaptso et al.	Ultrasound Probe Pretreatment	Ulloa and others (2015)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Peleg, 1st order, Kaptso et al., Weibull	Ultrasound Probe Pretreatment	Ulloa and others (2015)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Peleg	Temperature	Piergiovanni (2011)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg, Kumar	Temperature	Pramiu and others (2017)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg, Kumar	High hydrostatic Pressure	Pramiu and others (2017)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Peleg, 1st order, Kaptso et al., Weibull	Ultrasound Probe Pretreatment	López López and others (2017)
Common beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Peleg	High hydrostatic Pressure	Belmiro (2016)
Corn kernels	*Zea mays*	*Poaceae*	DCS	Becker	Temperature	Verma and Prasad (1999)
Corn kernels	*Zea mays*	*Poaceae*	DCS	Peleg, 1st order, Page, Ibarz et al., Two-terms	Ultrasound	Miano and others (2017a)
Corn kernels	*Zea mays*	*Poaceae*	DCS	Nicolin-Jorge	Temperature	Nicolin and others (2017)
Cowpeas	*Vigna unguiculata*	*Fabaceae*	DCS	Fick	Nothing	Sopade and Obekpa (1990)
Cowpeas	*Vigna unguiculata*	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
Cucumber seeds	*Cucumis sativus*	*Cucurbitaceae*	DCS	Peleg, Kaptso et al.	Temperature	Edith and others (2016)
Dark red kidney beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Kaptso et al.	Any evaluated	Miano and others (2017c)
Egyptian kidney beans	*Lablab purpureus*	*Fabaceae*	Sigmoidal	Kaptso et al.	Any evaluated	Miano and others (2017c)
Fava beans	*Vicia faba*	*Fabaceae*	DCS	1st order	Temperature	Haladjian and others (2003)
Fava beans	*Vicia faba*	*Fabaceae*	DCS	1st order	pH	Haladjian and others (2003)
Fava beans	*Vicia faba*	*Fabaceae*	Sigmoidal	Kaptso et al.	Nothing	Miano and others (2017c)
Garoua cowpeas	*Vigna unguiculata*	*Fabaceae*	Sigmoidal	Kaptso et al.	Temperature	Kaptso and others (2008)
Lentils	*Lens culinaris*	*Fabaceae*	DCS	Peleg, Kaptso et al. et al., Weibull, 1st order	Temperature	Oroian (2017)
Lentils	*Lens culinaris*	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)

(*Continued*)

Table 1–Continued.

Grain common name	Grain scientific name	Family	Hydration behavior	Model	Studied effect	Reference
Lentils (Green)	*Lens culinaris*	*Fabaceae*	Sigmoidal	Kaptso et al.	Nothing	Miano and others (2017c)
Lima beans	*Phaseolus lunatus*	*Fabaceae*	Sigmoidal	Not modeled	Growing season	Piergiovanni and others (2012)
Mung beans	*Vigna radiata*	*Fabaceae*	DCS	Peleg, Fick	Temperature	Sharanagat and others (2016)
Mung beans	*Vigna radiata*	*Fabaceae*	Sigmoidal	Kaptso et al.	Ultrasound	Miano and others (2016b)
Navy beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Ultrasound	Ghafoor and others (2014)
Navy beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Temperature	Ramaswamy and others (2005)
Navy beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Radiation (pretreated)	Ramaswamy and others (2005).
Navy beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	High hydrostatic Pressure	Ramaswamy and others (2005)
Ñuña beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
Panamito beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
Peanuts	*Arachis hypogaea*	*Fabaceae*	DCS	Fick	Nothing	Sopade and Obekpa (1990)
Pink kidney beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
Pinto beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Peleg	Pressure gradients	Zanella-Díaz and others (2014)
Pinto beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Not modeled	Nothing	Kinyanjui and others (2015)
Quinoa var. Blanca de Juli	*Chenopodium quinoa Willd*	*Amaranthaceae*	DCS	Peleg, Fick	Temperature	Ramos and others (2016)
Quinoa var. Kancolla	*Chenopodium quinoa Willd*	*Amaranthaceae*	DCS	Peleg, Fick	Temperature	Ramos and others (2016)
Quinoa var. Pasankalla	*Chenopodium quinoa Willd*	*Amaranthaceae*	DCS	Peleg, Fick	Temperature	Ramos and others (2016)
Quinoa var. Salcedo Inia	*Chenopodium quinoa Willd*	*Amaranthaceae*	DCS	Peleg, Fick	Temperature	Ramos and others (2016)
Red Kidney Beans	*Phaseolus vulgaris* L.	*Fabaceae*	DCS	Peleg	Temperature	Abu-Ghannam (1998)
Rice kernels	*Oryza sativa*	*Poaceae*	DCS	Fick	Temperature	Bello and others (2004)
Rice kernels	*Oryza sativa*	*Poaceae*	DCS	Nicolin-Jorge	Temperature	Nicolin and others (2017)
Rice kernels (parboiled)	*Oryza sativa*	*Poaceae*	DCS	Nicolin-Jorge	Temperature	Balbitoni and others (2018)
Rose coco beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Not modeled	Nothing	Kinyanjui and others (2015)
Sesame seeds	*Sesamum indicum* L.	*Pedaliaceae*	DCS	Peleg, Weibull, 1^{st} order, Khazaei	Temperature	Khazaei and Mohammadi (2009)
Sorghum kernels	*Sorghum* spp.	*Poaceae*	DCS	Peleg	Temperature	Kashiri and others (2010)
Sorghum kernels	*Sorghum* spp.	*Poaceae*	DCS	Peleg	Ultrasound	Patero and Augusto (2015)
Sorghum kernels	*Sorghum* spp.	*Poaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
Soybeans	*Glycine max*	*Fabaceae*	DCS	Fick	Nothing	Sopade and Obekpa (1990)
Soybeans	*Glycine max*	*Fabaceae*	DCS	Hsu	Temperature	Coutinho and others (2010)
Soybeans	*Glycine max*	*Fabaceae*	DCS	Hsu	Temperature	Nicolin and others (2012)
Soybeans	*Glycine max*	*Fabaceae*	DCS	Peleg	Temperature	Fracasso and others (2015)
Soybeans	*Glycine max*	*Fabaceae*	DCS	Peleg, Nicolin Jorge	Temperature	Borges and others (2017)
Squash seeds	*Cucurbita maxima*	*Cucurbitaceae*	DCS	Peleg, Kaptso et al.	Temperature	Edith and others (2016)
Squash seeds	*Cucurbita moschata*	*Cucurbitaceae*	DCS	Peleg, Kaptso et al.	Temperature	Edith and others (2016)
Tepary beans	*Phaseolus acutifolius A. Gray*	*Fabaceae*	DCS	Peleg	Pressure gradients	Zanella-Díaz and others (2014)
West cowpeas	*Vigna unguiculata*	*Fabaceae*	Sigmoidal	Kaptso et al.	Temperature	Kaptso and others (2008)
Wheat kernels	*Triticum* spp.	*Poaceae*	DCS	Peleg	Temperature	Maskan (2001)
Wheat kernels	*Triticum* spp.	*Poaceae*	DCS	Fick	Nothing	Igathinathane and Chattopadhyay (1997)
Wheat kernels	*Triticum* spp.	*Poaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
White Bambara groundnuts	*Voandzeia subterranea*	*Fabaceae*	Sigmoidal	Kaptso et al.	Temperature	Kaptso and others (2008)
White kidney beans	*Phaseolus vulgaris*	*Fabaceae*	DCS	Fick	High pressure	Naviglio and others (2013)
White kidney beans	*Phaseolus vulgaris*	*Fabaceae*	Sigmoidal	Kaptso et al.	Nothing	Miano and others (2017c)
White lupins	*Lupinus albus*	*Fabaceae*	DCS	Peleg	Nothing	Miano and others (2017c)
White lupins (roasted)	*Lupinus albus*	*Fabaceae*	Sigmoidal	Peleg	Temperature	Solomon (2009)
White-seed melons	*Cucumeropsis mannii*	*Cucurbitaceae*	DCS	Peleg, Kaptso et al.	Temperature	Edith and others (2016)
Yellow peas	*Pisum sativum*	*Fabaceae*	DCS	Peleg, Fick, Kaptso et al.	Temperature	Mercier and others (2015)

Table 2–Mathematical models used to describe the grains hydration process.

Model	Equation	Reference
	Downward concave shape behavior	
Fick's Second law	$\frac{\partial C_A}{\partial t} = D_{AB} \cdot \nabla^2 C_A$	Fick (1855)
Solutions for Fick's Second law	Rectangular coordinates: $\frac{M_t - M_\infty}{M_0 - M_\infty} = \sum_{i=1}^{\infty} \frac{2Bi}{\beta_i^2 + Bi^2 + Bi} \frac{\cos(\beta_i n)}{\cos(\beta_i)} \exp(-\beta_i^2 Fo)$ Cylindrical coordinates: $\frac{M_t - M_\infty}{M_0 - M_\infty} = \sum_{i=1}^{\infty} \frac{2Bi}{\beta_i^2 + Bi^2} \frac{J_0(\beta_i n)}{J_0(\beta_i)} \exp(-\beta_i^2 Fo)$ Spherical coordinates: $\frac{M_t - M_\infty}{M_0 - M_\infty} = \sum_{i=1}^{\infty} \frac{2Bi}{\beta_i^2 + Bi^2 + Bi} \frac{\sin(\beta_i n)}{\sin(\beta_i)} \exp(-\beta_i^2 Fo)$	Crank (1979)
First order	$\frac{M_t - M_\infty}{M_0 - M_\infty} = exp(-k_L \cdot t)$	Bera, Sahu, Mukherjee, Bargale and Sharma (1990)
Peleg	$M_t = M_o + \frac{t}{k_1 + k_2 \cdot t}$	Peleg (1988)
Modified Peleg	$M_t = \frac{t}{k_{1.1} + k_{2.1} \cdot t} + \frac{t}{k_{1.2} + k_{2.2} \cdot t}$	Paquet-Durand and others (2015)
Page	$\frac{M_t - M_\infty}{M_0 - M_\infty} = exp(-k_p \cdot t^n)$	Page (1949)
Ibarz et al.	$M_t = (\frac{k_{I1}}{k_{I2}}) - (\frac{k_{I1}}{k_{I2}} - M_0) \cdot \exp(-k_{I2} \cdot t)$	Ibarz, González and Barbosa-Cánovas (2004)
Two-steps hydration	$\frac{M_t - M_\infty}{M_0 - M_\infty} = p \cdot (1 - \exp(-k_{M1} \cdot t)) + (1 - p) \cdot (1 - \exp(-k_{M2} \cdot t)$	Miano, Ibarz and Augusto (2017a)
Weibull	$\frac{M_t}{M_\infty} = 1 - exp[-(\frac{t}{\beta})^\alpha]$	Hahn and Samuel (1967), Machado and others (1998)
Nicolin-Jorge	$\frac{d\rho_A}{dt} = \frac{A}{V} \cdot k_s \cdot (\rho_{eq} - \rho_A)$	Nicolin and others (2015)
Becker	$\frac{M_t - M_\infty}{M_0 - M_\infty} = \frac{2}{\sqrt{\pi}} \cdot \frac{A}{V} \cdot \sqrt{D \cdot t}$	Becker (1959)
Khazaei	$M_t = M_0 + M_{rel} \cdot (1 - \exp(-\frac{t}{t_{rel}})) + k_{rel} \cdot t$	Khazaei and Mohammadi (2009)
Hsu	$\frac{\partial M}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r}(r^2 \cdot D \cdot \frac{\partial M}{\partial r})$	Hsu (1983)
	Sigmoidal shape behavior	
Kaptso et al.	$M_t = \frac{M_\infty}{1 + exp[-k_k.(t - \tau)]}$	Kaptso, Njintang, Komnek, Hounhouigan, Scher and Mbofung (2008)
Ibarz and Augusto	$M_t = \frac{M_\infty}{1 + \frac{M_\infty - M_0}{M_0} exp[-k_{IA}.M_\infty.t]}$ $t_{(lag\ phase)} = \frac{1}{k_{IA} \cdot M_\infty} ln(\frac{M_\infty + M_0}{M_0})$	(Ibarz and Augusto 2015)

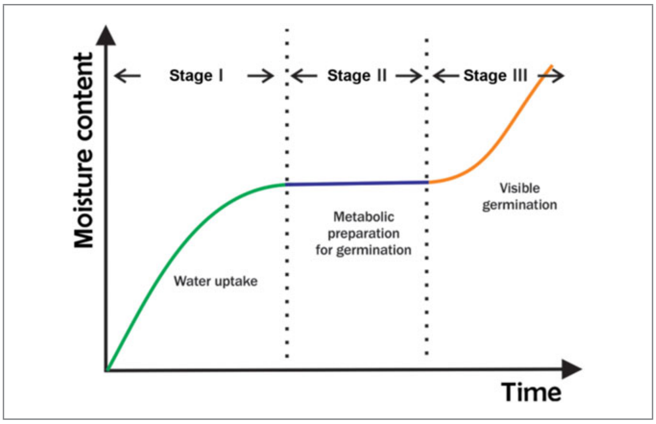

Figure 2–Seed water imbibition during the hydration – germination process. Adapted from Bewley and Black (1978). Stage I can have 2 different patterns, as described in Figure 6 and Section "Water uptake pathways and hydration behavior."

sizes, structure, composition, zones with varied permeability through which the water can flow. Therefore, the water does not only enter the grains by diffusion, but also by capillary flow. Thus, the hydration process is not as simple as it seems and involves not only mass transfer mechanisms, but also those of fluid flow.

Grain structure

Figure 3 and 4 show the structure of the most-widely consumed grains: cereals and legumes, respectively. The structures of cereal and legume grains are very different as a result of which they have different hydration behavior.

In fact, cereal grains, such as corn kernels, sorghum kernels, wheat kernels, and rice kernels are caryopses (that is, a small one-seeded dry indehiscent fruit, without pulp), whose pericarp is fused with the seed coat forming part of the bran (Bewley and Black 1978). The bran is the most external layer of cereal grains and is made up of many layers of different tissues (Figure 3). This structure is permeable to water (Syarief and others 1987) and so lets the water enter the grain by diffusion. The largest volume of cereal grains is occupied by the endosperm, in which the reserve components are stored. There are 2 kinds of endosperm: floury endosperm, which is more disorganized and easy to disintegrate during milling, and glassy or vitreous endosperm, which is more compact, organized and difficult to disintegrate (Kikuchi and others 1982). In addition, cereal grains have the germ, which is the main structure of germination. Some cereal grains, like corn kernels, have an external porous structure called tip cap, which contributes to the entry of water by capillarity (Ramos and others 2004; Miano and others 2017a).

Regarding structure of legume grains, the seed coat is the most external structure. In contrast to cereal grains, the seed coat

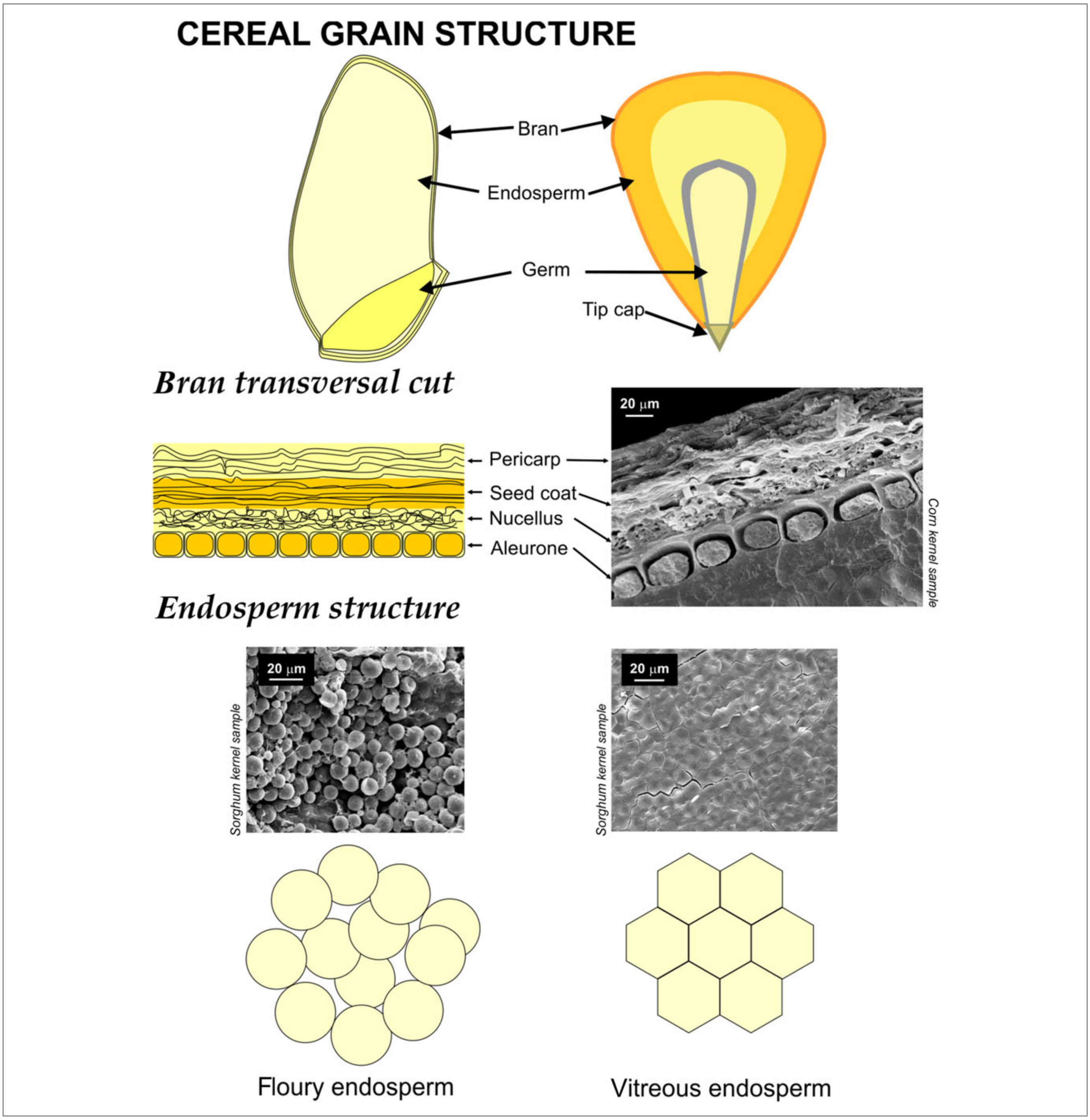

Figure 3–General structure of cereal grains. The figure is an adaptation of several schematizations and micrographs from Bewley and Black (1978), Evers and Millar (2002), Miano and others (2017a).

structure is more complex in legume grains. Firstly, this structure can be completely or partially impermeable to water, depending on its composition and structure. Figure 4 shows the general structure of the seed coat. There is an external layer of wax that makes the seed impermeable (Graven and others 1997). Then, there are 3 layers of tissues. The first layer is macrosclereid cells that form the palisade tissue. This tissue is very compact and has many hydrophobic components (Castillo and Guenni 2001). Consequently, it is a barrier to the entry of water. The second layer is formed of osteosclereid cells (bones-like cells), which have large intercellular spaces where water can flow by capillarity (Miano and others 2015b). The third layer of the legume grain seed coat is the sclerified parenchyma, made up of flat dead cells, which can be easily hydrated (Miano and others 2015b).

Other important structures of the legume grains are the raphe, hilum and micropyle. As the seed coat can be completely impermeable to water, many works have attributed these structures as the main entry path for water. For instance, Sefa-Dedeh and Stanley (1979), stated that the hilum is the main water entrance in cowpeas; Korban and others (1981), stated that the hilum, raphe and micropyle were the main water entrances in carioca and black beans; Varriano-Marston and Jackson (1981) and Miano and

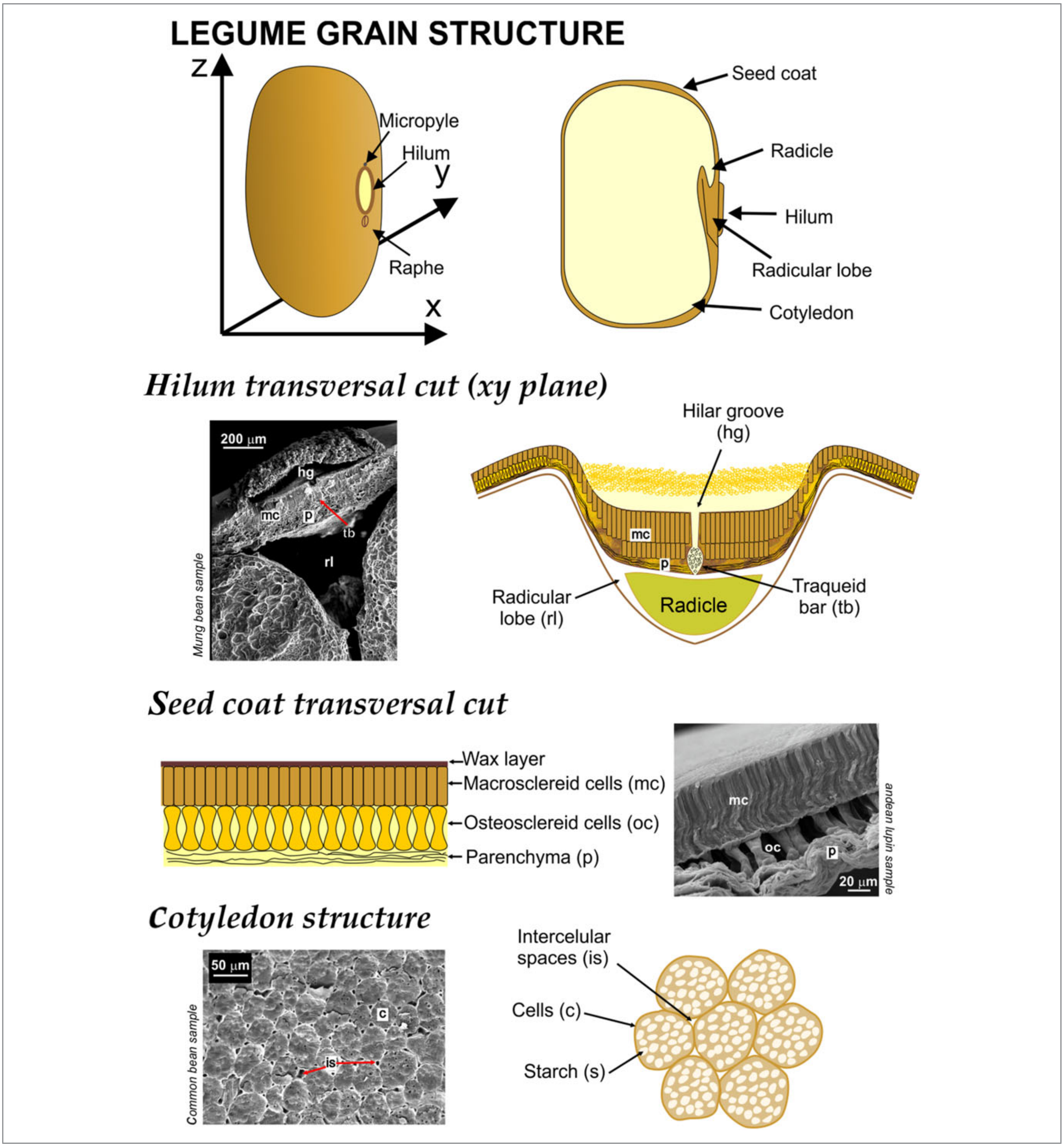

Figure 4–General structure of (a) cereal grains and (b) legume grains. The figure is an adaptation of several schematizations and micrographs from (Hyde 1954), Bewley and Black (1978), Lush and Evans (1980), Lersten (1982), Berrios and others (1998), Perissé and Planchuelo (2004), (Miano and others 2015b), Miano and others (2016b).

others (2015b) claimed that only the hilum is the main water entrance in black beans and Andean lupins. Most of the works attributed the main water entry to the hilum in legume grains. In fact, the hilum is considered as a valve, which controls the entry and exit of water (Hyde 1954). In a transversal cut of the hilum (Figure 4), it is observed that there is an aperture called the hilar groove, which is directly connected to a porous tissue called the traqueid bar (Lersten 1982). The traqueid bar facilitates gas exchange (Lersten 1982) and also water vapor exchange. Furthermore, the hilum area is larger than the micropyle and raphe. Therefore, the hilum is the best candidate for water entrance in grains from the *Fabaceae* family.

The cotyledon of legume grains is formed of the storage parenchyma tissue, whose structure is more homogeneous. It is made up of regular cells with small intercellular spaces (Figure 4), where water can cross. The main storage molecules by legume

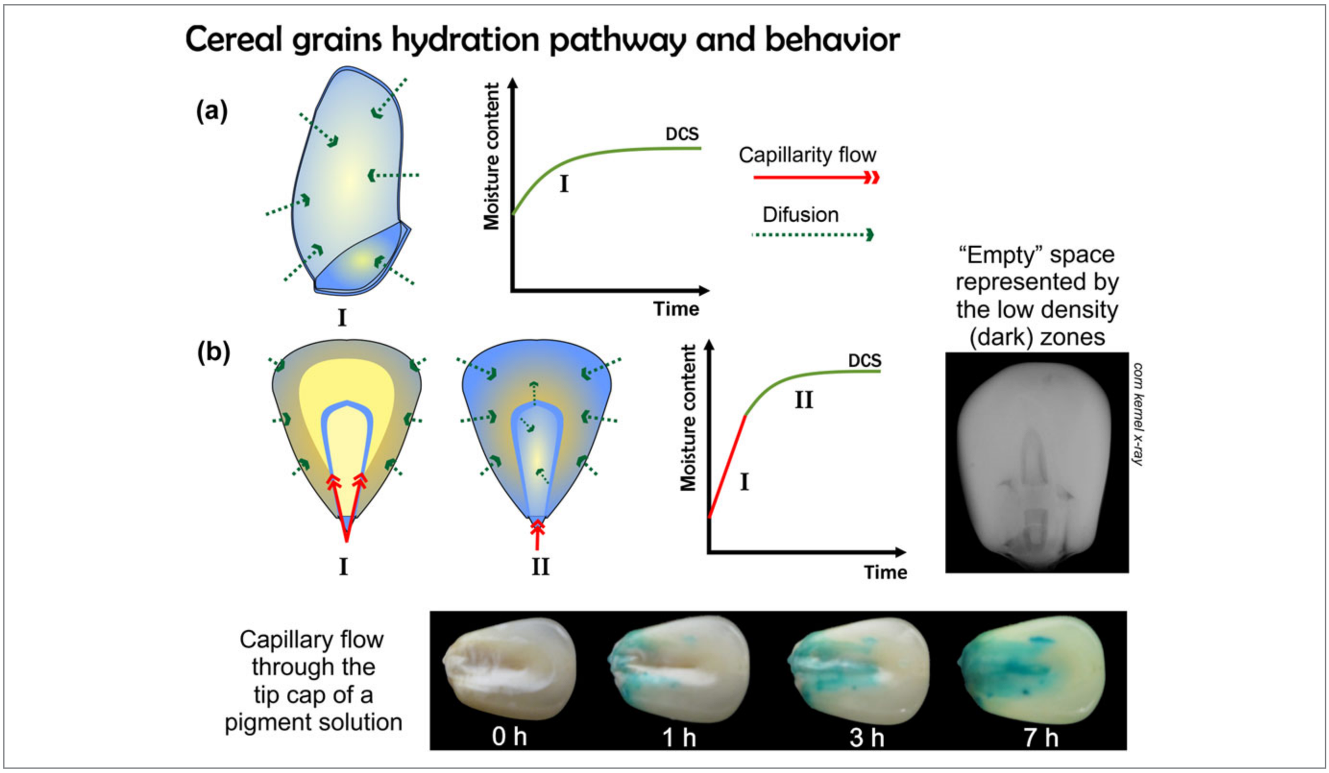

Figure 5–Water uptake pathway and hydration kinetics behavior of cereal grains. The arrows represent the direction and way water may enter: by diffusion or by capillary flow. (a) Downward concave shape behavior with 1 step – wheat kernel as an example. (b) Downward concave shape behavior with 2 steps – corn kernel as an example.

grains are starch, proteins, and lipids (Siddiq and others 2011), and depending on their relative quantities, water could enter more easily.

Water uptake pathways and hydration behavior

As stated, the hydration of grains is not a simple diffusional process. It means that water is not merely transported into the grain homogeneously. The water follows specific pathways to hydrate the grains, causing different hydration kinetics behavior, depending on the grain structure and composition.

One issue to be studied first is to determine where is the first entry of water into the grain. The seed coat in legume grains and the bran in cereal grains are the first structures that water were in contact with. Therefore, depending on the permeability of the seed coat, water can easily cross it or not. Therefore, water can flow through other grain structures. In fact, to determine the structure the water enters through, some structures can be waterproofed by using, for example, nail polish or contact glue (Ramos and others 2004; Miano and Augusto 2015; Miano and others 2017a) and then verify if the grain hydrates or if the hydration rate is reduced. By studying the internal structure of the grain (looking for "empty" spaces between the tissues) and the composition, the water uptake pathway and the hydration kinetics behavior can be determined. Another interesting way to follow the water entry pathway is by using Magnetic Resonance Microscopy (Mikac and others 2015). However, this technique is expensive and results in low-resolution images.

Cereal grain hydration kinetics. In cereal grains, as the bran is permeable to water, the water crosses all the surface area of the grains (Syarief and others 1987; Fast Seefeldt and others 2007). Therefore, the water is transferred by diffusion into the grain, making the water activity difference as the driving force. As starch is the main reserve component and the structure of the endosperm is usually compact, the total hydration process can take excessively long. In this case, the hydration kinetics behavior has a downward concave shape (DCS) (Figure 5). This behavior is characterized by a reduction in the hydration rate from a maximum value to zero when the grain absorbs the maximum water that it can hold (equilibrium moisture content). In other works, the driving force of mass transfer is reduced until the equilibrium is reached.

Some cereals can present "empty" spaces inside the grains and porous structures causing the water to be transferred by capillarity. For instance, corn kernels have a porous structure called tip cap and a space between the germ and the endosperm (Miano and others 2017a). Figure 5 shows that by immersing corn kernels in a pigment solution, the pigment follows a preferred pathway: the pigment enters by the tip cap and fills the space between the germ and the endosperm. In other words, the pigment solution enters by capillarity through the porous structures of corn. On the other hand, the endosperm is hydrated from the beginning of the process, suggesting that the water, besides entering by the tip cap, can cross the bran by diffusion without the pigment molecules (this probably because they are bigger molecules). This is one way to demonstrate that the grain hydration process can take place by diffusion and capillarity flow.

Therefore, depending on the structure, the DCS behavior can have a very high hydration rate at the initial part of the process due to the contribution of capillarity flow. Once the "empty" spaces of the grain are filled with water, the water hydrates the grain's other structures by diffusion. Consequently, the hydration kinetics have a DCS behavior with 2 steps (Figure 5): the first step with a high hydration rate due to capillarity is predominant; and the

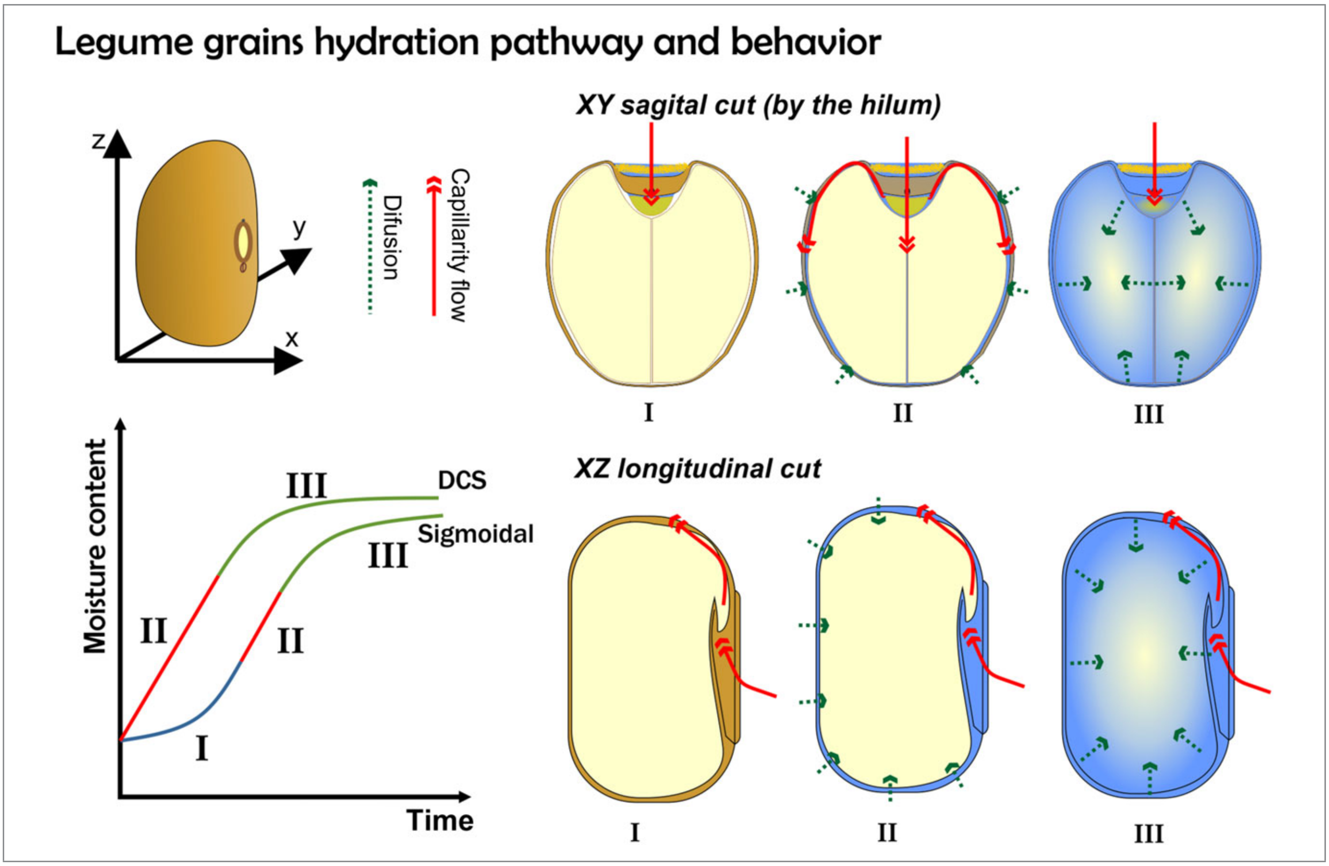

Figure 6–Water uptake pathway and hydration kinetics behavior of legume grains. The arrows represent the water pathway. The arrows represent the direction and way how water may enter: by diffusion or by capillary flow.

second, with a low hydration rated due to diffusion is predominant. This behavior is the main reason why some mathematical models, which consider hydration as only a diffusional process, do not fit or describe the hydration data well.

Legume grain hydration kinetics. Legume grains are more complex in structure than cereal grains. Therefore, besides the DCS behavior, the hydration kinetics behavior of legume grains can have a sigmoidal shape. This behavior has been little studied, especially regarding the search for suitable mathematical models to describe and predict it. As stated above, the seed coat of legume grains can be completely impermeable to water; therefore, water could only enter through the hilum. Figure 6 shows the water entrance pathway in legume grains and the possible hydration behavior divided into 3 stages. First (I): the water enters through the hilum, due to the impermeability of the seed coat, crossing the hilar groove and traqueid bar tissue to reach the radicle lob. Then, the water is distributed to the space between the internal face of the seed coat and the cotyledon. Second (II): once the seed coat is hydrated from inside, it becomes permeable to water (see section 2.3); thus, water starts to enter not only through the hilum but also crossing the seed coat by diffusion. Third (III): the cotyledon is hydrated by diffusion and capillarity depending on the structure and composition until reaching the equilibrium moisture. This water pathway causes a lag phase in the hydration kinetics, due to the impermeability of the seed coat, which limits the hydration process (Sayar and others 2001), giving this particular behavior (Miano and Augusto 2015). The legume grains with permeable seed coats have a DCS behavior of hydration kinetics, since the water pathway would start from the second (II) stage (Figure 6) skipping the lag phase. Table 1 shows different works describing legume grains with sigmoidal and DCS behavior. It is interesting to note that, despite the sigmoidal behavior, some works used mathematical models for DCS behavior, ignoring the actual sigmoidal behavior.

Effect of the Initial moisture content

The grain initial moisture content is an important condition that must be controlled to compare hydration kinetics among different samples. In fact, hydration behavior is affected by the initial moisture content, especially in legume grains (Tang and others 1994; Miano and Augusto 2015). Figure 7 shows how the initial grain moisture content influences its hydration behavior. Regarding grains with DCS behavior, the overall behavior is not changed, but the hydration rate is changed since the driving force of mass transfer is reduced (Figure 7a). However, in grains with sigmoidal behavior of the hydration kinetics, this turns into DCS as the initial moisture content increases. The explanation for this phenomenon is that the permeability to water of the seed coat increases with the increase in the water activity/moisture (Miano and Augusto 2015). This phenomenon can be explained with the glassy state theory (Ross and others 2013; Miano and Augusto 2015), which states that there is a condition (temperature or moisture content/water activity) when the seed coat components change from the glassy to rubbery state. Figure 8(a) is a general glass transition diagram, which shows that components change from the glassy state to the rubbery state by increasing their moisture content ($A \rightarrow B$) or their temperature ($A \rightarrow C$). When the components change from the glassy to the rubbery state, their properties (Fontana Jr and

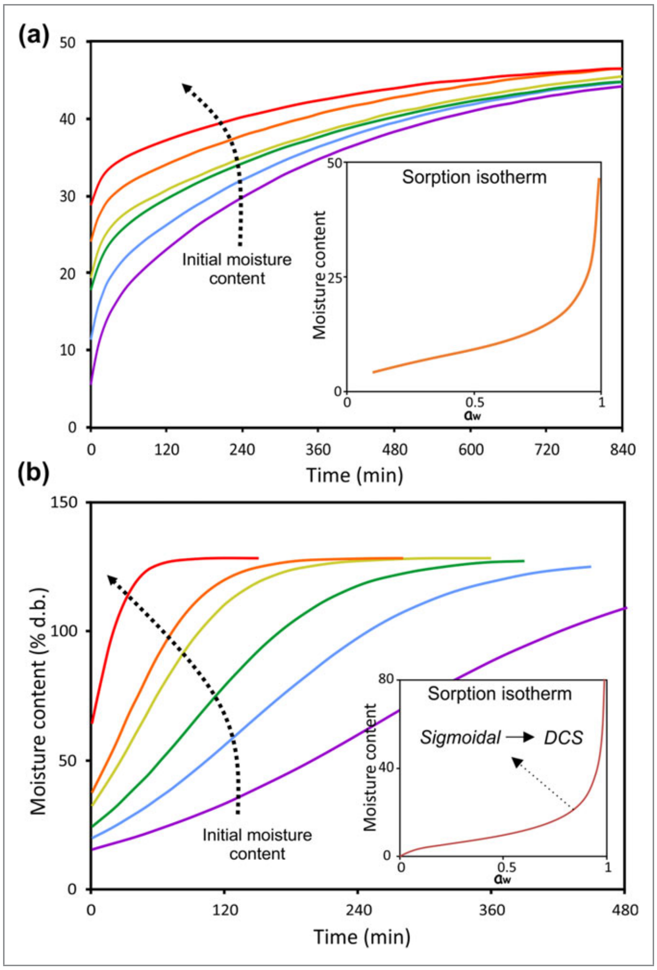

Figure 7–Effect of the initial grain moisture content with (a) Downward concave shape and (b) Sigmoidal behavior. Note the change from sigmoidal behavior to DCS at a certain initial moisture content.

others 2008), such as the permeability to water, are drastically modified. This also would be one of the causes why the hydration process is accelerated when performed at high temperatures. Ross and others (2008), state that the glassy transition temperature of the seed coat can be determined by Differential Scanning Calorimetry, measuring this property for the seed coat of different grains. However, as the seed coat is a multicomponent material, its exact determination is difficult as each component has a different glassy transition temperature.

In fact, there is a water activity/moisture content when the seed coat components change their state and become permeable to water. This moisture content could be estimated by the sorption isotherm of the grain (relation between moisture content and water activity).

The sorption isotherm (Figure 8b) has 3 characteristic zones in which the properties of water associated with each zone differ significantly (Reid and Fennema 2008). The moisture content where the sorption isotherm passes from zone II to zone III is attributed to the glassy state transition (Reid and Fennema 2008). In addition, this moisture content matches the initial moisture content when the hydration kinetics behavior changes from sigmoidal to DCS. Therefore, this "critical" moisture content can be estimated. It should be mentioned that this is 1 reason why the hydration kinetics behavior of the same grain is different in reported works.

Mathematical modeling

The hydration kinetics data can be fitted to a suitable mathematical model to predict the moisture content as a function of time and/or to study the process characteristics: the hydration rate, equilibrium moisture content and lag phase time (in sigmoidal behavior). Depending on the hydration kinetics behavior, there are many mathematical models available. Some of them are empirical and others are derivations from physical laws. The most widely-used models consider hydration as a purely diffusional process. However, as Sam Saguy and others (2005) stated, hydration and rehydration are governed by several mechanisms of imbibition in porous media. Furthermore, as described above, grains are not isotropic materials, being heterogeneous in both structure and composition.

Table 1 shows the different mathematical models used for many kinds of grains according to their hydration kinetics behavior. Note that there are many works in which, despite the grains having sigmoidal behavior, the authors used concave equations to fit the data (for example, see the discussion in Augusto and Miano (2017)). Next, some of the models most commonly used to describe the hydration kinetics of grains are presented.

Mathematical models for downward concave shape (DCS) hydration behavior. There are many different mathematical models

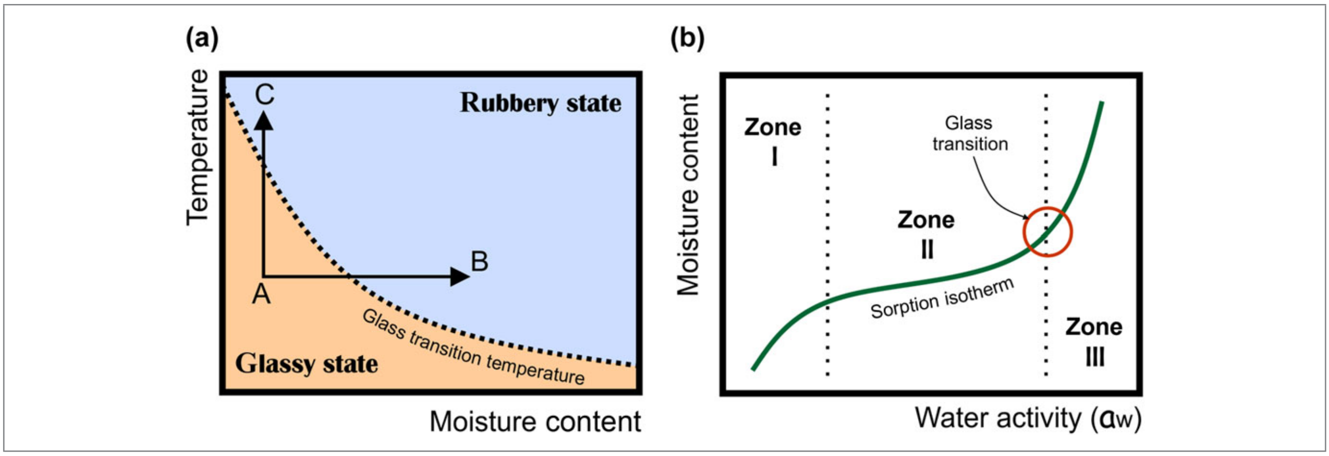

Figure 8–(a) General glass transition phase diagram: the component change from glassy stat to rubbery by increasing its moisture content or by increasing its temperature. Adapted from Bell and Labuza (2000). (b) General sorption isotherm diagram.

used to fit the data for the DCS hydration kinetics, the most used being Fick's Second law, the Peleg model and the first order kinetics equation. However, there are other mathematical models that are more specific for certain conditions that need to be better explored.

Fick's second law. Fick's Second law (Table 2) is used to describe the hydration process as an unsteady state purely diffusion process, being based on the diffusional works of Fick (Fick 1855). This equation has many solutions for regular geometries, such as infinite plates, infinite cylinders and spheres (Crank 1979), in the form of series with different terms. The main advantage of this equation is that only 1 parameter is estimated: the diffusivity (D_{AB}), which represents the diffusion of a component under isothermal and isobaric conditions (Laurindo 2016). Generally, it is described as an effective diffusivity (D_{eff}), a general diffusional coefficient considering all kinds of water transfer phenomena. The limitation of this equation is that some considerations must be assumed: (i) The geometry of the grain should be approximated to a regular geometry (plate, cylinder or sphere); (ii) The grain is an isotropic material, with homogenous composition and structure; (iii) The changes in volume and characteristic length during processing must be negligible; (iv) The water transfer only takes place by diffusion; (v) the diffusivity (D_{AB}), or effective diffusivity (D_{eff}), is a constant property of the grain (at each condition of temperature, for example), which does not change during processing and is the same in each part of the grain; (vi) Depending on the shape and process conditions, the solution of Fick's Second law becomes a sum of infinite terms, and a significant number of them have to be selected to estimate the diffusivity.

First-order model. This equation (Table 2) considers that the hydration of grains follows a first-order kinetics reaction (Abu-Ghannam and McKenna 1997a). In fact, the hydration rate is directly related to the difference between the equilibrium moisture content and the moisture reached at each process time (Bera and others 1990). This equation fits most of the grain hydration kinetics data with DCS behavior well. However, the structure of the grains must be very homogeneous to reach a suitable fitting, since this equation considers that the hydration kinetics is conducted mainly by diffusion. It means that it is assumed that all the tissues are hydrated at the same time and at the same hydration rate. In addition, this equation enabled us to estimate the equilibrium moisture content (M_∞) and the hydration rate (k_L).

Peleg model. This is the most commonly used equation for hydration and rehydration of different food products (Table 1). Although it is a semi-empirical equation (it was not derived from any physical law or diffusion theory), it fits the hydration of different products very well, using only 2 parameters (Peleg 1988) (Table 2). The advantage of this model is that both parameters have physical explanations for hydration process. The inverse of the value of k_1 is equivalent to the hydration rate and the sum of the initial moisture content plus the inverse of the value of k_2 is the equilibrium moisture content. Furthermore, during the data fitting, the initial moisture content can be set. Therefore, the predicted curve will always pass through the initial experimental moisture content. On the other hand, if the structure of the grain is too complex, this equation could not fit the experimental data well - as for corn kernels (Miano and others 2017a). Furthermore, new studies are enhancing the interpretation of the Peleg Model, such as by Kumar and others (2011), who stated that this equation can be defined according to a pseudo-second order kinetics expression.

Other models. There are many other models used to fit the hydration data of DCS grains (Table 2), some of them only used in certain conditions. For example, the model in Hsu (Hsu 1983) was developed considering the following assumptions: (i) spherical seeds, (ii) diffusion takes place only in a radial direction and (iii) the volume variation during hydration is negligible. Consequently, under these conditions, this equation can fit well for spherical grains like soybean, but this model is not suitable for other such grains as wheat kernel, rice kernels or beans.

Becker's model (Becker 1959) is an equation obtained by mathematical analysis of Fick's second law for arbitrary shapes. This equation was used for the drying and hydration of cereal grain like corn kernels and wheat kernels.

The Nicolin–Jorge model has demonstrated a suitable adjustment for grains including soybean, corn kernels and rice kernels. It was obtained from mass balance (Nicolin and others 2015). For fitting the hydration kinetics data, the grains should be considered to be regular geometry bodies, such as spheres, cylinders or parallelepiped.

Further, there are some models composed of 2 terms, which can be related to 2 mechanisms of hydration or 2 main structures. For example, the model proposed by Miano and others (2017a) to describe the hydration kinetics of corn kernels considers hydration as a 2-step process: the first where capillarity flow is predominant and the second where diffusion is the predominant water transfer phenomenon (Figure 5). In addition, the model proposed by (Paquet-Durand and others 2015) consists of a modified Peleg model with 2 terms. This equation was used to describe barley kernel hydration kinetics, where 1 term of the equation described the hydration of the bran, and the second the hydration of the endosperm.

The Page model (Page 1949) is widely used for drying processes, but it can be also used for hydration since it fits downward concave shape curves. This equation (Table 2) was empirically obtained, adding 1 more parameter (shape parameter) to the first order kinetics equation in order to adjust the data better. Even so, recently, Simpson and others (2017) stated that the Page equation parameters could have physical meaning, which can be interpreted using the fractional calculus approach: k_p would be related to the diffusion coefficient and the sample geometry and n would be related to the diffusion type and food microstructure.

In addition, Khazaei and Mohammadi (2009) proposed applying the same equation (Table 2) used to describe the creep of viscoelastic products (Rao and Steffe 1992) due to its similar curve shape. This model considers the hydration kinetics divided into 2 steps: the first with rapid water absorption, probably due to capillary mechanisms, and the second with a slow rate. It is interesting to note that this is the observed behavior for some grains, such as corn (Miano and others 2017a). However, this model considers that the grains do not reach an equilibrium moisture content and they absorb water linearly to infinite, which would be inadequate. Further, this model has 3 parameters: M_{rel} represents the quantity of water absorbed in the first part of the process, t_{rel} is the time needed to absorb 63% of the total water absorbed by the grain and k_{rel} is related to the water absorption rate in the relaxation phase. In fact, this model was used only in chickpeas and sesame seeds (Table 1).

Finally, another model used to fit the hydration kinetics is the Weibull distribution type equation (Machado and others 1998). This model has 2 parameters: ⟨ whose reciprocal is related to the process rate (it represents the time needed to reach 63% of the process) and β, known as a shape parameter (Marabi and others 2003).

Mathematical models for sigmoidal hydration behavior. Contrary to the DCS behavior, only 2 models are reported in the literature to describe the hydration of grains with sigmoidal behavior, probably due to this behavior being little studied and only found in certain legume grains. Consequently, there is still the need for more studies to find new mathematical models for describing the sigmoidal behavior of hydration, especially considering physical laws to deduce phenomenological models, but also considering empirical models with better adjustment, interpretation and/or convenience.

Kaptso et al. model. The first equation for sigmoidal behavior of hydration kinetics was proposed by Kaptso and others (2008). This semi-empirical equation has 3 interesting parameters that describe the sigmoidal behavior (Table 2). Parameter k_k is related to the hydration rate (curve slope), parameter τ is related to the lag phase time (inflexion point of the curve) and parameter M_∞ is the predicted equilibrium moisture. This equation successfully fitted the hydration kinetics data of many legume grains (Table 1).

Ibarz-Augusto model. This equation (Equation 5) was developed considering the hydration process as a second-order autocatalytic kinetics (Ibarz and Augusto 2015). In contrast to Kaptso et al. model, this equation is simpler since it has only 2 parameters: k_{IA},, which is related to a combination between the hydration rate and the lag phase time, and M_∞, which is the predicted equilibrium moisture content. However, with only 2 parameters, fitting the data could be challenging. Another drawback to this equation is that the lag phase time is not directly given by the equation. However, it can be calculated using another equation (Table 2).

Hydration Process Improvement

As the hydration process of grains is a batch and time-consuming process, its enhancement is very desirable. There are many technologies that have been used to accelerate grain hydration, such as the use of soaking water at elevated temperatures, high-power ultrasound and high hydrostatic pressure. Table 1 shows the technologies used for enhancing the hydration process of diverse kinds of grains and are described below.

Hydration at hot temperatures

This is the most frequently-used technology over the years for enhancing the hydration process. The use of soaking with hot water has effects not only on the hydration rate, but also on the equilibrium moisture content and the lag phase time. The main effects of this technology in each hydration characteristics are presented below.

All works (Table 1) have stated that the higher the temperature of the soaking water, the higher the hydration rate will be. This increase behaves exponentially; thus, the Arrhenius equation can be used to describe it and calculate the activation energy. The causes this improvement is attributed to are: (i) the increase in the reaction velocities; (ii) the reduction in water viscosity, which improves the capillary flow (Oliveira and others 2013); (iii) the dilatation of the tissues and pores (Oliveira and others 2013); (iv) the partial solution of some component, which increases the pore size.

In grains with sigmoidal behavior, the use of high temperatures reduced the lag phase time (Kaptso and others 2008; Piergiovanni 2011; Oliveira and others 2013; Miano and others 2015b). As the lag phase is related to the grain seed coat, the use of high temperatures reduces the lag phase time by: (i) accelerating the hydration of the seed coat by the mechanisms explained for the increase in the hydration rate (Oliveira and others 2013); (ii) reducing the minimum moisture content to change its component state (from glassy to rubbery), increasing its permeability to water (Figure 8a) (Ross and others 2013; Miano and Augusto 2015).

When high temperatures are used during the hydration process, the equilibrium moisture content can be increased, reduced or maintained without variation. This depends on the grain thermosensitivity and the evaluated temperature (temperatures higher than 60°C can drastically change the properties of such grain components as starch and proteins). In addition, the effect of the temperature on the parameter can be different depending on the temperature range evaluated. In other words, the parameter values can increase, reduce or stay constant depending on the temperature range studied. In most cases, the equilibrium moisture content increases (Verma and Prasad 1999; Maskan 2001; Bello and others 2004; Resio and others 2006; Khazaei and Mohammadi 2009; Kashiri and others 2010; Fracasso and others 2015; Miano and others 2015b; Montanuci and others 2015; Edith and others 2016; Ramos and others 2016) due to some mechanisms: (i) the pores and spaces inside the grains expand, enabling more water to be held; (ii) the solubility of the components increases, opening the pores. On the other hand, and in few cases, the use of high temperatures reduced the equilibrium moisture content (Abu-Ghannam and McKenna 1997b; Gowen and others 2007; Prasad and others 2010; Oliveira and others 2013; Johnny and others 2015). This can possibly be due to some mechanisms: (i) a high quantity of water-soluble components, which are lixiviated (Abu-Ghannam and McKenna 1997b); (ii) damage to cell membranes and walls (Oliveira and others 2013); (iii) the rapid saturation of the external layer of the grains with water, decreasing the driving force of mass transfer (Oliveira and others 2013). Finally, some grains are more stable at high temperatures and their equilibrium moisture content is not significantly changed using elevated water soaking temperatures (Kaptso and others 2008; Coutinho and others 2010). In fact, these results could be only considered as true in the temperature range studied. Perhaps in this range the equilibrium moisture content of the grain is not affected. Therefore, to give conclusive statements, wider ranges of temperature need to be studied.

Nevertheless, although the use of elevated temperatures enhances the hydration rate, it has the following disadvantages: (i) it can cause nutritional changes; (ii) component changes like starch gelatinization and protein denaturation; (iii) additional costs of heating the whole mass of grain and water, as well as the thermal isolation. Accordingly, other technologies for improving the hydration process of grains were also studied. Only isothermal hydration kinetics were evaluated, thereby, the hydration kinetics under non isothermal conditions need to be studied, for instance, using higher temperatures at the beginning of the process and lower ones at the end to avoid damage to thermosensitive grains.

Ultrasound technology

The ultrasound technology was recently used to improve the grain hydration process with successful results (Table 1). Ultrasound is acoustic waves with frequencies higher than 20 kHz, which can cause physicochemical changes in food, depending on the power used (Mason and others 1996). This technology was applied directly to the hydration (ultrasound assisted hydration process) using ultrasonic water baths (Yildirim and others 2010; Ranjbari and others 2013; Ghafoor and others 2014; Patero and

Augusto 2015; Miano and others 2016b; Miano and others 2017a) or as a pre-treatment using an ultrasonic probe (Ulloa and others 2015; López López and others 2017).

Some mechanisms of mass transfer enhancement have been attributed to ultrasound technology (Miano and others 2017b). Those reduced the internal resistance to mass transfer, once the external resistance (from the bulk water to the external grain layer) is negligible. Ultrasound can enhance the water flow through the grains by direct or indirect effects (Miano and others 2016a). The direct effects are related to the acoustic wave passing through the grains. This provokes alternative compression and expansion of the medium (tissues, air, water) causing pressure differences (Floros and Liang 1994; Miano and others 2016a) and an increase in the capillary flow thus enhancing the hydration. This is also called the "sponge effect" and "inertial flow". The main indirect effect of ultrasound is the structural changes due to acoustic cavitation, which consists of the implosion of micro water bubbles inside the tissues causing cell and tissue disruption and the formation of micro cavities (Mason and Peters 2004). These cavities can enhance water transfer in the grains. However, acoustic cavitation has more effect in foods with high water activity (Miano and others 2016a). Therefore, this mechanism is intensified as the grains are hydrated (water activity increase), which could explain why ultrasound has more effect in the last part of the hydration process.

In fact, ultrasound technology accelerates the hydration process by increasing the hydration rate and reducing the lag phase time due to its direct and indirect effects. In addition, the higher the ultrasonic power, the better the improvement in hydration is (Yildirim and others 2010; López López and others 2017). Regarding the equilibrium moisture content, some studies have reported that this characteristic is increased (Ghafoor and others 2014; Patero and Augusto 2015; López López and others 2017; Miano and others 2017a), and others that it is not changed (Ulloa and others 2015; Miano and others 2016b). The increase in the equilibrium moisture content is attributed to the pores opening and the micro cavities and channels forming by ultrasound, which allow the grain to hold more water.

Besides increasing the hydration rate by direct and indirect effects, ultrasound also reduces the lag phase time in grains with sigmoidal behavior. Among the direct effects, ultrasound pumps water from the hilum to the empty space between the cotyledon and the seed coat, causing faster hydration of the latter. The permeability of the seed coat to water is increased thus reducing the lag phase time (Miano and others 2016b).

Moreover, it is interesting to mention that the use of ultrasound in grain processing did not alter its starch (one of the main components) (Miano and others 2016b; Miano and others 2017a), increased its germination speed (Miano and others 2015a; Miano and others 2016b) and reduced cooking time (Yildirim and others 2013; Ulloa and others 2015). Therefore, the use of ultrasound technology has different advantages, being a promising technology for improving grain hydration.

High hydrostatic pressure (HHP)

High hydrostatic pressure is an emerging technology used for enhancing many processes. The effect of this technology on grain hydration has been studied in few works (Table 1). Consequently, the possible enhancement of the hydration process by high hydrostatic pressures is still uncertain. There are 6 works that have used this technology to date (Sangronis and others 2002; Ibarz and others 2004; Ramaswamy and others 2005; Ueno and others 2015; Belmiro 2016), all applied high pressures as a pre-treatment for chickpeas, adzuki beans, navy beans, carioca beans and black kidney beans. All these works used pressures from 33 MPa to 700 MPa and reported that this technology improved the hydration process by increasing the hydration rate. Furthermore, Ibarz and others (2004) stated that by using HHP above 550 MPa, the equilibrium moisture content of the grains was reduced due to the compactness of the structure. These works attributed this improvement to structural changes.

However, due to the experimental design, this improvement may not be only associated to HHP, as the HHP pretreatment caused the initial moisture content of the grains to increase in those studies. Therefore, the hydration process was affected by this initial moisture difference (See section 2.3). As this effect was not evaluated, it is difficult to identify the real contribution of HHP. The initial moisture content effect must be isolated to study better the HHP and further studies are called for to give conclusive results.

Other technologies

Irradiation is another technology studied in the hydration kinetics. To date, there has only been 1 work using this technology (Ramaswamy and others 2005). This work irradiated navy beans at 2 and 5 kGy using a ^{60}Co γ-ray source, and concluded that irradiation improved the hydration process when it is used for more than 60 min. In addition, irradiation caused the equilibrium moisture to increase, perhaps due to the breaking of starch molecules. However, no further information or description was given about using irradiation on the hydration of grains.

Furthermore, the work by Naviglio and others (2013) stated that the hydration process is enhanced by using cyclical pressure changes. It should be made clear that this technology is different from the High Hydrostatic Pressure, since cyclical pressure changes (not necessarily high) are applied throughout the process. The improvement in hydration with this technology makes sense since the water is better transferred inside the grain by improving the capillary flow (similarly to the ultrasound technology, which causes alternating changes in pressure). Therefore, this cyclical application causes pressure differences inside the grains, and so improved the capillary flow. Similarly to this work, Zanella-Díaz and others (2014) applied gradient pressures during the hydration process with low and high pressures. They stated that with this application, the gases trapped inside the grains are driven out, causing water to enter by bulk flow.

In addition, microwave output power has been used in the hydration of cowpeas. The hydration kinetics accelerated as the microwave output power increased (Demirhan and Belma 2015). The mechanisms were not explained. However, as in the drying process, microwaves might help to improve the hydration process by transferring energy to the water molecules, facilitating their movement.

As can be seen, more research is needed to assess the role of microwaves, irradiation, and cyclical application of pressure in grain hydration.

Final Remarks: Research and Industrial Needs and Future Trends

Description of grain hydration

The hydration of grains is a complex process, which depends on the structure and composition of the grains and the technology used. It has yet to be fully described. The role of each grain structure and component in the hydration is still unknown (for example, the seed coat impermeability for the sigmoidal behavior of hydration kinetics), as is the correlation between the grain components and the structure and hydration characteristics (hydration rate, equilibrium moisture content and lag phase time). These studies are very important since there are many mistaken ideas about hydration kinetics that need to be clarified and revised. For instance, Miano and others (2017c) demonstrated that the following statements are not always fulfilled: grains with dark colored seed coats hydrate slower than light colored ones; grains with sigmoidal behavior of the hydration kinetics hydrate slower than the ones with DCS behavior; bigger grains have a slower hydration kinetics compared to smaller ones. Therefore, the hydration of grains is a complex process where such physical characteristics as color, size, hardness and porosity, and chemical composition including the starch, protein and fat contents, and phenolics. cannot be individually attributed to a specific characteristic of the hydration process. All those intrinsic characteristics interact during hydration, giving the particular behavior to the grain. Consequently, evaluating the causes of the characteristics of the hydration behavior (lag phase, hydration rate and the equilibrium moisture content) is still a challenge.

Another study that needs to be carried out to complement the description of hydration is into whether the grain metabolism has an influence on the hydration kinetics. During the hydration of grains, their enzymes are activated and their respiration rate is increased (Bewley and others 2013). Perhaps these can enhance or hinder the hydration rate. As living cells are capable of controlling osmotic pressure, they could also control hydration. Therefore, the hydration of "dead" grains should be studied to verify whether the metabolism makes any contribution to the hydration kinetics.

Modelling hydration kinetics

Due to the complexity of the process, the hydration of grains must not be considered merely as a diffusional process, since capillary flow has a significant contribution for water transport. Almost all the mathematical models used to explain this process consider diffusion as the main phenomenon. Therefore, it is necessary to conduct more studies to describe the mechanisms involved and find new equations that consider diffusion, capillary flow, volumetric expansion, exit of trapped gases, glassy transition and structure relaxation.

Furthermore, Sam Saguy and others (2005) recommended including the porous media theory to improve the modelling of hydration. The drawback to use this approach is to determine some grain physical properties, such as the porosity, hydraulic conductivity, permeability and fluidity, as well as considering the whole grain being isotropic. For example, the porosity of the grain is not homogeneous due to it having different tissues with varying porosities (size, tortuosity). However, by using techniques like scanning electronic microscopy (SEM), X-ray, X-ray microtomography and others, the porosity and its tortuosity can be studied. The other physical properties could be determined using soil science techniques. However, these require long hydration processes, which can cause microbial spoilage, swelling and physical destruction of the food (Troygot and others 2011). In fact, there are some works on food rehydration using this approach, including Weerts and others (2003) with tealeaves and van der Sman and others (2014) with freeze dried carrots, in both cases, products had a relatively homogeneous structure. However, there are no works on the hydration kinetics of grains. Therefore, the possibility of using the porous media and capillarity approach with grains is still open.

Another approach that can be considered in grain hydration modelling studies is the use of the hydric potential theory. This approach is used for studying the water transfer from the soil to the plant (Bewley and others 2013). The hydric potential is considered the driving force for water transfer and considers many physical mechanisms besides diffusion. The total hydric potential is divided into 4 potentials: pressure potential, osmotic potential, matrix potential and gravitational potential (Laurindo 2016). The pressure potential is related to the absolute pressure that water is submitted to and the turgidity of the cells; the osmotic potential is related to the osmotic pressure due to the dissolved solutes in the cell water; the matrix potential is related to the food porosity and depends on the sorption forces and surface tension (capillarity flow); and the gravitational potential is related to the pressure generated by a fluid due to the height difference over a reference level. There is only 1 work on the water potential theory approach in salting processing of chicken breast (Schmidt and others 2008), but no work using it for grain hydration. Consequently, it would be interesting to study it for modelling.

Finally, another tool for modelling grain hydration is the use of fractional calculus, a powerful tool that was recently used to explain Page's model (Simpson and others 2017) and also to describe the viscoelastic properties of food (Guo and Campanella 2017), among other applications in food processing. According to Simpson and others (2013), the application of this tool can provide many applications for modelling mass and heat transfer. As the food mass transfer does not take place only by diffusion due to the complex structure of food, these authors proposed the use of fractional calculus to solve Fick's Law. The fractional order (α) explains how diffusional the process is, this being related to the heterogeneous structure of food. When $\alpha = 1$, the equation becomes a simple exponential equation which would represent purely diffusional processes. However, when the fractional order is different, the equation obtained shows that diffusion is not the only mechanism during mass transfer. In fact, if $0 < \alpha < 1$, the mass transfer is considered a sub-diffusional process. It means that the diffusant (the component that is diffusing) takes more time to travel than the diffusional theory predicts. On the other hand, if $1 < \alpha < 2$, the mass transfer is considered a super-diffusional process. It means that the diffusant travels faster than the diffusional theory predicts. Our interpretation is that when $\alpha \neq 1$, other mechanisms than diffusion are important. For example, the "super-diffusional process" ($1 < \alpha < 2$) may indicate the importance of capillarity.

As can be seen, there are many approaches that can be used for modelling grain hydration kinetics. Consequently, new phenomenological models should be deduced to describe the process.

Technologies exploration and scaling up

Some technologies used to enhance the hydration process need to be studied better. For instance, High Hydrostatic Pressure and Irradiation. The few works that used HHP as a pre-treatment in the hydration process have shown no conclusive results. It is difficult to conclude whether HHP as a pre-treatment enhanced the hydration process, since, during pre-treatment, the initial moisture content is increased and this also affects the hydration kinetics. Therefore, future works keeping the initial moisture content constant during HHP pre-treatment need to be performed. Furthermore, more works with the use of irradiation to improve the hydration process must be also conducted to determine which hydration characteristic is affected.

Other technologies may be explored for the grain hydration process. For example, the application of pulsed electric fields (PEF) and moderated electric fields (MEF). These technologies cause reversible permeabilization of the cell membranes, improving the mass transfer, as in drying, osmotic dehydration (Barba and others 2015) and extraction (Sensoy and Sastry 2004). In addition, the use of alternating increases and reductions of pressure may be a promising technology to improve grain hydration, as in the work by Naviglio and others (2013). However, more studies are necessary. Furthermore, the use of plasma can be a good technology to enhance the hydration process as it can affect the food components (Knorr and others 2011). For example, it can be applied only to the seed coat to change its composition, and thus its permeability to water. Finally, as ozone is used for microbial decontamination of grains (Tiwari and others 2010), its effect on the hydration kinetics should be studied.

Although hydration is accelerated by many technologies, the process still takes too long. However, this drawback can be turned into an advantage, for example, by using the hydration time to incorporate some component into the grains. There is 1 published work where grains were soaked in mineral solutions (iron and zinc) in order to fortify the grains and evaluate the effect on cooking and germination (Oghbaei and Prakash 2017). This work demonstrated that by soaking the grains in mineral solutions, they can be fortified. However, the effect of these solutions on the hydration kinetics has not yet been studied. In fact, this issue is being studied by our research group, considering both dissolved and microencapsulated nutrients.

Furthermore, it is important to state that the optimal use of these technologies must be determined. Therefore, optimization studies are required, for example: determining if it is necessary to apply a technology throughout the whole hydration process or only to a specific part; determining the optimum conditions (temperature, ultrasound power, pressure); and optimizing the equipment design. After performing these studies, scaling up would be the next step.

Regarding comparing all the novel technologies for enhancing the hydration process, we can conclude that ultrasound technology is the most widely studied and most promising. Therefore, the way to scale this up must be studied. As grain hydration is a batch process, it is performed in tanks with large quantities of grains. One way to use ultrasound would be setting the ultrasonic transducers on all the tank surfaces. Zhai and others (2017), demonstrated that by setting ultrasonic transducers in each of the tanks, the energy distribution was better than only placing them on the bottom as in most of the equipment. Therefore, this would be one way to begin the scaling up process. In addition, according to Patist and Bates (2008), there are some aspects that have to be considered and studied for the commercial scale: (i) High-power ultrasound equipment with a suitable wave distribution for large commercial operations; (ii) Energy-efficient equipment; (iii) systems that are easy to install and/or retrofit; (iv) Competitive energy cost in comparison with conventional technologies; (v) Low maintenance cost.

The future studies

Since space exploration is moving fast and manned voyages to other planets are being planned, the future of food supply and processing needs to be explored. For example, although current space food is based in pre-processed meals, when going further and longer, the system must be changed to the production of foods – which is the reality in the planning of expeditions to Mars (Perchonok and others 2012). In this case, grain hydration kinetics could be different in extra-terrestrial condition since many terrestrial considerations will be discarded or different. For example, the most important effect would be gravity. The gravitational pull on Mars is lower than on Earth. Therefore, this would change how capillarity takes places, maybe increasing the water uptake in grains. In addition, the atmospheric pressure is different, affecting the volume changes of the components. Evidently, considerably more works about the hydration of grains will need to be conducted.

Conclusion

The hydration of grains is an important process for their industrialization and direct consumption. Over time, the research works have demonstrated that there are 2 kinds of behavior in hydration kinetics: the downward concave shape and the sigmoidal shape behavior, the second being exclusively for legume grains. These behaviors depend on the grain structure and composition. This process is a mass transfer unit operation in which not only diffusion, but also capillary flow take place and many mathematical models have been used to successfully describe the hydration kinetics. However, to date, not all the water transfer mechanisms have been considered in the deduction of the model. Furthermore, many works have verified that grain hydration can be accelerated by many conventional (hot water soaking temperatures) and nonconventional technologies (ultrasound, high hydrostatic pressure, irradiation, and so on). Finally, more studies are recommended to complement the knowledge about this important process, such as to understand the relation between the grain structure/composition and the hydration behavior, looking for more technologies to improve the process, deducing better mathematical models and optimizing the process, as well as studies on scaling it up.

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Author Contributions

AC Miano and PED Augusto conceived and designed this critical revision, studying and evaluating the available information and redacting the manuscript.

Hydration kinetics determination

The hydration kinetics data of grains is easy to obtain in laboratory. The moisture in the grains during hydration can be obtained by taking samples every certain time and measuring their moisture content using the oven method. However, this is a destructive method. A higher number of samples would be needed and the sample/water relation should be controlled. In fact, it is assumed that the mass gained (weight) by the grain is only due to the absorbed water. Many works have demonstrated that the mass loss caused by leaching of water-soluble components is negligible. Consequently, the grain moisture over the processing time can be obtained by mass balance.

Therefore, a recommended way to obtain the hydration kinetics data is as follow:

- ☐ Determine the initial moisture content of the grain. This can be determined by the oven method or by using an infrared moisture analyzer.
- ☐ Weigh a certain quantity of grains (for example 10 g) and put them into a net bag. The net bag helps to remove the grains easily from soaking water.
- ☐ Hydrate the grains, and remove them to be weighed at certain times. It is recommended to record more data at the beginning of the process to avoid losing information if the grain has sigmoidal behavior.
- ☐ Determine the moisture content of the grains by mass balance each time. Ensure that the solid leaching from the grains is negligible.
- ☐ Perform the experiment until the mass increase is negligible (for example, <2% of difference).
- ☐ Plot the moisture content (on a dry basis) against time and use the most suitable model to fit the data.

The main advantage of this method is that it is not a destructive analysis. Therefore, the samples can be used for further analysis, such as for cooking or germination. In addition, this method reduces the error of each replicate as the same grains are always analyzed.

There are some special experiments that can help to describe the hydration kinetics and determine the water entry pathway of the grains.

- ☐ Determine the hydration kinetics by sealing some structures: the hilum, seed coat, micropyle, pericarp, tip cap, etc. This will enable the contribution of each structure to water entry to be established (Ramos and others 2004; Miano and others 2015b; Miano and others 2016b; Miano and others 2017a). Some examples of materials adequate for this are wax, nail polish or contact glue. Ensure that the material used is not lost in the water or by the expansion of the grain.
- ☐ Use some analyses to determine the grain structure. These include scanning electronic microscopy (SEM), X-ray, X-ray microtomography, etc. These analyses will help to determine if there is some "empty" space into the grains and the porosity of each structure (Friis and others 2007; Miano and others 2015a; Miano and others 2015b; Miano and others 2017a). These techniques require the samples to be dried. Other techniques, such as nuclear magnetic resonance (NMR), can be also used to follow the water entry pathway.
- ☐ Determine the hydration kinetics of the grain at different initial moisture contents. This allows us to know how the hydration behavior is affected by the initial moisture content, as well as the initial moisture content at which the seed coat becomes more permeable to water (Miano and Augusto 2015). In addition, the sorption isotherm of the grains would be useful for determining the approximate moisture content when the seed coat passes from the glass transition state to rubbery state.

Nomenclature

A	Area (Table 2) [m^2]
Bi	Biot number (Table 2) [dimensionless]
C_A	Component concentration (Table 2) [mol/m^3]
D	Diffusivity or diffusion coefficient (Table 2) [m^2/s]
D_{AB}	Diffusivity (Table 2) [m^2/s]
Fo	Fourier number (Table 2) [dimensionless]
J_i	Bessel functions of i order
k_1	Peleg model parameter related to the hydration rate (Table 2) [s]
$k_{1.1}, k_{1.2}, k_{2.1}, k_{2.2}$	Modified Peleg model parameters. Same meaning as Peleg model (Table 2) [different units]
k_2	Peleg model parameter related to the equilibrium moisture content (Table 2) [100kg d.b./kg]
k_{I1}, k_{I2}	Ibarz et al. Model parameters (Table 2) [different units]
k_{IA}	Hydration rate (Table 2) [1/s]
k_k	Hydration rate (Table 2) [1/s]
k_L	Hydration rate (Table 2) [1/s]
k_{M1}, k_{M2}	Two-steps hydration model parameters related to the hydration rate of each step (Table 2) [1/s]
k_p	Hydration rate of Page's model (Table 2) [1/s]
k_{rel}	Rate of absorption in the relaxation phase (Table 2) [kg/100kg d.b. · s]
k_s	Overall mass transfer coefficient (Table 2) [m^2/s]
M	Moisture content (Table 2) [kg/100kg d.b.]
M_0	Initial moisture content (Table 2) [kg/100kg d.b.]
M_{rel}	Moisture content reached in the first part of the absorption (Table 2) [kg/100kg d.b.]
M_t	Moisture content as a function of time (Table 2) [kg/100kg d.b.]
M_∞	Equilibrium moisture content (Table 2) [kg/100kg d.b.]
n	Page fitting parameter (Table 2) [dimensionless]
p	Percentage of hydration contribution of each hydration step (Table 2) [dimensionless]
t	Hydration time (Table 2) [s]
t_{rel}	Time to hydrate 63% of the total absorbed water (Table 2) [s]
V	Volume (Table 2) [m^3]
α	Shape parameter (Table 2) [dimensionless]
β	Rate parameter (Table 2) [s]
β_i	are the roots of the equation: $\beta_i \cdot tan\ \beta_i = Bi$ for rectangular coordinates; $\beta_i \cdot J_1(\beta_i)/J_0(\beta_i) = Bi$ for cylindrical coordinates; $\beta_i / tan\ \beta_i = 1 - Bi$ for spherical coordinates; (Table 2) [dimensionless]
∇	Nabla operator
ρ_A	Volumetric moisture content (Table 2) [kg/m^3]
ρ_{eq}	Volumetric equilibrium moisture content (Table 2) [kg/m^3]
τ	Lag phase time (Table 2) [min]

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APPENDIX B: CHAPTER 15 – OTHER MASS TRANSFER UNIT OPERATIONS ENHANCED BY ULTRASOUND

Chapter 15

Other Mass Transfer Unit Operations Enhanced by Ultrasound

Alberto C. Miano, Meliza L. Rojas, Pedro E.D. Augusto
University of São Paulo (USP), Piracicaba, Brazil

15.1 MECHANISMS FOR IMPROVING MASS TRANSFER IN FOOD WITH ULTRASOUND

Previous results prove that ultrasound has successfully enhanced different mass transfer unit operations such as drying, hydration, osmotic dehydration, and salting. These desirable results have been attributed to different mechanisms. These mechanisms are the results of the interaction, directly or indirectly, of the ultrasonic wave in the medium (gas, solid, or liquid). The main mechanisms cited in different studies are microchannel formation, acoustic cavitation, inertial flow, microjets, microstreaming, and so on, for drying processes (Sabarez et al., 2012; Fijalkowska et al., 2016; Rodríguez et al., 2014; Cárcel et al., 2011; Azoubel et al., 2010; Gamboa-Santos et al., 2014; Fernandes et al., 2008a), osmotic dehydration (Rodrigues and Fernandes, 2007; Fernandes et al., 2008a; Karizaki et al., 2013; Garcia-Noguera et al., 2010), extraction processes (Fernández-Ronco et al., 2013), salting and desalting processes (Kang et al., 2016; Ozuna et al., 2014; Ojha et al., 2016), and hydration processes (Yildirim et al., 2013; Ranjbari et al., 2013; Ulloa et al., 2015; Patero and Augusto, 2015; Ghafoor et al., 2014; Miano et al., 2017). Most of these works assumed that one or many of the mentioned mechanisms were the cause of the process improvement without demonstrating it.

The mass transfer in the different processes is a function of the external and/or internal resistance. Depending on the unit operation and the process conditions, one or both of those resistances can be depreciated. For example, the internal resistance is much more important in the hydration process than the external resistance. Fig. 15.1 shows the principal mechanism of mass transfer enhancement by ultrasound in relation to the mass transfer resistances.

Ultrasound: Advances in Food Processing and Preservation. http://dx.doi.org/10.1016/B978-0-12-804581-7.00015-4

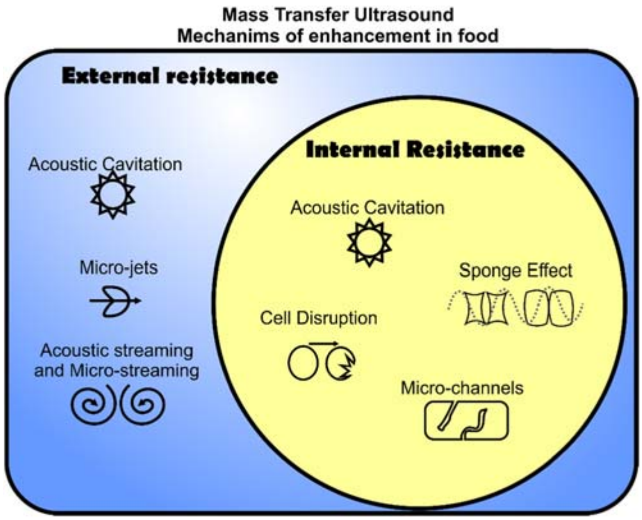


FIGURE 15.1 Principal mechanism of mass transfer enhancement by ultrasound in food.

15.1.1 Mechanisms That Reduce External Resistance

During mass transfer, there is an external resistance, which occurs in the bulk fluid (liquid or gas) around the food. Mass is convectively transferred by the bulk motion created by turbulences and by diffusion (with a small contribution) (Mittal, 2010). Conventionally, the external resistance is reduced by facilitating the bulk fluid motion using agitation of the liquid (Mavroudis et al., 1998; El-Nawawi and Shehata, 1987) or by increasing the gas velocity (Vega-Gálvez et al., 2012; Velić et al., 2004). The ultrasonic wave causes the bulk motion too, mainly by the generation of acoustic streaming and the action of acoustic cavitation.

Acoustic streaming is an oscillatory motion of a fluid caused by the ultrasonic wave traveling (Kuppa and Moholkar, 2010). The fluid is accelerated in the direction of the wave propagation, which means that the streaming flow speed increases with distance (Yasui, 2015). Another similar mechanism is microstreaming (Fig. 15.2), which takes place when the ultrasonic wave propagation reaches boundaries (container wall, food surfaces, and especially microbubbles), generating microturbulence (Yasui, 2015). These mechanisms generate agitation of the bulk flow, increasing the mass transfer by convection. In addition, microstreaming improves the mass diffusion in the bulk flow (Gould, 1974; Davidson, 1971).

Microjets are generated during acoustic cavitation in liquid medium, when microbubbles implode (Fig. 15.3). This mechanism takes place owing to the asymmetric collapse of the microbubbles, which can reach a speed on the order of 100 m/s (Wu et al., 2013). This causes the erosion of the food surfaces,

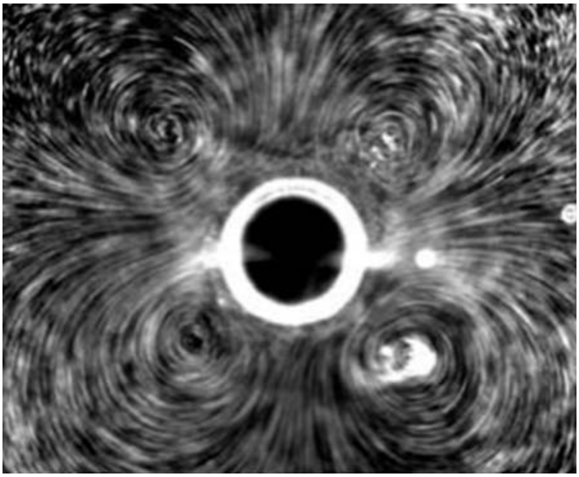


FIGURE 15.2 Streaming patterns around a 272-μm-radius bubble excited at an acoustic frequency of 3 kHz. *Reprinted with permission from Ooi, A., Tho, P., Manasseh, R., 2007. Cavitation microstreaming patterns in single and multiple bubble systems. The Journal of the Acoustical Society of America 122, 3051. Copyright (2007), Acoustic Society of America.*

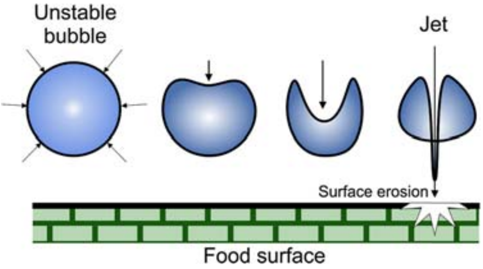


FIGURE 15.3 Schematic representation of the asymmetric collapse (implosion) of a vapor bubble caused by ultrasound. This takes place in a liquid medium near the food surface, forming a microjet. The *vertical arrow* represents the jet trajectory.

increasing the superficial area and reducing the external resistance to mass transfer. In addition, this mechanism is a possible cause for injection of liquid inside the food, for instance, brine in the salting process (Ojha et al., 2016).

15.1.2 Mechanisms That Reduce Internal Resistance

Inside the food, ultrasound enhances mass transfer according to other mechanisms. The ultrasonic wave can promote some direct and indirect effects (Miano et al., 2016). The intensity of these effects depends on the water activity, the porosity (Miano et al., 2016), and the glassy/rubbery state of the food (Muralidhara et al., 1985). These mechanisms reduce the internal resistance to mass transfer.

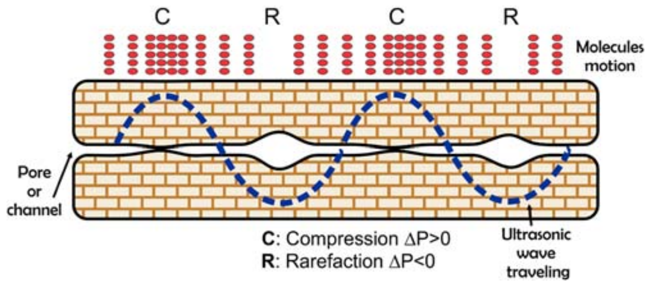


FIGURE 15.4 Schematic representation of the "sponge effect" caused by ultrasonic wave traveling.

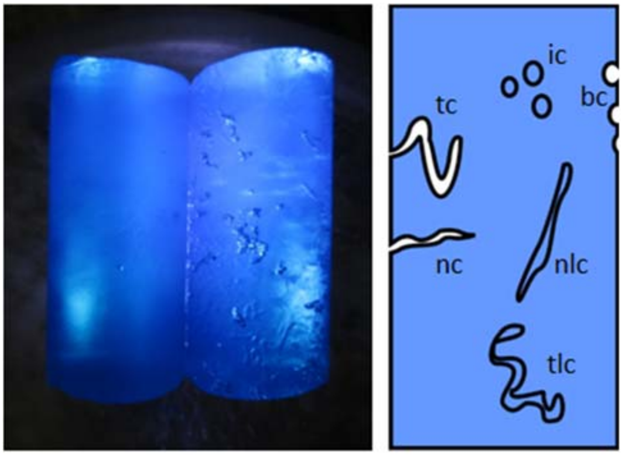
15.1.2.1 Direct Effects

These effects are mechanisms directly related to the ultrasonic wave travel. A sinusoidal pressure variation takes place in the medium due to the ultrasonic waves. In an elastic food matrix (e.g., rubbery state food), this pressure variation causes a rapid alternating compression and expansion of the tissue or structure, which is compared to a sponge being squeezed and released repeatedly, called the "sponge effect" (Floros and Liang, 1994; Mulet et al., 2003; Miano et al., 2016; Muralidhara et al., 1985) (Fig. 15.4). This mechanism facilitates the entrance of fluid from outside and the exit from inside (Mulet et al., 2003), reducing the internal resistance. Additionally, it helps to unblock the pores and spaces inside the food. On the other hand, if the structure of the food is rigid and compact (e.g., glassy state food), it could be difficult for this mechanism to take place. However, the pressure variation inside the pores could cause pumping of the fluids, which is called "inertial flow" (Patero and Augusto, 2015). In addition, these mechanisms benefit from the porosity of the food (Miano et al., 2016) because it facilitates the fluid flow caused by the direct effects.

15.1.2.2 Indirect Effects

The indirect effects are caused mainly by the action of acoustic cavitation, as it is an indirect effect of the acoustic wave propagation. The implosion of the microbubbles inside the food causes cell disruption, increasing the intracellular spaces. In addition, this mechanism can cause the cells to flatten and/or to lose their adhesion (Fernandes et al., 2008a, 2009), also increasing the intercellular spaces. These mechanisms take place apparently randomly; thus, the new spaces do not grow uniformly. During a long processing time, these spaces form microchannels, which can improve the mass transfer by the capillarity phenomena. However, as demonstrated by Miano et al. (2016), these microchannels can have different forms (Fig. 15.5), having different tortuosity, permeability, and diffusion, each improving or not the mass transfer in different ways (Warning et al., 2014).

The indirect effects are influenced by the water activity of the food. In fact, cavitation is directly related to the vapor pressure of the medium (Mason and



tlc: Tortuous channels with lack of connectivity
tc :Tortuous channels
ic: Isolated cavities
bc: Cavities formed in the boundary
nc: Non tortuous channels
nlc: Non tortuous channels with lack of connectivity

FIGURE 15.5 Photograph of agar gel cylinders untreated and treated with ultrasound (45 kHz and 28 W/L of volumetric power). The different kinds of microchannels are observed. *Based on Miano, A.C., Ibarz, A., Augusto, P.E.D., 2016. Mechanisms for improving mass transfer in food with ultrasound technology: describing the phenomena in two model cases. Ultrasonics Sonochemistry 29, 413–419; Warning, A., Verboven, P., Nicolaï, B., van Dalen, G., Datta, A.K., 2014. Computation of mass transport properties of apple and rice from X-ray microtomography images. Innovative Food Science and Emerging Technologies 24, 14–27.*

Peters, 2004). As the vapor pressure increases, the water activity increases and the acoustic cavitation takes place easily. This was demonstrated by Miano et al. (2016), who evaluated the effects of ultrasound during the hydration process of sorghum grains with two different water activities (0.6533 ± 0.0004 and 0.9851 ± 0.0029), showing that only at the higher water activity did the indirect effects take place and improve the process. In addition, the presence of solutes in the medium may hinder the indirect effects because this can reduce the water activity. Another factor that could influence the indirect effects is the hardness and compactness of the food, which can reduce the effect of the cavitation (Clemente et al., 2014; He et al., 2012) because it would be more difficult for the cell or matrix disruption to take place.

15.2 ULTRASOUND-ASSISTED DRYING OF FOODS

The process of drying food is one of the most used unit operations to increase the shelf life of food by reducing the water activity. Because hot-air convective drying is the most used method, several technologies have been studied to reduce the temperature and/or the time of the process, avoiding thermal effects on the food. For instance, ultrasound has obtained successful results in improving the drying process. However, because drying is a gas–solid operation, the efficiency of ultrasound is low. In fact, the effect of ultrasound is higher in liquid–solid operations than in gas–solid operation because of the difference in the acoustic impedances of the media (Mason et al., 2005). For that reason, it is desirable to improve the efficiency of the ultrasound during assisted drying.

Through ultrasound-assisted drying, the loss of the ultrasonic power is very likely to take place because of the reflection of the acoustic signal by air causing the transducer to heat up (Mason et al., 2005). Nevertheless, some kinds of systems were adapted to improve the efficiency of ultrasound in the drying process, such as siren and whistle systems, stepped plate systems, and vibrating cylinder systems (Mason et al., 2005). The most used system reported in food drying studies is the vibration cylinder (Gamboa-Santos et al., 2014; Rodríguez et al., 2014; Cárcel et al., 2011; Rodrigues et al., 2016; Santacatalina et al., 2016) because of its easy adaptation to traditional driers and to pilot scale (Mason et al., 2005).

In the drying process, the external and internal resistances are important; thus, several of the aforementioned mechanisms could take place during ultrasound application. Ultrasound reduces the external resistance due to the acoustic streaming in the drying air, enhancing the mass convection transfer of the moisture. In addition, it reduces internal resistance in the food by direct and indirect effects. In a reported study on raspberry, ultrasound reduced the drying time by 54% (100 W, 70°C) to almost 63% (200 W, 70°C) and to 79% if it was combined with microwave technology (Kowalski et al., 2016). Other works reported a 44% drying time reduction for strawberry (60 W, 60°C) (Gamboa-Santos et al., 2014) and 57% for apple slices (90 W, 45°C) (Sabarez et al., 2012). In addition, as the ultrasound power was increased, the intensity of these mechanisms was higher, enhancing the process even more (Fig. 15.6A). For example, Puig et al. (2012) stated that by keeping other conditions constant (temperature and air velocity), the drying process time was reduced as the ultrasonic power was increased (50% for 45 W and 74% for 90 W).

Regarding food structure, depending on the power, ultrasound can minimize food shrinkage, which avoids the increment of the internal resistance by this phenomenon. Sabarez et al. (2012) observed that sonication reduced cell deformation and structural collapse when ultrasound assisted in the drying process of apple (Fig. 15.7). Puig et al. (2012) stated that ultrasound reduces structural collapse until a certain power is obtained (45 W for eggplant), and at higher ultrasound power (90 W for eggplant), it can promote cell and tissue shrinkage. Despite that, the higher the ultrasound power is, the faster the drying process is. Therefore, this fact is very important to take into account for future rehydration processes.

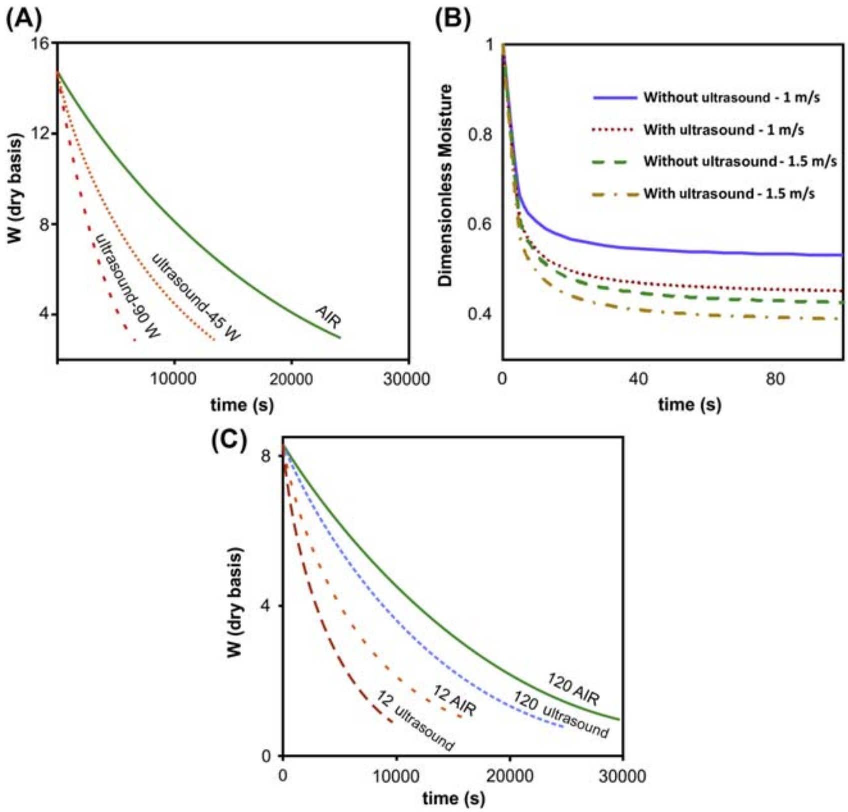


FIGURE 15.6 (A) Convective drying kinetics of eggplant cylinders (height 2 cm and diameter 2.4 cm) carried out at 40°C and 1 m/s of air velocity. Conventional (AIR) and ultrasound-assisted (AIR + Ultrasound) drying. (B) Effect of air velocity on the ultrasound mass transfer enhancement during the drying process of grape seeds. (C) Effect of the mass load on the AIR and ultrasound (75 W, 21.7 kHz) drying kinetics of carrot cubes (side 8.5 mm) at 40°C, 1 m/s of air velocity. *(A) Reprinted from Puig, A., Pérez-Munuera, I., Cárcel, J.A., Hernando, I., García-Pérez, J.V.T., 2012. Moisture loss kinetics and microstructural changes in eggplant (*Solanum melongena *L.) during conventional and ultrasonically assisted convective drying. Food and Bioproducts Processing 90, 624–622. Copyright (2012), with permission from Elsevier. (B) Data from Clemente, G., Sanjuán, N., Cárcel, J.A., Mulet, A., 2014. Influence of temperature, air velocity, and ultrasound application on drying kinetics of grape seeds. Drying Technology 32, 68–76. (C) Figure from Cárcel, J.A., Garcia-Perez, J.V., Riera, E., et al., 2011. Improvement of convective drying of carrot by applying power ultrasound—influence of mass load density. Drying Technology 29, 174–182, reprinted by permission of the publisher (Taylor & Francis Ltd., http://www.tandfonline.com).*

On the other hand, the enhancement of the drying process by ultrasound can be hindered by the drying conditions such as the temperature, mass load density, and air velocity. It is well known that as the drying air temperature increases, the drying time is reduced. Nonetheless, when air at high temperature is combined with ultrasound, the ultrasound effect is hindered (Fig. 15.8)

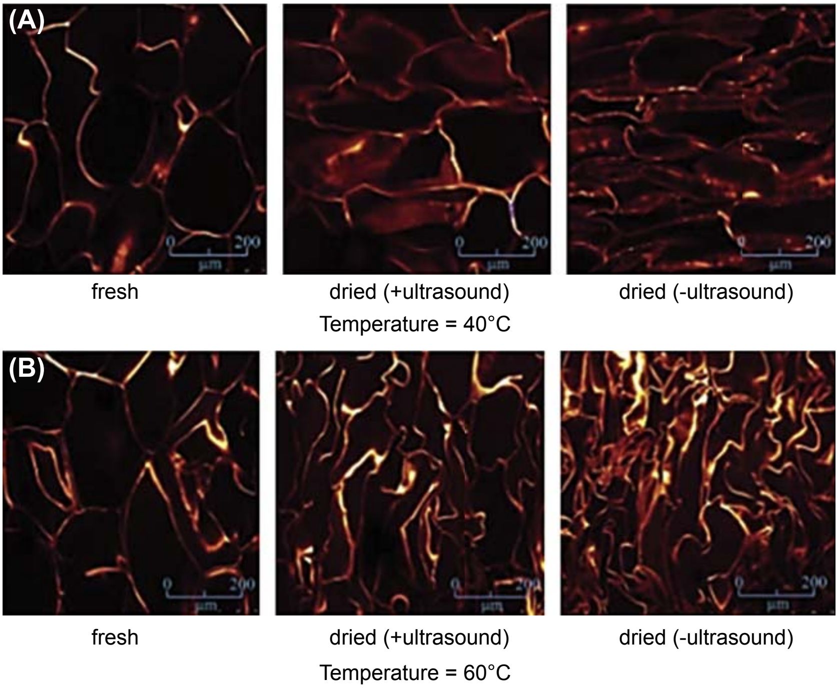


FIGURE 15.7 Confocal laser scanning microscope micrographs of apple tissues taken from the center of both fresh samples and samples dried with (+ultrasound) and without ultrasound (−ultrasound) at different temperatures (ultrasound power of 75 W): (A) 40°C, (B) 60°C. *Figure from Sabarez, H., Gallego-Juarez, J., Riera, E., 2012. Ultrasonic-assisted convective drying of apple slices. Drying Technology 30, 989−997, reprinted by permission of the publisher (Taylor & Francis Ltd., http://www.tandfonline.com).*

(Rodrigues et al., 2016; Sabarez et al., 2012; Clemente et al., 2014; Santacatalina et al., 2016). Ultrasound reduces the internal and external resistance during the drying process. However, as the air temperature is increased, the external resistance becomes negligible and the internal resistance is reduced. Therefore, the ultrasound enhancement is less evident (Sabarez et al., 2012). At high air temperatures, the ultrasound improvement totally disappears because the thermal effect reduces not only the external, but also the internal resistance.

Similarly, the increase in air velocity also hinders the ultrasound effect (Rodrigues et al., 2016; Clemente et al., 2014). As the air velocity increases, the external resistance during the drying process is reduced. Therefore, the ultrasound enhancement is less evident (Fig. 15.6B). In addition, higher air velocities increase the acoustic reflection reducing the ultrasound effect. However, in contrast to the air temperature, high values of air velocity do not affect the internal resistance. Consequently, the ultrasound would reduce only

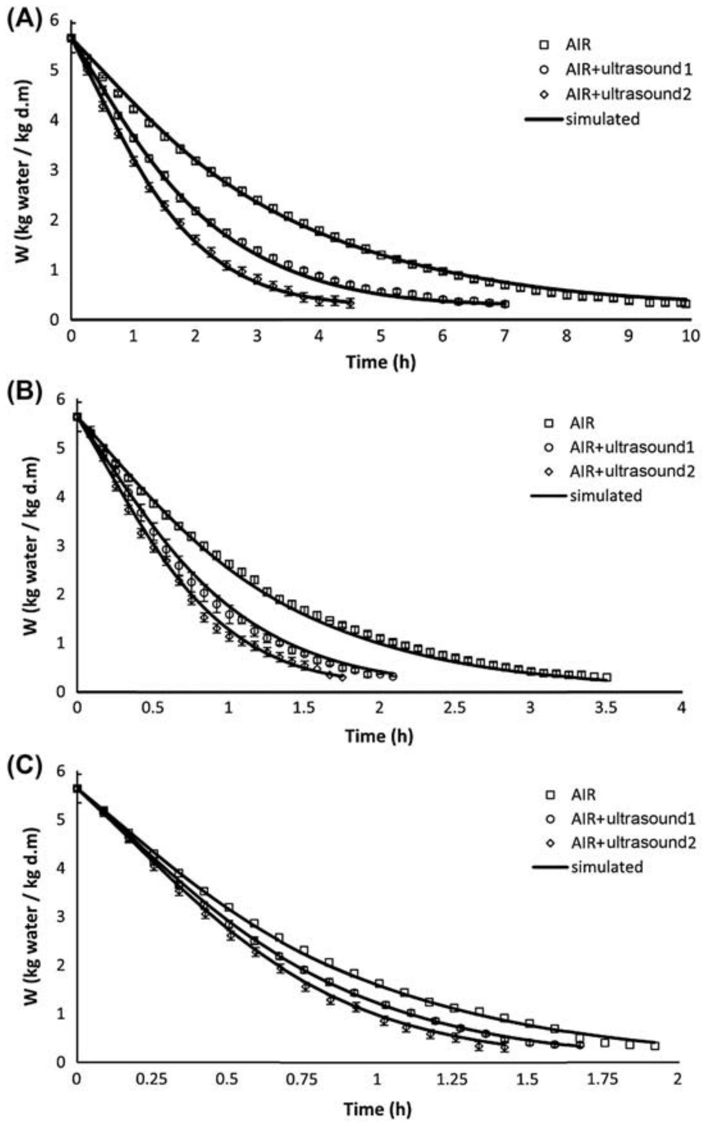


FIGURE 15.8 Experimental and simulated drying curves for apple without ultrasound (AIR) and with the application of ultrasound [AIR + ultrasound1 (18.5 kW/m^3) and AIR + ultrasound2 (30.8 kW/m^3)], at (A) 30°C, (B) 50°C, and (C) 70°C. *Reprinted from Rodriguez, Ó., Santacatalina, J.V., Simal, S., García-Pérez, J.V., Femenia, A., Rosello, C., 2014. Influence of power ultrasound application on drying kinetics of apple and its antioxidant and microstructural properties. Journal of Food Engineering 129, 21–29. Copyright (2014), with permission from Elsevier.*

the internal resistance, probably showing little improvement in the process no matter how high the air velocity is.

The mass load density increases the drying time because it hinders the homogeneous distribution of the air in the samples. However, apparently, this factor does not affect the ultrasound enhancement during the drying process (Cárcel et al., 2011) despite the fact that the acoustic energy is attenuated by the quantity of sample. Fig. 15.6C shows that when the mass load density is increased 10 times, the gap in enhancement that ultrasound caused to the drying process is not significantly altered. Probably, the reduction in the ultrasound enhancement by the mass load density is canceled out by the increase in bulk porosity in the mass load, which increases the direct effects of ultrasound.

15.3 ULTRASOUND-ASSISTED OSMOTIC DEHYDRATION OF FOODS

Osmotic dehydration is another unit operation that serves to reduce the water activity of food. However, in contrast to the drying process, the food does not reach very low water activity, making another complementary technology necessary for preservation. In fact, this unit operation is used mainly as a pretreatment before the drying process, to reduce the severity and improve the drying process. The main parameters that influence the velocity of this process are the osmotic solution concentration, the temperature, and the presence of agitation. Ultrasound has also been used to improve the process, causing an increase in both water loss and solid gain (Fig. 15.9A) (Cárcel et al., 2007b; Nowacka et al., 2014; Li et al., 2012; Corrêa et al., 2015; Athmaselvi and Arumuganathan, 2015; Barman and Badwaik, 2017; Simal et al., 1998; Luchese et al., 2015; Rosas-Mendoza et al., 2012).

Unlike the drying process, osmotic dehydration is a liquid–solid operation, thus the energy loss due to the ultrasound wave is almost null. In this case, other systems are used to treat the food with ultrasound: the ultrasonic bath (Nowacka et al., 2014; Barman and Badwaik, 2017) and the ultrasonic probe (Cárcel et al., 2007b; Luchese et al., 2015). Both systems are used to improve the osmotic dehydration by assisting the process or as a pretreatment. The ultrasonic bath is composed of one or several acoustic transducers (probes) at the bottom of the equipment. In this system, the ultrasound can be applied directly by placing the osmotic solution and the food inside the bath or indirectly by placing the osmotic solution and the food inside a flask that is immersed in the ultrasonic bath with distilled water. On the other hand, the ultrasonic probe is used to apply the ultrasound directly, whereby the osmotic solution and the food are placed in a flask as well as the probe.

Ultrasound was used to assist the process (Cárcel et al., 2007b; Li et al., 2012; Corrêa et al., 2015; Simal et al., 1998) and as a pretreatment (Rosas-Mendoza et al., 2012; Luchese et al., 2015; Barman and Badwaik, 2017;

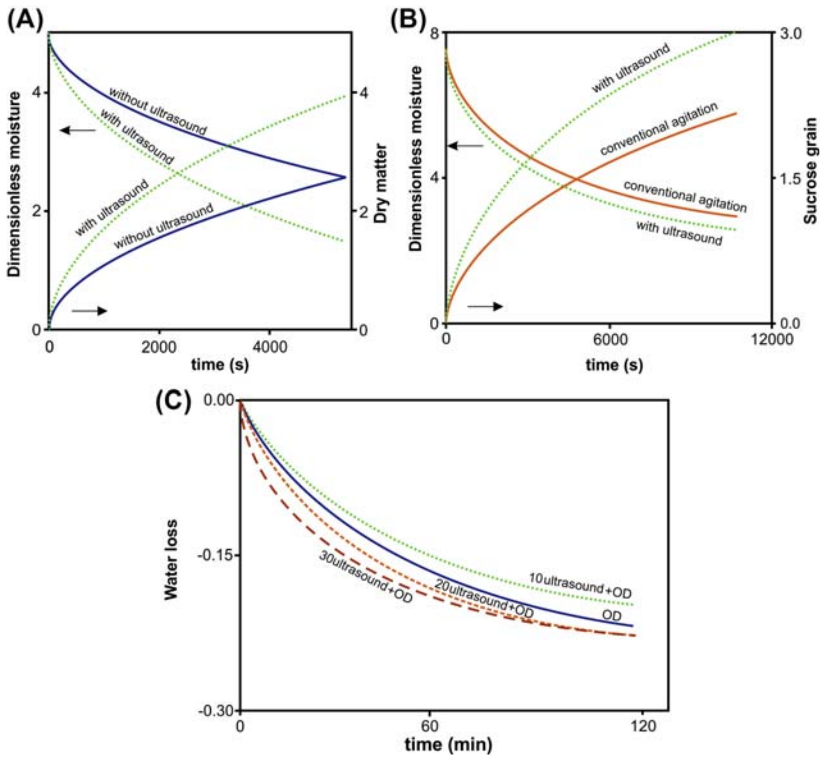


FIGURE 15.9 (A) Dimensionless moisture and increase in dry matter versus processing time of apple samples in a 30°Brix sucrose solution at 30°C with and without ultrasound treatment (11.5 W/cm^2 at 20 kHz). (B) Comparison of experiments carried out at 50°C using ultrasound and conventional agitation. (C) Water loss of kiwifruit at 25°C during osmotic dehydration (OD) without pretreatment and with 10, 20, and 30 min of ultrasound pretreatment. *(A) Reprinted from Cárcel, J.A., Benedito, J., Rosselló, C., Mulet, A., 2007b. Influence of ultrasound intensity on mass transfer in apple immersed in a sucrose solution. Journal of Food Engineering 8, 472–479. Copyright (2007), with permission from Elsevier. (B) Reprinted from Simal, S., Benedito, J., Sánchez, E.S., Rosselló, C., 1998. Use of ultrasound to increase mass transport rates during osmotic dehydration. Journal of Food Engineering 36, 323–336. Copyright (1998), with permission from Elsevier. (C) Reprinted from Nowacka, M., Tylewicz, U., Laghi, L., Dalla Rosa, M., Witrowa-Rajchert, D., 2014. Effect of ultrasound treatment on the water state in kiwifruit during osmotic dehydration. Food Chemistry 144, 8–25. Copyright (2014), with permission from Elsevier.*

Nowacka et al., 2014) during osmotic dehydration processing. When the ultrasound assists the osmotic dehydration, it causes the reduction of the internal and external resistance. The external resistance is reduced by the acoustic streaming and the microjets, enhancing the mass transfer by convection. Further, the internal resistance is reduced by the direct and indirect effects of ultrasound. For example, ultrasound technology could be more effective than conventional agitation (Fig. 15.9B) (Simal et al., 1998), because conventional agitation reduces only the external resistance (Fig. 15.10).

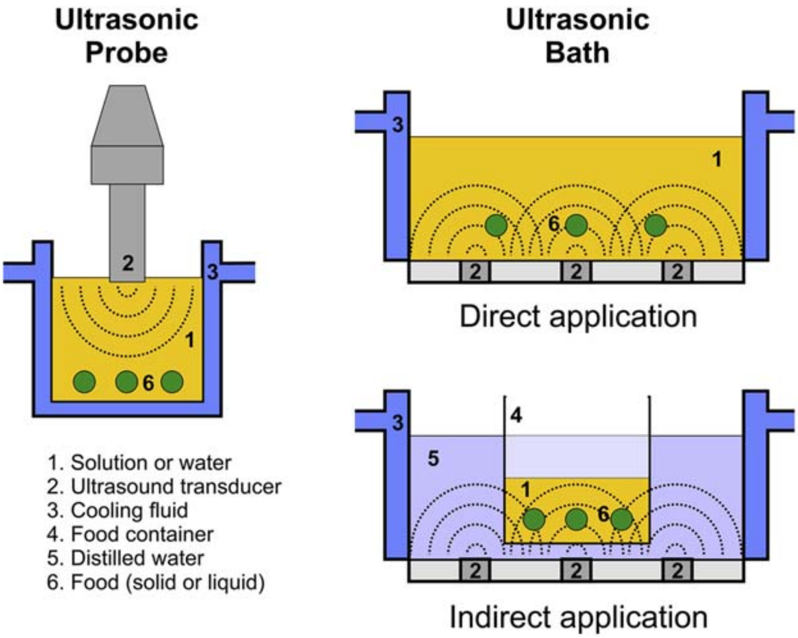


FIGURE 15.10 Different ways on how the ultrasound can be applied for improving liquid-solid operation of mass transfer.

On the other hand, when ultrasound is used as a pretreatment (the food is pretreated with ultrasound immersed in distilled water), ultrasound enhances osmotic dehydration by reducing only the internal resistance because it provokes structural changes and microchannel formation during the pretreatment. Nowacka et al. (2014) showed that the time of ultrasound pretreatment influences osmotic dehydration, a specific time of application being necessary to improve the process. This could be because the microchannels require time to be formed (Miano et al., 2016). Fig. 15.9C shows that when the ultrasound pretreatment was short in kiwifruit, the osmotic dehydration process was hindered, because at early times ultrasound causes additional water gain in the food. Nevertheless, pretreatment times longer than 10 min improve the osmotic dehydration process, probably because microchannels are formed (Nowacka et al., 2014).

Some factors that need to be considered in liquid−solid operations when ultrasound is applied are the temperature and the viscosity of the medium. As the temperature of the medium (water or osmotic solution) increases, the ultrasonic power is reduced because of the cushioning effect of the vapor present inside the cavitation bubbles (Raso et al., 1999). On the other hand, as the medium viscosity is increased, the ultrasonic power is increased. Although it is more difficult for the cavitation bubbles to be formed at higher viscosities, the collapse of the cavitation bubbles is more intense (Berlan and Mason, 1992; Raso et al., 1999).

It should be mentioned that it is difficult to compare ultrasound effects among the published works because the ultrasound power is expressed in different units and/or measured using different methods.

15.4 ULTRASOUND AS A PRETREATMENT TO DRYING OF FOODS

Ultrasound has also been applied as a pretreatment before drying processes to reduce the processing time. This application has also had successful results for different foods, especially vegetables and fruits (Azoubel et al., 2010, 2015; Fijalkowska et al., 2016; Garcia-Noguera et al., 2014; Fernandes et al., 2008a,b,c, 2009; Rodrigues et al., 2009a,b; Oliveira et al., 2011; Kek et al., 2013; Dehghannya et al., 2015; Shamaei et al., 2012; Liu et al., 2014; Jambrak et al., 2007). Most of the studies used ultrasonic baths for the pretreatment, although an ultrasonic probe could also be used.

Ultrasound pretreatment can be performed using distilled water as a medium or an osmotic solution; in both cases, the food is immersed. However, to evaluate the mechanisms involved, it can be vacuum packed before immersion to avoid the mass transfer between the food and the medium, thus isolating the ultrasound effect (Miano et al., 2016). When a food is immersed in distilled water with ultrasound as a pretreatment, there is water gain in and solid lost from the food owing to the direct effects of ultrasound (sponge effect and inertial flux). The water gain can cause the moisture content increase and the swelling of the cells. This takes place normally at early times of pretreatments (10 or 20 min, for example) (Fernandes et al., 2008a). As the time passes, the indirect effects can create microchannels due to cell flattening (needle-shaped cells) and/or cell disruption. These microstructural changes bring about the future enhancement of the drying process because water will have more pathways to exit the food. In addition, by the sponge effect, more solids are released during the pretreatment, obtaining, after the drying process, a final product with low sugar content (Kek et al., 2013; Fernandes et al., 2008b).

When food is pretreated with ultrasound-assisted osmotic dehydration, there is water loss and solid gain due to the direct effects (sponge effect and inertial flow). In addition, as the pretreatment time is increased, the indirect effects take place, forming microchannels, which are the main mechanism of enhancement for the following air-drying process. However, the solid gain can cause the pores and microchannels to clog, reducing the air-drying velocity (Azoubel et al., 2015). On the other hand, this pretreatment sometimes improves the nutritional retention. It should be mentioned that the longer the pretreatment is, the more severe is the food structural change. This can cause future drawbacks if the product has to be rehydrated because it would reduce the water-holding capacity. For that reason, an optimization of the process, looking for the best pretreatment time, is desirable to accelerate the drying process without compromising the rehydration of the food.

Furthermore, as previously discussed, when ultrasound is applied as a pretreatment, the use of high temperature or high air velocity during the drying process can hinder the ultrasound effect.

15.5 ULTRASOUND-ASSISTED HYDRATION AND REHYDRATION OF FOODS

Hydration of food is a unit operation used prior to other operations such as cooking, germination, malting, extraction, and fermentation. The main dried foods are the grains, which are found in this condition as a raw material. In addition, this process is called rehydration, when the food was previously dried. This operation is carried out by immersing the food inside a tank with water and it is mainly a discontinuous and time-consuming process (for example, some grains can take 14 h to be completely hydrated). Heating is the principal conventional technology used to enhance the process; however, high temperature can carry some difficulties such as the degradation of nutritional components. For that reason, other technologies such as ultrasound have been used to improve the process, and like most of the mass transfer unit operations, successful results were obtained (Yildirim et al., 2010; Ranjbari et al., 2013; Ghafoor et al., 2014; Patero and Augusto, 2015; Ulloa et al., 2015; Miano et al., 2017). The hydration process was improved by assisting the process with ultrasound and by using ultrasound as a pretreatment. However, the ultrasound-assisted process was more efficient.

During the hydration process, the external resistance to mass transfer is negligible, thus this process is driven by the internal resistance. Consequently, the ultrasound enhancement is due to direct and indirect effects. In addition, because the ultrasound enhancement is more evident at high water activity (Miano et al., 2016), the difference between the hydration kinetics of a process without ultrasound and one with ultrasound becomes larger over time (Fig. 15.11). In the first part of the process (low water activity), the enhancement is probably due to only direct effects, and as the food gains water (increasing its water activity), the indirect effects can take place and create microchannels. Therefore, in the final part of the process, the microchannels increase the porosity of the food, also increasing the direct effects. Moreover, some studies have shown that ultrasound reduces the hydration time almost 35% for corn kernels without affecting its starch properties (Miano, 2015). In addition, the germination of grains is enhanced by ultrasound (Miano et al., 2015; Liu et al., 2016); therefore, using this technology to hydrate seeds would be desirable. In addition, as in other unit operations, high temperatures hinder the ultrasound effect on the process (Fig. 15.11), because the temperature effect is much higher than the ultrasound effect.

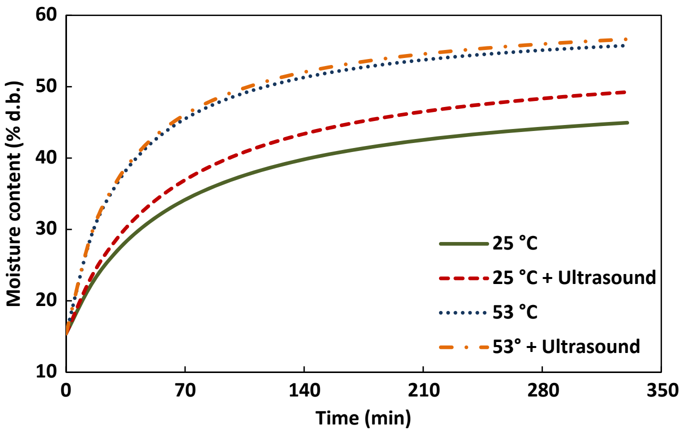


FIGURE 15.11 Hydration behavior of sorghum grains at room temperature (25°C), with heating (53°C), using the ultrasound technology (25°C + ultrasound), and combining both technologies (ultrasound + 53°C). *Reprinted from Patero, T., Augusto, P.E.D, 2015. Ultrasound (US) enhances the hydration of sorghum (*Sorghum bicolor*) grains. Ultrasonics Sonochemistry 23, 11–15. Copyright (2015), with permission from Elsevier.*

Regarding the rehydration process of a dried food, ultrasound has not been studied in detail. As of this writing, good results have been obtained during ultrasound assisted-rehydration (Zhang et al., 2016). Furthermore, it was demonstrated that when food is dehydrated using ultrasound, its further rehydration process is improved. Ultrasound enhances the food structure, avoiding the shrinkage process (the main problem during the drying process) (Fijalkowska et al., 2016). During the drying process with ultrasound (assisted or as a pretreatment), microchannels are formed, which help the rehydration; however, if the ultrasound application is too severe, the food structure can collapse, increasing the internal resistance to mass transfer of all processes. Therefore, an optimization of the whole process, selecting the suitable time of ultrasound application to reduce drying time and enhance the rehydration process, is very desirable.

15.6 OTHER UNIT OPERATIONS OF MASS TRANSFER

The salting (brining) process is an osmotic process used to reduce the water activity of food, especially in meat and fishery products. Regarding the desalting process, the main objective is to remove the excessive quantity of salt from the food (previously used to preserve the food) before its consumption or following processing. Both processes fulfill the same functions as osmotic dehydration. This means that when put into an osmotic solution (in this case a brine) the food loses water and gains solids. Ultrasound technology has been also used to accelerate the process in different kinds of food such as pork meat, cheese, cod, and peppers (Sánchez et al., 1999; Ojha et al., 2016; Ozuna et al., 2013, 2014; Gabaldón-Leyva et al., 2007; Cárcel et al., 2007a; Kang et al., 2016).

As in the osmotic dehydration process, ultrasound-assisted salting and desalting enhancement is due to the same mechanisms: acoustic streaming, microjets, microchannel formation (structure changes), sponge effect, and inertial flow. These mechanisms reduce the internal and external resistance of the process. In addition, because this process is applied more for meats, it was demonstrated that ultrasound causes an increase in the myofibril spaces and their rupture (a kind of microchannels compared to other kinds of food), which enhances the process (Kang et al., 2016). In addition, the results of Kang et al. (2016) demonstrated that the ultrasound effect was increased with NaCl concentration (from 3% to 6%), showing a possible synergic effect in beef. This was explained by the protein denaturation and the increase in intermyofibril spaces by ultrasound, which allows the faster entry of the salt solution. In addition, the microjets probably help to inject the salt solution into the food.

15.7 INDUSTRIAL PERSPECTIVES AND COMMERCIAL USE

Although ultrasound has shown excellent results in the scientific literature for enhancing mass transfer unit operations, industrial application is still limited. Therefore, more studies need to be performed, especially on optimization and scale-up. For that, three issues need to be considered: which ultrasound system generator is more suitable for each unit operation, what is the best processing time, and how much energy is necessary in comparison to the conventional process.

To use ultrasound for enhancing mass transfer in some unit operations in industry, the most suitable ultrasound system generator must be selected. As explained, depending on the type of operation (gas–solid or liquid–solid), the conditions of the ultrasound application to increase the efficiency are different. Ultrasonic baths and probes are better for liquid–solid processes (extraction, hydration, and osmotic dehydration) and the siren and whistle systems, stepped plate systems, and vibrating cylinder systems for gas–solid processes (air drying).

As explained, the water activity and porosity of the food affect how the ultrasound enhances the process by reducing the internal resistance. In addition, the viscosity, density, temperature, and other conditions of the external medium (air, distilled water, osmotic solution) also affect the ultrasound power. For example, during the hydration process of grains, the application of ultrasound would be more effective after the grains reach a certain water activity (higher than the initial). Another example is that ultrasound has better effect in food drying when a low drying temperature and low air velocity are used. For these reasons, more studies on optimization need to be performed to take the next step—scaling up the process to industry.

15.8 CONCLUSIONS

Ultrasound has shown very promising results for improving different mass transfer unit operations, such as drying, osmotic dehydration, hydration, extraction, and brining, among others. Several mechanisms are responsible for this improvement, by reducing the external and/or the internal resistance to mass transfer. Further, ultrasound can be applied directly and indirectly, assisting the process or as a pretreatment. In addition, in this chapter, the influence of some process parameters such as the medium (gas or liquid), temperature, agitation, and viscosity were discussed. Finally, some considerations were listed, recommending future studies for optimization and scale-up.

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APPENDIX C: HYDRATION KINETICS OF CEREAL AND PULSES: NEW DATA AND HYPOTHESIS EVALUATION

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Hydration kinetics of cereal and pulses: New data and hypothesis evaluation

Alberto Claudio Miano | Viviane Deroldo Sabadoti | Jéssica da Costa Pereira | Pedro Esteves Duarte Augusto

Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil

Correspondence

Pedro Esteves Duarte Augusto, Avenida Pádua Dias, 11 – Piracicaba/SP – 13418-900, Brazil.

Email: pedro.ed.augusto@usp.br

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Abstract

This work described the hydration kinetics of several grains from the legumes and cereal families, which many of them were studied for the first time. In addition, some comparisons among the different hydration kinetics were performed to corroborate some hypothesis about this process. By comparing the hydration kinetics of the studied grains and fitting their data to suitable equations, the idea that this process is very complex was reinforced. It is difficult to say how intrinsic properties of the grains (color, size, specie, family, structure or composition) affect the hydration process. Some reported hypothesis about the hydration process were argued. For instance, it was demonstrated that some hypotheses about the grains hydration are not completely true: the grains with darker color seed coat do not always hydrate slower than the lighter color ones; the grains with sigmoidal behavior can hydrate faster than the grains with downward concave shape behavior; the bigger size grains sometimes hydrate slower than the smaller ones. It was concluded that the hydration process behavior and velocity are affected by many intrinsic properties of the grains (composition and structure, in a complex interaction) acting together to give the representative hydration kinetics behavior of each curve.

Practical applications

From the academic point of view, the results of this work will help to avoid misunderstandings during the study of the grains hydration process, as many hypotheses were reanalyzed and argued. Furthermore, it provides new data, in special for sigmoidal behavior grains, which is very scarce in the literature. From the industrial point of view, this work provides data about the hydration kinetics of several grains, which is very useful for the process design.

1 | INTRODUCTION

Cereal and pulses are raw materials with huge importance for the human consumption and nutrition. Cereals, such as corn, wheat, barley, sorghum and others are important source of carbohydrates as starch. Pulses, as many beans, peas, lentils and others are good source of vegetable proteins, besides its carbohydrate contribution. After their harvest, both cereal and pulses are dried to increase their shelf life during storage. Consequently, a future hydration is necessary. In fact, the hydration process of these raw materials is very important before cooking, germination, extraction, fermentation and malting.

The hydration process of cereal and pulses has been widely studied, demonstrating that this is not a simple process as it seems. Their structure are very complex with different tissues and presence of pores, which involves different mechanisms of mass transfer (water transfer) (Miano, García, & Augusto, 2015). For that reason, each cereal or pulse has its own hydration kinetics behavior, which can be a downward concave shape (DCS) behavior or a Sigmoidal Shape behavior (Ibarz & Augusto, 2014; Miano & Augusto, 2015). The DCS behavior of hydration is characterized by a rapid hydration at the beginning of the process and a gradually decreases of the hydration rate along the time until reaching the equilibrium moisture content. Several mathematical models are used to fit the data; however, the Peleg model (Peleg, 1988) is the most used due to its simplicity and interpretation. However, the sigmoidal shape behavior is characterized by an initial slow hydration rate of the grain, which accelerates until certain point (inflexion point). Then, the hydration rate is reduced until reaching the equilibrium moisture content. Only two mathematical models are reported

TABLE 1 Grains used to study the hydration kinetics behavior

Name			Dimensions (mm)[a]			
Common	Scientific	Family	Length	Width	Thickness	Obtained from
Adzuki bean	*Vigna angularis*	*Fabaceae*	7.69 ± 0.42	4.33 ± 0.28	3.20 ± 0.22	Brazil
Barley kernels	*Hordeum vulgare*	*Poaceae*	7.98 ± 0.19	2.58 ± 0.38	3.41 ± 0.18	Brazil
Black kidney bean	*P. vulgaris*	*Fabaceae*	9.64 ± 0.69	6.60 ± 0.72	4.79 ± 0.51	Brazil
Caballero bean	*P. vulgaris*	*Fabaceae*	11.89 ± 0.84	9.48 ± 0.66	8.10 ± 0.70	Peru
Canary bean	*P. vulgaris*	Fabaceae	11.29 ± 0.69	7.85 ± 0.41	6.39 ± 0.46	Brazil
Carioca kidney bean	*P. vulgaris*	*Fabaceae*	10.66 ± 0.55	7.28 ± 0.40	5.17 ± 0.40	Brazil
Chick pea	*Cicer arietinum*	*Fabaceae*	8.80 ± 0.30	7.58 ± 0.34	7.58 ± 0.34	Canada
Corn kernels	*Zea mays*	*Poaceae*	12.68 ± 0.82	8.45 ± 0.46	4.27 ± 0.46	Brazil
Cowpea	*Vigna unguiculata*	*Fabaceae*	9.24 ± 0.57	6.79 ± 0.28	4.89 ± 0.20	Brazil
Dark red kidney bean	*P. vulgaris*	*Fabaceae*	10.91 ± 0.86	7.22 ± 0.52	6.21 ± 0.70	Brazil
Egyptian kidney bean	*Lablab purpureus*	*Fabaceae*	11.39 ± 0.74	8.29 ± 0.59	5.79 ± 0.31	Peru
Fava bean	*Vicia faba*	*Fabaceae*	24.60 ± 1.35	16.64 ± 1.37	8.98 ± 0.81	Peru
Green Lentils	*Lens culinaris*	*Fabaceae*	4.55 ± 0.22	4.55 ± 0.22	2.29 ± 0.10	France
Lentils	*Lens culinaris*	*Fabaceae*	6.48 ± 0.27	6.48 ± 0.27	2.39 ± 0.17	Brazil
Mung bean	*Vigna radiata*	*Fabaceae*	5.11 ± 0.24	3.80 ± 0.22	3.62 ± 0.16	Brazil
Ñuña bean	*P. vulgaris*	*Fabaceae*	13.37 ± 1.07	8.64 ± 0.47	7.64 ± 0.48	Peru
Panamito bean	*P. vulgaris*	*Fabaceae*	8.60 ± 0.57	5.91 ± 0.32	5.05 ± 0.34	Peru
Pink kidney bean	*P. vulgaris*	*Fabaceae*	9.93 ± 0.64	6.38 ± 0.29	3.78 ± 0.32	Brazil
Sorghum kernels	*Sorghum bicolor*	*Poaceae*	4.80 ± 0.18	3.89 ± 0.16	2.92 ± 0.13	Brazil
Wheat kernels	*Triticum* spp.	*Poaceae*	6.03 ± 0.33	2.39 ± 0.24	2.93 ± 0.23	Brazil
White kidney bean	*P. vulgaris*	*Fabaceae*	16.97 ± 0.74	8.12 ± 0.59	6.46 ± 0.31	Brazil
White lupin	*Lupinus albus*	*Fabaceae*	11.39 ± 1.07	8.29 ± 0.42	5.79 ± 0.46	Brazil

[a]Average ± standard deviation.

in the bibliography, the Kaptso et al. model (Kaptso et al., 2008) and the Ibarz–Augusto model (Ibarz & Augusto, 2015), that have explainable parameters and a suitable fitting of the data from this hydration behavior.

It should be mentioned that the sigmoidal behavior is slightly studied and only recently reported. As a consequence, for example, some grains hydration kinetics are not satisfactorily described as sigmoidal, being reported as DCS behavior (Augusto & Miano, 2017). For that reason, it is very important to discriminated which grains have sigmoidal or DCS behavior, as well as provide further data about grains with the sigmoidal behavior.

Furthermore, some hypotheses about the hydration process of grains are reported in the literature, although they have not been demonstrated yet. For example, Powell, Oliveira, and Matthews (1986), hypothesized that grains with colored seed coat hydrate slower than grains with white seed coat. Another hypothesis is that grains with sigmoidal hydration behavior always hydrate slower than grains with DCS behavior (Piergiovanni, 2011). In addition, Montanuci, Jorge, and Jorge (2015), hypothesized that as the grain size increases, the water absorption is reduced. All these hypotheses are being considered as true in several studies about hydration, without proving them and resulting in wrong interpretations of the process. Therefore, the comparison of the hydration kinetics of many grains, at similar conditions, should be performed to verify those hypotheses.

Consequently, this work aimed to describe the hydration behavior as well as to perform some comparisons among the different hydration kinetics to verify some hypothesis about this process.

2 | MATERIALS AND METHODS

2.1 | Raw material

Twenty-two different commercial grains were used: 19 species from the *Fabaceae* Family (pulses) and 4 from the *Poaceae* Family (cereals), obtained from local markets from different countries (Table 1). Ten of those grains have never been studied before (Caballero beans, Fava beans, Dark red kidney beans, Egyptian kidney beans, Canary beans, Green lentils, Pink kidney beans, Ñuña beans, Black kidney beans and

Panamito beans). The grains were previously selected excluding the damaged grains before the hydration process.

2.2 | Hydration process

To perform the hydration process, approximately 10 g of grains, placed in a net bag, were soaked inside a Beaker with 250 ml of distilled water at $25 \pm 1\,^{\circ}$C. The temperature was controlled using a water bath (Dubnoff MA 095 MARCONI, Piracicaba, Brazil). During the hydration process, the samples were periodically drained, superficially dried and their moisture content were obtained by mass balance using the initial moisture content (determined using a Moisture Analyzer MX-50 AND, Tokyo, Japan). The sampling was carried out every certain time (e.g., each 30 min or each 15 min depending on the behavior or the hydration rate) until reaching constant mass. The hydration process was performed in triplicate.

2.3 | Mathematical model fitting

The grains hydration kinetics data were fitted using the DCS equation of Peleg (Equation 1; Peleg, 1988) and the sigmoidal equation of Kaptso et al. (Equation 2; Kaptso et al., 2008). For that purpose, the dry basis moisture content of the grains (M % d.b.) versus the hydration time (min) was tabulated for each grain. The data were fitted to the mathematical model with a confidence level of 95% using the Levenberg–Marquardt algorithm in Statistica 12.0 (StatSoft, Palo Santo, California) software.

$$M_t = M_0 + \frac{t}{k_1 + k_2 \cdot t} \tag{1}$$

$$M_t = \frac{M_\infty}{1 + \exp[-k.(t-\tau)]} \tag{2}$$

The equilibrium moisture content of the grains with DCS was calculated according Equation 3 (Peleg, 1988):

$$M_\infty = M_0 + \frac{1}{\boldsymbol{k}_2} \tag{3}$$

Finally, the goodness of fit was evaluated by the determination coefficient (R^2), the root-mean-square deviation values (RMSD, Equation 4), the normalized RMSD (NRMSD, Equation 5) and by plotting the moisture content values obtained by the model (M_{model}) as a function of the experimental values ($M_{experimental}$). The regression of those data to a linear function (Equation 6) results in three parameters that can be used to evaluate the description of the experimental values by the model, that is the linear slope (a, which must be as close as possible to one), the intercept (b, which must be as close as possible to zero) and the coefficient of determination (R^2; that must be as close as possible to one).

$$\mathrm{RMSD} = \sqrt{\frac{\sum_{i=1}^{n}\left(M_{\mathrm{experimental}} - M_{\mathrm{model}}\right)^2}{n}} \tag{4}$$

$$\mathrm{NRMSD} = 100 \cdot \frac{\mathrm{RMSD}}{\left(M_{\mathrm{experimental}}\right)_{\mathrm{maximum}} - \left(M_{\mathrm{experimental}}\right)_{\mathrm{minimum}}} \tag{5}$$

$$M_{\mathrm{model}} = a \cdot M_{\mathrm{experimental}} + b \tag{6}$$

2.4 | Hierarchical cluster analysis (HCA)

A Hierarchical Cluster Analysis was performed to classify the studied grains according to their hydration characteristics similarities. This analysis was performed for the DCS and Sigmoidal hydration behavior separately, considering their respectively model parameters. The used Amalgamation rule was the "Single linkage." Statistica 12.0 (StatSoft) software was used.

3 | RESULTS AND DISCUSSION

Table 2, Figures 1 and 2 show the hydration kinetics behavior and the data fit of the different studied grains. Both, Kaptso et al. model for sigmoidal behavior and Peleg model for DCS behavior had an excellent fit with determination coefficients higher than 97%, "a" values close to one, "b" values close to zero and low RMSD and NMSD values. This corroborates the suitability of these equations to describe the hydration kinetics of grains. In addition, although there are only two kinds of hydration kinetics behaviors, there is a great variety of curves types: some grains absorb more quantity of water and/or at different rates. The water absorption characteristics are interesting to observe. There are grains that can absorb almost 20 times of their initial moisture content and others only four times. This characteristic is frequently attributed to the grain composition. However, by observing the hydration kinetics of the studied grains, the composition would not be the only factor that would affect the process. The structure could have also an important influence. For example, green lentils and carioca beans have similar composition; however, lentils absorb more water and hydrates faster than carioca beans.

The sigmoidal behavior is characterized by a slow hydration at the first part of the process. This is caused by the structural characteristics of the grains, especially by the seed coat (Miano & Augusto, 2015; Miano et al., 2015). Figure 1 shows that this behavior is observed only during the hydration of grains from the *Fabaceae* family (considering only the *Fabaceae* and *Poaceae* family, corresponding to most of edible grains). In contrast to the *Poaceae* family grains, the grains from the *Fabaceae* family have a seed coat with a more complex structure (Lush & Evans, 1980; Miano et al., 2015; Perissé & Planchuelo, 2004). The *Poaceae* family grains have a pericarp, since their kernels are part from a fruit called caryopsis (Marcos Filho, 2005). The pericarp is very permeable to water (Fernández-Muñoz, Acosta-Osorio, Gruintal-Santos, & Zelaya-Angel, 2011; Miano et al., 2017; Singh & Eckhoff, 1996) while the seed coat from *Fabaceae* family grains can be completely impermeable depending of the variety, composition and moisture content (Miano & Augusto, 2015; Sefa-Dedeh & Stanley, 1979). However, the velocity of the hydration process and the maximum water holding capacity probably is mainly affected by the internal structure and composition of the grain (Prasad, Vairagar, & Bera, 2010; Sobukola & Abayomi, 2011). Therefore, the effect in combination of the grains different intrinsic properties (seed coat or pericarp structure and

TABLE 2 Hydration kinetics data fit using the Peleg Model (Equation 1) and the Kaptso et al. model (Equation 2) for DCS and Sigmoidal behaviors, respectively

Sigmoidal behavior—Kaptso et al. model									
Grain	M_0 (% d.b.)	M_∞ (% d.b.)	k (min^{-1})	τ (min)	R^2	RMSD	NMSD	a	b
Adzuki bean	15.0 ± 0.1	134 ± 1	0.0100 ± 0.0003	284 ± 6	.993	0.780	0.733	1.02	2.14
Mung bean	13.3 ± 0.1	140 ± 1	0.0103 ± 0.0001	249 ± 2	.997	0.563	0.499	1.01	0.84
Caballero bean	15.9 ± 0.2	113 ± 3	0.0097 ± 0.0009	193 ± 28	.999	0.207	0.228	1.00	−0.03
White kidney bean	13.1 ± 0.1	114 ± 1	0.0112 ± 0.0006	189 ± 12	.999	0.245	0.260	1.00	−0.23
Fava bean	13.4 ± 0.1	127 ± 1	0.0142 ± 0.0016	163 ± 14	.992	0.732	0.620	1.00	−0.16
Dark red kidney bean	14.2 ± 0.2	111 ± 4	0.0132 ± 0.0011	130 ± 12	.998	0.418	0.436	1.01	−0.35
Egyptian kidney bean	13.4 ± 0.2	124 ± 2	0.0175 ± 0.0012	107 ± 7	.991	1.080	0.939	1.02	−1.65
Canary bean	15.7 ± 0.2	108 ± 1	0.0180 ± 0.0013	81 ± 4	.994	0.808	0.845	1.01	−0.98
Carioca kidney bean	16.0 ± 0.1	112 ± 1	0.0224 ± 0.0009	76 ± 1	.997	0.644	0.646	1.00	−0.46
Green lentils	10.4 ± 0.1	117 ± 1	0.0432 ± 0.0023	39 ± 1	.990	1.556	1.439	1.03	−3.01
DCS behavior—Peleg model									
Grain	M_0 (% d.b.)	M_∞ (% d.b.)[a]	k_1 (min % d.b.$^{-1}$)	k_2% d.b.$^{-1}$)	R^2	RMSD	NMSD	a	b
White lupin	10.7 ± 0.1	237 ± 7	1.2575 ± 0.0410	0.0044 ± 0.0001	.999	0.309	0.229	0.99	0.86
Pink kidney bean	13.6 ± 0.1	175 ± 1	1.0431 ± 0.0215	0.0062 ± 0.0001	.996	0.696	0.627	0.98	−1.24
Lentils	10.3 ± 0.1	170 ± 6	0.4391 ± 0.0400	0.0063 ± 0.0002	.992	2.258	1.955	0.97	−2.30
Cowpea	11.8 ± 0.1	165 ± 2	0.3768 ± 0.0311	0.0065 ± 0.0001	.995	0.750	0.566	1.00	0.94
Chick pea	10.9 ± 0.2	148 ± 1	0.5913 ± 0.0454	0.0073 ± 0.0001	.997	0.645	0.559	0.98	1.55
Ñuña bean	11.7 ± 0.1	142 ± 2	0.9171 ± 0.0276	0.0077 ± 0.0001	.995	0.654	0.631	0.99	1.36
Black kidney bean	17.1 ± 0.1	141 ± 4	0.5936 ± 0.0522	0.0081 ± 0.0002	.996	1.032	0.984	1.00	0.41
Panamito bean	15.1 ± 0.2	141 ± 0	0.6975 ± 0.0510	0.0080 ± 0.0001	.995	0.739	0.711	1.02	−2.37
Barley kernels	10.0 ± 0.1	77 ± 0	1.6274 ± 0.0262	0.0148 ± 0.0001	.993	0.378	0.753	1.01	1.00
Wheat kernels	11.3 ± 0.1	67 ± 0	1.9406 ± 0.0375	0.0181 ± 0.0001	.981	0.547	1.213	1.03	2.08
Corn kernels	12.6 ± 0.1	57 ± 1	4.6615 ± 0.1412	0.0224 ± 0.0006	.990	0.271	0.744	1.03	1.51
Sorghum kernels	11.8 ± 0.1	40 ± 2	1.6227 ± 0.3261	0.0354 ± 0.0020	.970	0.362	1.459	0.97	0.94

[a]Calculated using Equation 3.

composition and cotyledon or endosperm structure and composition) are the cause of the different hydration kinetics shapes, which are shown in Figures 1 and 2.

In this work, ten new grains varieties were studied for the first time: Caballero beans, Fava beans, Dark red kidney beans, Egyptian kidney beans, Canary beans, Green lentils, Pink kidney beans, Ñuña beans, Black kidney beans and Panamito beans. Six of those beans showed a hydration kinetics with sigmoidal behavior, which few studies are in the literature. The other grains showed DSC behavior, which is the most studied and reported behavior.

By performing comparisons among the hydration kinetics of the different grains (with very similar initial moisture content), it is possible to verify that some hypothesis reported in the literature are not always satisfied. Figure 3 highlights those comparisons.

For example, the sigmoidal behavior sometimes is related to a slow hydration process (Piergiovanni, 2011). However, Figure 3a shows that a grain with sigmoidal behavior can reach the equilibrium moisture content faster than a grain with DCS behavior (in Figures 1 and 2 more examples can be seen). As stated before, the hydration behavior is related to the seed coat structure and the hydration velocity and the maximum water holding capacity is related to the internal structure and composition. Therefore, grains with sigmoidal behavior not have always slow water uptake rate during processing. Thus, they can reach the equilibrium moisture content faster despite their initial lag phase.

Another hypothesis about the hydration process is that the seed coat color is related to the hydration velocity, observing that beans with white seed coat hydrate faster than beans with dark seed coat (Powell et al., 1986). Figure 3b shows the comparison between the hydration behavior of a bean with white seed coat and a bean with black seed coat (both are from the same species, *Phaseolus vulgaris*). This clearly shows that the dark seed coat bean hydrates faster than the white seed coat bean. Therefore, the individual effect of the seed

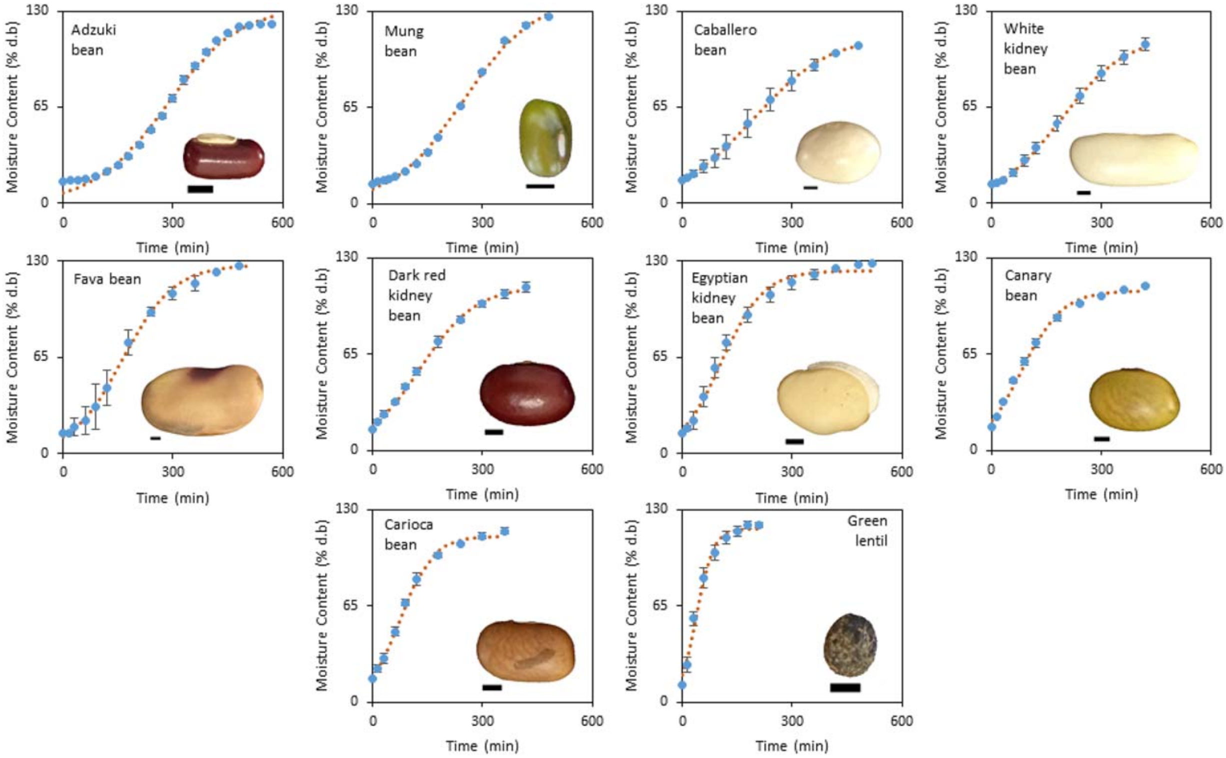

FIGURE 1 Hydration kinetics of grains with sigmoidal behavior. The dots are the experimental values; the vertical bars are the standard deviation and the discontinuous curves are the values obtained from the Kaptso et al. model (Equation 2). The horizontal bar close to the grains image represents 2 mm

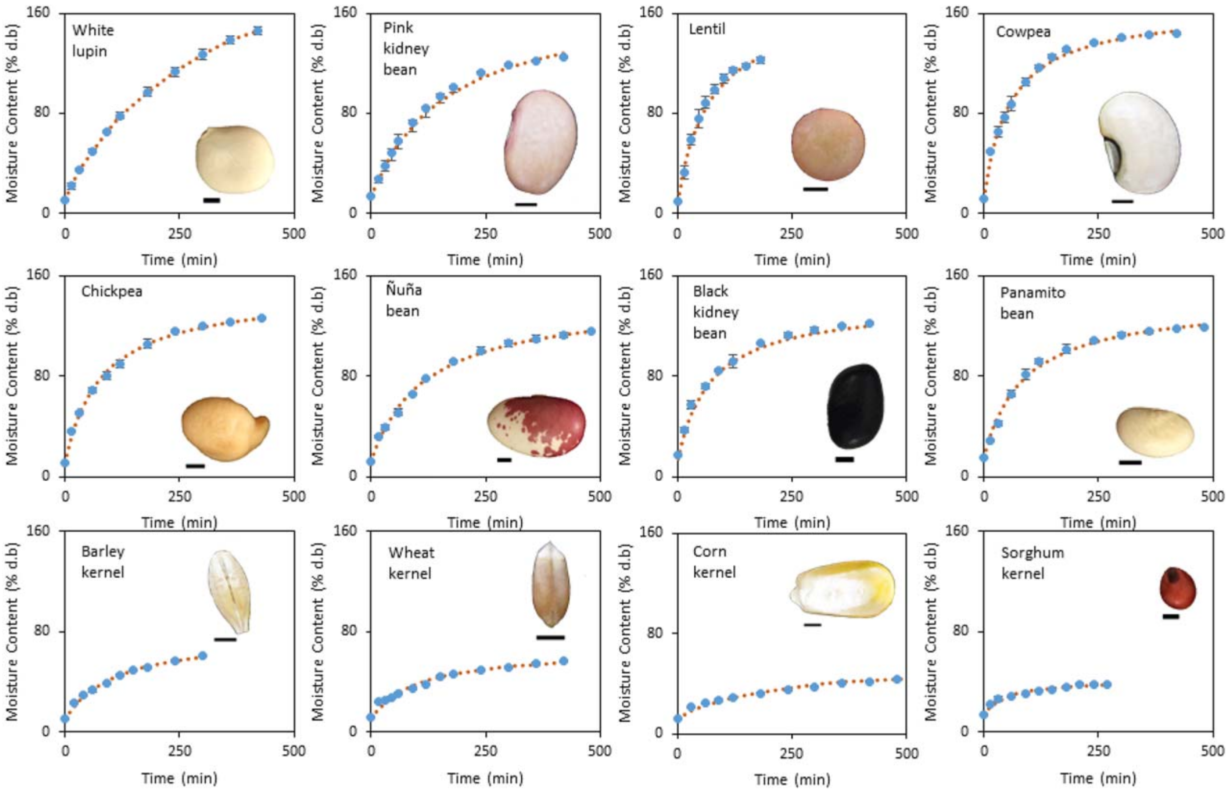

FIGURE 2 Hydration kinetics of grains with DCS behavior. The dots are the experimental values; the vertical bars are the standard deviation and the discontinuous curves are the values obtained from the Peleg model (Equation 1). The horizontal bar close to the grains image represents 2 mm

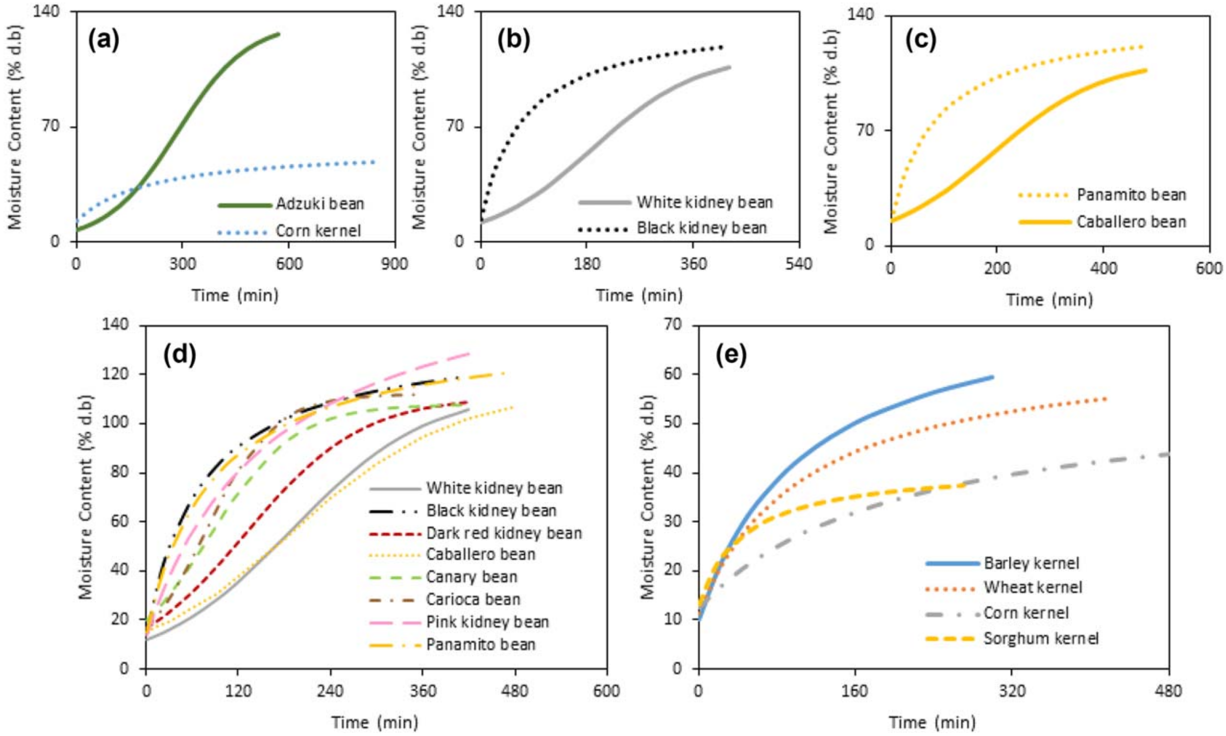

FIGURE 3 Relevant comparisons among the hydration kinetics behavior of some grains. (a) The sigmoidal behavior hydration is not always slower than the DCS behavior. (b) Comparison between two different color of beans from the same species. (c) Comparison between two different size of beans from the same species and color. (d) Hydration kinetics of different cultivars of *P. vulgaris* beans. (e) Hydration kinetics of grains from *Poaceae* family. The curves correspond the values obtained from the Peleg model (Equation 1) or the Kaptso et al. model (Equation 2)

coat color in not always the cause of the hydration velocity. Despite the color differences, the seed coat composition and/or structure also affect the hydration kinetics.

Other reported hypothesis is the idea that the smaller the grain is, the faster the hydration process is. This is stated because the surface area per unit mass of small grains is larger for the water transfer (Montanuci et al., 2015). This statement would be true if only diffusional mechanisms take place during the hydration and the seed coat is completely permeable to water. Nevertheless, other mechanisms of mass transfer, in especial the capillarity are important during the hydration process—being function of the sample microstructure. Furthermore, some grains, especially the sigmoidal behavior grains, have partially or completely impermeable seed coats, which need to be hydrated from inside of the grain (by the water that enter by the hilum and/or micropyle) to become permeable to water (Miano & Augusto, 2015). Thus, grains with larger relative area could hydrated slower because the seed coat would have more area to be hydrate, delaying the process. In Figure 3c, two beans from the same species (*P. vulgaris*), with the same seed coat color (White), but with different sizes are compared. The obtained data demonstrate that the bigger grain (Caballero bean) hydrates slower than the smaller grain (Panamito bean), fulfilling the hypothesis since is probable that the seed coat from Caballero beans is permeable to water. However, regarding only sigmoidal behavior hydration kinetics grains: mung bean hydrates slower than canary bean, despite the first is much smaller than the second one, being probably explained by the impermeability of the seed coat. Additional comparisons can be performed using Figures 1 and 2 demonstrating the unsatisfying of this hypothesis. Therefore, the fact that the grain size directly affects the hydration process is not always satisfied. This reinforces the idea that the hydration process is intrinsically affected by a vast number of factors that act simultaneously hindering the hypotheses demonstration and generalization.

Figure 3d shows the hydration behavior of different varieties of the same species of grains (*P. vulgaris*). All those grains are different in size, color, composition and structure, which causes different hydration behavior. For instance, Panamito beans and Black kidney beans have the same hydration kinetics despites their difference in color and size. Similarly, the hydration kinetics of Caballero beans and White kidney beans, grains with similar color, but different in size.

Furthermore, Figure 3e shows the hydration behavior of grains from the *Poaceae* family. The grains from this family are called as cereals and are characterized by their high quantity of starch. All of the studied grains have a DCS behavior. However, despite this behavior has a rapid hydration at the beginning of the process, the time to reach the equilibrium moisture content can be very long. In addition, they do not absorb as much water as the grains from the *Fabaceae* family. The *Fabaceae* family grains have higher quantity of protein (16–25%) and shorter quantity of starch (16–45%) (Siddiq and Uebersax, 2012), which could allow to reach higher equilibrium moisture content than *Poaceae* family grains (8–12% of protein and 55–70% of starch (Koehler &

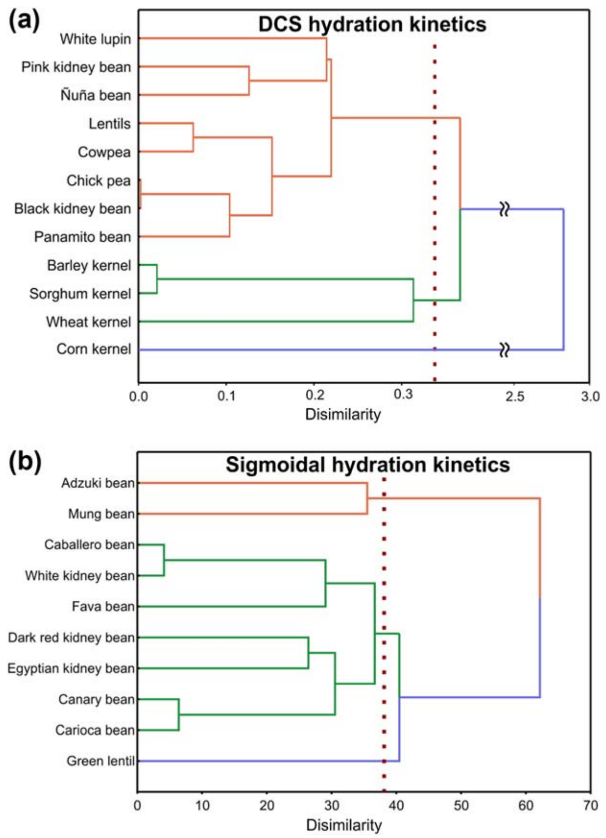

FIGURE 4 Hierarchical Cluster Analysis of 22 grains classified by their hydration kinetics behavior. (a) DCS hydration kinetics. (b) Sigmoidal shape hydration kinetics. The vertical discontinuous line indicates the suggested stopping location, which determined the formed clusters

Wieser, 2013)). This is due to the higher affinity to water that proteins have.

Figure 4 shows a Hierarchical Cluster Analysis of all the studied grains classifying them into groups depending on their hydration characteristics. Regarding DCS behavior (Figure 4a), the grains were classified in three groups. As expected, cereal grains (*Poaceae* family) and legumes (*Fabaceae* family) were discriminated, which means that despite having the same hydration behavior, legumes are characterized by different hydration rate and different equilibrium moisture content than cereals (legumes have different composition, physical properties and structure than cereal grains). Furthermore, among the four studied cereals, wheat kernels, barley kernels and sorghum kernels were joined in a group separately from corn kernels due to their hydration kinetics similarities. Corn kernels hydration kinetics is very different from all the grains being the slowest process.

Regarding sigmoidal behavior grains (Figure 4b), which all are legumes, there were classified in three groups. It is interesting to highlight that the groups were discriminated by the genre (taxonomic classification). The first group is formed by *Vigna* genre (Adzuki beans and mung beans), the second group is formed mainly by the *Phaseolus* genre (except by Egyptian, which is from the *Lablab* genre and Fava bean from the *Vicia* genre) and the third group is formed by *Lens* genre represented by lentils. The studied genres of legumes have similar chemical composition, but different external structure as the hilum form and size, probably causing the differences among the hydration kinetics.

As can be seen, the hydration behavior is a complex process to study. There are several intrinsic properties such as size, color, composition and structure of the grains, which together affect the hydration process. Therefore, the comparison among the obtained results about hydration kinetics cannot be performed with precision between families, species, even, varieties, since those intrinsic properties are very difficult to control.

In fact, there are works in the literature focusing on stablished how structure (Lush & Evans, 1980; Miano et al., 2015; Sefa-Dedeh & Stanley, 1979) and composition (e.g., the initial moisture content (Miano & Augusto, 2015)) affect the hydration process of grains. However, many doubts still exist, and the exactly mechanisms involved in this process are still unknown. For example, how the composition affect structure, and how both composition and structure affect the grains hydration, as well as the exactly mass transfer mechanism that takes place at each processing period. Therefore, it is important to perform more studies about the relation of the composition, structure, physical properties and hydration process to stablish how those intrinsic properties affects the processing.

4 | CONCLUSIONS

The hydration of grains is a complex process, which is affected by many intrinsic properties of the grains. Those properties cause the different kind of behaviors: the DCS, which was already widely studied, and the sigmoidal behavior, which has very few studies. This work described the hydration behavior of ten new grains, six of them demonstrating the sigmoidal behavior. Furthermore, by comparing the hydration kinetics of 22 grains, some hypothesis about the hydration process related to the color, size and hydration behavior were evaluated, finding that they are not always satisfied. In addition, the studied grains were classified according to their hydration characteristics (behavior, hydration rate, and equilibrium moisture) finding a possible relation between the hydration kinetics and the taxonomic classification of the grains. Despite the difficulty of evaluating each individual factor isolated from the others, future studies are recommended to stablish how the grains intrinsic properties affect their hydration kinetics.

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NOMENCLATURE

a	Linear model parameter (slope) (Equation 3) [Different unit]
b	Linear model parameter (ordinate axis intercept) (Equation 3) [Different unit]
k	Kaptso et al. kinetic parameter related to water absorption rate (Equation 2) [% d.b.$^{-1}$]
M_∞	Equilibrium moisture content (Equation 2) [% d.b.]
$M_{experimental}$	Experimental moisture content value (Equations 4 and 5) [% d.b.]
M_{model}	Model moisture content value (Equations 4 and 5) [% d.b.]
M_o	Initial moisture content (Equation 1) [% d.b.]
M_t	Moisture content over the time t (Equations 1 and 2) [% d.b.]
NRMSD	Normalized root-mean-square deviation values (Equation 5) [%exp]
RMSD	Root-mean-square deviation values (Equation 4) [% d.b.]
t	Time (Equations 1 and 2) [min]
τ	Kaptso et al. kinetic parameter related to lag phase (Equation 2) [min^{-1}]

ORCID

Alberto Claudio Miano http://orcid.org/0000-0002-2874-3012

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APPENDIX D: CORRELATING THE PROPERTIES OF DIFFERENT CARIOCA BEAN CULTIVARS (*Phaseolus vulgaris*) WITH THEIR HYDRATION KINETICS

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Correlating the properties of different carioca bean cultivars (*Phaseolus vulgaris*) with their hydration kinetics

Alberto Claudio Miano[a,*], Erick Saldaña[a], Luciano Henrique Campestrini[a], Alisson Fernando Chiorato[b], Pedro Esteves Duarte Augusto[a,*]

[a] Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil
[b] Agronomic Institute (IAC), Campinas, SP, Brazil

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ABSTRACT

This work explained how the intrinsic properties of beans affects the hydration process. For that, different properties of six cultivars of carioca bean (a variety of common bean) were analyzed to verify the correlation with their hydration kinetics characteristics (hydration rate, lag phase time and equilibrium moisture content), using a Multiple Factorial Analysis (MFA): the chemical composition (starch, protein, lipids, minerals (Mg, P, S, K, Ca, Mn, Fe, Cu, Zn), functional groups from the seed coat analyzed by FT-IR), physical properties (size, 1000 grain weight, seed coat thickness, energy to penetrate the bean) and microstructure. Only few properties correlated with the hydration kinetics characteristics of the studied bean, comprising both composition and structure. The fat content, potassium content, specific surface, and the protein to lipids ratio correlated with the lag phase time, which is related with the seed coat impermeability to water. The necessary energy to perforate the seed coat correlated negatively with the hydration rate. It was concluded that the hydration of beans process is a complex phenomenon and that despite being from the same variety of legume, any change due to agronomic enhancement may affect their hydration process kinetics.

1. Introduction

The legumes are a very important source of nutrients such as protein, dietary fiber, starch, mineral and vitamins for human consumption (Siddiq, Butt, & Sultan, 2011). Therefore, their production and industrialization is a significant entry in the world. Due to preservation and logistic reasons, the legumes are mainly commercialized as dried food. Consequently, their hydration is needed before being processed (cooking, germinating, malting, fermenting and/or extracting some components). In addition, over time, several agronomic and genetic enhancements have been performed in order to produce grains with some environmental or pathologic resistance and/or to produce nutritional enriched grains. These enhancements could change the process behavior of the grains, for instance the hydration behavior due to the grain chemical and/or physical changes.

The hydration process is a mass transfer unit operation, which is conducted, for instance, by immersing the food in water. Despite the hydration is, in general, considered a simple process, it is in fact a complex phenomenon, especially in grains such as legumes and cereals. The food with heterogeneous structure and different properties, causes the mass transfer to be not only by diffusion, but also by capillarity and with a specific pathway to the water flow. In addition, different intrinsic and extrinsic factors also affect the hydration behavior. The hydration kinetics is characterized by the water uptake rate, equilibrium moisture (maximum water holding capacity), and, in some cases, the necessary time to end the lag phase (related with the seed coat impermeability) (Kaptso, Njintang, Komnek, Hounhouigan, Scher, & Mbofung, 2008; Miano, García, & Augusto, 2015). Depending on the existence of the lag phase, the curve of the hydration kinetics can be downward concave or sigmoidal shaped.

Intrinsic factor such as the structure (Swanson, Hughes, & Rasmussen, 1985), water activity (Miano & Augusto, 2015) and chemical composition affect the velocity and/or hydration kinetics behavior (downward concave shape or sigmoidal behavior (Albert Ibarz & Augusto, 2015)). Extrinsic factors such as the soaking water temperature (Miano et al., 2015; Oroian, 2015) accelerate the process, as well as the use of different technologies, such as ultrasound (Miano, Ibarz, & Augusto, 2017; Yildirim, Öner, & Bayram, 2010) and high hydrostatic pressure (A. Ibarz, González, & Barbosa-Cánovas, 2004; Ueno, Shigematsu, Karo, Hayashi, & Fujii, 2015).

However, there are not enough researches giving the relation and/or correlating the intrinsic factors or properties of the grain with its

* Corresponding authors at: Avenida Pádua Dias, 11, Piracicaba, SP, 13418-900, Brazil.
E-mail addresses: cmiano@usp.br (A.C. Miano), pedro.ed.augusto@usp.br (P.E.D. Augusto).

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Nomenclature

a and b	Linear model parameter (slope) (Eq. 5) [Different unit]
k	Kaptso et al. kinetic parameter related to water absorption rate (Eq. 4) [% d.b.$^{-1}$]
L	Length (Eqs. 2 and 3) [m]
M_e	Equilibrium moisture content (Eq. 4) [% d.b.]
$M_{experimental}$	Experimental moisture content value (Eqs. 5, 6, and 7) [% d.b.]
M_{model}	Estimated moisture content value by Eq. 4 (Eqs. 5, 6, and 7) [% d.b.]
M_t	Moisture content over the time t (Eqs. 1 and 2) [% d.b.]
$NRMSD$	Normalized root-mean-square deviation values (Eq. 6) [% exp]
$RMSD$	Root-mean-square deviation values (Eq. 5) [% d.b.]
S	Surface (Eqs. 1 and 3) [m^2]
S_{sp}	Specific Surface (Eq. 1) [m^2/m^3]
t	Time (Eq. 4) [min]
T	Thickness (Eqs. 2 and 3) [m]
τ	Kaptso et al. kinetic parameter related to lag phase (Eq. 4) [min^{-1}]
V	volume (Eqs. 1 and 2) [m^3]
W	Width (Eqs. 2 and 3) [m]

hydration kinetics. In fact, it is important to know which intrinsic property of the bean affects its hydration kinetics since any change on these properties would change the processing conditions. Therefore, knowing which intrinsic property is related to the hydration kinetics behavior would be interesting. For those reasons, this work aimed to find which of the intrinsic properties (microstructure, chemical composition and physical characteristics) of one variety of common bean (*Phaseolus vulgaris*) are correlated with the hydration kinetics characteristics, considering six different cultivars with specific agronomic improvements.

2. Materials and methods

2.1. Raw materials

For the present study, six cultivars of carioca bean (*Phaseolus vulgaris*), developed by the Agronomic Institute (IAC), Brazil, were used: IAC Imperador (IMP), IAC Milênio (MIL), IAC 45/57-7-3-1/4 (45), IAC C10-2-4/41 (C10), IAC Sintonia (SIN) and IAC Eté (ETE) (Table 1, Fig. 1). All of them were cultivated and stored at the same conditions after harvesting. The initial moisture content of all the cultivars was considered as 13.17 ± 0.12% (d.b.) since no significant difference ($p > 0.05$) was found among the actual values.

2.2. Chemical analysis

For the following analysis, a sample of beans were milled using an analytical mill (IKA A11 basic analytical mill, Germany) and sieved (0.5 mm of screen).

Proximate composition (ash, lipid by Soxhlet, and proteins by Kjeldah) were determined according to AOAC (2010). The starch content was obtained by an enzymatic method (modified AOAC Method 996.11, AACC Method 76–13.01 and RACI Standard Method by Megazyme, USA). The mineral contents (Mg, P, S, K, Ca, Mn, Fe, Cu, Zn) were determined using an energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-720, Japan) following the methodology of Tezotto et al. (2013). The carbon and nitrogen content were determined by an organic elemental analyzer (CHNS-O; Flash 2000 model, Thermo Fisher Scientific Inc. – Delft, Netherland). For that, 10 mg of milled sample was placed in a small pan to be placed inside the equipment. All the analyses were performed in triplicate.

2.3. Physical analysis

For the physical characterization of the grains, the 1000 grain weight (1000 w), specific surface, seed coat thickness and work or energy necessary to perforate 3 mm of the grain were determined.

The 1000 grain weight was performed by weighting 100 grains of each cultivar in a semi-analytical scale and multiplying the result by ten. This analyses was performed in triplicate.

To calculate the specific surface (S_{sp}), volume (V) and surface (S), the length (L), width (W) and thickness (T) were determined using a micrometer (Mitutoyo MDC-25SB, Japan), and the following equations were used to estimate the specific surface area (S_{sp}) and the volume (V) (Gupta & Das, 1997):

$$S_{sp} = \frac{S}{V} \tag{1}$$

$$V = \frac{1}{6}\pi \bullet L \bullet W \bullet T \tag{2}$$

$$S \approx 4\pi \bullet \sqrt[1.6075]{\left(\frac{\left(L \bullet W/_4\right)^{1.6075} + \left(L \bullet T/_4\right)^{1.6075} + \left(T \bullet W/_4\right)^{1.6075}}{3}\right)} \tag{3}$$

Since the carioca bean can be considered geometrically as an ellipsoid, the Eq. 3 was used to estimate the surface area of ellipsoids, which is the Knud Thomsen approximation. According to Xu et al.

Table 1
Agronomic information of six cultivars of Carioca beans (*Phaseolus vulgaris*).

Characteristics	Cultivar					
	IAC Milênio (MIL)	IAC Imperador (IMP)	IAC 45/57–7–3-1-4 (45)	IAC C10–2-4/41 (C10)	IAC Sintonia (SIN)	IAC Eté (ETE)
Leaf color	Dark green	Light green	Light green	Light green	Green	Light green
Plant habit	Erect	Semi-erect	Erect	Erect	Semi-erect	Erect
Cycle	92 days (Normal)	75 days (Premature)	88 days (Semi-premature)	92 days (normal)	88 days (Semi-premature)	92 days (normal)
Dry tolerance	Intermediate	Tolerant	Intermediate	Intermediate	Intermediate	Tolerant
High temperature tolerance	Intolerant	Tolerant	Intermediate	Intermediate	Tolerant	Tolerant
Anthracnose resistance	Intermediate	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Angular leaf spot resistance	Susceptible	Susceptible	Intermediate	Susceptible	Susceptible	Resistant
Bacteriosis resistance	Susceptible	Susceptible	Intermediate	Intermediate	Intermediate	Resistant
Grain color	Light	Light	Light	Intermediate	Dark	Dark
Grain Size	High	Medium	Medium	Medium	High	Medium
Grain darkness tolerance	Tolerant	Tolerant	Intermediate	Intermediate	Intolerant	Intolerant
Productive capacity	High	Medium	Medium	High	High	Medium

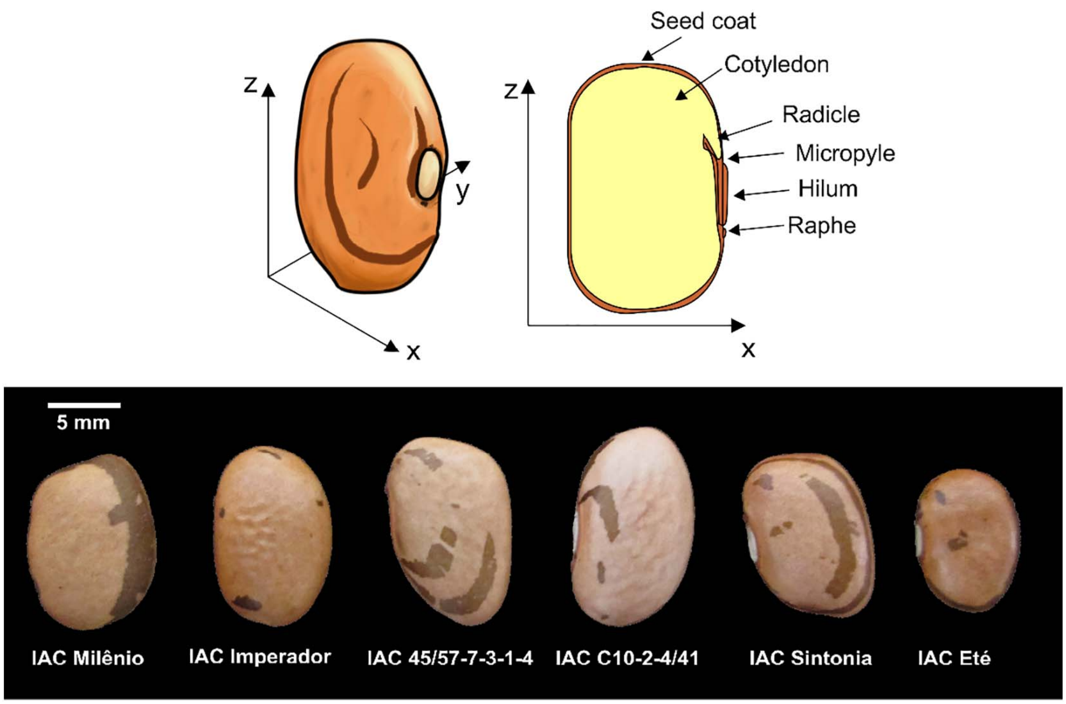

Fig. 1. Morphology of Carioca bean (*Phaseolus vulgaris*) and photography of the used cultivars.

(2009) this approximation has a relative error of approximately 1%. In addition, despite this equation is limited for ellipsoids, its used gave very good approximation in many grains (Fıratlıgil-Durmuş, Šárka, Bubník, Schejbal, & Kadlec, 2010; Ghafoor, Misra, Mahadevan, & Tiwari, 2014; Kara, Sayinci, Elkoca, Öztürk, & Özmen, 2013). These analyses were performed for fifty grains.

The seed coat thickness of twenty grains was measured using a micrometer (Mitutoyo MDC-25SB, Japan). For that, the seed coat from the abaxial sides (y-axis considering the hilum as the ventral part of the grain; Fig. 1) of the dried grains (initial moisture content) were carefully extracted to be measured.

The work or energy to perforate a hydrated grain (grain at the equilibrium moisture content) was analyzed using a Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 5 kg-f (49,03 N). The grain was perforated at 0.2 mm·s^{-1} in the abaxial side (y-axis) using a 0.2 cm cylindrical probe (P/2) until the distance of 3 mm. The force measured by the equipment as a function of the penetration depth was then used to calculate the necessary work to penetrate the sample. The area bellow the curve from the initial of the process until the first peak (this indicates the seed coat rupture) was considered as the work necessary to perforate the seed coat (W sc). The remaining area until the first peak corresponding to the seed coat rupture was considered as the work necessary to perforate approximately half of the cotyledon (W c) (Fig. 2).

2.4. Microstructure

For the microstructural analysis, the samples were cut with a scalpel blade to see the different tissues (seed coat, cotyledon, and external surface) and dehydrated in a sealed container using silica gel for 3 days. Then, they were sputtered with a 30 nm gold layer. Finally, the samples were observed in a scanning electronic microscope (SEM) operated at an acceleration voltage of 20 kV (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England).

2.5. FT-IR seed coat analysis

Since the seed coat is the most important structure that changes the behavior of the hydration kinetics, an FT-IR analysis was performed. For that, after the hydration process of each cultivar, the beans were dehulled and the seed coats were dried at 35 °C for 12 h. Them, the samples were milled and sieved (sieve 80, 180 mm). Approximately, 1 mg of sample and 9 mg of KBr (analytical grade) were mixed and then pressed for obtaining each pellet. The latter was introduced in the FT-IR infrared spectrometer (BIORAD – Hercules – CA – USA) and analyzed in the spectral band from 400 to 4000 cm^{-1} of wavelength number with a resolution of 8 cm^{-1} and 16 scans. This analysis was performed in quintuplicate and the data were evaluated using the equipment own software recording the absorbance at each wavelength number.

2.6. Hydration process

To perform the hydration process, approximately 15 g of grains placed in a net bag were soaked in 250 mL of distilled water at 25 ± 1 °C contained into a beaker. The temperature was controlled using a water bath (Dubnoff MA 095 MARCONI, Brazil). During the hydration process, the samples were periodically drained, superficially

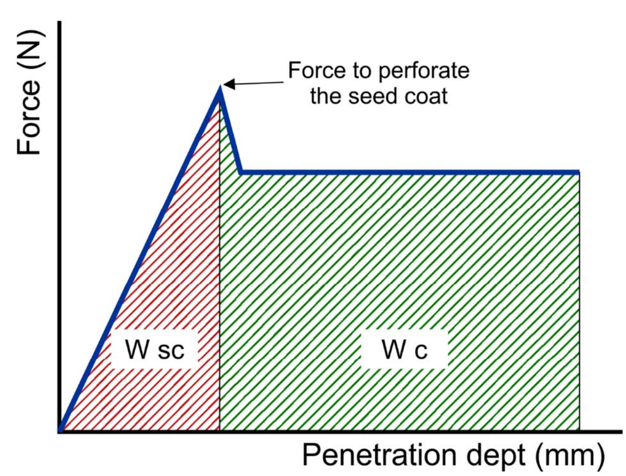

Fig. 2. Schematic representation of the carioca bean penetration test.

dried and their moisture content were obtained by mass balance considering the initial moisture content previously determined. The initial moisture content of the samples was determined using a moisture analyzer (MX-50 AND, Japan) at 105 °C by infrared heating. The sampling was carried out every 15 min in the first hour, every 30 min for the latter 2 h and then every hour until constant weight. Each moisture content was calculated on dry mass. The hydration process was performed in triplicate.

The carioca beans hydration kinetics was modeled using the sigmoidal equation proposed by Kaptso et al. (2008) (Eq. 4). For that purpose, the dry basis moisture content of the grains (M % d.b.) versus the hydration time (min) was tabulated. The data were fitted to the equation with a confidence level of 95% using the Levenberg-Marquardt algorithm in Statistica 12.0 (StatSoft, USA) software.

$$M_t = \frac{M_e}{1 + exp[-k.(t - \tau)]} \quad (4)$$

Being M_t the sample moisture content (% d.b.) at each time t; M_e is the equilibrium moisture content; τ describes the necessary time to reach the inflection point of the curve, being thus related to the lag phase; and k is the water absorption rate kinetic constant. Finally, the goodness of fit of the models was evaluated by the R^2 regression value, the root-mean-square deviation values (RMSD, Eq. 5), the normalized RMSD (NRMSD, Eq. 6) and by plotting the moisture content values obtained by the model (M_{model}) as a function of the experimental values ($M_{experimental}$). The regression of those data to a linear function (Eq. 7) results in three parameters that can be used to evaluate the description of the experimental values by the model, i.e. the linear slope (a, which must be as close as possible to one), the intercept (b, which must be as close as possible to zero) and the coefficient of determination (R^2; that must be as close as possible to one).

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n} (M_{experimental} - M_{model})^2}{n}} \quad (5)$$

$$NRMSD = 100\bullet\frac{RMSD}{(M_{experimental})_{maximun} - (M_{experimental})_{minimun}} \quad (6)$$

$$M_{model} = a\bullet M_{experimental} + b \quad (7)$$

2.7. Statistical analysis

Statistical analysis was performed through analysis of variance (ANOVA) and Tukey's test (5% of significance), using the software Statistica 12.0 (StatSoft, USA). In addition, Multiple Factor Analysis (MFA) was performed to evaluate which factors (grain chemical composition, physical characteristic and seed coat functional groups) correlate with the hydration characteristics (parameters of Eq. 4). Four groups of variables were considered: chemical composition, physical characteristics, seed coat functional groups and parameters of hydration (supplementary group). This analysis was performed using the XLSTAT software (Addinsoft, New York, USA).

3. Results and discussion

3.1. Hydration kinetics

All cultivars of Carioca beans showed a sigmoidal hydration behavior (Fig. 3). Consequently, they probably follow the specific water entrance pathway as in others legumes (Miano et al., 2015; Miano & Augusto, 2015) i.e. as the seed coat is impermeable at lower water activities, it needs to be hydrated to increase its permeability and accelerate the process. For that, the water firstly enters by the hilum and/or micropyle and hydrates the seed coat from inside (the wax presented outside the seed coat avoid its hydration) until reaching a certain water activity when the water starts to permeate more through the seed coat (end of the lag phase). Finally, the water hydrates the cotyledon until reaching the equilibrium moisture content.

In addition, Fig. 3 and Table 2 shows that the Kaptso et al. model successfully fit the hydration kinetics data of all cultivars of carioca beans. It is interesting to highlight that despite the grains studied are from the same variety of bean (carioca bean, *Phaseolus vulgaris*), the cultivars have different sigmoidal shapes and hence, hydration behaviors, but with the same equilibrium moisture content. According to Table 2 (which can also be verified on Fig. 3), the cultivars differ in the lag phase time (τ) and the hydration rate (k). For example, the IAC Eté cultivar has much longer lag phase than the IAC Imperador cultivar. Since all cultivars presented non-statistical difference ($p > 0.05$) among their equilibrium moisture contents, the average of all the obtained values (129 ± 2% d.b.) was considered as constant in the equation fit process. It is important to mention that, despite having a successfully fit, the Kaptso et al. model has as limitation the lack of fitting at the initial moisture content. Therefore, future models for hydration process with sigmoidal behavior need to be developed.

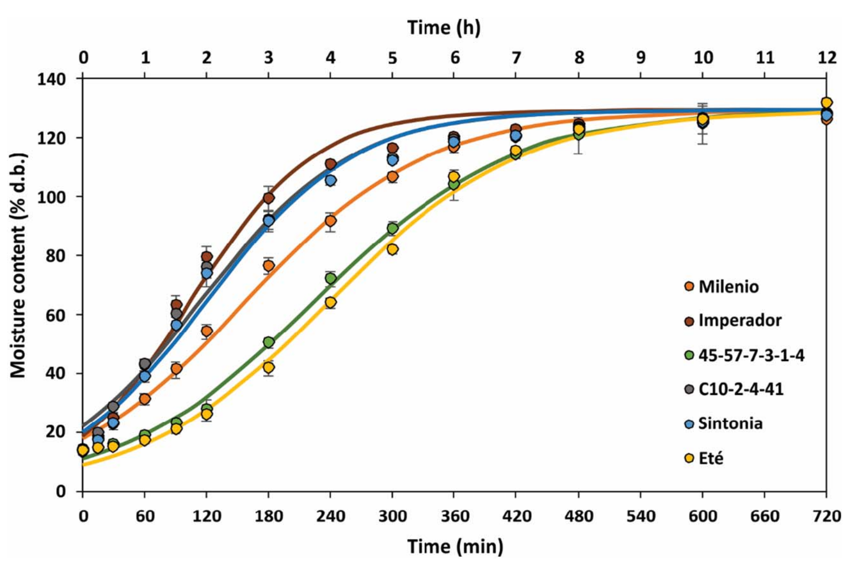

Fig. 3. Hydration kinetics of the six studied cultivars of Carioca beans (*Phaseolus vulgaris*). The dots are the experimental data, the curves are the fitted data to Kaptso et al. model (Eq. 4) and the vertical bars represent the standard deviation.

Table 2
Hydration characteristics of the six studied cultivar of Carioca beans: values for the Kaptso et al. model (Eq. 4) parameters. All cultivars presented the same equilibrium moisture content (M_e = 129 ± 2% d.b.) The results are represented by the mean ± standard deviation. Letters represent Tukey Test mean comparison (p < 0.05) after performing ANOVA analysis.

Cultivar	k (min^{-1})	τ (min)	R^2
IAC Milenio (MIL)	0.011 ± 0.001c	159 ± 5b	0.996
IAC Imperador (IMP)	0.017 ± 0.002a	105 ± 7c	0.988
IAC 45/57–7–3-1/4 (45)	0.011 ± 0.001c	226 ± 6a	0.998
IAC C10–2-4/41 (C10)	0.014 ± 0.001b	115 ± 6c	0.983
IAC Sintonia (SIN)	0.014 ± 0.001b	121 ± 5c	0.984
IAC Eté (ETE)	0.011 ± 0.001c	240 ± 4a	0.996

3.2. Bean cultivars microstructure and correlation with their hydration kinetics

In this study, the legume variety, the growth conditions, and the initial moisture content were the same for all the grains. Therefore, the structure, chemical composition and physical characteristics were correlated with the hydration kinetics.

Firstly, the microstructure of each cultivar was evaluated. It is important for the hydration kinetics since the different configuration of the pores, with different connectivity, tortuosity and permeability (Warning, Verboven, Nicolaï, van Dalen, & Datta, 2014) can affect the water transport (mass transfer) inside the grains. Fig. 4 and Fig. 5 show the seed coat external surface and transversal cut microstructure respectively. The external surface of all cultivars of carioca beans have the same structure showing a homogeneous surface without porosity similarly to other legumes as cowpeas (Sefa-Dedeh & Stanley, 1979), common bean (Swanson et al., 1985), adzuki beans (Miano & Augusto, 2015), Andean lupin (Miano et al., 2015) and mung bean (Miano, Pereira, Castanha, Júnior, & Augusto, 2016). The transversal cut of all the seed coats has similar structure showing the layer of macrosclereid cells, the osteosclereid cells and the parenchyma tissue. In contrast to other legumes such as Andean Lupin (Miano et al., 2015), the thickness of the osteoclereid layer is narrow and almost non-visible. Further, the seed coat thickness is similar in all carioca beans cultivars. Despite the differences in hydration kinetics, the seed coat did not show conclusive differences related to those behaviors. Nevertheless, it should be

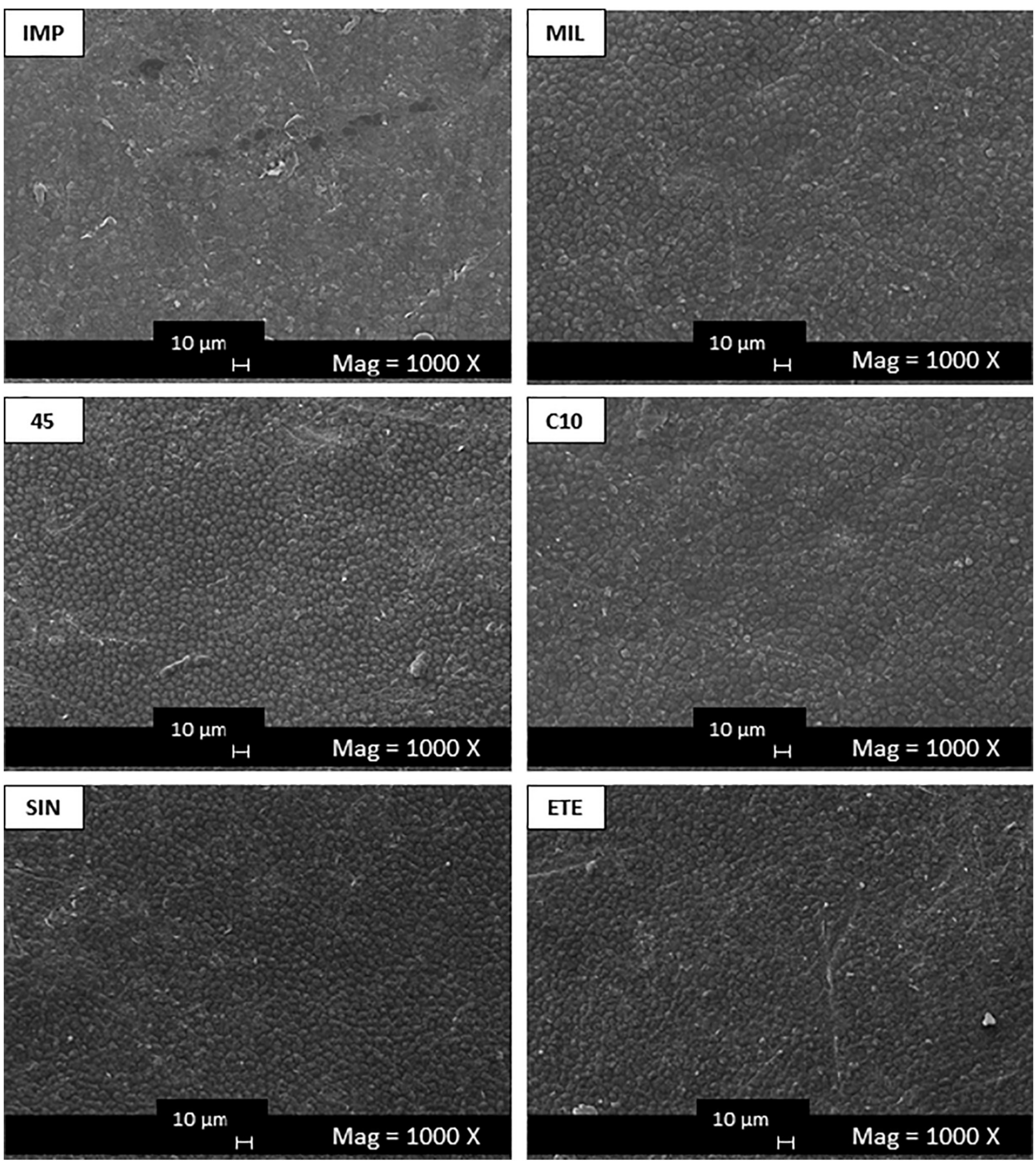

Fig. 4. Microphotography of the seed coat superficial surface of carioca bean cultivars (*Phaseolus vulgaris*) (SEM, 20 kV; 1000× of magnifications – the 10 µm bar is shown in the figures).

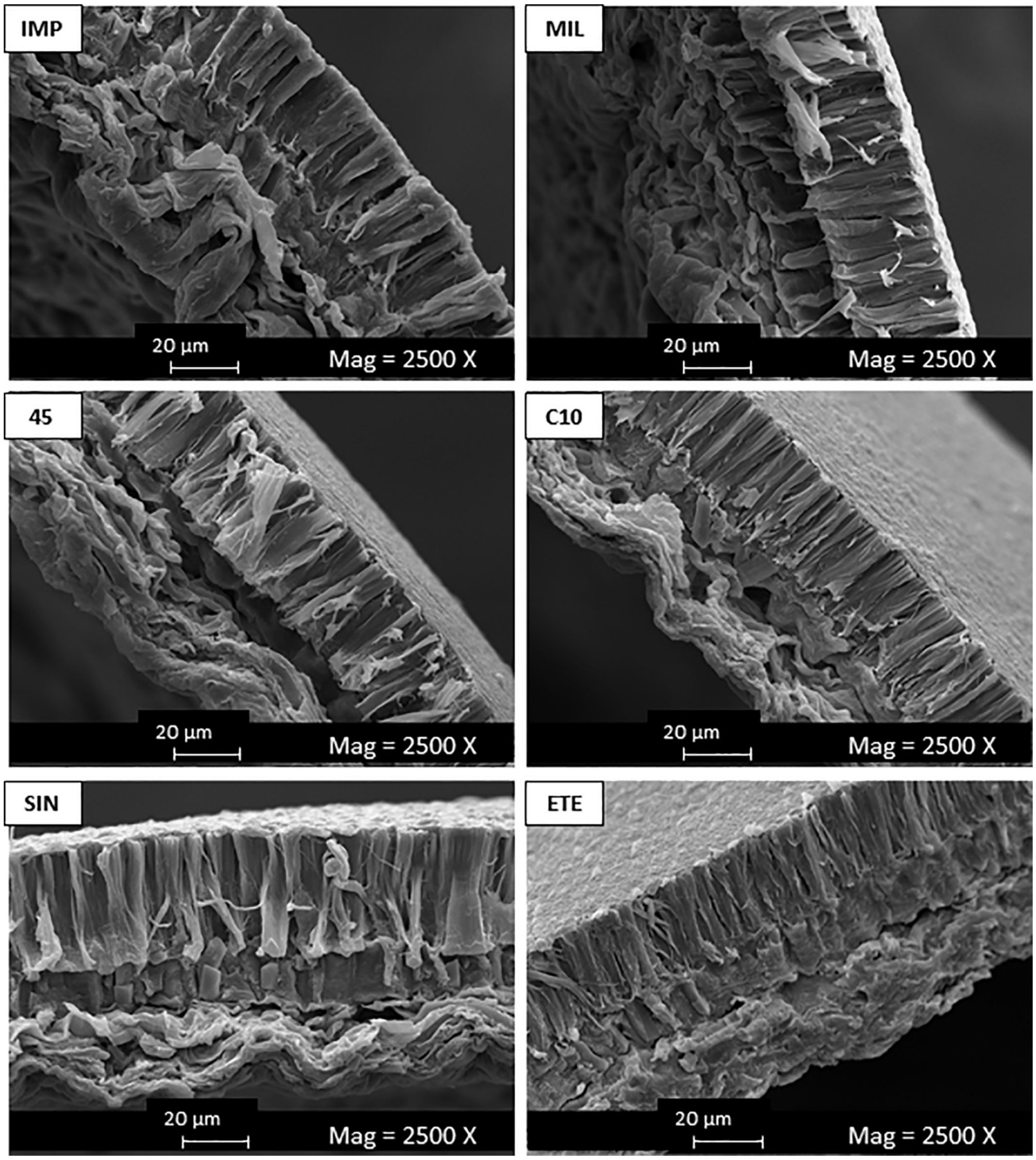

Fig. 5. Microphotography of the seed coat transversal cut of carioca bean cultivars (*Phaseolus vulgaris*) (SEM, 20 kV; 2500× of magnifications – the 20 µm bar is shown in the figures).

highlighted that the seed coat structure of the IAC Eté cultivar apparently is more compact than the other seed coats. This could be the cause of the longest lag time (τ) and the lowest hydration rate (k) (Table 2).

The first structures that are in contact with water are the seed coat, the hilum, and the micropyle. Depending on the moisture content of the seed coat, its permeability to water can change (Miano & Augusto, 2015). In most cases, when the bean is at the equilibrium water potential with that of the environment (i.e., dried, low water activity), the seed coat is impermeable to water. Therefore, the water enters by the micropyle and/or the hilum at the beginning of the hydration process. However, the water pathway entrance is still controversial and apparently depends on the legume variety. For example the hilum is the principal water entrance for cowpeas (Sefa-Dedeh & Stanley, 1979), while other works stated that the entrance of water is the micropyle and hilum in Carioca beans and black beans (Korban, Coyne, & Weihing, 1981), or only the hilum in black beans (Varriano-Marston & Jackson, 1981) and Andean lupin (Miano et al., 2015). A further discussion of this topic is provided by Miano and Augusto (2018).

In this work, the structure of both the hilum and the micropyle of every cultivar of carioca beans was evaluated. Fig. 6 shows that the hilum of all cultivars is similar, being a very porous structure. Some of the images show damage in the hilum, which is normally due to the post-harvest processing causing the hilar fissure exposure. This damage is not very important in the hydration process, since this structure is very porous, allowing the water to pass immediately through the hilar fissure, which controls the water entrance. Fig. 7 shows that the porosity of the hilum is similar for all the cultivars of carioca beans. According to Fig. 8, the micropyle of all the studied cultivars have similar shape, some of them are apparently open (IAC Milenio, IAC 45/57-7-3-1/4 and IAC C10-2-4/41). However, according to Fig. 3, the fact that the micropyle could be opened, does not indicate a faster hydration of those beans. Although there is a probability that the water enters the grain through the micropyle (Korban et al., 1981), the hilum can be considered as the main water entrance (Miano et al., 2015; Miano & Augusto, 2015; Sefa-Dedeh & Stanley, 1979), since the hilum has a significant larger area in comparison to the micropyle.

Finally, the microstructure of the cotyledon of all cultivars of carioca beans was observed (Fig. 9). The cotyledon of the cultivars shows cells with plenty of big oval starch granules and with non-visible intercellular spaces as characterized in many beans such as common

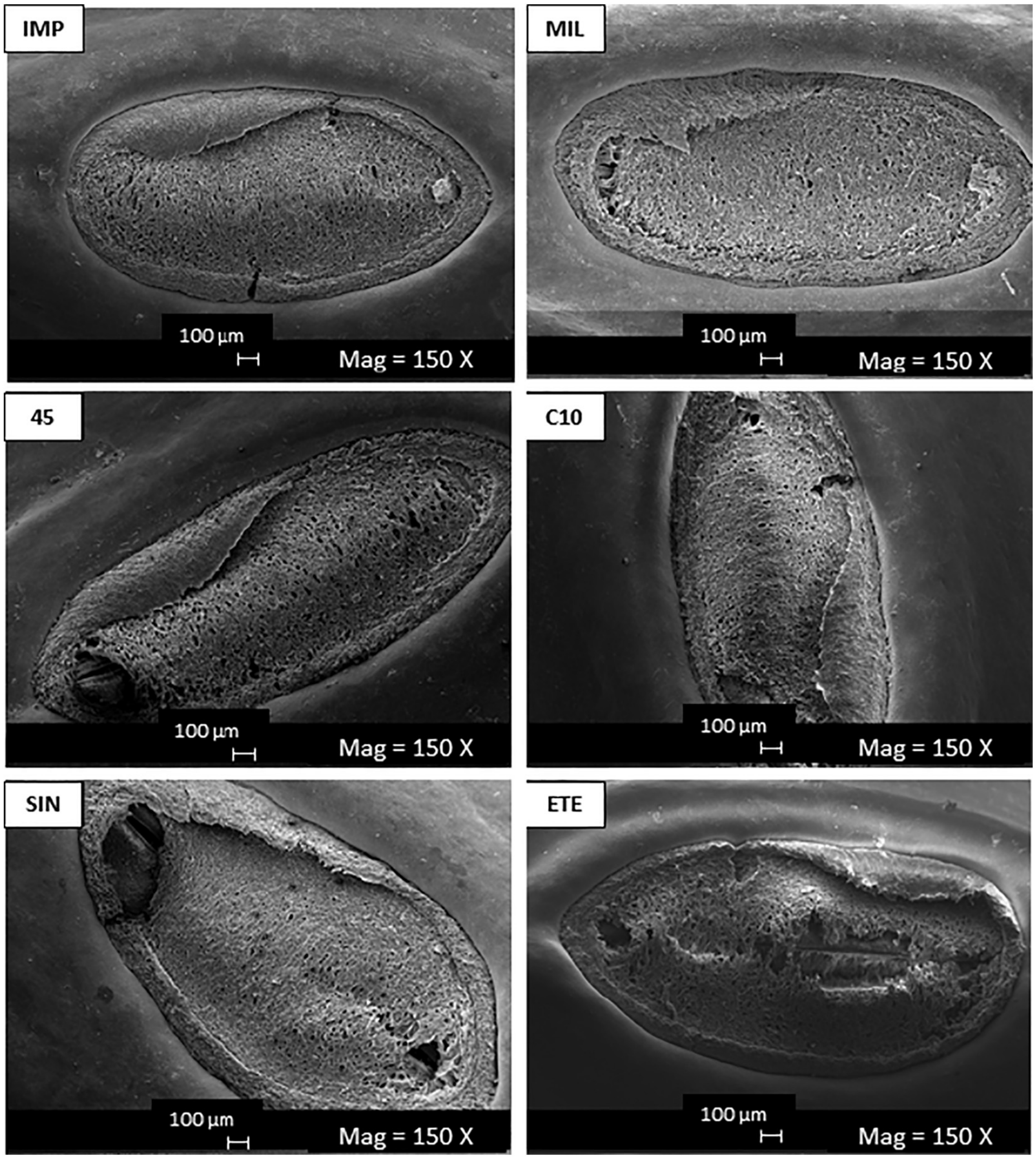

Fig. 6. Microphotography of the hilum of carioca bean cultivars (*Phaseolus vulgaris*) (SEM, 20 kV; 150× of magnifications – the 100 μm bar is shown in the figures).

beans (Swanson et al., 1985), adzuki beans (Miano & Augusto, 2015) and mung beans (Miano et al., 2016).

As observed, only little differences were detected: the seed coat of IAC Eté cultivar showed a difference in its compactness probably causing the longer lag phase time; and some cultivar showed differences in the aperture of the micropyle. However, no relation with the hydration kinetics was found. Despite having little microstructure differences, all cultivars showed different hydration kinetics (Table 2). For that reason, some chemical and physical characteristics were evaluated.

3.3. Correlation of the physical and chemical characteristics with the hydration kinetics

Table 3 shows the evaluated physical and chemical characteristics of all cultivars of carioca beans. Almost all the characteristics differ among the cultivar, being the seed coat thickness the only characteristic than does not differ.

Regarding chemical composition, there are significant difference ($p < 0.05$) among the cultivars. Table 3, shows that all the cultivars are mainly composed by starch and proteins, which is characterized by this variety of legume. The bean with the highest starch quantity was the IAC C10–2-4/41 cultivar, and the bean with the highest protein quantity was IAC Sintonia. These components are important for the technological properties of the flour. In addition, the beans have a considerable quantity of total fat, being the IAC Eté the bean with the highest quantity of this component. By simply comparing these results with the hydration kinetics (Fig. 2), it can be deduced that the hydration kinetics did not depended on the protein and starch contents and/or starch/protein ratio. In contrast, the bean with the highest total fat content has the slowest hydration being an important component that influences the process.

In addition, all carioca beans showed the presence of many kind of minerals with nutritional importance, such as Mg, Fe, Ca, Zn, etc. in difference quantities among the cultivars ($p < 0.05$) due to the different agronomic enhancements. For example, the iron quantity varies from 38.59 mg/kg (d.b.) (IAC C10-2-4/41) to 49.39 mg/kg (d.b.) (IAC Milenio). All the studied cultivar have less iron content compares to bio-fortified beans (Brigide, Canniatt-Brazaca, & Silva, 2014). Even so, they can be considered as a good source of iron, since the recommended dietary intake (RDI) of iron is 9.1 mg/day for men and 19.6 mg/day for

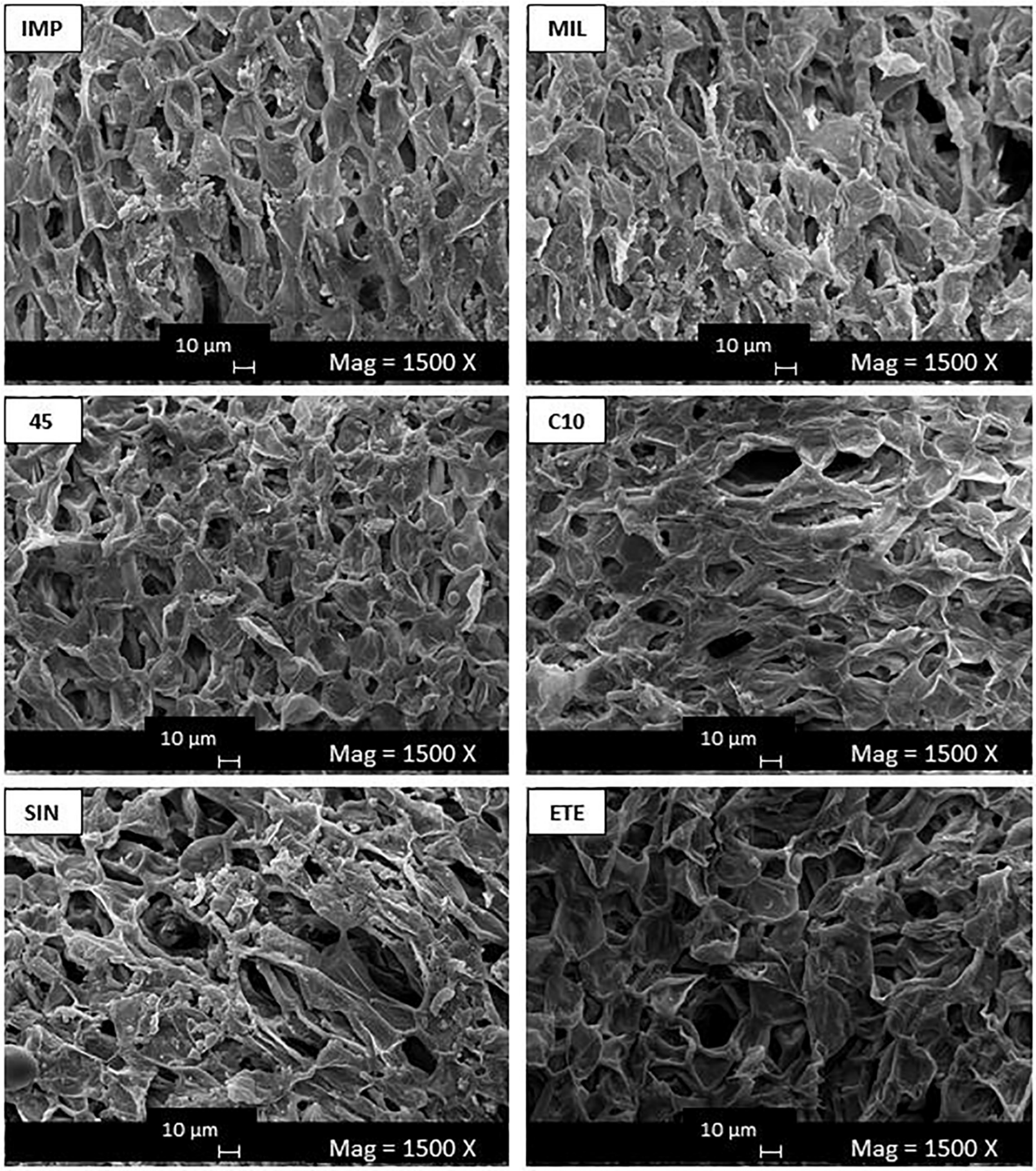

Fig. 7. Microphotography of a high magnification of the hilum of carioca bean cultivars (*Phaseolus vulgaris*) (SEM, 1500× of magnifications – the 10 µm bar is shown in the figures).

women (FAO/WHO). On the other hand, regarding Zn content (another important mineral), the studied cultivars have quantities higher that some fortified beans (Brigide et al., 2014). For instance, the cultivar IAC C10-2-4/41 has the higher quantity of zinc (49.50 mg/kg), being also and interesting source knowing that the recommended dietary intake (RDI) of zinc is 11 mg/day for men and 8 mg/day for women (FAO/WHO). However, different bioavailabilities can change the relative nutritional importance of these sources.

Further, the differences of mineral quantity could affect the hydration kinetics; however, comparing these results with the hydration kinetics is difficult through naked eye. Therefore, a multi factorial analysis (MFA) was subsequently used.

Regarding the physical characteristics (Table 3), each cultivar differs from others by the size, being the IAC Eté cultivar the smallest bean, which is related with the highest specific surface and the lowest weight from one hundred seed. On the other hand, the IAC C10-2-4/41 is the biggest bean. Further, the necessary work or energy to penetrate the bean (seed coat and cotyledon) is different for each cultivar, which could suggest differences about the hardness, compactness and/or elasticity of the beans than can also affect the hydration kinetics.

The seed coat is mainly related with the lag phase of the hydration process (Miano et al., 2015) and allows the beans to control the hydration rate and to maintain water for the germination (Bewley & Black, 1978). Therefore, FT-IR of the seed coat was evaluated using MFA to determine which functional groups would be related with the hydration characteristics. Fig. 10 shows the FT-IR profile of seed coat of all the Carioca bean varieties and their general description is presented below. A band of absorption at 903 cm^{-1} is clearly visible in the spectrum, representing the presence of dominant β-glucosidic bonds between the sugar units in these hemicellulosic fractions (Jiang et al., 2013). The stretching of C–O bonds in ether groups and in β-1,4 glycosidic bonds. Peaks at 1060 cm^{-1} are related to the C–O–C pyranose ring skeletal vibration and at 1160 cm^{-1} are related to the asymmetric stretching of C–O–C in sugar unities of cellulose and hemicellulose (Landim et al., 2013; Liu, Ni, Fatehi, & Saeed, 2011; Silvério, Flauzino Neto, Dantas, & Pasquini, 2013). Peaks around 1334, 1375 and 1443 cm^{-1} can be attributed to a typical signal of cellulose (Jin, Katsumata, Lam, & Iiyama, 2006). The band in the spectra near 1736 cm^{-1} is assigned mainly to the C–O stretching vibration of the carbonyl and acetyl groups in the xylan component of hemicelluloses

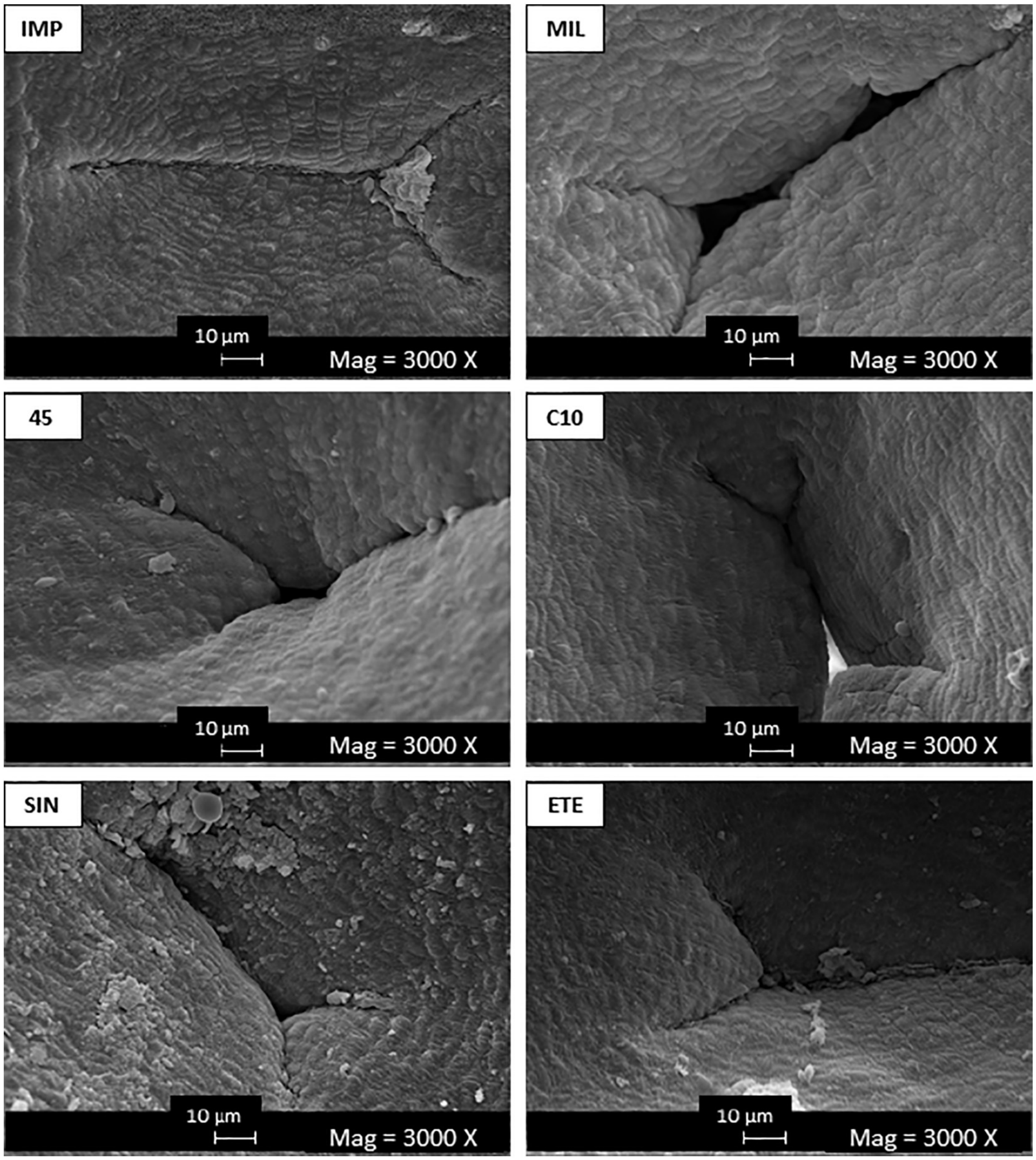

Fig. 8. Microphotography of the micropyle of carioca bean cultivars (*Phaseolus vulgaris*) (SEM, 20 kV; 3000× of magnifications – the 10 µm bar is shown in the figures).

and in the lignin. In the same spectrum, the band near 1250 cm^{-1} could correspond to the axial asymmetric strain of ═C─O─C, which is commonly observed when ═C─O─ is presented, for instance in ether, ester, and phenol groups (Silvério et al., 2013; Siqueira, Bras, & Dufresne, 2010). In the carbonyl stretching region, the peak at 1644 cm^{-1} is attributed to the absorbed water (Jin et al., 2006). The region around 1200 cm^{-1} could correspond to the C-O-C of the ring bonds stretching and the region between 1230 and 1270 cm^{-1} may be represented by the C═O bond stretching in hemicellulose. The peak close to 3390 cm^{-1} could correspond to the absorption caused by the OH group stretching, which is caused by the inter and intra molecular hydrogen bonds. The peak close to 2900 cm^{-1} may correspond to the C─H absorption, which contains the CH, CH_2 and CH_3 bonds stretching and vibration (Jiang et al., 2013). The weak band at 1519 cm^{-1} can be an indicative of the presence of lignin, which can be attributed to the C═C aromatic skeletal vibration (Silvério et al., 2013; Sun, Xu, Sun, Fowler, & Baird, 2005; Xiao, Sun, & Sun, 2001).

Although many peaks, which represent different functional groups, only six of them had significant difference ($p < 0.05$) among the evaluated cultivars. These peaks are represented in Fig. 10 (~2924, ~1722, ~1643, ~1519, ~1442, ~1057 cm^{-1}).

Though, many characteristics are significantly different among the carioca bean cultivars, only some of them were correlated with the hydration kinetics characteristics. For that, the Multiple Factor Analisys (MFA) was performed to find these correlations. This analysis reduced all the dimensions (variables) into two dimensions, which, in this study, represented 63.36% (explained variance) of the whole information. Regarding (MFA) (Fig. 11), only few characteristics strongly correlate with the hydration kinetics characteristics. According to Evans (1996), values of the correlation coefficient higher than |0.6| represent a strongly correlation. Thus, intrinsic factors whose correlation coefficients (with the hydration characteristics) were less than |0.6|, did not have suitable correlation to be considered. The properties with strongly correlation were the total fat content, the relation protein/lipid, the potassium content, the specific surface and the energy to perforate the seed coat.

The MFA states that the total fat content has positive correlation with the lag phase hydration parameter ($r = 0.71$), which means that the higher the total fat content is, the longer the lag phase is. The lag phase time is related with the seed coat permeability and this

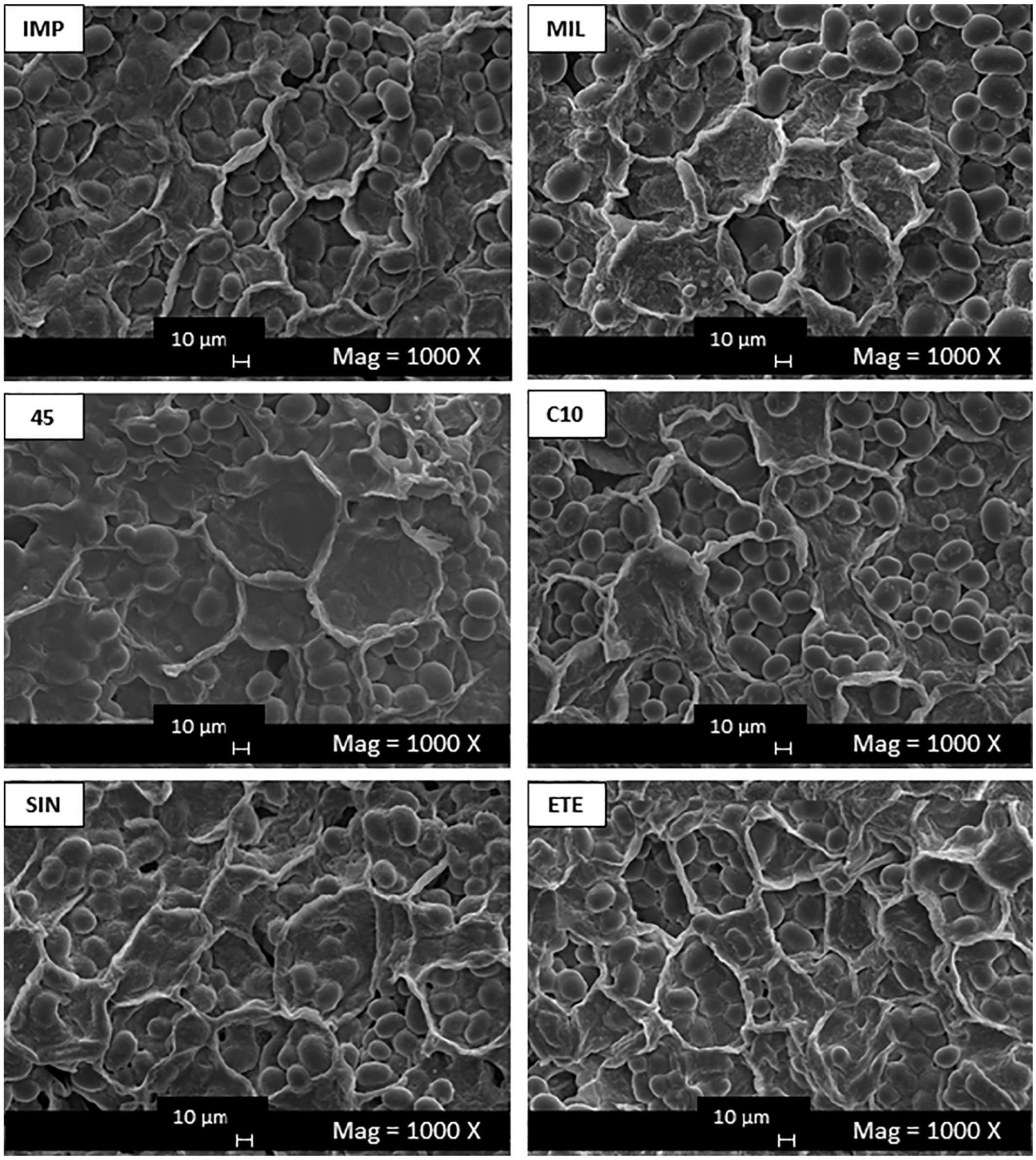

Fig. 9. Microphotography of the cotyledon of carioca bean cultivars (*Phaseolus vulgaris*) (SEM, 20 kV; 1000× of magnifications – the 10 μm bar is shown in the figures).

characteristic gives to the hydration kinetics the sigmoidal behavior (Miano & Augusto, 2015). In fact, the longer the lag phase is, the more sigmoidal the hydration kinetics is. The seed coat permeability depends on its composition and water activity. For instance, the seed coat has an external layer of wax (Graven, de Koster, Boon, & Bouman, 1997), which increases the impermeability to water as this layer is thicker. Further, as the water enters by the hilum, the total fat presented in the cotyledon could avoid the quick distribution of water to the seed coat delaying its hydration and permeability increase. This can be corroborated by comparing the Table 3 and the Fig. 3 where it can be seen that the IAC Eté cultivar is the bean with the highest value of total fat and the longest lag phase time.

The MFA (Fig. 11) also shows that the protein content is not correlated with the hydration characteristics, despite these molecules have good water affinity which could give the idea of improving the hydration process. This was probably due to the lipid presence which avoid the possible water absorption by the proteins. Therefore, the relation protein lipid (P/L) was analyzed. This relation was significantly correlated with the hydration characteristics. This parameter has negative correlation with the lag phase time ($r = -0.69$). It means that by having higher protein content in relation to the lipid content, the lag phase time will be shorter and the hydration process will be faster, as the protein has better affinity to water (Sefa-Dedeh & Stanley, 1979) and its quantity in relation to the lipid quantity is enough to favor the water entrance.

The MFA also correlated the potassium content (K) positively with the lag phase time ($r = 0.70$). The presence of potassium in the seed is important for some reasons such as activating many germination enzymes and controlling the pH enhancing the enzyme activity (Sodek, Lea, & Miflin, 1980). After germination, potassium increases the water use efficiency of seedlings (Bradbury & Malcolm, 1977) and enhance osmotolerance of the generated plant (Rascio et al., 2001). In addition, addition, potassium helps reduce the hard-to-cook phenomena by reducing the formation of calcium and magnesium pectates (Srilakshmi, 2007). All these effects could be desirable for the seed quality. However, for the hydration kinetics could be or not related with the hydration kinetics. Unfortunately, this work did not find an explanation of this results. Therefore, futures studies relating potassium and hydration kinetics of grains could be performed.

Another significant factor is the specific surface which was

Table 3
Chemical composition and physical characteristics of the studied cultivars of carioca beans (*Phaseolus vulgaris*). The results are represented by the mean ± the standard deviation. Letters represent Tukey Test mean comparison ($p < 0.05$) after performing ANOVA analysis.

Cultivar	IAC Milenio (MIL)	IAC Imperador (IMP)	IAC 45/57–7–3-1/4 (45)	IAC C10–2-4/41 (C10)	IAC Sintonia (SIN)	IAC Eté (ETE)
Whole bean chemical composition						
starch (% d.b.)	35.9 ± 1.2^{b}	36.3 ± 0.3^{b}	36.9 ± 0.4^{b}	39.2 ± 0.3^{a}	34.6 ± 0.9^{bc}	33.1 ± 0.6^{bc}
protein (% d.b.)	19.5 ± 0.4^{c}	19.5 ± 0.2^{c}	20.3 ± 0.2^{bc}	20.4 ± 0.3^{b}	21.9 ± 0.4^{a}	20.5 ± 0.3^{b}
fat (% d.b.)	1.5 ± 0.1^{c}	1.6 ± 0.1^{bc}	1.6 ± 0.1^{b}	1.6 ± 0.1^{bc}	1.6 ± 0.1^{bc}	1.9 ± 0.1^{a}
Starch/Protein (S/P)	2.1 ± 0.1^{ab}	2.1 ± 0.1^{ab}	2.1 ± 0.04^{b}	2.2 ± 0.1^{a}	1.8 ± 0.1^{c}	1.8 ± 0.1^{c}
Protein/Lipid (P/L)	12.9 ± 0.5^{b}	12.3 ± 0.3^{b}	12.5 ± 0.2^{b}	13.2 ± 0.6^{ab}	14.1 ± 0.3$_{a}$	10.6 ± 0.2^{c}
ash (% d.b.)	5.5 ± 0.1^{a}	5.4 ± 0.1^{ab}	5.4 ± 0.1^{b}	5.1 ± 0.1^{c}	5.1 ± 0.1^{c}	5.4 ± 0.1^{ab}
C (% d.b.)	40.0 ± 0.1^{c}	40.5 ± 0.2^{bc}	40.2 ± 0.1^{c}	40.4 ± 0.2^{bc}	40.8 ± 0.3^{ab}	40.9 ± 0.1^{a}
N (% d.b.)	3.29 ± 0.02^{c}	3.33 ± 0.04^{c}	3.42 ± 0.04^{bc}	3.51 ± 0.03^{b}	3.68 ± 0.05^{a}	3.50 ± 0.05^{b}
Mg (g/kg d.b.)	0.92 ± 0.04^{a}	0.87 ± 0.03^{ab}	0.89 ± 0.04^{a}	0.94 ± 0.03^{a}	0.78 ± 0.03^{c}	0.78 ± 0.02^{bc}
P (g/kg d.b.)	3.48 ± 0.07^{c}	3.91 ± 0.04^{b}	4.26 ± 0.04^{a}	4.19 ± 0.05^{a}	3.30 ± 0.06^{d}	3.86 ± 0.02^{b}
S (g/kg d.b.)	1.40 ± 0.05^{bc}	1.47 ± 0.02^{ab}	1.48 ± 0.02^{a}	1.54 ± 0.02^{a}	1.35 ± 0.01^{c}	1.47 ± 0.03^{ab}
K (g/kg d.b.)	8.07 ± 0.07^{b}	8.10 ± 0.11^{ab}	8.10 ± 0.13^{ab}	7.89 ± 0.17^{b}	7.68 ± 0.06^{c}	8.38 ± 0.03^{a}
Ca (g/kg d.b.)	0.93 ± 0.08^{a}	0.87 ± 0.11^{ab}	0.73 ± 0.08^{ab}	0.58 ± 0.06^{b}	0.76 ± 0.15^{ab}	0.76 ± 0.14^{ab}
Mn (mg/kg d.b.)	13.78 ± 0.43^{de}	28.5 ± 0.11^{a}	24.79 ± 1.16^{b}	22.13 ± 1.29^{c}	15.39 ± 0.91^{d}	12.94 ± 1.24^{e}
Fe (mg/kg d.b.)	49.39 ± 0.81^{a}	40.05 ± 0.64^{b}	39.85 ± 3.19^{b}	38.59 ± 1.46^{b}	36.98 ± 0.9^{b}	39.90 ± 0.68^{b}
Cu (mg/kg d.b.)	3.18 ± 0.17^{ab}	2.58 ± 0.27^{bc}	2.69 ± 0.32^{bc}	2.47 ± 0.08^{bc}	3.48 ± 0.47^{a}	2.19 ± 0.23^{c}
Zn (mg/kg d.b.)	33.38 ± 2.5^{b}	39.93 ± 5.85^{ab}	41.36 ± 5.96^{ab}	49.50 ± 1.56^{a}	33.01 ± 5.37^{b}	33.15 ± 4.1^{b}
Seed coat FT-IR (absorbance related to wave number peaks)						
~2924 cm^{-1}	0.240 ± 0.001^{ab}	0.191 ± 0.001^{c}	0.150 ± 0.002^{d}	0.252 ± 0.018^{a}	0.155 ± 0.001^{d}	0.232 ± 0.002^{b}
~1722 cm^{-1}	0.029 ± 0.001^{a}	0.022 ± 0.000^{c}	0.017 ± 0.000^{e}	0.027 ± 0.002^{b}	0.021 ± 0.001^{c}	0.026 ± 0.000^{b}
~1643 cm^{-1}	0.172 ± 0.002^{b}	0.136 ± 0.001^{c}	0.113 ± 0.001^{d}	0.188 ± 0.013^{a}	0.129 ± 0.001^{c}	0.168 ± 0.001^{b}
~1519 cm^{-1}	0.260 ± 0.002^{b}	0.211 ± 0.001^{c}	0.168 ± 0.002^{d}	0.287 ± 0.020^{a}	0.178 ± 0.001^{d}	0.253 ± 0.002^{b}
~1442 cm^{-1}	0.203 ± 0.002^{b}	0.166 ± 0.001^{c}	0.128 ± 0.001^{d}	0.220 ± 0.016^{a}	0.133 ± 0.001^{d}	0.191 ± 0.001^{b}
~1057 cm^{-1}	0.197 ± 0.001^{b}	0.172 ± 0.001^{c}	0.129 ± 0.001^{d}	0.218 ± 0.016^{a}	0.124 ± 0.001^{d}	0.188 ± 0.001^{b}
Physical properties						
Specific Surface – SS (m^2/m^3)	0.88 ± 0.04^{b}	0.87 ± 0.04^{b}	0.84 ± 0.03^{c}	0.80 ± 0.03^{d}	0.85 ± 0.04^{bc}	1.03 ± 0.07^{a}
1000 grain weight – 1000w (g)	257 ± 5^{c}	269 ± 5^{c}	289 ± 5^{b}	330 ± 6^{a}	266 ± 3^{c}	187 ± 3^{e}
Seed Coat Thickness (mm)	0.09 ± 0.07^{a}	0.10 ± 0.02^{a}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.11 ± 0.02^{a}	0.08 ± 0.01^{a}
Work sc - Wsc (N·mm)	12 ± 2^{ab}	8 ± 1^{d}	13 ± 2^{a}	9 ± 1^{cd}	11 ± 2^{bc}	9 ± 1^{cd}
Work c - Wc (N·mm)	19 ± 2^{b}	19 ± 2^{bc}	15 ± 4^{c}	24 ± 2^{a}	19 ± 3^{bc}	21 ± 3^{ab}

positively correlated with the lag phase time ($r = 0.60$). Therefore, the higher the specific surface is, the slower the hydration process will be since the lag phase will be longer. However, Montanuci, Jorge, and Jorge (2015), stated the opposite, as the grain specific surface increases, the hydration of a bean is faster. Nevertheless, this premise was stablished during the hydration of a cereal grain (barley kernels), being not demonstrated. It is well-known that the larger the area is, the faster the mass transfer is. This statement is fulfilled when the mass transfer is

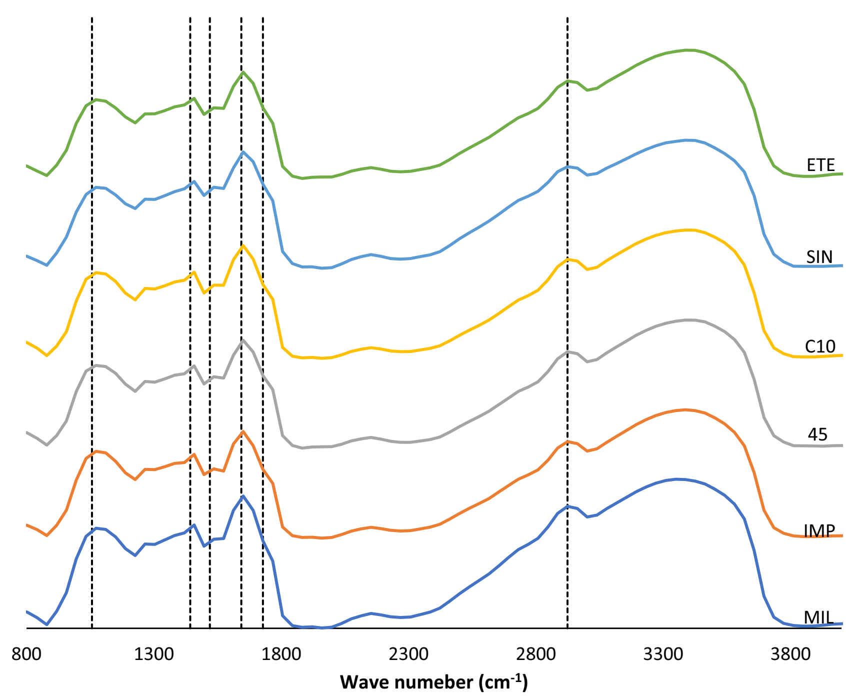

Fig. 10. Absorption FT-IR spectra in the 4000–400 cm^{-1} region of the of carioca bean cultivars seed coat (each spectrum represents the mean of five replicates). The discontinuous lines represent the peaks wave number where there was significant difference among the bean cultivars (~2924, ~1722, ~1643, ~1519, ~1442, ~1057 cm^{-1}).

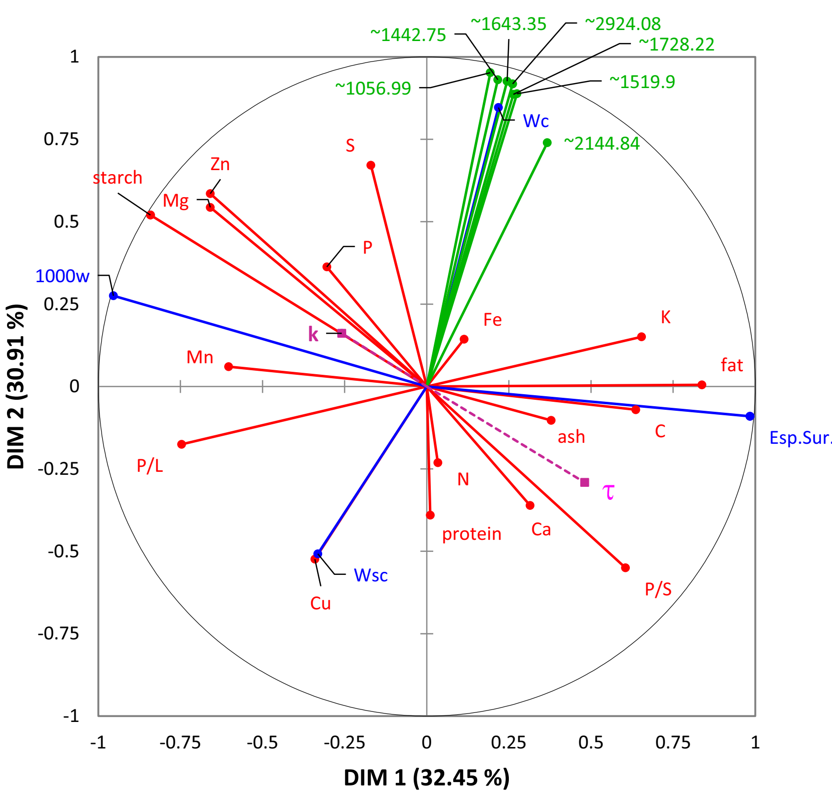

Fig. 11. Multiple factor analysis (MFA) represented on two principal components (63.39% of explained variance). Scatter plot of variables: chemical composition, physical characteristics, seed coat components functional groups and hydration equation parameters (legend is presented in Table 3). Red lines represent the chemical composition components, blue lines represent the physical characteristics, green lines represent the functional groups and purple lines represent the hydration kinetics characteristics. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

homogenous through all the surface. However, most of the pulses have an impermeable seed coat being a barrier to water (Miano & Augusto, 2018). As explained, the seed coat would need to be hydrated especially from the inside by the water that enters by the hilum to increase its permeability to water. Therefore, having high specific surfaces means that there is more area of seed coat to be hydrated delaying the whole process. In contrast, cereal grains have a permeable pericarp (seed coat) allowing water to pass. In this case, since the seed coat is not a barrier, having high specific surfaces would enhance the hydration process corroborating the hypothesized by Montanuci et al. (2015).

The necessary energy to perforate the cotyledon was negatively correlated with the hydration rate (k) ($r = -0.70$). During the penetration assay, the probe has to perforate the macrosclereid layer, which has long cells positioned parallel to the probe, and it has to perforate the osteoesclereids cells, which are also long cell parallel to the probe, as well as the parenchyma formed by dead cell perpendicular to the probe. It is expected that the probe can perforate the macroesclereid and osteclereid layer easily, unless their cells are well joined together. Further, the parenchyma layer might give resistance to be perforated. Therefore, it is probably that the seed coats, with less necessary energy to be perforated, have less joined cells, facilitating the pass of water once the seed coat become permeable. Thus, a seed coat with more joined cells would reduce the hydration rate (k).

As can be seen, all these results make clear the fact that the hydration of legumes is a complex process. Firstly, the presence of an impermeable seed coat cause the sigmoidal behavior (lag phase presence) of the hydration behavior, which is probably due to its composition (wax or other components) and structure (cells junction and compactness) can cause differences on the curve shape. Then, the differences on the composition (protein/lipid relation) and physical properties (specific surface) of the whole bean can also change the hydration behavior. Further, the lag phase hydration kinetics parameter was the most correlated with the intrinsic properties, suggesting the importance of the seed coat in the process. Consequently, there is the need of studying other intrinsic properties focusing in this structure as it is responsible for the sigmoidal behavior. Moreover, in spite of the significant differences among physical and chemical characteristics, the equilibrium moisture content was not changed. However, there could be more intrinsic factors related to this parameter. Finally, the hydration rate was different among the studied cultivars and, as observed, whatever the agronomic enhancement is performed (for producing new cultivars with higher resistances or higher quantity of some nutrients), it affects the hydration kinetics, which is an important property to be studied, considering the pulse industrialization and consumption.

4. Conclusions

Although being from the same species and variety of legume, the different carioca bean cultivars have different hydration kinetics behavior. All of them have a sigmoidal shape of hydration, but with different intensities (different lag phase time and hydration rate). It was not found strong relation between the beans microstructure and the hydration kinetics. However, some chemical and physical properties strongly correlated with the hydration kinetics: the total fat content, the protein/lipid relation, the potassium content, specific surface and necessary energy to perforate the seed coat. Most of them correlated with the lag phase time – related to the seed coat. The total fat content, the potassium content and the specific surface correlated positively with the lag phase time. Further, the protein/lipid relation correlated negatively with the lag phase time. In addition, the necessary energy to penetrate the seed coat correlated negatively with the hydration rate. A logic explanation of these correlations was given, except for the potassium content, which no explanation was found. It was concluded that probably these intrinsic factors are the most important during the hydration process of carioca beans.

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APPENDIX E: USING ULTRASOUND FOR IMPROVING HYDRATION AND DEBITTERING OF ANDEAN LUPIN GRAINS

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Using ultrasound for improving hydration and debittering of Andean lupin grains

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Using ultrasound for improving hydration and debittering of Andean lupin grains

Alberto Claudio Miano, Meliza Lindsay Rojas and Pedro Esteves Duarte Augusto*

Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil

* **Information for contact:**

Telephone: +55 (19) 34478692

Fax: : +55 (19) 34294288

E-mail: cmiano@usp.br (AC Miano), mrojas@usp.br (ML Rojas), pedro.ed.augusto@usp.br (PED Augusto)

Avenida Pádua Dias, 11 - Piracicaba/SP - 13418-900 Brazil

Short title: US accelerates hydration and debittering of pulses

Abstract

This work presents results about the potential use of ultrasound technology on the debittering process of Andean lupin grains (*Lupinus albus* Sweet.). The hydration process was conducted conventionally and also assisted by the ultrasound technology (ultrasonic water bath, 25 kHz, 41 W/L). Further than the hydration behavior, the alkaloid content was evaluated. As results, the hydration kinetics of this grain showed a sigmoidal behavior, which was accelerated almost 40% using ultrasound. The lag phase was reduced about 13%, and the equilibrium moisture content was increased about 14%. Finally, by using ultrasound, the alkaloid extraction was also improved after the hydration process, extracting 21% more compared to the conventional process. It was concluded that ultrasound technology accelerates not only the hydration process but also the extraction of alkaloids.

Practical Applications

Andean lupin is a pulse with potential importance for human and animal alimentation due to its high quantity of proteins. However, this pulse has a considerable quantity of undesirable alkaloids, that are bitter and toxic. Therefore, a debittering process is required. Further, before debittering, hydration of the grains is required, which is a long and batch process. This work demonstrated that both the hydration and the debittering processes can be enhanced by ultrasound. Consequently, ultrasound technology would have a double application during hydration process of Andean lupin grains.

Keywords: hydration, ultrasound, *Lupinus mutabilis*, debittering, legumes

1. Introduction

Andean lupin (*Lupinus mutabilis* Sweet) is a grain that growths in the Andean region of South America. This grain is characterized by its high protein content (44.3 g/100g) and fat content (16.5 g/100g), with important unsaturated fatty acids (omega-9, omega-6 and omega-3) (Jacobsen and Mujica, 2006). Therefore, this is a promising protein source for both animal and human nutrition (Güémes‐Vera et al., 2008). However, this grain has a high quantity of alkaloids that are toxic, which makes it unsuitable for direct consumption (Ruiz, 1978). For that reason, the debittering process is necessary before the grains processing and consumption. This process is conducted in different stages, being the hydration the first stage for facilitating the alkaloid extraction in the following steps (Carvajal-Larenas et al., 2013). However, the hydration is a time consuming and discontinuous process. Consequently, its enhancement is very desirable.

The ultrasound technology has shown good results enhancing different mass transfer processes, especially the grain hydration process. For instance, this technology accelerated the hydration of chick peas (Yildirim et al., 2011), common beans (Ulloa et al., 2015), sorghum kernels (Patero and Augusto, 2015), navy beans (Ghafoor et al., 2014), corn kernels (Miano et al., 2017a), mung beans (Miano et al., 2016b), white kidney beans (Miano et al., 2018) and carioca beans (Miano and Augusto, 2018b). Ultrasound consists of using acoustic waves with frequencies between 20 kHz and 500 kHz and power higher than 1 W/cm^2, which by both direct and indirect effects causes physical-chemical and structural changes on food (Awad et al., 2012). Further, it also affects the mass transfer phenomena. The direct effects of using this technology are related to the acoustic wave traveling, which causes pressure differences in the food (Miano et al., 2017b). By contracting and expanding the tissues, the “sponge effect” takes place (Floros and Liang, 1994). Furthermore, as proposed by (Patero and Augusto, 2015), an “inertial flow” can be expected through the microchannels. On the other hand, the indirect effects are related with changes on the food structure caused by the acoustic cavitation, as tissue and cell disruption, with the consequent formation of micro cavities and micro channels, improving the mass transfer phenomena (Miano et al., 2016a, Rodrigues et al., 2009).

Although there are some works improving the hydration process using the ultrasound technology, there is neither any work in Andean lupin hydration, nor about the extraction of alkaloids using this promising technology. Therefore, the aim of this work was to study the effect of ultrasound technology on the hydration kinetics of Andean lupin grains leading to improve the alkaloid extraction.

2. Material and Methods

2.1 Raw Material

Peruvian Andean lupin (*Lupinus mutabilis* Sweet) grains were acquired in Trujillo (Perú). The grains presented a moisture of 3.65 ± 0.01% dry basis, dimensions of 6.02 ± 0.35 mm, 8.39 ± 0.41 mm and 9.98 ± 0.64 mm of thick, width and length respectively. The grains were sorted, eliminating cracked and stained grains, before the experiments.

2.2 Hydration Process

2.2.1 Conventional hydration

The methodology proposed by Miano and Augusto (2018a) was used. This methodology is based on determining the hydration kinetics from the moisture increment measurement during hydration process by mass balance. The hydration process was performed at 25 ± 1 °C using a thermostatic water bath (Dubnoff MA 095 MARCONI, Brazil). It is important to highlight that any mass of solid extraction during hydration process was negligible. This process was performed in triplicate.

2.2.2 Ultrasound assisted hydration

For the ultrasound assisted hydration process, an ultrasonic bath that consisted of nine piezoelectric elements arranged underneath the container (Q13/25, Ultronique Brazil) was used. The ultrasonic bath operated at 25 kHz of frequency and 41 W/L volumetric power (determined using the calorimetric method (Mason and Peters, 2004)). The process was performed at 25 ± 1 °C using 4 L of distilled water and 160 g of grains maintaining the same sample/water ratio as the conventional processing. The grains were placed in net bags at the bottom of the ultrasonic bath. The data were obtained as in the conventional hydration process.

2.2.3 Mathematical description

As the Andean lupin hydration kinetics followed a sigmoidal behavior, the Kaptso et al. Model (Equation 1; (Kaptso et al., 2008)) was used. The experimental data were fitted to Equation 1 using the Levenberg-Marquardt algorithm in Statistica 12.0 (StatSoft, USA) software at a significance level of 5%.

$$M_t = \frac{M_{eq}}{1+exp[-k\cdot(t-\tau)]} \tag{1}$$

Where M_t is the grain moisture in dry basis (% d.b.) at hydration time (t); M_{eq} is the equilibrium moisture; τ (min) is the lag phase, defining the necessary time to reach the inflection point of the curve; and k (min^{-1}) is the parameter of water absorption rate.

Finally, the determination coefficient (R^2) and the plot of the calculated data (M_{model}) from Equation 1 versus the experimental data ($M_{experimental}$) were used to evaluate the fitting (Motulsky and Christopoulos, 2004). The model fitting was also evaluated through the linear regression of those data (Equation 2) and the obtained parameters (the slope “a”, where values close to one is desired, the intercept “b”, where values close to zero is desired and the coefficient of determination - R^2).

$$M_{model} = a \cdot M_{experimental} + b \qquad (2)$$

2.3 Alkaloid content determination

The alkaloid content of the grains, hydrated with and without ultrasound technology, was determined using the Ecuadorian Standard Method (INEN 2 390:2004) (INEN, 2005). This method is based on extracting the alkaloids using chloroform as solvent, which are then volumetrically quantified by titrating with Sulphur acid.

2.4 Statistical analysis

When pertinent, analysis of variance (ANOVA), Tukey’s means comparison test and t-student mean comparison test were performed to the treatments using Statistica 12.0 (StatSoft, USA) software.

3. Results and discussion

As expected, the Andean lupin grains showed a sigmoidal hydration behavior (Miano et al., 2015). This behavior is caused by the variable seed coat permeability to water (Figure 1A). Figure 1B schematizes how the water enters into Andean lupin grains. As most of the legume grains has seed coats with partial permeability to water, the water has to firstly enter by other structures such as the hilum and/or micropyle (Step I). Then, as the seed coat become hydrated from inside, its permeability change letting the water to cross by diffusion (Miano and Augusto, 2015) and reaching the maximum hydration rate of the process (Step II). Finally, the cotyledon is hydrated until reaching the maximum water holding capacity (equilibrium moisture content) (Step III). Consequently, this water pathway of entrance is the cause of the sigmoidal behavior (Miano and Augusto, 2018a), successfully fitting the data to the equation of Kaptso et. al. (Table 1).

Kaptso et al. equation has three parameters, which characterized the hydration kinetics of beans. The parameter k is associated to the hydration rate, represented by the inclination of the curve (Figure 1B). In the case of Andean lupin the value of this parameter was 0.013 ± 0.001 min^{-1}, similar to many legume grain as dark red kidney beans, fava beans and white kidney beans (Miano et al., 2017c). The parameter τ is related to the necessary time to change the permeability of the seed coat (Figure 1B), when it become completely permeable to water due to the change on its components from the glassy to the rubbery state (Miano and Augusto, 2015). This characteristic only happens in beans with sigmoidal behavior of hydration kinetics as Andean lupin. In this grain the value of τ was 193 ± 12 min. The parameter M_{eq} represents the equilibrium moisture content, whose value was 140 ± 3% d.b. for Andean lupin. This value is high comparing to other legume grains and cereals (Miano et al., 2017c) probably due to the high quantity of proteins of this grain (Jacobsen and Mujica, 2006) which have more affinity to water.

Furthermore, Figure 1 shows that ultrasound technology (41 W/L and 25 KHz) significantly improved the hydration process of Andean lupin. This technology reduces the hydration process time almost 40% at 25 °C from 480 min to 300 min. This value is similar to the work of Miano et al. (2017a), that reduced 35% of the processing time (25 °C) for corn kernels (*Zea mays)*; Patero and Augusto (2015), that reduced 40% of the processing time (25 °C) for sorghum kernels (*Sorghum sp*); Ghafoor et al. (2014) that reduced 45% of the processing time (16 °C) for navy beans (*Phaseolus vulgaris*); Miano et al. (2016b) that reduced 25% of the processing time (25 °C) for mung beans (*Vigna radiata*); and to the work of Miano et al. (2018) that reduced 30% of processing time (30 °C) for white kidney beans (*Phaseolus vulgaris)*. It should be mentioned, that this result depends on the applied volumetric power of ultrasound, since as the power is increased, the hydration is accelerated (Yildirim et al., 2011). In addition, the grain structure-composition is responsible for the hydration kinetics characteristics (velocity and maximum absorbed water) (Miano et al., 2017c), which can affect how ultrasound enhances the process.

In addition, it is notice in Figure 1 that the hydration improvement is better as the process time passes. The first explanation for this behavior is discussed by (Miano et al., 2016a), as the acoustic cavitation is more probable to take place at higher water activity. Furthermore, the acoustic impedance difference between water and food can also explain this behavior. In fact, when the acoustic impedance difference between two mediums is very different (e.g. gas to solid or water to solid), the acoustic wave is reflected (Mason et al., 2005). Consequently, at the beginning of the process, the grains are very compact solid causing probably the acoustic wave reflecting. However, as the grains gain water, their acoustic impedance is closer to the acoustic impedance of water. Consequently, the acoustic energy losses less energy and the effect of ultrasound can be higher.

Therefore, this work corroborates the effectiveness of ultrasound to enhance the hydration process in grains with sigmoidal behavior. Now it is presented how ultrasound affected the hydration behavior characteristics.

The lag phase time (τ) of the hydration process was reduced ($p<0.05$) from 193 ± 11 min to 169 ± 8 min (Table 1). This lag phase is mainly caused by the seed coat, whose water permeability is function of the water activity, resulting in a slow and specific water pathway into the grain (Swanson et al., 1985, Miano et al., 2015, Meyer et al., 2007). Therefore, the ultrasound technology probably accelerates the internal hydration of the seed coat due to the already explained direct mechanisms. As the ultrasound wave passes through the grain, its pores and spaces expand and compress due to the pressure differences, pumping the water to the inside. It is particularly important in the space between the grain cotyledon and seed coat. This caused a rapid filling of this space with water, thus reducing the lag phase. Another ultrasound mechanism that improves the mass transfer phenomenon is formation of micro channel due to mechanical stress of the food structure due to ultrasound and the acoustic cavitation. However, as this mechanisms is more likely to happen in food with high water activities (Miano et al., 2016a), the micro channel formation is probably negligible for the lag phase time reduction.

In addition, the equilibrium moisture content (M_{eq}) of the Andean lupin grain was significantly ($p<0.05$) increased by ultrasound, from 140 ± 3% d.b. to 160 ± 3% d.b (Table 1). This result was similar for corn kernels (Miano et al., 2017a), chick peas (Yildirim et al., 2011) and sorghum kernels (Patero and Augusto, 2015), white kidney beans (Miano et al., 2018) and carioca beans (Miano and Augusto, 2018b). As the grains absorb water, their water activity increases, increasing the probability of the acoustic cavitation takes place and forming micro channels. The increase of M_{eq} can be a result of different phenomena. For example, the micro channel formation results in more sites to absorb and keep the water, as well as the micro channel expansion and compression can unblock the grain pores (Miano et al., 2017a) and also expel the entrapped air, allowing more water to be hold. Further, previous works observed that the ultrasound increased the solubility of legume proteins (Jambrak et al., 2009, Jiang et al., 2014), which can also explain the increment on the water holding capacity.

The hydration rate (k) was not significantly changed ($p>0.05$), although Table 1 shows this parameter tends to increase. In fact, the increase in the water influx rate due to the ultrasound technology was demonstrated for other grains, such as corn kernels (Miano et al., 2017a), chick peas (Yildirim et al., 2011), sorghum kernels (Patero and Augusto, 2015), common beans (Ulloa et al., 2015) and navy beans (Ghafoor et al., 2014), all of them with downward concave shape behavior fitted to Peleg equation (Peleg, 1988). Furthermore, the increase of the hydration rate by ultrasound was demonstrated for mung beans (Miano et al., 2016b), white kidney beans and carioca beans, all of them with sigmoidal behavior fitted with the equation proposed by Kaptso et al. (2008). In addition, the lack of significance was probably due to the high standard deviation for the conventional hydrated grains, since despite the controlled conditions of the process, there are intrinsic factors of each grain, that result in differences among them (Marcos Filho, 2005). However, apparently, ultrasound homogenized the grains properties, as is observed in Figure 1, which could be an interesting future study.

Finally, Figure 2 shows that the conventional hydration process reduced almost 45% of the grain alkaloid content, from 3.46 ± 0.16% d.b. to 1.91 ± 0.08% d.b. However, when the grains were hydrated with ultrasound, the alkaloid content was reduced to 1.51 ± 0.09% d.b.. Therefore, the ultrasound enhanced the process almost 21%. This is an interesting result since it means that if the ultrasound process is used in the following alkaloid extraction stages, the debittering process time could be reduced, as well as the use of water.

4. Conclusions

The ultrasound technology, besides the enhancement of the hydration process, has the potential to improve the debittering process of Andean lupin grains. In the present work, this technology reduced 40% the hydration process time, as well as enhanced the alkaloid extraction almost 21%, when compared to the conventional process. These results prove the possible use of ultrasound in many processes of grains industrialization.

Acknowledgments

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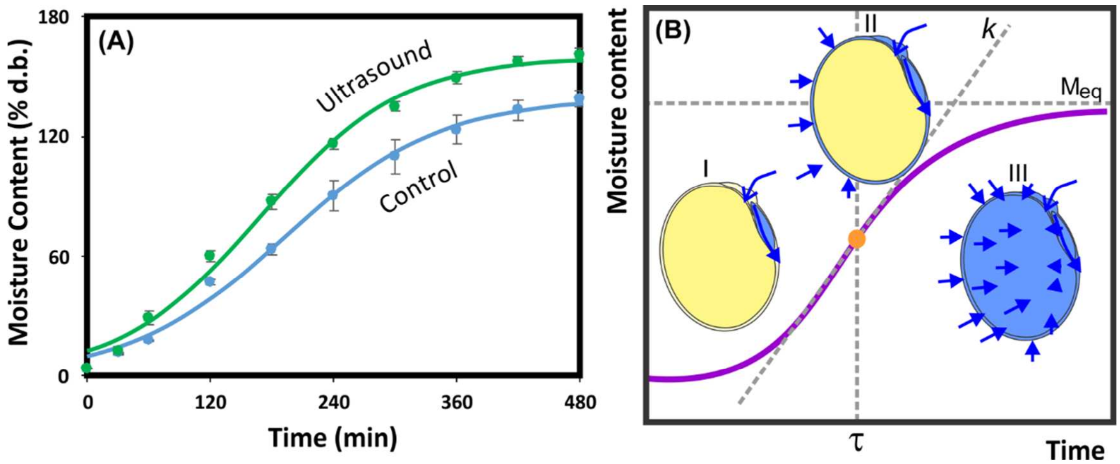

Figure 1. (A) Hydration of Andean lupin grains with (US) and without (CONTROL) ultrasound (25 °C, 41 W/L and 25 kHz). The dots are the experimental values; the bars are the standard deviation and the curves are the model values. **(B)** Schematic representation of the water pathway entrance in Andean lupin grains during the hydration process: the continuous line represents the hydration kinetics and the discontinuous lines represent the Kaptso et al model (Equation 1) parameter.

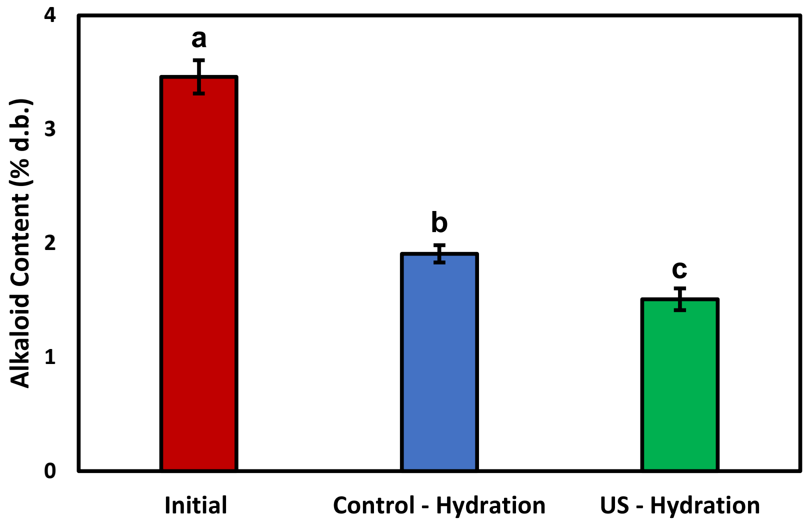

Figure 2. Alkaloid content before and after the hydration process with (US) and without ultrasound (Control) of Andean lupin. The vertical bars are the standard deviation and the letters represent Tukey test mean comparison ($p < 0.05$).

Table 1. Parameters of Kaptso et al. model (Equation 1) (mean ± standard deviation) and fitting statistics obtained from the hydration kinetics of Andean lupin grains. Lowercase letters indicate significant difference between the treatments with 95% of confidence.

Parameter	Control	Ultrasound
M_{eq} (% d.b.)	140 ± 3^b	160 ± 3^a
K (min^{-1})	0.013 ± 0.001^a	0.015 ± 0.001^a
τ (min)	193 ± 12^a	169 ± 8^b
R^2	>0.99	>0.99
a	0.98 ± 0.01	0.97 ± 0.01
b	-1.92 ± 0.40	-2.67 ± 0.09

APPENDIX F: ENHANCING MUNG BEAN HYDRATION USING THE ULTRASOUND TECHNOLOGY: DESCRIPTION OF MECHANISMS AND IMPACT ON ITS GERMINATION AND MAIN COMPONENTS

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Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components

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Alberto Claudio Miano, Jessica da Costa Pereira, Nanci Castanha, Manoel Divino da Matta Júnior & Pedro Esteves Duarte Augusto

The ultrasound technology was successfully used to improve the mass transfer processes on food. However, the study of this technology on the grain hydration and on its main components properties was still not appropriately described. This work studied the application of the ultrasound technology on the hydration process of mung beans (*Vigna radiata*). This grain showed sigmoidal hydration behavior with a specific water entrance pathway. The ultrasound reduced ~25% of the hydration process time. In addition, this technology caused acceleration of the seed germination – and some hypothesis for this enhancement were proposed. Moreover, it was demonstrated that the ultrasound did not change both structure and pasting properties of the bean starch. Finally, the flour rheological properties proved that the ultrasound increased its apparent viscosity, and as the starch was not modified, this alteration was attributed to the proteins. All these results are very desirable for industry since the ultrasound technology improves the hydration process without altering the starch properties, accelerates the germination process (that is important for the malting and sprouting process) and increases the flour apparent viscosity, which is desirable to produce bean-based products that need higher consistency.

The hydration process is an important step before many others grain process such as cooking, germination, extraction, malting and fermenting. It is a discontinuous and time spender process, being limiting in the industrial processing. Therefore, its improvement is very desirable.

In fact, many works have used higher soaking temperatures to enhance this process[1–8]. However, the use of high temperatures can change the properties of the grains components and alter their nutritional composition. In addition, temperatures can bring additional use of water for the heating system, as well as the amount of energy. Consequently, other technologies are being studied to improve the hydration process, being the ultrasound technology one of the most promising.

The ultrasound technology has been successfully used in many mass transfer processes in food, such as in drying, extraction, osmotic dehydration, desalting and hydration. The enhancement of the mass transfer by ultrasound is attributed to its direct and/or indirect effects, which depend on the food properties (porosity and water activity)[9]. The direct effects are related to the ultrasonic wave traveling through the food, which causes the expansion and compression of the medium. These effects are the called "sponge effect" (when the cells or the food matrix is compared to a sponge squeezed and released repeatedly) and the inertial flow (mass flow due to the wave propagation). The indirect effects are related to changes in the product structure caused by the acoustic cavitation, resulting in cell and matrix disruption, and then creating micro cavities (or micro channels) that improve the mass transfer[9–11].

In fact, the ultrasound technology was successfully used to enhance the hydration process of foods. However, it was studied only for a small number of grains, such as sorghum grains[12], navy beans[13], chickpeas[14], common

Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, 13418900, Brazil. Correspondence and requests for materials should be addressed to A.C.M. (email: cmiano@usp.br) or P.E.D.A. (email: pedro.ed.augusto@usp.br)

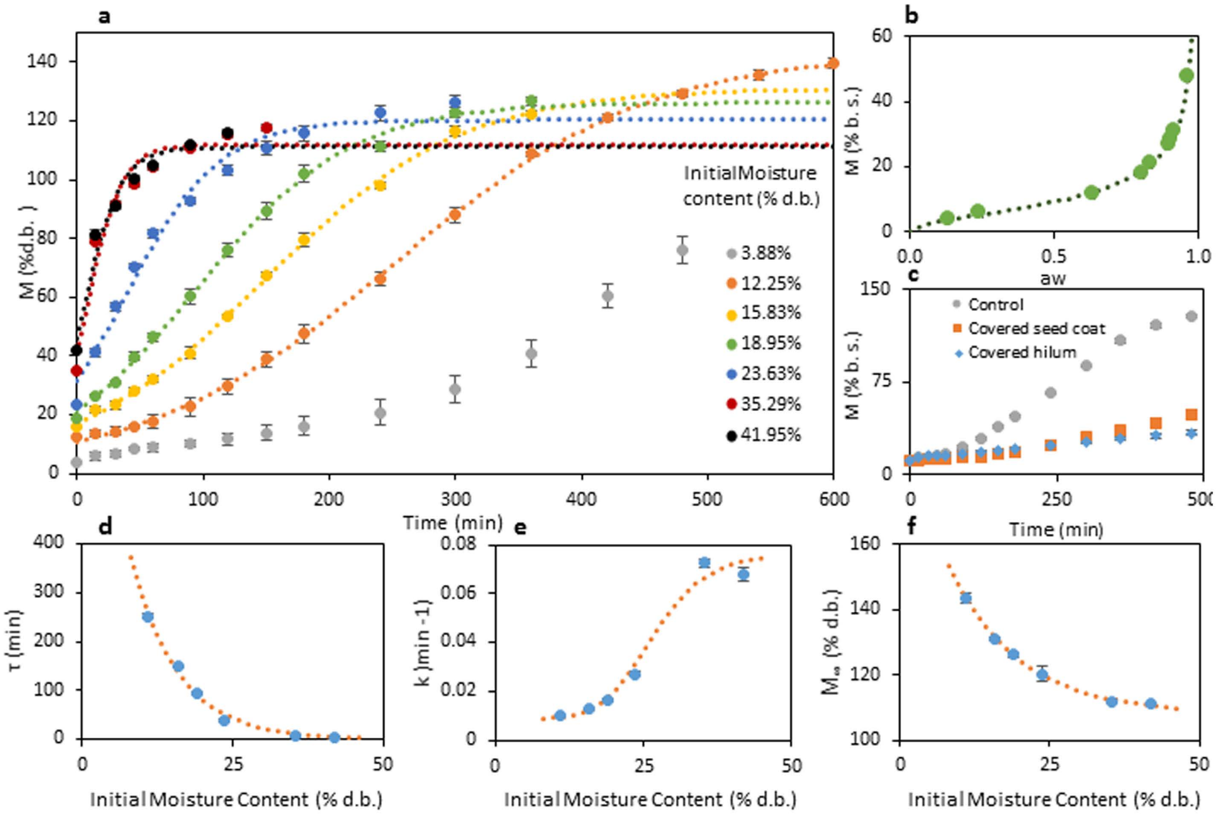

Figure 1. Mung bean hydration at 25 °C as function of its initial moisture content. The dots are the experimental values; the vertical bars are the standard deviation and the curves are the values obtained from the models. (**a**) Mathematical modeling using Kaptso *et al.* model (Equation 2) at different initial moisture contents. (**b**) Adsorption isotherm of mung bean (25 °C) (the data were modeled using the Oswin Model (Equation 1)). (**c**) Hydration (at 25 °C; 12.25% d.b) of mung bean under different treatments to explain the function of the seed coat and the hilum on the hydration kinetic. Effect of the initial moisture content on the Kaptso *et al.* parameters: (**d**) τ (Equation 3) (**e**) k (Equation 4) and (**f**) M_∞ (Equation 5).

beans[15] and corn kernels[16], as well as on the rehydration of other kind of food such as sea cucumber[17]. Even so, the application of this technology should still be studied, in special for grains, where the hydration process is the limiting step during the industrial processing.

Most importantly, once the positive effect of the ultrasound technology on the hydration process was already demonstrated for some foods, it is now necessary to conduct studies not only for further products, but also for those with different behaviors and purposes. Consequently, to demonstrate the involved mechanisms, and to evaluate the impact of this technology on selected properties and components of the product. For example, although the hydration of grains can show two behaviors (the downward concave shape (DCS) and the sigmoidal behaviors[18] – see further discussion), several grains with the downward concave shape hydration behavior and only one with sigmoidal hydration behavior grain were studied. Thus, highlighting the importance of studying this technology in grains with the sigmoidal behavior.

In this work, the mung bean (*vigna radiata*) hydration assisted by the ultrasound technology was studied. It was used since it has a sigmoidal behavior and due to its importance as a food for direct consumption and from sprouting[19,20]. Consequently, this work aimed to study the effect of ultrasound technology not only on the hydration process of mung bean, but also on the possible structural and functional properties of its flour and starch.

Results and Discussion

Mung bean hydration behavior description. Depending on the seed coat permeability, grains can hydrate following two different behaviors: Downward concave shape (DCS) and Sigmoidal shape[21]. Figure 1a shows that mung bean has sigmoidal behavior during hydration under its normal (equilibrium with environment) initial moisture content (25 °C, 12.25% d.b.), similarly to other pulses such as Andean lupin[4], Adzuki beans[3,21,22], Cowpea[8] and Italian Lima beans[23]. Further, its hydration behavior changes to the DCS when the initial moisture is increase.

The low permeability of the seed coat depends on its composition and its moisture content. The presence of callose, suberin and phenolic compounds in the seed coat can reduce its permeability[24,25]. In addition, the permeability of the seed coat increases when its moisture content is increased, changing the hydration behavior from sigmoidal shape to Downward Concave Shape (DCS) (Fig. 1a)[21]. This change on the seed coat permeability has two possible hypotheses. Firstly, when the moisture content of the bean is reduced, it can cause the shrinkage of cells, reducing the space between the seed coat and the cotyledon, and the closure of the hilum, avoiding the water entrance[26]. Secondly, the low moisture content may cause that the seed coat components pass from the rubbery state to the glassy state reducing its permeability[27].

The state transition of the grain components is related to the grain's water activity. Based on recent works[21,27], there is a critical moisture content (due to a critical water activity value) when the hydration changes its behavior. According to Reid and Fennema[28], the relation between the moisture content and the water activity (sorption isotherm; Fig. 1b) shows the different conditions that water has, depending on how the water is bound in the structure of the food, dividing the curve in three zones. Moreover, they state that the water activity when the water pass from the zone II to the zone III indicates the plasticization of the food structure, consequently the state transition. According to the sorption isotherm of mung bean (Fig. 1b, Equation 1) and this classification, the change of behavior would take place at approximately 0.83 of water activity, corresponding to approximately 23% d.b. of moisture content. This result agrees with Fig. 1, where the change of the hydration behavior (from sigmoidal to DCS) can be observe after ~23% d.b. of initial moisture content. In addition, it is interesting to highlight that the parameter values of the Oswin model (A and B)[29] were similar to the obtained for Adzuky beans (A = 9.75 and B = 0.46)[21], which means that the values could be similar for aleuro-amylaceous grains.

$$M = 9.34\left[\frac{a_w}{1 - a_w}\right]^{0.49} \tag{1}$$

Water pathway during mung bean hydration. As all beans from the *fabaceae* family, mung bean has a complex structure (Fig. 2). Therefore, water may have a specific entrance route during the process and the mass transfer phenomena, as diffusion and capillarity, may take place together. The seed coat surface of this grain (Fig. 2c) does not have cracks or pores that permit the water to enter. In addition, the transversal cut of seed coat (Fig. 2d) shows the presence of the macroesclereids cells, common on this family of grains. Thus, all these structures give some degree of impermeability to the seed coat[30]. Further, osteosclereids cells are presented in the seed coat, which have large intercellular spaces probably contributing to the water lateral distribution[25]. Figure 2e shows the hilum, micropyle and raphe of the grain. The hilum is very porous, which probably allows the water to pass through. The transversal cut of the hilum (Fig. 2f) shows that this structure has direct contact with the radicle. In other words, this structure might cause the rapid hydration of the radicle to assure the activation of the germination process. The water would pass through the hilar fissure to the radicle, which has a porous structure (Fig. 2g) allowing the rapid water absorption. Figure 2e shows that the cotyledon is formed by a great quantity of starch covered by a protein matrix, which probably has a high affinity to water. In addition, the cotyledon structure has some intercellular spaces that can allow water to pass through. Therefore, once the water reaches the radicle and the cotyledon, they hydrate faster. However, this hydration might follow a specific path, starting from the radicle side until the rest of the grain.

The role of each grain structure in the water entrance is still controversial. For example the hilum is the principal water entrance for cowpeas[31], while for Carioca beans and black beans, the entrance of water is by the micropyle, the raphe and the hilum[32] despite this is more by the hilum. On the other hand, another works considered the hilum as the principal water entrance, as for black beans[33] and for Andean lupin[4]. Although there is a probability that the water enters through the micropyle or raphe, the current work considers the hilum as the main water entrance. This was based on the observed microstructure (Fig. 2e and f), as the hilum has a significant larger area in comparison to the micropyle and raphe.

Furthermore, some treatments that describe the contribution of the seed coat and the hilum to the hydration process were performed (Fig. 1c), by covering (waterproofing) specific structures to know their participation in the process. When one of the structures (hilum or seed coat) was covered, the hydration rate was sharply reduced. When the hilum was covered, the hydration took place only by the seed coat; however, due to the low permeability of it, the process was very slow. Further, when the seed coat was covered, the hydration took place by the hilum. The hydration process was very slow despite the porosity of this structure. Due to the small area of this structure, the mass transfer through it is very low. In addition, it can be clearly seen that both structures have a synergic effect on the global hydration process (uncovered beans) since the sum of both hydration kinetics did not reach the uncovered bean hydration curve. It means that both structures work together to hydrate the whole bean. The water that enters by the hilum helps to accelerate the hydration of the seed coat, causing the change of its permeability, consequently accelerating the hydration process.

Similarly to previous works with soybean[34], Andean lupin[4] and adzuki beans[21], the mung bean could have a similar water entrance pathway. The water probably enters through the hilum by capillarity and by the seed coat by diffusion depending on its moisture content.

With all the information explained above, the hydration pathway of this bean would be as follow: Firstly, the water mainly enters by the hilum (due to its porosity), hydrating the radicle slowly (due to its small area) to prevent drowning and assuring the metabolic activation. In addition, the osteoesclereids cells cause the lateral hydration of the bean (between the cotyledon and the seed coat) and the homogeneous distribution of water in the bean[25]. This first part is related to the initial lag phase of the process. Once the grain reaches approximately 23% d.b. of moisture content, the seed coat permeability changes drastically as it reaches the glassy transition moisture content, accelerating the hydration process. Finally, the water is distributed to the entire cotyledon until reaching the equilibrium moisture.

Hydration process mathematical modeling. The hydration kinetics and the effect of the initial moisture content on the hydration behavior were mathematical modeled. Since mung bean has a sigmoidal behavior, Kaptso *et al.* model (Equation 2; ref. 8) was used at each moisture content obtaining a successful fit (Table 1). This

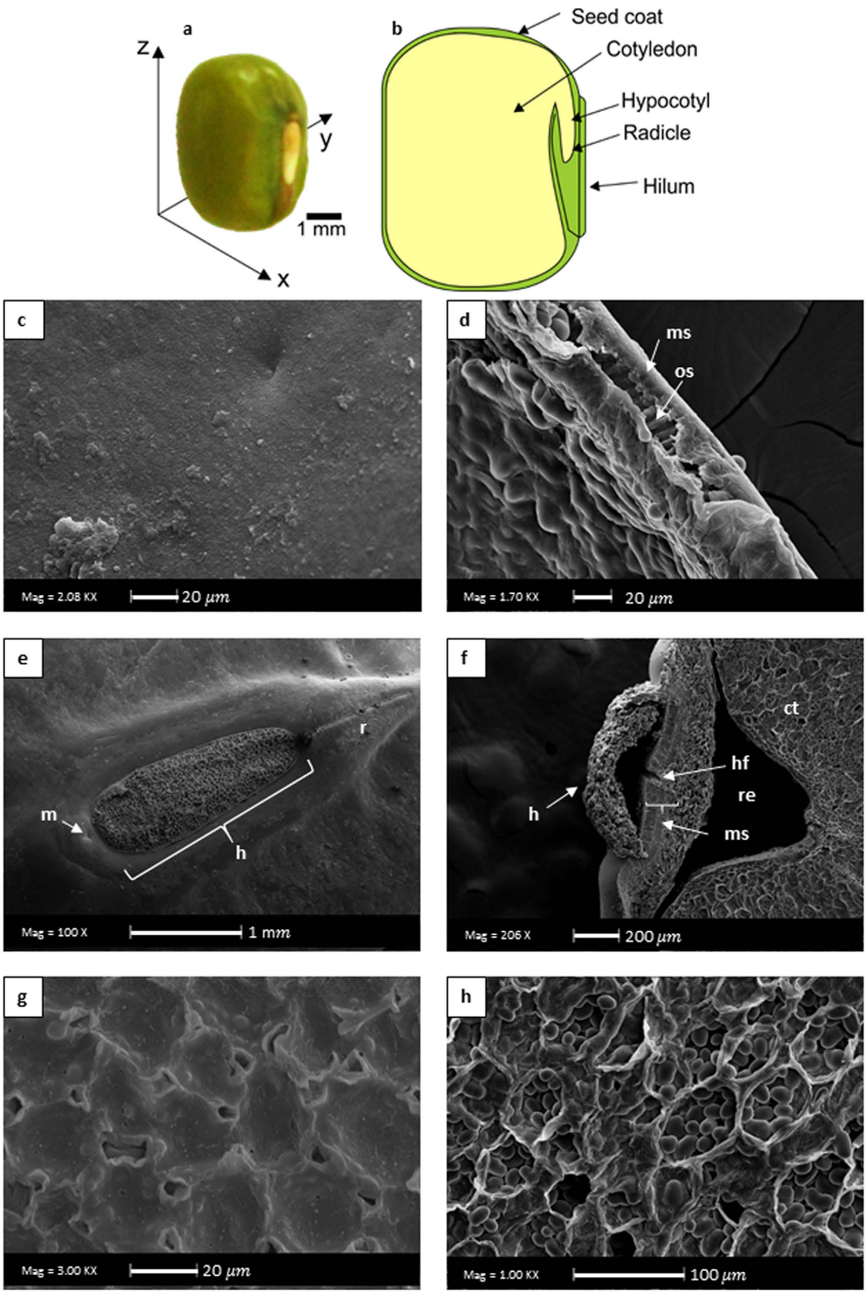

Figure 2. Morphology and microstructure (SEM, 20 kV; the magnifications are shown in the figures) of mung bean (*Vigna radiata*). (**a**) Real photo, scale bar and reference axes. (**b**) Representation of the longitudinal cut (xz plane) of the bean, with selected morphological structures. (**c**) External surface of seed coat. (**d**) Transversal cut of seed coat: ms. Macrosclereids, os. Osteosclereids. (**e**) h. Hilum, m. Micropyle, r. Raphe. (**f**) Transversal cut of the hilum: h. Hilum, re. Radicle space, ct. Cotyledon. hf. Hilar fissure. (**g**) Transversal cut of the radicle. (**h**) Cotyledon.

model has explainable parameters with physical meaning. Therefore, they were useful to explain the behavior change of the hydration process.

$$M_t = \frac{M_\infty}{1 + \exp[-k.\,(t - \tau)]} \tag{2}$$

where M_t is the sample moisture content (% d.b.) at each time t; M_∞ is the equilibrium moisture content; τ describes the necessary time to reach the inflection point of the curve, being thus related to the lag phase; and k is the water absorption rate kinetics parameter.

The parameter τ represents the lag phase duration. As the initial moisture content of the beans is increased, the value of this parameter exponentially decreases (Fig. 1d). This parameter tends to zero when the initial moisture content of the grain is higher than ~23% d.b., which means that the lag phase desapears and the sigmoidal

Initial Moisture Content (% d.b.)	τ (min)	k (min^{-1})	M_∞ (% d.b.)	R^2	RMSD (% d.b.)	NRSMD (%)
12.25	252.2 ± 4.7	0.0099 ± 0.0003	143.5 ± 1.7	0.99	1.0	0.8
15.83	148.0 ± 2.0	0.0128 ± 0.0003	131.1 ± 0.5	0.99	3.0	2.7
18.95	95.5 ± 3.3	0.0165 ± 0.0006	126.2 ± 0.9	0.99	3.4	3.2
23.63	38.1 ± 1.9	0.0270 ± 0.0010	120.3 ± 2.5	0.98	7.6	8.6
35.29	7.6 ± 0.1	0.0724 ± 0.0017	111.8 ± 0.8	0.96	13.3	18.8
41.95	5.0 ± 0.2	0.0681 ± 0.0028	111.1 ± 0.4	0.96	12.2	18.8

Table 1. Parameter values from the mathematical model that evaluate the effect on the initial moisture content on the hydration kinetic of Mung bean (mean ± standard deviation).

hydration behavior turns into DCS behavior. This result was similar to Adzuki beans[21]. Consequently, an exponetial equation was used to model the effect of the initial moisture content on this parameters obtaining the Equation 3 (R^2 of 0.99; Fig. 1d).

$$\tau = 1635.3 \cdot e^{-0.152 \cdot M_o} \tag{3}$$

The parameter *k* represents the water absorption rate of the process. The higher the initial moisture content of the grain was, the higher the value of this parameter was; however, in a more complex pattern. It has a constant value at lower initial moisture content of the grain since the main entrance of water is the hilum, limiting the hydration rate. However, when the beans reach ~23% d.b, the *k* value sharply increases. From this moisture content, the seed coat is very permeable to water, allowing the water enters not only by the hilum, but also by the seed coat, which involves the increment of the value of this parameter. Further, when the initial moisture content of the beans is close to the equilibrium moisture content (very high), the value of the parameter *k* is reduced until constant rate. This happens probably because of the mass transfer driving force (water activity difference) is reduced, reducing the hydration rate. In this case, a sigmoidal model was used to explain the behavior of this parameter (Equation 4; R^2 of 0.97; Fig. 1e).

$$k_k = 0.009 + \left(\frac{0.059}{1 + 24072 \cdot 10^3 \cdot M_o^{-5.16}} \right) \tag{4}$$

Finally, the equilibrium moisture content parameter (M_∞) value decreased as the initial moisture content increased. However, this result was not presented for Adzuki beans[21] nor lentils[26]. This can be explained according the following hypothesis. Mung bean is characterized by a fast germination[35], and due to the germination enzymes are more active at relative high initial moisture content (up to 20% d.b. of moisture content the enzyme are activated[36]), the radicle growth could start earlier. Therefore, if the radicle starts to grow faster, additional water will be absorbed, and the stage I will finish earlier reducing the equilibrium moisture content of this stage (see the section 2.4; Fig. 3a). Beans with high initial moisture content have the moisture homogeneously distributed in the bean. Thus, enzymes are more active in whole bean, triggering the germination process (radicle growth). In contrast, beans, which reach high moisture contents by the hydration process of dry beans (12.25% d.b. of moisture content), have a heterogeneous distribution of the moisture, having a higher moisture content in the external parts and a lower moisture content in the internal parts of the bean. Consequently, the enzymes of the internal layer of the embryo are not activated, delaying the germination process until the complete hydration of the bean. Due to the observed pattern, the equilibrium moisture content was fitted to a composed exponential equation (Equation 5; R^2 of 0.99; Fig. 1f).

$$M_\infty = 124.3 \cdot e^{-0.107 \cdot M_o} + 109.5 \tag{5}$$

Ultrasound assisted hydration of mung beans and impact on its germination. Mung bean can be used as a grain or as a seed, depending of its finality. When the germination process is involved, mung bean can be considered as a seed; when germination is not involved, it is a regular pulse and grain[37].

During the germination process, the seed hydration follows a tree-stage water uptake pattern (Fig. 3a). The first stage consists of the hydration process itself (as described in sections 2.1), when the seed absorbs the necessary water to activate its metabolism. In this stage, the seed arises the first signs of metabolism reactivation[37]. The second stage consists of reserves digestion and new molecules synthesis. In this stage, the hydration of the seed is negligible (it can be considered as the equilibrium moisture content of the hydration process of grains, i.e., the M_∞ of stage I – as described above). Stage III takes place when the radicle starts to grow and many structural components are synthetized; thus, water is required in many metabolic processes, resulting in more water absorption[37]. Therefore, the hydration process in the third stage is mainly due to biological phenomena.

In the case of grains, used as food, only stage I is important for their processing. Thus, during the hydration study of grains, only the stage I is evaluated (which can be widely observed in the literature). However, in the present work, although the hydration modeling (section 2.5) was conducted only in the stage I, the process was evaluated until stage III, when a small, but visible radicle proves the start of germination. Further, as previously described, the food hydration does not have only a DCS behavior, but in some cases, it also shows a sigmoidal behavior. Therefore, Fig. 3a was complemented, highlighting the two possible hydration behavior at stage I. As

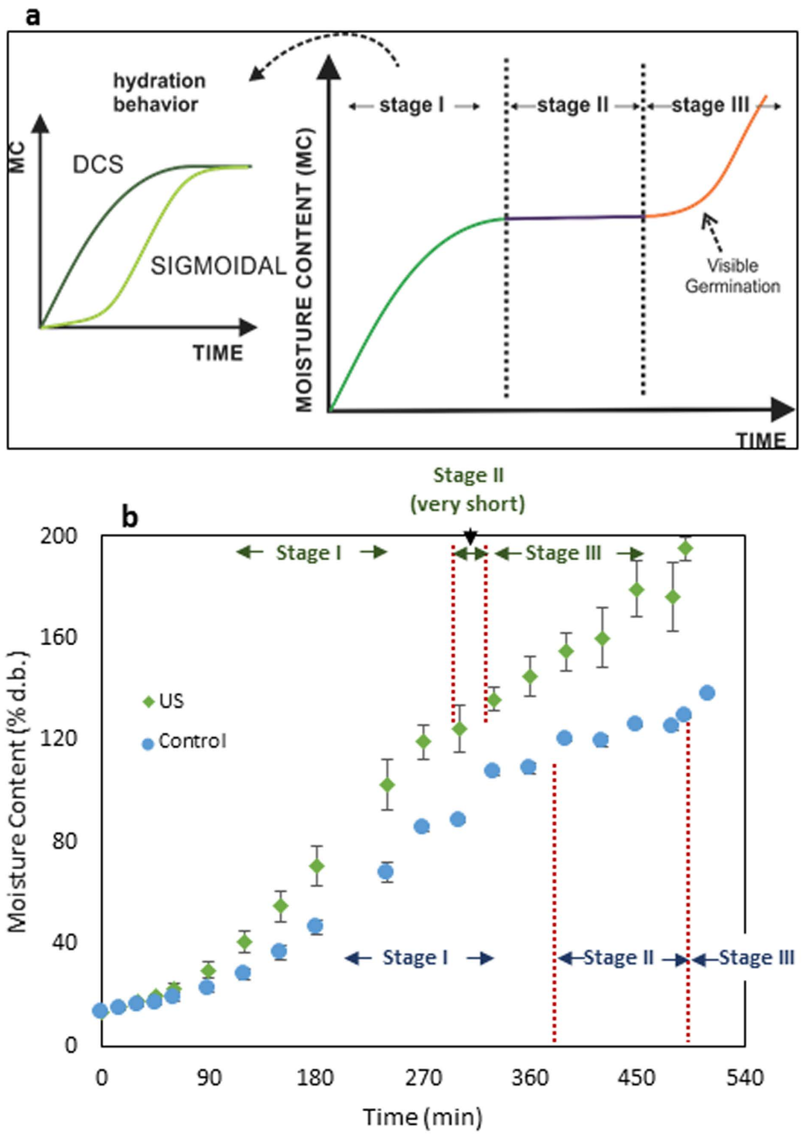

Figure 3. (**a**) Stages of the seeds germination as function of the moisture content. Adapted from Bewley and Black[30] and ref. 18. (**b**) Ultrasound assisted hydration process increases the hydration and germination velocity of mung bean. The dots are the experimental values and the vertical bars are the standard deviation.

mung bean has a short phase II, of approximately 2 h[35], the germination process is very fast. This may explain the reduction of the equilibrium moisture content when a bean with high initial moisture contents were hydrated. At high initial moisture contents, the beans are metabolically more active, reducing the minimum moisture content to germinate and the length of stage I and stage II.

Figure 3b shows the effect of the ultrasound technology (41 W/L, 25 kHz of frequency) on the hydration process of mung beans. It can be clearly seen that the ultrasound enhanced the hydration process, reducing approximately 25% of the time to reach the equilibrium moisture (i.e., the stage I duration, reaching the stage II). Besides this successful result, ultrasound also accelerated the germination process of this bean by reducing the stage I length and almost disappearing the stage II.

In fact, the ultrasound has improved the hydration process of other grains such as chickpeas[14,38], navy beans[13], sorghum grains[12], common beans[15], and corn kernels[16]. Most of these works attributed the improvement to the direct and indirect effects of ultrasound on mass transfer processes[9] – strictly physical mechanisms of mass transfer improvement.

However, it was demonstrated that the ultrasound technology enhances the seeds vigor, probably by enhancing its metabolism[39]. Consequently, the hydration process may also be enhanced not only by physical phenomena, but also due to metabolic/biological phenomena (also accelerating the germination). In fact, although it was still not described, it is a possibility that can explain the observed behavior. In fact, this possibility must be further evaluated; unfortunately, it cannot be proved in the present work.

At stage I, the moisture content of the beans is low and, consequently, the water activity too. Therefore, the enzyme activity in the grain is also low, being increased when the moisture content increases. In this part of the process, the main improvement by the ultrasound technology may be physical, due to its direct and indirect effects. The direct effects are the inertial flow and the sponge effect, which by taking advantage of the porosity of the bean, increases the water intake by pumping the water into the tissues and by unblocking the pores[9,16]. In addition, the traveling of the ultrasonic waves probably caused the change of the beans pores size or shape. As the beans moisture content is increased, probably the indirect effects gain strength, since the water vapor is increased, facilitating the acoustic cavitation and the formation of micro cavities and micro-channels[9]. Consequently, both the ultrasonic direct (inertial flow and sponge effect) and indirect effects (micro-channels formation) could take place at the final part of the stage I, improving the hydration process.

Figure 4 shows the microstructure of mung bean hydrated with and without ultrasound. There was not any significant visible difference among the structures of the bean (seed coat, hilum and cotyledon) hydrated with and without ultrasound. In addition, Fig. 4g and h demonstrated that the structure of the starch was not modified (the effect of ultrasound on the mung bean starch is discussed in the following section). In conclusion, as other previous works[9,16], it is demonstrated that the ultrasound technology (at the used conditions of power and frequency) did not cause significant changes on the grains structure. Although the micro-channels formation was demonstrated for sorghum grains[9], the Scanning Electronic Microscopy probably is not a suitable analysis for detecting the formed micro-channels. Probably, the micro-channels are too small or difficult to identify. In addition, the sample preparation of this technique (as the grain must be dried, which definitely affects its structure) could have more effect on the microstructure than the process, which hinders the possible changes that ultrasound could have caused. Therefore, other techniques could be studied in future researches. However, SEM analysis gave us an idea that ultrasound did not change the overall structure, and that the modifications are slight.

Furthermore, the ultrasound reduced the stage II (Fig. 3a and b), causing the bean germination, leading to the stage III. In fact, the ultrasound technology has improved the germination process of other seeds, such as barley[39,40], switchgrass[41], pea[42] and grass seeds[43]. Most of those works gave as the possible effect of ultrasound on the germination process, the increasing in the nutrient mobility, the respiration rate and/or the water availability for metabolic reactions. During stage II the reserve components digestion takes place, as well as the nutrient transport and the synthesis of some components[37]. Therefore, ultrasound could have improved those processes for mung bean, helping the reserve molecules catabolism and the transport of molecules to the radicle (mass transfer improvement), reducing the stage II duration. In fact, Liu *et al.*[43], demonstrated that the ultrasound technology increased the metabolic activity of aged grass seed, enhancing the germination percentage, attributing this improvement to the cited reasons and the increment of the porosity of the seed by the acoustic cavitation. In addition, the vibration caused by ultrasound could have caused the increment of the metabolism activity, accelerating the germination process as it was demonstrated that a sinusoidal vibration enhances the germination process[44].

It is interesting to highlight that the acceleration of the mung bean germination is a desirable result, as this grain is widely consumed as a sprout. Consequently, the ultrasound technology can be useful for the mung bean sprout (also called as Moyashi) production, by accelerating both the hydration and germination.

Ultrasound assisted hydration of mung beans: impact on mass transfer and modeling. Finally, the hydration process with and without ultrasound was modeled using the Kaptso *et al.* model[8] and its parameters were evaluated (Fig. 5). It should be mentioned that for the ultrasound assisted hydration, the data of the phase III were not considered, using the data until the beginning of stage II since this model only describes the hydration process (stage I). Therefore, the value of the equilibrium moisture content (M_∞) was fixed and considered the same to the control treatment. Despite this consideration, the Kaptso *et al.* model successfully fitted the experimental data (R^2 of 0.99 for both treatments). The parameters k and τ had significant difference ($p < 0.05$) when ultrasound was applied for the hydration process.

The parameter k, which is related to the hydration rate, increased when the beans were hydrated with ultrasound, from $0.0104 \pm 0.0004\,\text{min}^{-1}$ to $0.0150 \pm 0.0016\,\text{min}^{-1}$ (an increase of ~44%). It means that the ultrasound decreases the internal resistance for the water flow through the bean. As described, this enhancement could be probably caused by the direct effects at the first part of the process (due to the low moisture content of the beans), and probably by both direct and indirect effects at the final part of the hydration process (due to the high moisture content of the beans), increasing the total hydration rate.

The parameter τ, which is related to the lag phase of the hydration process, decreased almost 28% when the ultrasound technology was applied, from 243.7 ± 9.6 min to 174.5 ± 14.2 min. The lag phase of the hydration process ends when the seed coat is enough hydrated to increase its permeability[21,26]. Therefore, the ultrasound technology caused the rapid entry of water in the first part of the process, hydrating faster the seed coat, increasing its permeability and accelerating the hydration process. It is very likely that this improvement has been due to the direct effects, helping the lateral hydration of the bean through the space between the seed coat and the cotyledon and through the osteosclereids cells of the seed coat.

There is not any work in the literature relating the ultrasound-assisted hydration of sigmoidal behavior hydration beans. Thus, it is the first work that demonstrated that ultrasound reduces the lag phase of the hydration process. However, further studies should be performed to determine whether higher power ultrasound could further reduce the lag phase.

All these results demonstrate that the ultrasound is a promising technology, which can be implemented in the industries since it reduces the hydration process time and, in some cases, could accelerate the germination process of seeds, which is very desirable for the sprouting and malting process.

Effect of the ultrasound assisted hydration on the properties of mung bean flour and starch. Figure 4g and h shows the SEM microphotographs of the isolated starches from the beans hydrated

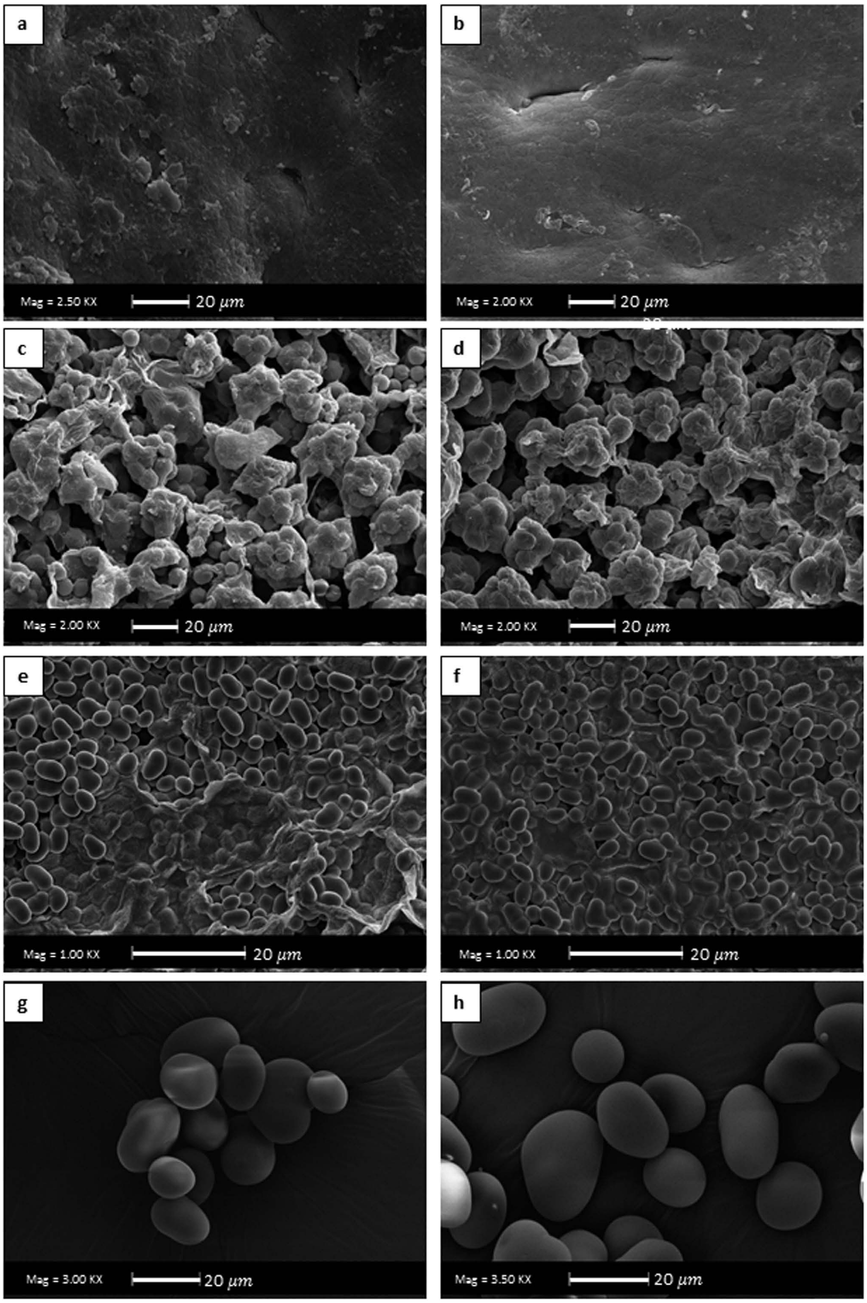

Figure 4. Microphotography SEM (20 kV; the magnifications are shown in the figures) of the different structures and starch of mung bean . (**a**,**c**,**e** and **g**) are the seed coat surface, hilum, cotyledon and starch, respectively, from conventionally hydrated beans. (**b**,**d**,**f** and **h**) are the seed coat surface, hilum, cotyledon and starch, respectively, from from ultrasound assisted hydrated beans.

without and with ultrasound, respectively. Both shows an oval to spherical shaped with smooth surface without fissures as verified by Rupollo *et al.*[45]. Therefore, ultrasound did not change the structure of the starch grain. In some works were shown that ultrasound changed the starch microstructure modifying its technological properties[46,47]. In addition, the Rapid Viscosity Analysis (RVA) profile of the starch suspensions (Fig. 6) also demonstrated that the ultrasound technology did not alter the pasting properties of starch isolated from the hydrated beans (for all the evaluated parameters: peak, trough, breakdown, setback and final apparent viscosity), reinforcing the results of SEM. This result was different in comparison with those carried out using isolated starch granules in suspension, such as the work of Zuo *et al.*[48]. They demonstrated that the ultrasound technology reduces the apparent viscosity of the starch suspensions. However, it is necessary to clarify that in the mentioned works, the results obtained in the SEM and RVA analysis were acquired with isolated starches treated with ultrasound, in contrast to the present work, where the starch was still inside the grains (cotyledon), fiscally protected, when the ultrasound technology was applied. This result is also in accordance with the work of Miano[16].

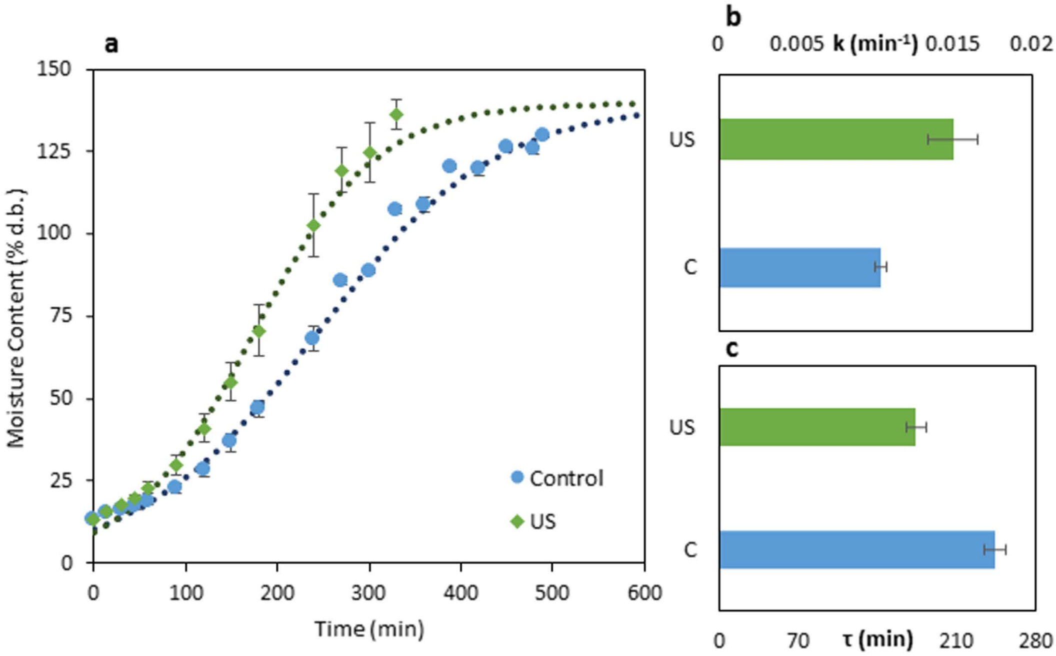

Figure 5. (**a**) Mathematical modeling of the hydration process with and without the ultrasound technology. The dots are the experimental values; the bars are the standard deviation and the curves are the model values. (**b**) Effect of the ultrasound technology on *k* parameter of Kaptso *et al.* model (Equation 2). (**c**) Effect of the ultrasound technology on τ parameter of Kaptso *et al.* model (Equation 2).

Figure 6 also shows the force-displacement graphic obtained from the gel texture evaluation. The gel strength is associated with starch constituents (amylose and amylopectin) and the interaction between them[49]. Therefore, any changes in the starch gel texture (keeping constant all the other parameters, such as temperature, concentration, etc.) would be due to the molecular depolymerization and amylose molecular size reduction, which are directly associated with starch retrogradation and its ability to form gels[50,51]. There was not significant change ($p < 0.05$) between the gel strength of the starch isolated from the mung bean hydrated without and with ultrasound. This means, therefore, that there was not significant change in the starch molecular structure when the beans were hydrated using the ultrasound technology.

Finally, Sepharose CL 2B gel permeation chromatograms of bean starches are shown in Fig. 6. The first peak corresponds to amylopectin and the second one (determined by the blue value of the iodine) corresponds to amylose[52,53]. These results suggested any important change in the molecular weight and structure of the starches isolated from mung beans hydrated with and without ultrasound – reinforcing the previous results.

The obtained results demonstrate that the ultrasound technology did not affect both the starch structure and technological properties during mung bean hydration process, which is highly relevant for the starch industry.

On the other hand, the RVA profile of the beans flour (Fig. 7) demonstrated that the ultrasound caused an increment of the apparent viscosity. Higher apparent viscosity could be beneficial for some food industries, considering bean based products that need higher consistency. Similar results were obtained by Ghafoor *et al.*[13] who demonstrated that the apparent viscosity of flour from navy beans hydrated with ultrasound was higher than the hydrated without ultrasound. However, they attributed this change to the starch modification by ultrasound, even though, that work has only evaluated the flour. Nevertheless, as the present work performed the RVA profiles of both starch and flour, and as there was not any difference in the starch structure and properties (Fig. 6), it can be demonstrated that the ultrasound changed the protein properties, instead of the starch properties (Fig. 7). Ultrasound technology might have altered the beans protein structure, modifying the accessibility of water molecules to the binding sites of the protein chains. O'Sullivan *et al.*[54] observed that the ultrasound reduces the aggregates size of legume protein increasing the solubility of them[55,56]. Therefore, the proteins solubility increasing can explain the increment of the beans flour apparent viscosity.

Conclusions

Mung bean hydration process has a sigmoidal behavior and, similarly to other pulses, this behavior changed to the Downward Concave Shape behavior when the initial moisture of the grain is approximately 23% d.b. The route of water entrance of this bean was established according to the seed coat permeability and the water absorption participation of the hilum and the seed coat. Furthermore, it was demonstrated that the ultrasound technology improved the hydration process of mung bean, reducing the total process time almost 25% (reducing the lag phase time ~28% and increasing the water absorption rate ~44%). In addition, it was demonstrated that this technology accelerated the germination process of this bean, which is a very desirable result for sprouting or malting. Finally, it was concluded that the ultrasound technology did not alter the starch properties (structural

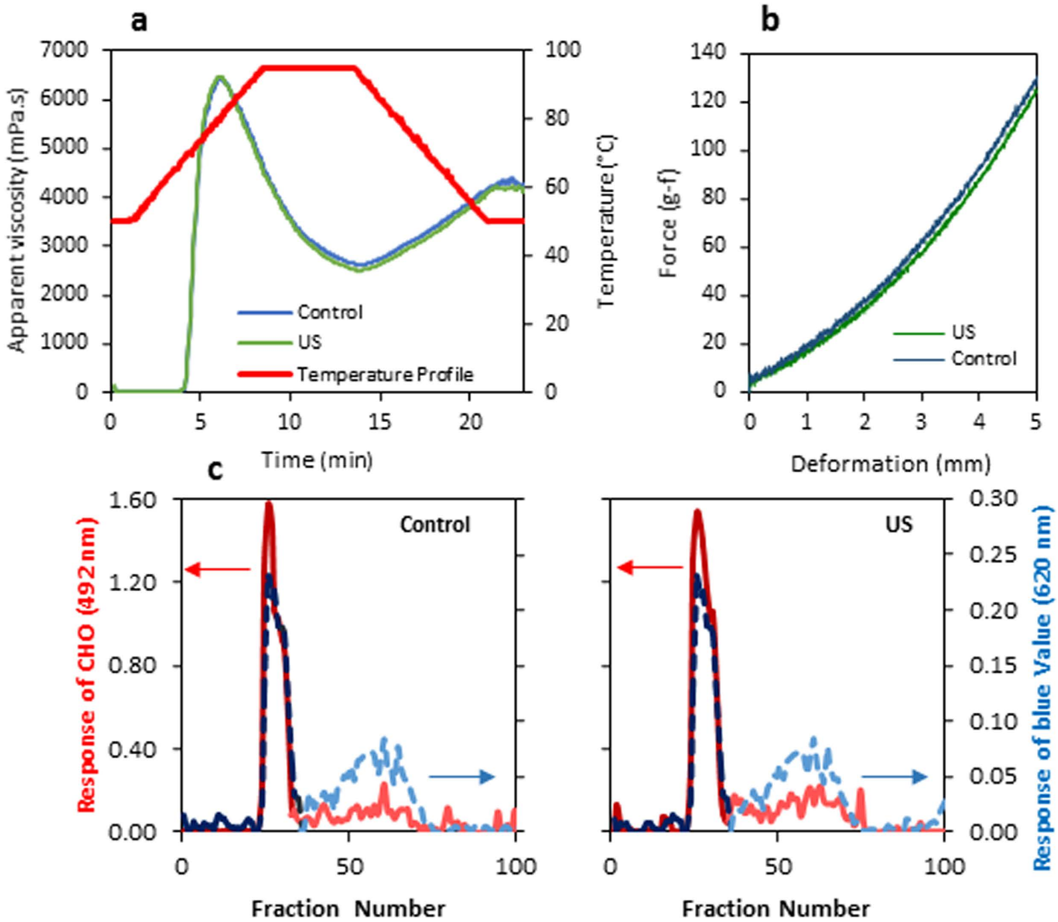

Figure 6. Evaluation of the starch properties extracted from mung beans hydrated without (Control) and with ultrasound (US). (**a**) RVA profile. (**b**) Texture of the starch gel. (**c**) Sepharose CL 2B gel permeation chromatograms: Continuous red curves represent the response of CHO and the dot curves represent the response of blue value. In addition, darker colors represent the higher molecular weight region (amylopectin) and lighter colors represent the lower molecular region (amylose).

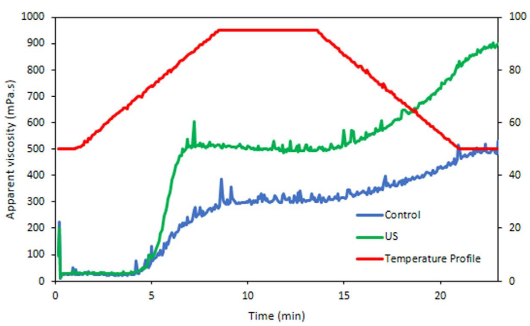

Figure 7. Rapid viscosity analysis profile (RVA) of the flour of mung beans hydrated with (US) and without (Control) ultrasound.

and rheological). However, this technology increased the apparent viscosity of the whole bean flour, which was attributed to the proteins changes. Considering everything, the ultrasound technology can be used to accelerate the hydration process of this bean without altering its starch. In addition, depending on the purpose, the ultrasound can be used to accelerate the germination process, being these results very desirable for pulses industry.

Materials and Methods

Raw Material. Mung bean (*Vigna radiata;* 12.25 ± 0.53% d.b (g water/100 g of dry matter) of moisture content; 5.12 ± 0.24 mm length, 3.81 ± 0.22 mm width and 3.62 ± 0.16 mm thick) obtained at a local market of Campinas - Brazil was used.

Conventional hydration process description. For the hydration process, 10 g of pre-selected grains (without any damage) were placed into net bags and soaked in 4 L of distilled water (to avoid water be a limiting in the process) at 25 ± 1 °C during all kind of treatments. During the hydration process, the grains were periodically drained, superficially dried and their moisture content were obtained by mass balance using the initial moisture content (determined using a Moisture Analyzer MX-50 AND, Japan) (after verifying the possibility to neglect the solid loss to the water). Then, the grains were soaked again to continue the process. The grains were weighted every 15 min for the first hour, every 30 min for the latter two hours and every hour from then on. The hydration process was performed at constant temperature using a water bath (Dubnoff MA 095 MARCONI, Brazil) and in triplicate.

Effect of the initial moisture content on the hydration behavior. To study the effect of the initial moisture content on the hydration behavior, generating subsidies for better understand the hydration mechanisms, samples with different initial moisture content were prepared. To obtain samples with a higher initial moisture content (15.83, 18.95, 23.63, 35.29 and 41.95% d.b.) the grains were hydrated for a specific time at 25 ± 1 °C. Then, these grains were put into sealed containers for a week at 5 ± 1 °C to homogenize the moisture into the grains. The lower initial moisture content sample (3.88% d.b.) was prepared by placing the grains in a desiccator with silica gel for 2 weeks until obtaining the required moisture content. The initial moisture content of the samples was then obtained by a mass balance using the moisture content of the original sample[21].

Further, the sorption isotherm was also elaborated. Beans with different moisture contents (prepared using the procedure described above) were ground using a cutter mill prior to determining their water activity at 25 °C using a water activity meter (AquaLab 4TE, Decagon Devices, Inc USA). The sample moisture content (% d.b.) was then plotted as function of the water activity. The obtained curve was modeled using the Oswin Equation (Equation 6) since it is recommended for starchy food[29]. In this equation, M is the moisture content of the product (% d.b.), a_w is the water activity of the product and A and B are model parameters related to the curve shape.

$$M = A\left[\frac{a_w}{1 - a_w}\right]^B \tag{6}$$

Study of the water entrance route. In this case, the hydration process was performed with some covered structures. In addition, the microstructure of the grains was studied to observe the different structures of the grain (according to Fig. 2).

To verify the water entrance, the seed coat or the hilum were covered using a varnish (nail polish; Risqué – Cosmed Industry Brazil) as a sealant, similar to Ramos *et al.*[57]. This treatment allowed determining the contribution of these structures to the hydration process.

For the microstructural analysis, the samples were cut with a scalpel blade to see the different tissues (seed coat, cotyledon, and external surface) and dehydrated in a sealed container using silica gel for 3 days. Then, they were sputtered with a 30 nm gold layer. Finally, the samples were observed in a scanning electronic microscope operated at an acceleration voltage of 20 kV (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England).

Ultrasound assisted hydration. During the experiments, an ultrasonic bath with a frequency of 25 kHz and a volumetric power of 41 W/L (Q13/25, Ultronique Brazil; determined following the method described by Tiwari *et al.*[58]) was used. This bath has its piezoelectric elements arranged below the tub. It generates the mechanical waves that are transmitted through the water to the product. The ultrasonic waves distribution in the water bath was determined by the method of the aluminum foil[59,60]. Further, the other good practices described by[59,60] were also verified. Thus, the samples were placed in the parts where the waves had the highest and most homogeneous intensity.

The ultrasound-assisted hydration was performed in the ultrasonic water bath with 4 L of water at 25 ± 1 °C. The grains were placed into net bags and placed on the bottom of the ultrasonic water bath. The data were collected as the control hydration process explained above.

Modeling of the hydration process. The Mung bean hydration kinetics was modeled using the sigmoidal equation of Kaptso *et al.* (Equation 2; ref. 8). For that purpose, the dry basis moisture content of the grains (M% d.b.) versus the hydration time (min) was tabulated for each initial moisture. The data were fitted to the mathematical model with a confidence level of 95% using the Levenberg-Marquardt algorithm in Statistica 12.0 (StatSoft, USA) software.

Finally, the goodness of fit of the models was evaluated by the R^2 regression value, the root-mean-square deviation values (RMSD, Equation 7), the normalized RMSD (NRMSD, Equation 8) and by plotting the moisture content values obtained by the model (M_{model}) as a function of the experimental values ($M_{experimental}$). The regression of those data to a linear function (Equation 9) results in three parameters that can be used to evaluate the description of the experimental values by the model, i.e. the linear slope (a, which must be as close as possible to one), the intercept (b, which must be as close as possible to zero) and the coefficient of determination (R^2; that must be as close as possible to one).

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n}(M_{experimental} - M_{model})^2}{n}} \tag{7}$$

$$NRMSD = 100 \cdot \frac{RMSD}{(M_{experimental})_{maximun} - (M_{experimental})_{minimun}} \quad (8)$$

$$M_{model} = a \cdot M_{experimental} + b \quad (9)$$

Starch and flour evaluation. The starch of the mung beans (hydrated with and without ultrasound) was extracted as follow: The hydrated beans (with and without ultrasound) were milled (with distilled water) using a blender and sieved (60 and 325 mesh). The supernatant was washed two times with distilled water. The filtrate was centrifuged at 3200 g for 5 min, for them separating the starch from the rest of the components (water, proteins and lipids). Finally, the starch was dried at 35 °C for 12 h in a flat tray and was softly milled using a mortar and pestle.

The flour of the mung beans (hydrated with and without ultrasound) was obtained by grinding them after the hydration process using a cutter mill.

To evaluate if the obtained starch or flour was affected by the ultrasound, the following evaluation was performed.

The mung bean flour (i.e., the whole grain milled) and starch pasting properties were evaluated in a Rapid Visco Analyzer (RVA-S4A; Newport Scientific, Warriewood, NSW, Australia) using 3 g of sample (corrected for 14% of moisture) in 25 g of water. The suspension was first held at 50 °C for 1 min and then heated to 95 °C at a rate of 6 $°C \cdot min^{-1}$. The sample was then held at 95 °C for 5 min, followed by cooling to 50 °C at a rate of 6 $°C \cdot min^{-1}$, and finally holding it at 50 °C for 2 min. As the starch was evaluated separately from the flour, the differences between their rheological profiles could be related with the changes on the product proteins (the two main components of the grain: 31.1% of starch[61] and 23.8% of protein[20] as average).

Microstructure of the starch was evaluated using scanning electronic microscopy in similar way as the beans analysis. The starch was placed on the stubs using a dry brush and passing directly to the sputtering process.

The mechanical properties of the starch gel were also analyzed by instrumental texture. The gel strength was determined using a Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 5 kg-f (49,03 N). The gel obtained after the RVA determination was stored in a 40 × 20 mm (diameter × height) plastic cup for 24 h at room temperature to stay solid before evaluation. To ensure uniform moisture of the samples, they were held in a desiccator with water at the bottom. A 0.5 cm cylindrical probe (P/0.5 R) was used to compress the samples until the distance of 5 mm at 1 mm s^{-1}. The force measured by the equipment as a function of the penetration depth was then used to evaluate the gel strength.

The molecular mass distribution profiles of the starch samples were determined by gel permeation chromatography, using a GE XK 26/70 column (2.6 cm diameter and 70 cm high), packed with Sepharose CL-2B gel (Sigma, Sweden). 10 mL of dimethylsulfoxide (DMSO; 90%, Labsynth, Brazil) was added to 0.1 g of starch and heated in boiling water bath for 1 h, then remaining for 24 h at 25 °C under constant stirring. An aliquot of 3 mL (30 mg of starch) was then mixed with 10 mL of absolute ethanol to precipitate the starch, being the suspension centrifuged for 30 min at 3000 g. The precipitated starch was dissolved in 9 mL of boiling distilled water and put in boiling water bath for 30 min[52]. An aliquot of 4 mL was then eluted in the chromatographic column upwardly. A solution containing 25 $mmol \cdot L^{-1}$ of NaCl and NaOH 1 $mmol \cdot L^{-1}$ was used as eluent at a rate of 60 $mL \cdot h^{-1}$. Fractions of 4 mL were collected (Gilson model FC203B, Middleton, England) and analyzed for total carbohydrate content at 490 nm by the phenol sulfuric method (Dubois *et al.*, 1956) and blue value at 620 nm (Juliano, 1971), using a microplate reader (Asys Expert plus, Biochron, England).

Statistical evaluation. When relevant, statistical analysis was performed to the treatments through analysis of variance (ANOVA) and Tukey's test ($P \leq 0.05$), using the software Statistica 12.0 (StatSoft, USA).

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Author Contributions

A.C.M. and P.E.D.A. conceived and designed the experiments, evaluated the data and wrote the manuscript. A.C.M. and J.C.P. performed the experiments. N.C. and M.D.M. Jr. performed the experiments with starch and flour, also evaluating these data. P.E.D.A. directed and managed the team. All the authors discussed and approved the manuscript.

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APPENDIX G: ENHANCING THE HYDRATION PROCESS OF COMMON BEANS BY ULTRASOUND AND HIGH TEMPERATURES: IMPACT ON COOKING AND THERMODYNAMIC PROPERTIES

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Enhancing the hydration process of common beans by ultrasound and high temperatures: Impact on cooking and thermodynamic properties

Alberto Claudio Miano*, Viviane Deroldo Sabadoti, Pedro Esteves Duarte Augusto*

Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil

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ABSTRACT

Although many technologies have been applied to enhance the hydration process of grains, some combinations and mechanisms still need better description. Consequently, this work studied how the combination of ultrasound and temperature affected the hydration process of one variety of legume grain, as well as described their thermodynamic properties of hydration and the impact on cooking kinetics. White kidney beans were hydrated using ultrasound technology (28 W/L of volumetric power and 45 kHz of frequency) at four different temperatures (25, 35, 45 and 55 °C). In addition, the softening kinetics during cooking at boiling temperature was studied by penetration analysis. Further, the thermodynamic properties, such as activation enthalpy, Gibbs free energy and entropy were determined. It was demonstrated that despite both technologies improve the hydration process individually, hot temperatures hinder the ultrasound effect. Further, the thermodynamic properties of the hydration phenomenon were affected by ultrasound, suggesting how the molecules rearrangement is affected by this technology during hydration. Finally, the use of both technologies did not affect softening kinetics of the bean during cooking process.

1. Introduction

Pulses are an important food source due to their nutritional and agronomic advantages. Besides being a mainly protein, iron and fiber source, pulses are easy to grow and help to fertilize the soils (FAO, 2016). For being consumed and facilitating their digestion, pulses must pass for previous processes such as cooking, germination, fermentation, etc. (Siddiq et al., 2011). However, as they are dried food, a hydration process is needed as a first step. Further, as the grain hydration is a slow and batch process, its study and improvement is desirable.

The hydration kinetics of pulses is a complex phenomenon, involving different mechanisms and presents two possible behaviors: downward concave shape (DCS) and sigmoidal shape. The first behavior is the most common in all kind of grains. Consequently, it is the most studied. The DCS behavior presents a high hydration rate from the beginning of the process due to the water activity difference between the grain and the soaking water. This rate decreases until reaching the equilibrium moisture content e.g. the maximum quantity of water that the grain can hold. In contrast, the second behavior seems to be exclusive for *Fabaceae* family grains and it was still slightly studied. This behavior is caused by the water permeability of the seed coat, which changes widely with the water activity and then limits and defines the water flow. This is represented by a lag phase which ends when the seed coat is hydrated and becomes permeable to water (Miano and Augusto, 2015). Finally, the water uptake continues until reaching the equilibrium moisture content. For both behaviors, some technologies have been studied to accelerate the hydration process, and in each behavior the enhancement mechanisms are different.

The classical way to accelerate the hydration process is to increase the water soaking temperature. This technique has demonstrated satisfactory results for different pulses and cereal grains, such as corn kernels (Verma and Prasad, 1999), lentils (Oroian, 2017), adzuki beans (Oliveira et al., 2013), barley kernels (Montanuci et al., 2015), Andean lupin (Miano et al., 2015), among many others. However, the use of elevated temperatures has as drawbacks the possibility of components degradation as well as the increase of process cost (heating and isolation). Therefore, other technologies have been studied, as ultrasound technology.

* Corresponding authors. Avenida Pádua Dias, 11, Piracicaba, SP, 13418-900 Brazil.
E-mail addresses: cmiano@usp.br (A.C. Miano), Pedro.ed.augusto@usp.br (P.E.D. Augusto).

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Ultrasound technology has demonstrated excellent results for improving different mass transfer unit operations. This technology consists of using acoustic energy, with frequencies higher than 20 kHz, to cause physicochemical changes on biological products, to increase their quality, safety or to improve the processing (Mason et al., 1996). Regarding hydration process, this technology has improved not only the DCS hydration kinetics behavior (Ghafoor et al., 2014; Miano et al., 2017a; Patero and Augusto, 2015; Ranjbari et al., 2013; Ulloa et al., 2015; Yildirim et al., 2013), but also the sigmoidal one (López et al., 2017; Miano et al., 2016b), reducing the lag phase time and increasing the hydration rate. It should be mentioned that these works represent all the studies using ultrasound to enhance the hydration of beans.

Both, the use of hot soaking water and ultrasound technology has demonstrated their efficiency on enhancing the hydration process of pulses, but isolated. Nonetheless, any work has used the combination of both, especially for grains with sigmoidal behavior of hydration. For that reason, this work evaluated if ultrasound and hot soaking water in combination can accelerate the hydration process of sigmoidal behavior hydration kinetics pulses. Further, the effect of both technologies was evaluated in the cooking kinetics of the beans and in the thermodynamic properties of hydration.

2. Materials and methods

2.1. Raw material

White kidney beans (*Phaseolus vulgaris;* 15.82 ± 0.26% d.b (g water/100 g of dry matter) of moisture content; 16.97 ± 0.74 mm of length, 8.12 ± 0.59 mm of width and 6.46 ± 0.31 mm of thick) were obtained at a local market of Piracicaba - Brazil. This pulse was chosen because it presents the sigmoidal behavior of hydration (Miano et al., 2017c), being frequently consumed all around the world.

2.2. Hydration process

For the hydration process, 10 g of pre-selected grains were placed into net bags and soaked in 2 L of distilled water at 25, 35, 45 and 55 °C. The excess of water avoids water be a process limitation. During the hydration process, the samples were periodically drained, superficially dried with towel paper and their moisture content was obtained by mass balance (after verifying the possibility to neglect the solid loss to the water). The sampling was carried out every 15 min for the first hour, every 30 min for the latter two hours and every hour from then on. The hydration process was performed at constant temperature and in triplicate.

Further, to describe the water flow pathway, the permeability of the seed coat was evaluated by covering the hilum and micropyle of the beans using nail polish (Miano et al., 2016b; Ramos et al., 2004) and then comparing the hydration kinetics of the covered beans with the uncovered beans.

2.3. Ultrasound assisted hydration

During the experiments, an ultrasonic bath (USC-1400, Unique Brazil) with a frequency of 40 kHz and a volumetric power of 28 W/L (determined following the calorimetric method described by Kimura et al. (2007)) was used for the ultrasound assisted hydration. As in the conventional hydration process, 10 g of pre-selected grains (placed into net bags) were placed at the bottom of the ultrasonic bath in order to be subjected by the waves with the highest and more homogeneous intensity. This process was performed with 2 L of water at 25, 35, 45 and 55 °C. The data were obtained in the same way as in the conventional hydration process. It should be mentioned that the ultrasonic waves distribution in the water bath was determined by the method of the aluminum foil (Mason, 1991; Vinatoru, 2015).

2.4. Hydration data description

The white kidney beans hydration kinetics data was fitted using the sigmoidal equation proposed by Kaptso et al. (Equation (1) (Kaptso et al., 2008);). For that purpose, the grain moisture content (M, in dry basis, % d.b.) versus the hydration time (min) was tabulated for each treatment. Each replication data was fitted using a generalized reduced gradient algorithm, which is implemented in the 'Solver' tool of software Excel 2016 (Microsoft, USA). Different initial guesses of the three parameters were assessed to detect possible local convergence.

$$M_t = \frac{M_\infty}{1 + exp[-k\cdot(t-\tau)]} \tag{1}$$

Furthermore, the individual effect of the temperature on the Kaptso model parameter was evaluated by fitting the parameter value as a function of the temperature (Kelvin) on a suitable equation. For the parameter k and τ the Arrhenius equation was used (Equation (2)), due to its exponential behavior. Thus, the activation energy (E_a) was obtained for the conventional and the ultrasound assisted hydration process.

$$P_T = A\cdot\exp\left(\frac{-E_a}{R\cdot T}\right) \tag{2}$$

For the equilibrium moisture content, the effect of temperature was explained by an empirical equation due to its specific behavior (Equation (3)).

$$M_{\infty\ T} = M_{\infty\ 25^\circ C} - b\cdot exp(c\cdot T) \tag{3}$$

2.5. Thermodynamics properties

The activation enthalpy ($\Delta H^{\#}$), the activation Gibbs free energy ($\Delta G^{\#}$) and the activation entropy ($\Delta S^{\#}$) were obtained for both the hydration rate (k) and time lag parameters (τ), using the following equations (Sánchez et al., 1992; Silva et al., 2017):

$$\Delta H^{\#} = E_a - R\cdot T \tag{4}$$

$$\Delta G^{\#} = -R\cdot T\cdot ln\left(\frac{P_T\cdot h}{k_B\cdot T}\right) \tag{5}$$

$$\Delta S^{\#} = \frac{\Delta H^{\#} - \Delta G^{\#}}{T} \tag{6}$$

The activation enthalpy ($\Delta H^{\#}$) is related to the necessary energy to form the activated complex before the reactants form the products. The free energy ($\Delta G^{\#}$) is related to the spontaneity of the transition state formation and the activation entropy ($\Delta S^{\#}$) is related to this activated molecule complexity (Al-Zubaidy and Khalil, 2007; Vikram et al., 2005).

2.6. Cooking kinetics

Approximately 80 g of beans were cooked in a Becker with 1.6 L of boiling distilled water (98 °C). Each 10 min, a sample of 10 beans were removed, stored in a closed container to avoid dehydration and cooled until reaching room temperature (~25 °C) before performing the penetration test. The penetration force profile (force versus time) of the cooked beans was obtained, and the maximum peak was considered to study the cooking kinetics. Since this force is reduced during the cooking time, this analysis could be considered as the softening kinetics evaluation.

The penetration force of each bean was measured using a Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kg-f (49,03 N). The bean was perforated in the center of mass of its top side (considering the hilum as lateral side) using a cylindrical probe with 0.2 cm of diameter (P/2) until the distance of 3 mm at 0.5 mm s^{-1}. The force measured by the equipment as a function of the penetration depth was recorded, being the maximum peak considering for cooking description.

This evaluation was performed in triplicate for beans hydrated at 25 °C and 55 °C with and without using ultrasound. It should be mentioned that the moisture content of the beans before being cooked was the same to isolate the effects of temperature and ultrasound without being affected by the moisture content. For that, according to their respectively hydration kinetics, the beans of each treatment were hydrated enough time to reach the same equilibrium moisture content of the control sample (25 °C).

The maximum penetration force against the cooking time was plotted and fitted using Equation (7). This equation let to estimate the maximum softening of the grains.

$$F_t = F_\infty + (F_0 - F_\infty)\cdot e^{-k_F\cdot t} \tag{7}$$

2.7. Statistical evaluation

When relevant, statistical analysis was performed to the treatments through analysis of variance (ANOVA) and Tukey's test, using the software Statistica 12.0 (StatSoft, USA).

3. Results and discussion

3.1. Ultrasound and temperature effect on the hydration kinetics

White kidney beans hydration kinetics had a sigmoidal behavior (Fig. 1) and the sigmoidal equation proposed by Kaptso et al. (2008) (Equation (1)) showed a suitable adjustment for all the treatments (Figs. 1 and 2, $R^2 > 0.97$). This model has three parameters, which explain the main characteristics of this hydration kinetics behavior: the hydration rate k, the lag phase time τ and the equilibrium moisture content M_∞. The sigmoidal behavior is characterized by a lag phase presence, which is related to the seed coat as barrier to water entrance (Miano and Augusto, 2015). Therefore, as the legume's seed coat permeability is low and in some cases completely impermeable, the water is forced to enter by the hilum and/or the micropyle (Fig. 1A) (Korban et al., 1981; Miano et al., 2015; Sefa-Dedeh and Stanley, 1979) until the seed coat is completely hydrated. By covering the hilum and micropyle, the hydration kinetics of white kidney beans was significantly slowed down (Fig. 1B), reducing the hydration rate (k) ~22% and increasing the lag phase time (τ) ~25%. However, unlike other beans with high seed coat impermeability (such as the Adzuki Beans (Miano and Augusto, 2015)), the seed coat of white kidney beans proves to be permeable to water at the conditions of environmental equilibrium. This result highlighted the importance of the hilum and micropyle in the hydration process, as well as that each grain behaves different during its hydration due to its structure and/or composition.

Fig. 2 shows the hydration kinetics of white kidney beans at different temperatures with and without ultrasound. As expected, the higher the soaking water temperature is, the faster the hydration is. These results are in accordance with other works for different beans with both hydration behaviors: DCS behavior (Abu-Ghannam, 1998; Fracasso et al., 2015; Gowen et al., 2007; Jideani and Mpotokwana, 2009; Resio et al., 2006) and sigmoidal behavior (Kaptso et al., 2008; Miano et al., 2015; Oliveira et al., 2013). As the water soaking temperature is increased, the hydration rate is increased, the lag phase is reduced and the equilibrium moisture content is slightly reduced. On the other hand, Fig. 2 also shows that the used of ultrasound improved the hydration kinetics as in other works, not only for sigmoidal behavior, but also for DCS behavior (Miano et al., 2016b, 2017a; Patero and Augusto, 2015; Yildirim et al., 2013). This technology also increased the hydration rate, decreased the lag phase time and slightly increased the equilibrium moisture content.

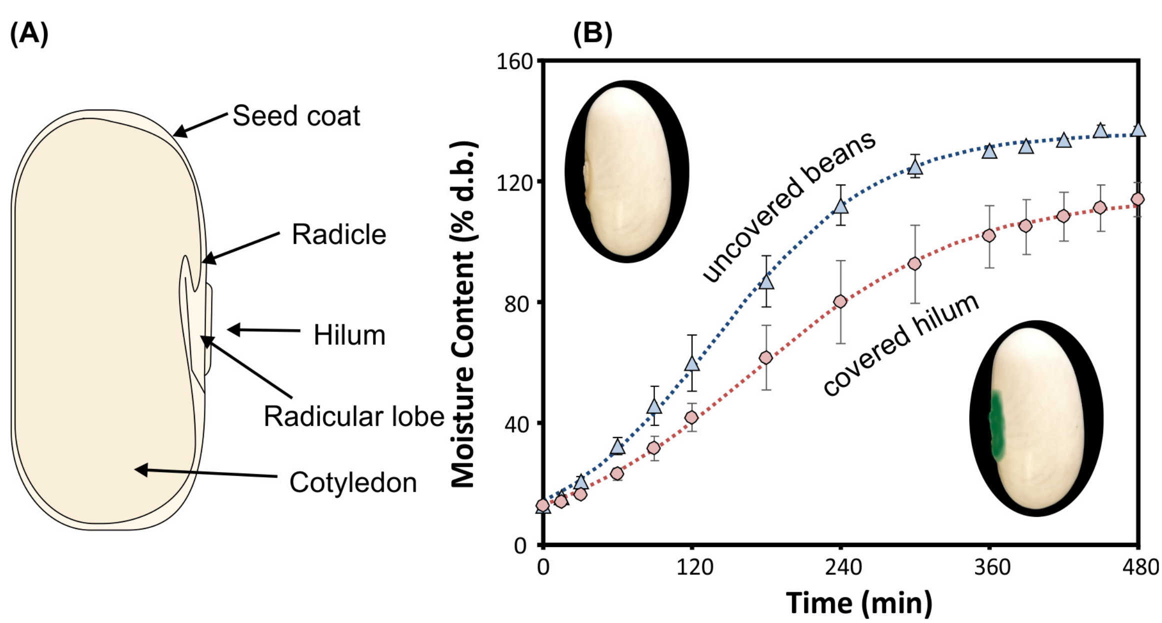

Fig. 1. (A) Morphology of white kidney bean (*Phaseolus vulgaris*). (B) Hydration kinetics of white kidney beans (*Phaseolus vulgaris*) with and without covering the hilum. The dots are the experimental data, the vertical bars the standard deviation and the discontinuous lines are the Kaptso et al. (2008) model (Equation (1)).

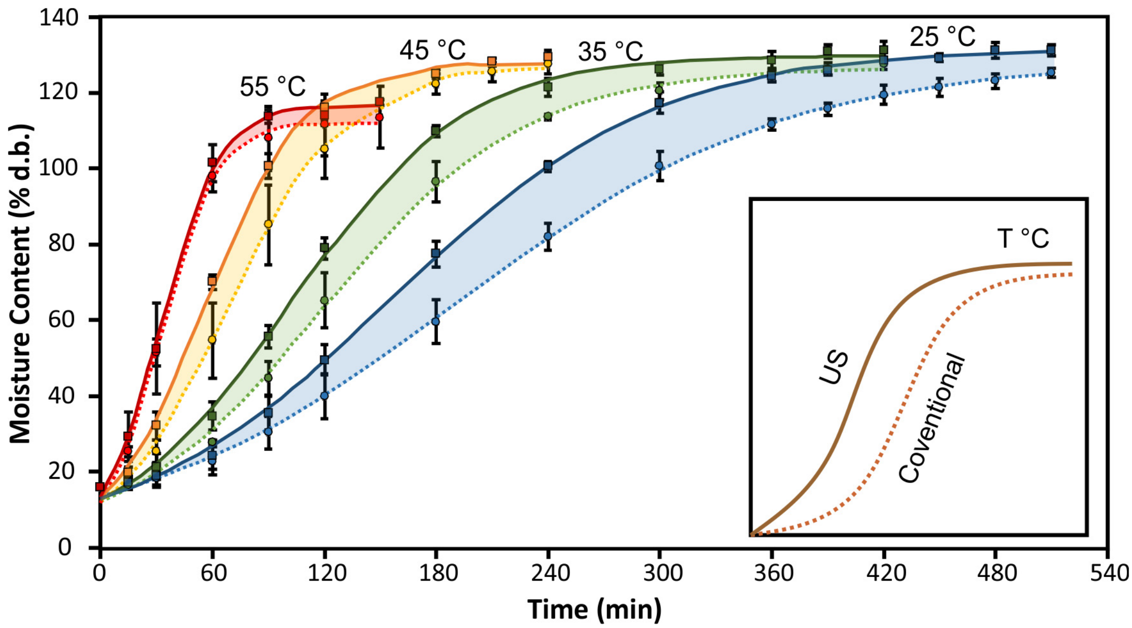

Fig. 2. Hydration kinetics of White kidney beans (*Phaseolus vulgaris*) at different soaking water temperatures with and without ultrasound technology (28 W/L of volumetric power and 40 kHz of frequency). The dots are the experimental data, the vertical bars the standard deviation, the discontinuous lines are the Kaptso et al. (2008) model. The shaded area represents the enhanced caused by the ultrasound.

The effect of using high temperatures was higher than the effect of ultrasound in the evaluated conditions, i.e., the use of hot soaking water improved better the hydration process than the use of ultrasound. Moreover, the high temperatures reduced the ultrasound effect. In fact, the effect of the temperature was too strong that every improvement by the ultrasound could be hindered. This results were also observed by Patero and Augusto (2015) in sorghum kernels (a cereal grain). In addition, when soaking water temperature is increased, the ultrasonic power can be reduced due to the cushioning effect of the vapor inside the cavitation bubbles (Raso et al., 1999) explaining the obtained results.

The Kaptso et al. model parameters were significantly affected by both the temperature and the ultrasound. Fig. 2 shows how temperature and ultrasound affected these parameters.

The parameter k, which is related to the hydration rate, was affected by both the temperature and the ultrasound. The value of this parameter was exponentially increased (Fig. 3A) by the increment of temperature ($p < 0.01$), accelerating the hydration process almost 6 times at 55 °C when compared to the hydrated samples at 25 °C. As the temperature increases, the water viscosity is reduced, improving the water flow into the beans. In addition, higher temperatures increase some reactions velocities as the cell wall carbohydrate degradation, facilitating the water uptake and increasing the hydration rate (Martínez-Manrique et al., 2011; Miano et al., 2015; Oliveira et al., 2013; Siddiq et al., 2011).

Further, ultrasound technology also increased the hydration rate (k), but much less than temperature ($p < 0.08$). This technology enhances the mass transfer during hydration process by two main mechanisms: the direct and indirect effects (Miano et al., 2016a). The direct effect is directly related to the ultrasonic wave traveling through the food, which causes the alternative compression and expansion of the medium causing the water entrance into the grain pores by pumping (Patero and Augusto, 2015). Further, this mechanism also causes the compression and expansion of the tissues, which behave as a sponge, squeezing the water (Floros and Liang, 1994; Mulet et al., 2003). In fact, Naviglio et al. (2013) observed that the hydration process is enhanced by applying cyclical pressure differences, which can facilitate the occluded air to exit and the water to enter the grain. On the other hand, indirect effects of ultrasound are related to structural modifications due to the acoustic cavitation, which causes the cell and tissue disruption. This mechanism forms microchannel, thereby improving the mass transfer (Miano et al., 2017b). The ultrasound increased the hydration rate (k) by almost 25% at 25 °C.

The activation energy (Ea) from the hydration rate parameter (k) was calculated for both treatments, with and without ultrasound (Table 1), using Equation (1). Ultrasound reduced the activation energy from 54.1 kJ/mol to 44.2 kJ/mol, suggesting the reduction on the energetic barrier to form the final products (hydrated beans). In other words, ultrasound probably reduced the maximum potential energy that atoms reach during their rearrangement (atomic distances and bond angles (Atkins and de Paula, 2009)) (Please see Equation (8) in section 3.3). In addition, the pre-exponential factor (or frequency factor, related to the collisions rate irrespective to their energy (Atkins and de Paula, 2009)) was increased by ultrasound, which means that this technology increase the collision frequency among the molecules for the activated complex formation.

Regarding the lag phase time (τ), this parameter was significantly affected by the temperature ($p < 0.01$), ultrasound ($p < 0.01$) and the interaction temperature-ultrasound ($p < 0.02$). This is the main characteristic of sigmoidal behavior hydration kinetics related to the seed coat. The lag phase ends when the seed coat is hydrated until certain moisture (water activity) causing its state changing (from glassy state to rubbery state) (Miano and Augusto, 2015). This state transition can also be caused by the temperature increase (Ross et al., 2013). Consequently, at higher water soaking temperatures, the seed coat state transition happened in a lower moisture content, reducing the lag phase time. The permeability of seed coat is also increased when it reaches the rubbery state (Miano and Augusto, 2015), thus accelerating the hydration. For that reason, the lag phase is reduced when higher soaking water temperatures are used. This result matches with the result obtained by Oliveira et al. (2013) for adzuki beans, Miano et al. (2015) for Andean

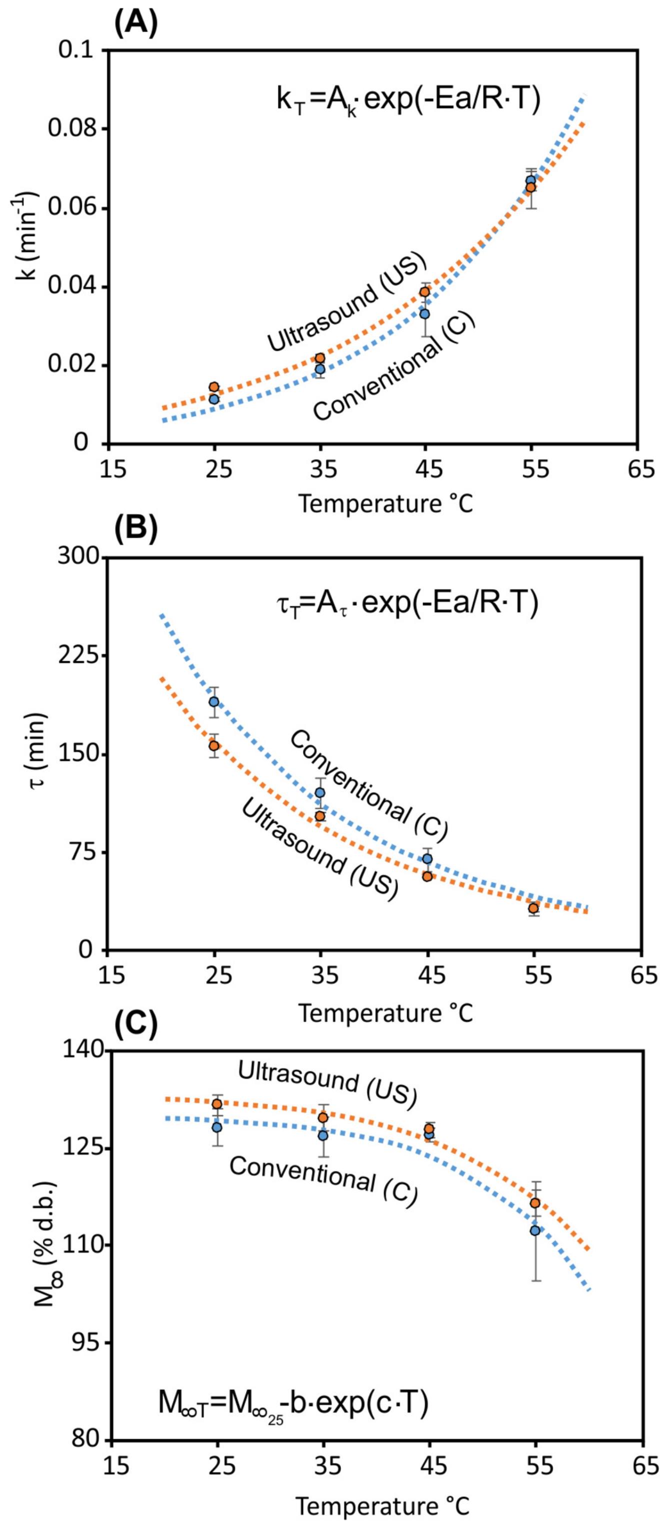

Fig. 3. Effect of temperature on the hydration parameters of the sigmoidal equation proposed by Kaptso et al. (2008). The dots are the experimental data, the vertical bars the standard deviation and the discontinuous lines the adjusted data.

lupin and Kaptso et al. (2008) for cowpea and Bambara groundnuts – all grains with sigmoidal behavior.

The ultrasound also reduced the lag phase time (τ). The relative improvement was higher than in the hydration rate (k). Therefore, the ultrasound mainly accelerates the hydration process of beans by reducing the lag phase. In fact, as the seed coat of the white kidney bean has certain degree of water impermeability (Fig. 1B), the sponge effect and inertial flow (direct effects) could accelerate the entrance of water through the hilum and micropyle, as well as the water distribution between the internal face of the seed coat and the cotyledon. Consequently, the seed coat was hydrated faster, increasing its permeability to water and accelerating the water uptake by the seed coat. Similar result was observed for mung beans (Miano et al., 2016b) using ultrasound to enhance their hydration process.

In addition, the interaction temperature-ultrasound had significant effect of the lag phase time ($p < 0.02$). Although, the individual effect of each technology is positive, the combination of both technologies has antagonist effect. In fact, as the temperature is increased, the ultrasound effect on the lag phase (τ) is reduced. Both factors, ultrasound and temperature, facilitates the glass transition of the seed coat. Higher temperatures do it directly, since the glass transition temperature of the components is reached. In contrast, ultrasound facilitates the glass transition of the components by accelerating the hydration of the seed coat, which is a slower process than heating. Consequently, the use of high temperatures is more effective to reduce the lag phase.

The activation energy (Ea) from the lag phase (τ) was also calculated for both treatments, with and without ultrasound (Table 1), using Equation (1). Ultrasound did not affect significantly the activation energy of this parameter. However, the pre-exponential factor (collision rate constant) was increased by ultrasound, which mean that ultrasound increased the collision rate of the molecules during the activated complex formation. This could have improved the molecular arrangement for the state transition of the seed coat components.

The equilibrium moisture content was affected by the soaking water temperature ($p < 0.01$). In fact, the equilibrium moisture content of white kidney beans was maintained at 25, 35 and 45 °C. However, at 55 °C this characteristic drastically drops, following equation (3). Depending on the grains and the studied temperature range, the soaking water temperature can increase, decrease or keep constant the equilibrium moisture content. Few works have reported equilibrium moisture content reduction as the temperature is increasing, such as for chickpeas (Gowen et al., 2007), red kidney beans (Abu-Ghannam and McKenna, 1997) and adzuki beans (Oliveira et al., 2013). As the temperature is increased, some reactions velocities are increased, for instance some structural components degradations as pectin and cellulose from cell walls, affecting the cell integrity (Oliveira et al., 2013). In addition, some thermosensitive proteins can denature, decreasing their water holding capacity. Another possible explanation is the rapid saturation of water in the seed edges and external layers, reducing the driving force for mass transfer (Oliveira et al., 2013).

On the other hand, the ultrasound technology slightly increased the equilibrium moisture content ($p = 0.06$). This result was also obtained for two cereal grains, sorghum kernels (Patero and Augusto, 2015) and corn kernels (Miano et al., 2017a), using ultrasonic baths. There are not works reporting the increase of the equilibrium moisture content using ultrasonic baths in pulses. In fact, there are only two works, one with navy beans (Ghafoor et al., 2014) and another with mung beans (Miano et al., 2016b) stating that ultrasound did not change the equilibrium moisture content. Consequently, due to the complexity of the grains (structure-composition), studying the hydration of more pulses using ultrasound would be important to confirm this parameter variation.

Fig. 3, shows that the equilibrium moisture content decreases as the soaking water temperature increased following Equation (3) in both treatments, with and without ultrasound (Table 1). This behavior predicts that the equilibrium moisture content is

Table 1
Effect of the soaking water temperature on the sigmoidal hydration kinetics parameters (Equation (1)). The parameters k and τ were fitted using Equation (2) and the parameter M_∞ using Equation (3).

Parameter	Conventional Hydration	R^2	Ultrasound Assisted Hydration	R^2
k	$k_T = 2.77\cdot 10^7\cdot exp\left(\frac{-54145}{R\cdot T}\right)$	0.98	$k_T = 6.98\cdot 10^5\cdot exp\left(\frac{-44162}{R\cdot T}\right)$	0.99
τ	$\tau_T = 1.09\cdot 10^{-5}\cdot exp\left(\frac{-(-41342)}{R\cdot T}\right)$	0.97	$\tau_T = 1.86\cdot 10^{-5}\cdot exp\left(\frac{-(-39541)}{R\cdot T}\right)$	0.98
***M*∞**	$M_{\infty\,T} = 128 - 1.1\cdot 10^{-25}\cdot exp(0.18\cdot T)$	0.81	$M_{\infty\,T} = 131 - 1.3\cdot 10^{-18}\cdot exp(0.13\cdot T)$	0.94

significantly affected when the soaking water temperature is higher than 45 °C. In contrast, Oliveira et al. (2013), found a linear decrease of this parameter as the soaking water temperature is increased, meaning that the used grains (adzuki beans) are more thermosensitive than white kidney beans. While the equilibrium moisture content of adzuki beans decreases from 30 °C, the equilibrium moisture content of white kidney beans started to decrease at 45 °C, thus proving its higher tolerance to “high” temperatures in comparison with the adzuki beans.

3.2. Thermodynamic properties of the hydration process

Table 2 shows the thermodynamic properties of the hydration process with and without ultrasound, considering the hydration rate (k) and the lag phase (τ) parameters.

The hydration rate parameter (k) is related to the velocity of the bean hydration. Its activation entropy ($\Delta S^{\#}$) had negative values (Table 2) in both the conventional hydration and the ultrasound-assisted hydration. Consequently, the activation free energy variation ($\Delta G^{\#}$) for the hydration rate parameter has positive sign, indicating that the reaction is nonspontaneous. Similar results were obtained in the works of Borges et al. (2017) for soybean hydration and Jideani and Mpotokwana (2009) for Botswana Bambara, observing no spontaneity. In fact, it seems to be contradictory to consider hydration as a nonspontaneous process. However, the evaluated thermodynamic properties might be related to a reaction with intermediates formation (activated complex) and not to the final product. Therefore, to explain these results, the activated complex formation theory was considered as in other works (Huang et al., 2016).

During the reactions, the reactants must come into contact before forming the products. During this contact, the molecules structure are distorted: some atoms are exchanged, the interatomic distances and bond angles changes and so on (Atkins and de Paula, 2009). All these mechanisms cause the potential energy to reach the maximum values (called activation energy - Ea) and cause the molecules to be close each other forming an activated complex. In fact, additional given energy to this point sends the reactants to form the products.

Let us assume that the hydration of the dry bean follows equation (8): during the hydration of a dry bean (DB), the activated complex ($[DB - H_2O]$) is formed before obtaining the hydrated bean (WB). For the activated complex formation, many mechanism take place, as molecular rearrangement, bond angles, interatomic distances changes, and so on, involving energetic and organization changes (enthalpy, entropy and Gibbs free energy of activation) (Atkins and de Paula, 2009).

$$DB + H_2O \leftrightarrow [DB - H_2O] \rightarrow WB \tag{8}$$

The activation enthalpy ($\Delta H^{\#}$) for the hydration rate parameter (k) has positive sign, which means an endothermic reaction when the activated complex ($[DB - H_2O]$) is formed (Al-Zubaidy and Khalil, 2007). It means that the formation of this complex is improved by adding energy, such as temperature or ultrasound, which matches with the obtained results. The value of $\Delta H^{\#}$ is reduced almost 20% by the ultrasound application in the studied temperature range (25 °C–55 °C). This means that ultrasound contributes with a quantity of energy to form the activated complex, accelerating the bean hydration rate.

The activation free energy variation ($\Delta G^{\#}$) for the hydration rate parameter has positive sign, while the activation entropy variation ($\Delta S^{\#}$) had negative values (Table 2) in both the conventional hydration and the ultrasound-assisted hydration. Consequently, the activated complex formation is a nonspontaneous reaction. This means that during the formation of the activated complex, the molecular structure is more organized. During the hydration, the H bonds between water and other molecules are more organized than the ones among water molecules, leading to the reduction of entropy (Walstra, 2002). Ultrasound technology turns $\Delta S^{\#}$ even more negative, but with a small influence on $\Delta G^{\#}$, suggesting that the

Table 2
Thermodynamic properties of the hydration process of white kidney beans with and without ultrasound application.

Hydration rate (k)						
Temperature (K)	Conventional hydration			US assisted hydration		
	$\Delta H^{\#}$ (kJ/mol)	$\Delta G^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (kJ/K·mol)	$\Delta H^{\#}$ (kJ/mol)	$\Delta G^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (kJ/K·mol)
298	51.7	84.1	−0.109	41.7	83.5	−0.140
308	51.6	85.7	−0.111	41.6	85.3	−0.142
318	51.5	87.1	−0.112	41.5	86.7	−0.142
328	51.4	88.0	−0.111	41.4	88.0	−0.142
Lag phase time (τ)						
Temperature (K)	**Conventional hydration**			**US assisted hydration**		
	$\Delta H^{\#}$ (kJ/mol)	**$\Delta G^{\#}$ (kJ/mol)**	**$\Delta S^{\#}$ (kJ/K·mol)**	**$\Delta H^{\#}$ (kJ/mol)**	**$\Delta G^{\#}$ (kJ/mol)**	**$\Delta S^{\#}$ (kJ/K·mol)**
298	−43.8	60.0	−0.348	−42.0	60.5	−0.344
308	−43.9	63.2	−0.348	−42.1	63.7	−0.343
318	−44.0	66.8	−0.349	−42.2	67.4	−0.345
328	−44.1	71.1	−0.351	−42.3	71.1	−0.346

organization of the activated complex gets more difficulty. As the ultrasound technology causes the chaotic and turbulence movement of the molecules, the molecules organization during the activated complex formation would be more difficult. Therefore, the process needs more reduction of entropy to form the activated complex. In addition, another possible cause of the $\Delta S^{\#}$ increment is the sonolisys of water, which form free radicles (Mason and Peters, 2004), hindering the organization.

The lag phase (τ) is related to the state transition of the seed coat components. As described, the seed coat permeability to water change when its components pass from the glassy to the rubbery state due to the increase in moisture content and/or temperature (Miano and Augusto, 2015; Ross et al., 2008). Therefore, the thermodynamic properties calculated for this parameter (Table 2) might be related to the state transition of the seed coat components. This phenomenon could be explained by Equation (9): the seed coat components in glassy state (G_{SC}) react with water forming the "activated complex" ($[G_{SC}-H_2O]$) in order to change the components to the rubbery state (R_{SC}).

$$G_{SC}+H_2O\leftrightarrow[G_{SC}-H_2O]\rightarrow R_{SC} \tag{9}$$

The activation enthalpy, entropy and free energy of the lag phase parameter were not significantly affected neither by the temperature nor ultrasound in the evaluated conditions. It can be noted that this state transition (Equation (9)) is an exothermic process due to the negative sign of the activation enthalpy ($\Delta H^{\#}$). In addition, the positive value of the activation free energy ($\Delta G^{\#}$) demonstrates that this transition is nonspontaneous and the negative value of the activation entropy ($\Delta S^{\#}$) give us an idea of molecular organization during this transition. In fact, the thermodynamic properties of the activated complex are slightly depended on temperature (Espenson, 1995). Therefore, despite high temperatures accelerated the state transition of the seed coat components, the results suggest this did not affect the organization, spontaneity and involved energies to form the activated complex. Further, not only temperature, but also ultrasound helped water molecules and seed coat molecules to be close. Finally, it is important to consider that the seed coat is a multi-component structure. Consequently, there is not an exactly state transition temperature (Ross et al., 2008), since each component change at different temperatures and forming different activated complexes. Thus, the calculated thermodynamic properties would represent and average value.

3.3. *Cooking process*

Fig. 4 shows the softening kinetics during cooking process (98 °C) of beans hydrated with 25 °C and 55 °C, with and without ultrasound. As can be seen, neither temperature nor ultrasound application during hydration affected the cooking process of white kidney beans. It should be mentioned that the cooking process was evaluated using beans at the equilibrium moisture content since this is the common practice in both industry and home cooking. In addition, the same moisture content for all the treatments was used in order to avoid the effect of the moisture content on the bean softening kinetics (Ibarz et al., 2004).

The work of Ulloa et al. (2015) studied the cooking process of bean hydrated after ultrasound pre-treatment by determining the cooking time using sensory tests. They stated that ultrasound pretreatment (10, 20 and 30 min with an ultrasonic bath of 40 kHz of frequency and 26 W/L of volumetric power) reduced the cooking time up to 43%. However, they evaluated the sensorial texture of beans cooked after the beans have reached their equilibrium moisture content in every treatment (with and without ultrasound). Therefore, the moisture content of the beans before being cooked was different and this influenced the final cooked beans texture. For that reason, the present work assured to keep the same moisture content before cooking the beans in order to isolate the possible effect of the ultrasound and/or temperature of hydration. Consequently, the supposed improvement of the cooking process by ultrasound or other technology could be due to the increase in the equilibrium moisture content. Further studies need to be carried out in order to verify the effect of ultrasound in the cooking time.

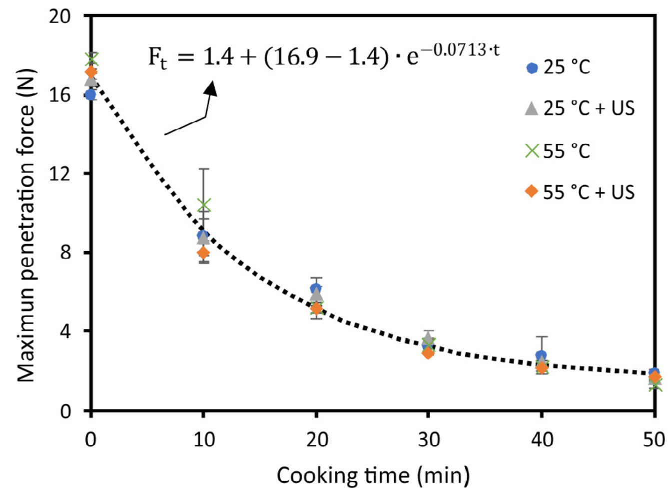

Fig. 4. Softening kinetics during cooking (98 °C) of beans hydrated at 25 °C and 55 °C, with and without using ultrasound (US). The dots are the experimental data and the vertical bars the standard deviation. As there was no significant different among the treatments, the discontinuous line represents the regression to Equation (7) for all the data.

4. Conclusions

The hydration process of white kidney beans was studied using elevated temperatures of soaking water and/or ultrasound. Both the temperature and the ultrasound technology improved the hydration process by increasing the hydration rate and reducing the lag phase (characteristic of hydration kinetics with sigmoidal behavior). However, when both technologies were used in combination, the higher the temperature of the soaking water was, the lower the ultrasound effect was. In addition, the thermodynamic properties of the process with and without ultrasound were evaluated, observing that the ultrasound reduced the required energy (activation enthalpy) to form the activated complex during the bean molecules hydration. Finally, the softening kinetics of the beans during cooking was evaluated, perceiving that the evaluated treatment with both ultrasound and elevated temperatures did not affect the cooking process of white kidney beans.

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Nomenclature

A	preexponential parameter of Arrhenius equation [$J \cdot mol^{-1}$] (Equation (2))
b, c	empirical parameters [different units] (Equation (3))
E_a	Activation energy [$J \cdot mol^{-1}$] (Equations (2) and (4))
F_0	Penetration force before cooking process [N] (Equation (7))
F_t	Penetration force as function of cooking time [N] (Equation (7))
F_∞	Minimum penetration force that beans reached during cooking process [N] (Equation (7))
H	Plank's constant (6.6262×10^{-34} J/s) (Equation (5))
k	hydration rate [s^{-1}] (Equation (1))
k_B	Boltzmann's constant (1.3806×10^{-23} J/K) (Equation (5))
k_F	Softening rate during cooking process [s^{-1}] (Equation (7))
M_t	moisture content as function of time in dry basis [% d.b.] (Equation (1))
M_∞	equilibrium moisture content in dry basis [% d.b.] (Equation (1))
$M_{\infty\ T}$	equilibrium moisture content in dry basis as function of temperature [% d.b.] (Equation (3))
P_T	parameter or property as function of temperature [different units] (Equation (2))
R	Gases constant [$8.314\ J\ mol^{-1} \cdot K^{-1}$] (Equations (2), (4) and (5))
T	Temperature [K] (Equations (2)–(6))
$\Delta G^\#$	Activation Gibbs free energy variation [$J \cdot mol^{-1}$] (Equations (5) and (6))
$\Delta H^\#$	Activation enthalpy variation [$J \cdot mol^{-1}$] (Equations (4) and (6))
$\Delta S^\#$	Activation entropy variation [$J \cdot K^{-1} \cdot mol^{-1}$] (Equation (6))
τ	lag phase, or time to reach the inflexion point of the hydration curve [s] (Equation (1))

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APPENDIX H: THE ULTRASOUND ASSISTED HYDRATION AS AN OPPORTUNITY TO INCORPORATE NUTRIENTS INTO GRAINS

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The ultrasound assisted hydration as an opportunity to incorporate nutrients into grains

Alberto Claudio Miano, Pedro Esteves Duarte Augusto*

Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil

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ABSTRACT

Hydration is an important but long step in processing beans. Consequently, any ways of taking advantage of this processing time are desirable. One possibility is to fortify the beans during the hydration process, especially with water-soluble nutrients. This work studied the incorporation of iron into beans during hydration with and without ultrasound, describing the kinetics of water and iron uptake, the entrance pathway and its effect on germination and the cooking process. For that, carioca beans were soaked in ferrous sulfate solution (0.271% w/v) with and without ultrasound (91 W/L; 25 kHz) at 25 °C. It was demonstrated that iron could be incorporated during the hydration process, describing a similar kinetics behavior to the water uptake. In addition, ultrasound accelerated this process, achieving 60.1 mg Fe/100 g w.b. after 510 min of process, in contrast to 34.4 mg Fe/100 g w.b. when the beans were hydrated without ultrasound. Finally, by hydrating the beans with ferrous sulfate, the cooking process was accelerated, which is desirable. However, the capacity for germination of the beans was reduced. In conclusion, the hydration process time can be used to fortify the beans with iron (and, possibly, other water-soluble nutrients). Nevertheless, future studies must be performed to determine if the incorporated iron is bioavailable and bioaccessible, as well as how relevant this approach is as a nutritional policy.

1. Introduction

Pulses are an important source of nutrients, especially proteins, fiber and some minerals, and are consumed worldwide (Singh, 2017). This food is harvested almost dried, this being a great advantage for its transport and storage since pulses have long shelf lives. Therefore, before being processed, pulses must be hydrated (Siddiq, Butt, & Sultan, 2011). However, the hydration process can take several hours to reach the maximum water holding capacity, this being performed in batches. Consequently, accelerating this process is very desirable, especially from an industrial point of view, and some technologies to achieve this were studied.

The use of soaking water at high temperatures was the most widely-used way to accelerate the hydration process (Miano & Augusto, 2018). Another technology used with growing interest is ultrasound, which is the application of acoustic energy with frequencies higher than 20 kHz. This technology has demonstrated positive results in accelerating the hydration of pulses and other grains, such as navy beans (Ghafoor, Misra, Mahadevan, & Tiwari, 2014), corn kernels (Miano, Ibarz, & Augusto, 2017), mung beans (Miano, Pereira, Castanha, Júnior, & Augusto, 2016), sorghum kernels (Patero & Augusto, 2015) and chickpeas (Yildirim, Öner, & Bayram, 2011).

However, although the hydration of pulses can be accelerated, this still takes too long. Even so, it is possible to take advantage of this time to fortify the pulses. Fortification is defined as the addition of a nutrient to a food in order to prevent or correct a demonstrated deficiency of that nutrient in the population. This term is different from enrichment, which means restoring the nutrients lost in a process (FAO/WHO, 1994). Fortification or enrichment can be performing by adding the nutrients in a free form or protected by encapsulation (Sauvant, Cansell, Sassi, & Atgié, 2012). Furthermore, the mass influx in the food matrix during processing can be used as an approach to incorporate nutrients.

For example, Salazar-López, Jiménez, Salazar, and Azuara (2015) demonstrated the incorporation of microcapsules into pineapple during osmotic dehydration, while vacuum impregnation has already been well studied with this purpose (for example: Gras, Vidal, Betoret, Chiralt, and Fito (2003)). In fact, Oghbaei and Prakash (2017) proposed the incorporation of iron and zinc into green grains during hydration and germination. The authors observed a remarkable increase in the content and bioavailability of these minerals after germination. However, they did not study the kinetics of mineral incorporation before germination, or the effect of the germination kinetics on the softening

* Corresponding author at: Avenida Pádua Dias, 11, Piracicaba, SP 13418-900, Brazil.
E-mail addresses: cmiano@usp.br (A.C. Miano), pedro.ed.augusto@usp.br (P.E.D. Augusto).

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of beans during cooking.

In fact, the incorporation of a water-soluble component, such as minerals, is interesting as it is transported by the soaking water. The incorporation of iron would be desirable since this mineral is important in many biological processes as an oxygen carrier in hemoglobin and myoglobin, energetic metabolism, DNA synthesis, hydrogen peroxide destruction and so on (Massarioli, Melo, & Matias de Alencar, 2018). Consequently, this work aimed to describe the kinetics of iron incorporation during the hydration of carioca beans with and without ultrasound. Furthermore, the effect of this mineral on the germination and cooking process was also evaluated.

2. Materials and methods

2.1. Raw material

Carioca kidney beans (*Phaseolus vulgaris)* from the IAC Imperador cultivar (Chiorato et al., 2012) were used. This variety of pulse was developed and kindly provided by the Agronomic Institute of Campinas (IAC), Brazil. The batch used had an initial moisture content of 12.80 ± 0.16% d.b. (g water/100 g of dry matter), a starch content of 36.33 ± 0.3% d.b., a protein content of 19.54 ± 0.21% d.b., a total fat content of 1.59 ± 0.03% d.b., an ash content of 5.44 ± 0.06% d.b. and an iron content of 51.4 ± 0.6 mg/kg d.b. In addition, the average size of the grains was 10.8 ± 0.3 mm in length, 7.3 ± 0.3 mm wide and 5.1 ± 0.4 mm thick.

2.2. Hydration kinetics

For the study of the hydration kinetics, 10 g of pre-selected grains (without damage) was placed in net bags and soaked in 4 L of distilled water with and without ultrasound application. During the hydration process, the samples were periodically drained, superficially dried with paper towels and their moisture content was obtained by mass balance (verifying the possibility of neglecting the solid loss to the water). The sampling was carried out every 15 min for the first hour, every 30 min for the next 2 h and every hour from then on. The hydration process was performed at 25 ± 1 °C and in triplicate.

The ultrasound application was performed using an ultrasonic bath (Ultronique Q13/25 - Brazil) with a frequency of 25 kHz and a volumetric power of 91 W/L (determined following the calorimetric method described by Kimura, Kobori, Rodriguez-Amaya, and Nestel (2007)). The samples (beans in net bags) were placed at the bottom of the ultrasonic bath in order to be subjected to the waves with the highest and most homogeneous intensity. The temperature of the process was kept constant at 25 ± 1 °C throughout the process using heat exchangers.

Furthermore, to describe the water flow pathway, the permeability of the seed coat was evaluated by coating the hilum and micropyle of the beans with nail polish (Miano, Pereira, et al., 2016; Ramos, Pezet-Valdez, O'Connor-Sánchez, Placencia, & Pless, 2004). Then, the hydration kinetics of the coated and uncoated beans were compared.

In addition, as the kinetics of iron incorporation were studied, the influence of the presence of ferrous sulfate on the hydration kinetics was studied, by using the concentration studied (0.271%m/v) and twice this (0.542%m/v). The hydration kinetics of the beans soaked in distilled water and sulfate ferrous solution were compared.

Finally, the data of the beans hydration kinetics were fitted using the sigmoidal equation proposed by Kaptso et al. (2008) (Eq. (1)). For that purpose, the grain moisture content (*M*, in dry basis, % d.b.) versus the hydration time (min) was tabulated for each treatment.

$$M_t = \frac{M_\infty}{1 + \exp[-k \bullet (t - \tau)]} \quad (1)$$

2.3. Iron incorporation kinetics

For the kinetics of iron incorporation, a sample of beans was soaked in a ferrous sulfate ($FeSO_4$) solution (0.271%w/v equivalent to 100 mg of Fe/100 g of solution) in the proportion of 1:20 of beans/solution. Ferrous sulfate was chosen because it is soluble in water and is one of the most common sources of iron for fortifying food (Martınez-Navarrete, Camacho, Martınez-Lahuerta, Martınez-Monzó, & Fito, 2002). At certain time intervals (30, 60, 120, 300 and 510 min), a sample of 10 g of beans was removed from the solution, rinsed three times with distilled water and dried at 40 °C for 12 h. Then, each sample was milled using a stainless-steel analytical mill (IKA A11 basic analytical mill, Germany) before determining the iron content. The iron content was measured with an energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-720, Japan) following the methodology of Tezotto et al. (2013). All the experiments were performed in triplicate.

In addition, other treatments were performed to determine where the iron was accumulated in the bean and its flow pathway. For that, the seed coat and the cotyledon of beans were separated and their iron content was determined after soaking them in water and ferrous sulfate solution for 510 min (the final part of the hydration process). In addition, as in the hydration kinetics process (Section 2.2), the hilum of the beans was sealed using nail polish to evaluate whether iron passes through the seed coat. The iron content of the seed coat and the cotyledon of the covered grains was also determined.

Then, the iron incorporation kinetics were studied using ultrasound as in the hydration kinetics. The proportion of beans/solution was keep at 1:20 considering 4 L of solution in the ultrasonic bath and at 25 °C.

2.4. Cooking process

Approximately 80 g of beans were cooked in a Becker with 1.6 L of boiling distilled water (98 °C). Every 15 min, a sample of 10 beans was removed, stored in a closed container to avoid dehydration and cooled to room temperature (~25 °C) before performing the penetration test. The penetration force profile (force versus time) of the cooked beans was obtained, and the maximum peak was considered to study the cooking kinetics. Since this force is reduced over the cooking time, this analysis was considered as the softening kinetics.

The penetration force of each bean was measured using a Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kg-f (49,03 N). Each bean was perforated in the center of mass of its top side (considering the hilum as the lateral) using a 0.2 cm-diameter cylindrical probe (P/2) to a depth of 3 mm at 0.5 mm·s^{-1}. The force measured by the equipment as a function of the penetration depth was recorded, being the maximum peak considering for cooking description.

This evaluation was performed in triplicate for all the hydration treatments performed (soaked in distilled water and ferrous sulfate solution with and without ultrasound). It should be mentioned that the moisture content of the beans before cooking was the same, since it affects the cooking time (Ibarz, González, & Barbosa-Cánovas, 2004). For that, according to their respective hydration kinetics, the beans from each treatment were hydrated long enough to reach the same equilibrium moisture content as the conventional hydrated samples.

The maximum penetration force over cooking time was plotted and fitted using Eq. (2), which describes an asymptotic decay in the penetration force through a first order kinetics. This equation allows the maximum softening of the grains to be estimated.

$$F_t = F_\infty + (F_0 - F_\infty) \bullet e^{-k_F \bullet t} \quad (2)$$

2.5. Germination process

Three replications of 48 beans were placed in rolled paper towels moistened with water at a proportion of 2.5 times the dry substratum weight and left to germinate at 25 °C in the dark for 4 days. Every certain time, the germinated beans in the rolled paper towels were counted. The data of the total germinated beans against time was tabulated and fitted to a sigmoidal equation (Eq. (3)).

$$G_t = \frac{G_{max}}{1 + \exp[-G_r \bullet (t - G_{50})]} \tag{3}$$

2.6. Statistical evaluation

For the nonlinear regression of the data in the different equations, each replication datum was fitted using a generalized reduced gradient algorithm, which is implemented in the 'Solver' tool in the Excel 2016 software (Microsoft, USA). Different initial guesses of the three parameters were assessed to detect possible local convergence.

Furthermore, when relevant, a statistical analysis was performed on the treatments through analysis of variance (ANOVA) and Tukey's test, using the Statistica 12.0 software (StatSoft, USA).

3. Results and discussion

3.1. Hydration kinetics

As expected, the hydration kinetics of the carioca beans, Imperador cultivar, showed sigmoidal behavior (Fig. 1A), which is a characteristic of some pulses (Miano & Augusto, 2017) due to the structure and composition of the seed coat, which is not completely permeable to water at low moisture contents. This forces the water to enter the grain by the hilum and/or micropyle in order to hydrate the seed coat from inside the bean until its moisture content is increased enough to become permeable to water. In fact, this phenomenon is attributed to the physical state of the seed coat components: when the seed coat reaches a certain moisture content, its components change from a glassy or rubbery state to become more permeable to water (Miano & Augusto, 2015; Ross, Arntfield, & Cenkowski, 2013). This partial permeability was also demonstrated by covering the hilum. Fig. 1A shows that when the hilum is covered, the hydration process is slowed down. In fact, the hydration rate is reduced by ~56% and the lag phase is increased by ~77% (Table 1) reinforcing the idea of partial permeability of the seed coat. Even so, in contrast with Adzuki beans whose seed coat water permeability was negligible (Miano & Augusto, 2015), the seed coat of this Carioca bean was slightly permeable.

Fig. 1B, shows that by soaking the beans in ferrous sulfate solution, the hydration kinetics were not altered (Table 1). In fact, the hydration kinetics could be affected by the pH or ionic strength of the soaking water (Haladjian et al., 2003; Oladele, Agbetoye, Osundahunsi, & Augusto, 2017). However, the concentration of ferrous sulfate used was too low to modify either the pH or ionic strength of the soaked water. Therefore, this could be the reason why the hydration kinetics were not affected – which is an interesting result in the context of this work.

Table 1
Data fitting of the hydration, cooking and germination process of Carioca beans at different treatments. Average ± standard deviation.

Hydration process	Parameters of Eq. (1): $M_t = \frac{M_\infty}{1+\exp[-k \bullet (t-\tau)]}$		
	M_∞ (% d.b.)	k (min^{-1})	τ (min)
Conventional (distilled water)	132 ± 1.2	0.016 ± 0.001	148 ± 5.2
Covered hilum	128 ± 3.1	0.007 ± 0.001	262 ± 36.1
With $FeSO_4$ (0.271% w/v)	129 ± 2.4	0.015 ± 0.001	142 ± 8.9
With $FeSO_4$ (0.542% w/v)	128 ± 2.8	0.016 ± 0.001	143 ± 11.1
Ultrasound with water	136 ± 1.1	0.022 ± 0.001	103 ± 5.4
Cooking process of beans hydrated for 510 min in:	Parameters of Eq. (2): $F_t = F_\infty + (F_0 - F_\infty) \cdot e^{-k_F \cdot t}$		
	F_∞ (N)	k_F (min^{-1})	F_0 (N)
Distilled water	177 ± 14.9	0.094 ± 0.011	1491 ± 20.1
With $FeSO_4$ (0.271% w/v)	114 ± 3.7	0.136 ± 0.002	1460 ± 34.1
Ultrasound	201 ± 11.3	0.097 ± 0.004	1493 ± 3.2
With $FeSO_4$ and ultrasound	86 ± 2.4	0.125 ± 0.007	1302 ± 23.7
Germination of beans hydrated for 510 min in:	Parameters of Eq. (3): $G_t = \frac{G_{max}}{1+\exp[-G_r \bullet (t-G_{50})]}$		
	G_{max} (%)	G_r (h^{-1})	G_{50} (%)
Distilled water	97 ± 1.5	0.335 ± 0.061	30 ± 1.4
With $FeSO_4$ (0.271% w/v)	50 ± 16.1	0.209 ± 0.062	32 ± 1.3
Distilled water and ultrasound with water	28 ± 4.4	0.257 ± 0.101	39 ± 5.4
With $FeSO_4$ and ultrasound	0	0	0

3.2. Ultrasound assisted hydration and iron incorporation

After demonstrating that the addition of ferrous sulfate in the soaking water did not affect the hydration kinetics of the carioca bean, the quantity of iron that entered the bean and the effect of ultrasound were studied.

Firstly, the effect of ultrasound on the hydration kinetics was studied. Fig. 2A shows that ultrasound (25 kHz of frequency and 91 W/L of volumetric power) enhanced the hydration process, increasing the hydration rate (k) ~37%, reducing the lag phase (τ) ~30% and increasing the equilibrium moisture content (M_∞) ~3%. This technology has also

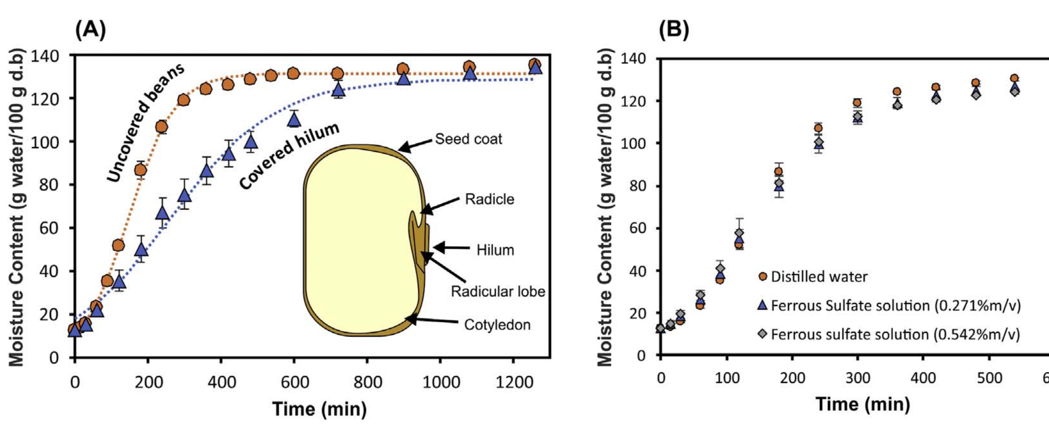

Fig. 1. (A) Hydration kinetics of carioca beans with covered and uncovered hilum. In the detail, the morphology of the grain. (B) Effect of ferrous sulfate concentration on the hydration kinetics of carioca beans. The dots represent the mean of the experimental data, the vertical bars the standard deviation and the lines the data from Kaptso et al. model (Eq. (1)).

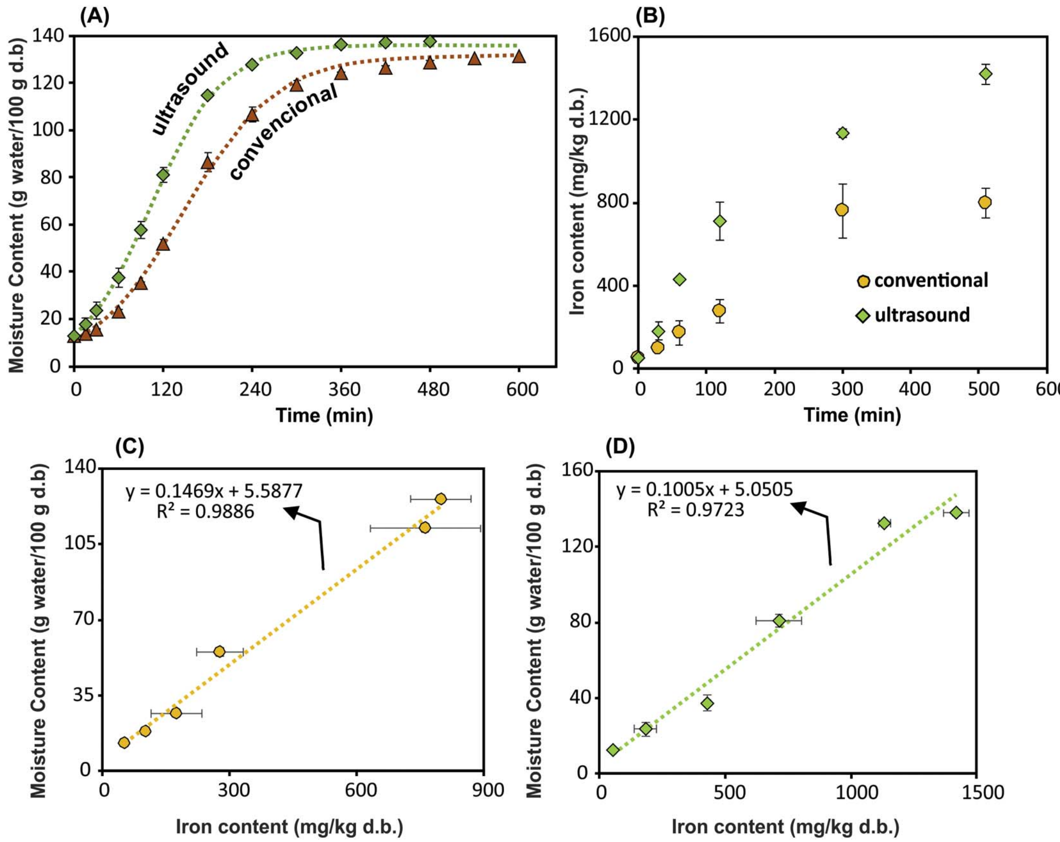

Fig. 2. (A) Hydration kinetics of carioca beans with and without ultrasound fitted to Kaptso et al. model (Eq. (1)). (B) Iron incorporation kinetics in beans soaked in iron sulfate solution with and without ultrasound. (C) Correlation between moisture content and iron content during the hydration process without ultrasound. (D) Correlation between moisture content and iron content during the hydration process with ultrasound. The dots represent the mean of the experimental data, the vertical and horizontal bars are the standard deviation.

enhanced the hydration process of other grains with sigmoidal behavior such as mung beans: increasing the hydration rate by ~44% and reducing the lag phase by ~25% (Miano, Pereira, et al., 2016); and white kidney beans: increasing the hydration rate by ~25% and reducing the lag phase by ~17% (Miano, Sabadoti, & Augusto, 2017). Furthermore, this technology enhanced the hydration kinetics of grains with downward concave behavior such as corn kernels (reducing the process time by ~35%) (Miano et al., 2017), sorghum kernels (reducing the process time by ~40%) (Patero & Augusto, 2015), navy beans (reducing the process time by ~60%) (Ghafoor et al., 2014) and chickpeas (reducing the process time by ~20%) (Yildirim et al., 2011). It is important to highlight that all the works mentioned used ultrasonic bath, with similar systems, but with different volumetric power and/or frequency.

Many mechanisms enhance the mass transfer using ultrasound technology. Some of them reduce the external resistance and others reduce the internal resistance (Miano, Rojas, & Augusto, 2017). However, in the hydration process, the water transfer is only controlled by the internal resistance. Therefore, ultrasound could enhance the hydration process by mechanisms such as inertial flow and the sponge effect (the food matrix behaves like a sponge due to the alternative squeezing and relaxing of the structure), which are caused by the pressure differences inside the beans due to the travel of the acoustic waves. In addition, ultrasound can form micro channels and cavities inside the beans, accelerating the hydration process and increasing the equilibrium moisture content. It is important to mention that some of these mechanisms, especially the acoustic cavitation and micro channel formation, are improved by the water activity. As the water activity in the bean increases, the higher the ultrasound effect becomes (Miano, Ibarz, & Augusto, 2016). This is why the enhancement of the hydration process rises with the process time (Fig. 2A).

Despite ultrasound technology accelerating the hydration of beans, this process still takes too long finish. However, this time can be exploited by incorporating some nutrients into the beans. Fig. 2B demonstrates that the iron concentration in the beans increases with the hydration process time. In addition, when ultrasound is used, the incorporation of iron is higher. After 510 min of the process, the beans increase their iron content from 51.4 mg/kg d.b. to 798.2 mg/kg d.b. without ultrasound and to 1418.6 mg/kg d.b. with ultrasound.

The enriched carioca beans obtained provide 34.4 mg Fe/100 g w.b. when they were hydrated without ultrasound and 60.1 mg Fe/100 g w.b. with ultrasound. The recommended iron intake is 9.1 mg/day for men and 19.6 mg/day for women (FAO/WHO). Therefore, consuming from 15 to 31 g of this product per day would supply the recommended iron intake. However, we highlight the need for nutritional studies such as into how bioavailable the incorporated iron is. Furthermore, it is important to mention that values below 10–20 mg Fe/kg of body mass are not toxic for human consumption (Davila-Hicks, Theil, & Lönnerdal, 2004; Ellenham & Barceloux, 1988). Consequently, the quantity of iron added to the beans with and without ultrasound would not be toxic and the hydration process can be a simple approach to enhancing the iron content of beans.

Fig. 2B shows also that kinetics of iron incorporation have sigmoidal behavior, similar to the hydration kinetics. This suggests that the iron mainly enters the beans with water, through capillarity. Fig. 2C and D validate this, since there is a very strong positive correlation between the gain in moisture content and the incorporation of iron, when the beans were soaked without and with ultrasound. In fact, it is known that water is transferred into the bean by diffusion and capillarity: water enters through the hilum by capillarity and through the seed coat by diffusion (Miano, García, & Augusto, 2015). Accordingly, some treatments were performed to verify if iron was transferred by both mechanisms.

Table 2
Iron content of Carioca beans hydrated at different conditions. Average ± standard deviation. The iron sulfate solution 0.271% w/v was used.

Treatment	Iron content (mg/kg d.b.)		
	Whole bean	Seed coat	Cotyledon
Conventional hydrate for 510 min	51.4 ± 3.8	79.9 ± 2.1	35.7 ± 1.2
Beans soaked in ferrous sulfate solution for 510 min	798.2 ± 71.8	5403.2 ± 523.3	182.7 ± 12.2
Covered hilum beans soaked in ferrous sulfate solution for 510 min	558.1 ± 34.6	n.a.	152.9 ± 10.3
Beans soaked in ferrous sulfate solution with US for 510 min	1418.6 ± 49.9	n.a.	n.a.

n.a. not analyzed.

The hilum of the beans was coated to evaluate whether iron can pass through the seed coat. According to Table 2, the quantity of iron in the cotyledon of the coated beans increased from ~35.7 to ~152.9 mg/kg d.b. during this treatment, demonstrating that iron passed through the seed coat. Since the seed coat structure is compact, without significant intercellular spaces, this work suggests that iron could pass through it by diffusion.

When uncoated beans were processed, the iron content of the cotyledon was higher (Table 2), proving that part of the iron is also transferred by capillarity through the hilum, and filling the space between the seed coat and the cotyledon.

Table 2 shows that most of the iron incorporated is fixed in the seed coat. In fact, most of the natural iron in the beans studied is stored in the seed coat (Takahashi et al., 2009). Griffiths (1982) stated that the seed coat of beans has more iron binding components, such as tannins and phytates, than the cotyledon. Therefore, this is one of the reasons the seed coat has more iron and why it retains the incorporated iron (Table 2). However, tannins act as natural chelating agents, decreasing the availability of iron (Gibson, Bailey, Gibbs, & Ferguson, 2010; Reddy, Pierson, Sathe, & Salunkhe, 1985). Consequently, despite the beans being enriched with iron, future studies of bioavailability must be conducted. Furthermore, the large area of the seed coat improves the iron binding with the components of the coat.

In summary, iron incorporation in beans during the hydration process was possible and enhanced by ultrasound. This nutrient was transferred into the beans by capillarity and diffusion, crossing both the hilum and the seed coat. Furthermore, most of the iron incorporated was stored in the seed coat due to the presence of iron binding components, and the iron content of the cotyledon was increased by a factor of almost six. Consequently, not only iron, but also other minerals could be incorporated during hydration, taking advantage of the fact that this process is time-consuming.

3.3. Cooking process

One important quality property of beans is their cookability (cooking process characteristics). Therefore, this property was evaluated for each treatment. Fig. 3 shows that the way the beans were hydrated affected their softening kinetics during cooking.

When carioca beans were hydrated using ultrasound, the softening kinetics were not significantly affected (Fig. 3A). Although this technology could cause structural changes, improving the hydration kinetics (Fig. 2), these were not great enough to affect the bean texture and cooking.

Ulloa et al. (2015) stated that using ultrasound as a pretreatment for 10 to 30 min can reduce the cooking time of common beans by almost 43%. However, they did not perform the cooking process at the same final moisture content after hydration, as in the present work. In fact, the higher the moisture content of the bean at the beginning of cooking is, the shorter the cooking process is (Ibarz et al., 2004). Consequently, it is hard to understand if the results in Ulloa et al. (2015) were due to the ultrasound technology or the different moistures. Furthermore, the authors determined the cookability through sensory methods, while the present work uses a more objective approach.

On the other hand, when carioca beans were soaked in ferrous sulfate solution, the cooking time was reduced, because they become soft faster (Fig. 3). Many published works state that the presence of ions in the cooking water can accelerate the cooking process of beans, or correct the hard-to-cook defect (which appear when beans are stored at high temperatures and high moisture content causing the formation of insoluble components that prevent them from softening) (Ávila et al., 2015). For instance, Reyes-Moreno, Paredes-López, and Gonzalez (1993) stated that the hard-to-cook phenomenon of beans can be corrected by adding some salts to the cooking water. These salts cause ion exchange and chelation of such insoluble components as pectates and phytates turning them into soluble components and enhancing the cell separation during cooking. In fact, calcium pectates and phytates are the main components that prevent the beans from softening during cooking, acting as an adhesive in the middle lamella (Muller, 1967). Therefore, the incorporated iron probably replaces the calcium ions that are bound with pectates and phytates, since iron ions have more affinity for these salts than calcium ions (Oberleas, 1973). Therefore, iron helps to reduce the insoluble calcium salts thus improving the softening of the beans.

Finally, when the beans where hydrated in ferrous sulfate solution with and without ultrasound technology, they softened faster than when they were hydrated with water, with and without ultrasound (Fig. 3). As stated, iron ions probably replaced the calcium ions in the pectates and phytates salts, making it easier for the beans to soften during cooking. In addition, ultrasound might have accelerated this process by breaking up some molecules in the middle lamella by acoustic cavitation, generating reactive molecules, which could bind with iron – although note that we have not evaluated this. Furthermore, ultrasound accelerated the iron transfer, increasing the quantity of this reactant. It is important to highlight that when the beans were soaked in iron solution with ultrasound before cooking (Fig. 3B), the beans were softened, suggesting a severe structural change or weakening due to the combination of ultrasound and ionic exchange caused by the ferrous sulfate salts.

3.4. Germination process

Fig. 4 shows the germination kinetics (germination time courses (Bewley, Bradford, Hilhorst, & Nonogaki, 2013)) of the carioca bean Imperador cultivar. This bean shows an almost complete (97 ± 2%) and uniform germination. However, the hydration treatments on these beans affected their germination significantly.

When the beans were hydrated with ultrasound, the maximum germination percentage decreased sharply (Table 1 and Fig. 4B) to almost 50%. This result is contradictory to previous published studies, which stated that ultrasound enhanced the germination of seed. For instance, this technology has demonstrated an improvement in the germination for the seeds of rice (Ding et al., 2018), grass (Liu et al., 2016), Arabidopsis (López-Ribera & Vicient, 2017), barley (Miano, Forti, et al., 2015; Yaldagard, Mortazavi, & Tabatabaie, 2008) and mung beans (Miano, Pereira, et al., 2016) chickpeas, and wheat, pepper and watermelon seed (Goussous, Samarah, Alqudah, & Othman, 2010). These works used ultrasonic volumetric powers from 13 to 41 W/L and frequencies between 20 and 45 kHz. In addition, the seeds were processed for 5 to 60 min, except in the works by Miano, Forti, et al. (2015) and Miano, Pereira, et al. (2016) that applied ultrasound to barley seeds and mung beans for 5 h. These works reported that ultrasound enhances

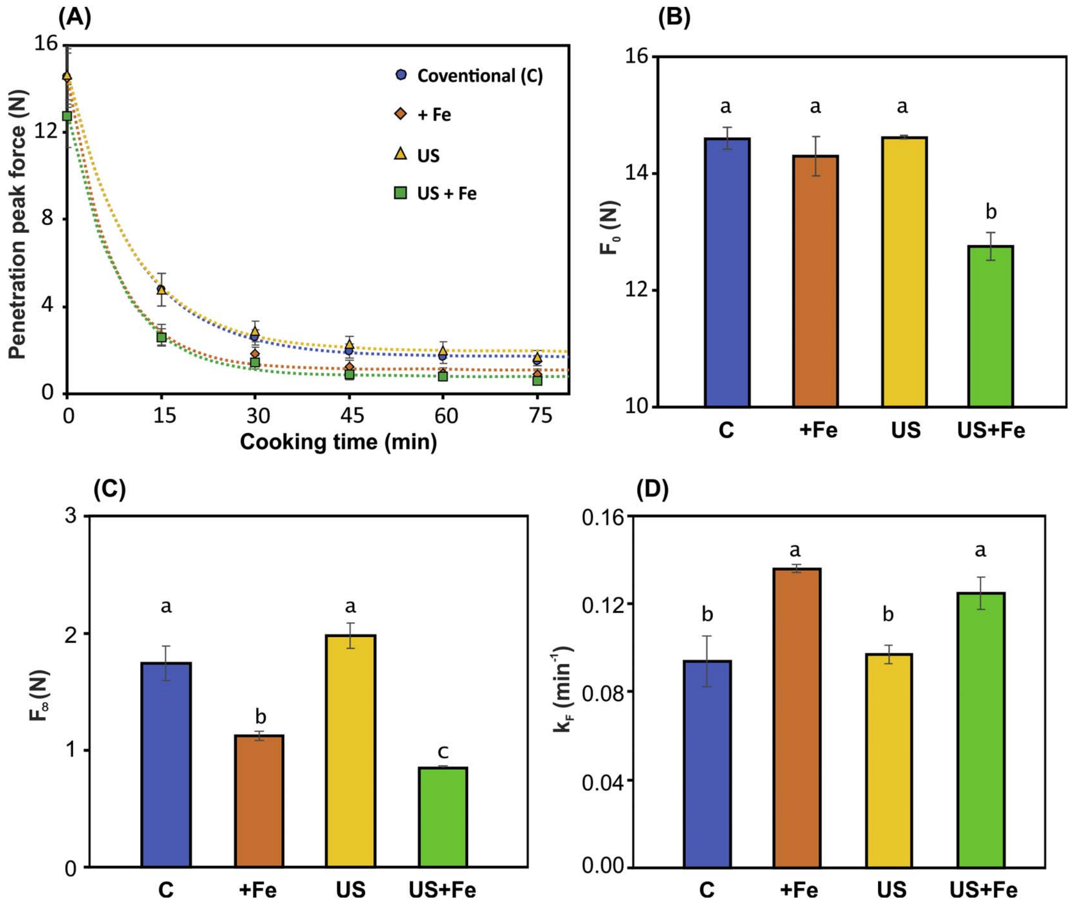

Fig. 3. (A) Softening kinetics during cooking process of Carioca beans hydrated at different treatments: in distilled water (C), in ferrous sulfate solution (+Fe), in distilled water with ultrasound (US) and in ferrous sulfate solution with ultrasound (US + Fe). The dots represent the mean of the experimental data and the lines the data from Eq. (2). (B) Effect of the soaking process on the penetration force of carioca beans. (C) Effect of the soaking process on the minimum penetration force during cooking of carioca beans. (D) Effect of the soaking process on the softening rate (cooking velocity) of carioca beans. For all this figures, the vertical bars represent the standard deviation.

the germination by two main mechanisms: the improvement in mass transfer, which enhances the transport of water, gases and nutrients, and the activation of germination enzymes.

However, the present work applied ultrasound with a volumetric power of 91 W/L, frequency of 25 kHz and for 510 min. Consequently, the energy provided to the carioca beans was much higher than in the published works. This result means that depending on the intensity (power and time), ultrasound can enhance or hinder the process, as in enzyme activation/inactivation (Rojas, Hellmeister, & Augusto, 2016). The high intensity used could cause excessive cell disruption, damaging germination tissues like the radicle. Moreover, the enzymes of germination could be inactivated by ultrasound, thus hindering the germination process. There is a possibility that this negative effect is specific to the bean studied. Consequently, different grain germinations using high-energy ultrasound should be studied to obtain conclusive results.

When the beans were soaked in radicle iron solution, germination was also hindered (Fig. 4), reducing the maximum germination percentage to 28 ± 4%. An excess of iron in the seed is toxic to the embryo (Grillet, Mari, & Schmidt, 2013), reducing the percentage of germination (El Rasafi, Nouri, Bouda, & Haddioui, 2016), even killing the seeds (Nozoe, Tachibana, Uchino, & Yokogami, 2009). So, although fortified beans were obtained, germination was negatively affected. However, this result is not a problem when the objective is to fortify the beans for direct consumption.

Furthermore, when the beans were hydrated in the iron solution using ultrasound, the beans did not germinate (Table 1, Fig. 4). In this case, germination was not only affected negatively by ultrasound, but also synergistically by the toxicity of iron. Ultrasound causes a large quantity of iron to enter the beans, thus increasing its toxic effect and promoting cell disruption and tissues damage. It should be mentioned that for industrial bean cooking, germination would be not important, but it would be in sprout production. Consequently, future studies of optimization of the iron solution concentration and ultrasonic intensity (power – time) should be conducted to obtain fortified beans without losing their capacity to germinate.

3.5. Final consideration

The present work has demonstrated that the hydration of beans could be used to incorporate water-soluble nutrients into the grain. However, it should be mentioned that this work aimed to study how a nutrient enters the bean, focusing on the mass transfer phenomenon: the quantity, kinetics, and entrance pathway of iron were all studied. Although this target was met, future studies must be performed to determine if the incorporated iron is bioavailable and bioaccessible.

Consequently, this work does not aim to be a reference from the nutritional point of view nor does it propose that ferrous sulfate is the best source of iron for humans or recommend incorporating nutrients into grains as a nutritional policy. Even so, we highlight the importance of this work as a prospective study of using the hydration process to obtain products with better quality, taking advantage of the process drawbacks.

Furthermore, the germination analyses demonstrated that the incorporated iron hindered this process. However, the cooking process was improved through accelerated bean softening. Therefore, optimization studies are also recommended to incorporate enough of a

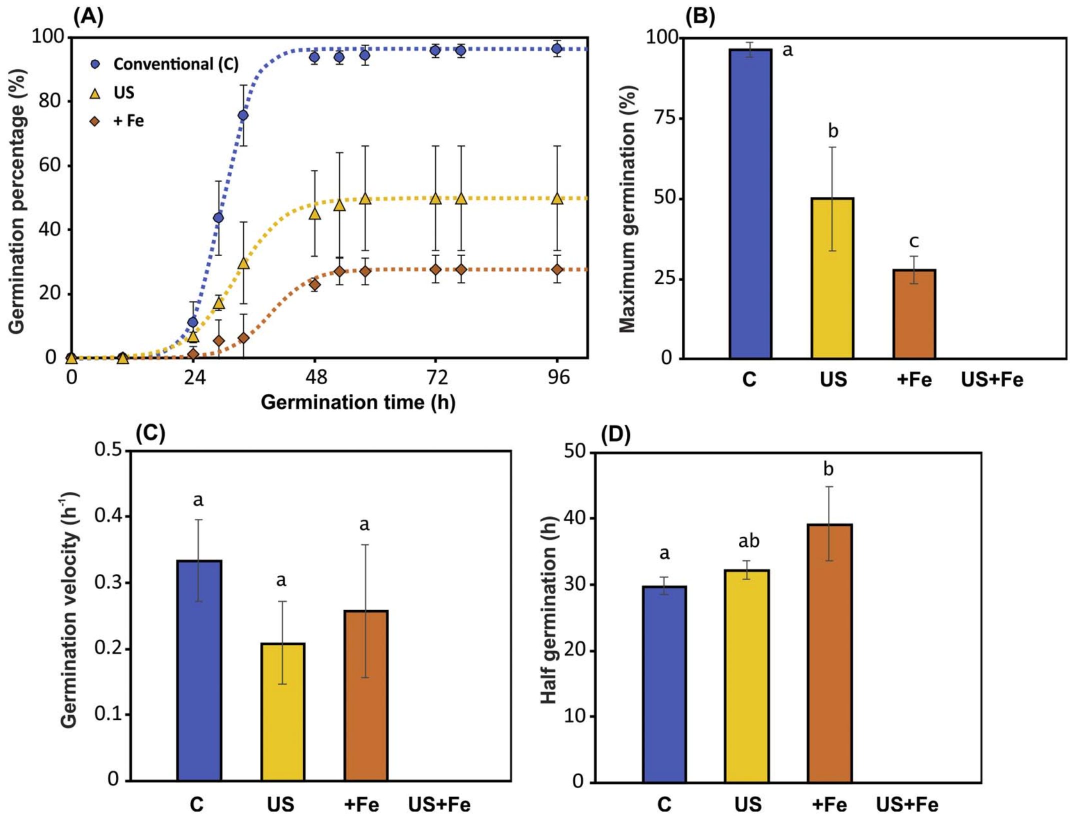

Fig. 4. (A) Germination kinetics of Carioca beans hydrated at different treatments: in distilled water (C), in ferrous sulfate solution (+Fe), in distilled water with ultrasound (US) and in ferrous sulfate solution with ultrasound (US + Fe). The dots represent the mean of the experimental data and the lines the model from Eq. (3). (B) Effect of the soaking process on the maximum germination percentage of carioca beans. (C) Effect of the soaking process on the germination rate of carioca beans. (D) Effect of the soaking process on the time to reach half of the maximum germination of carioca beans. For this entire figure, the vertical bars represent the standard deviation.

nutrient to obtain the maximum advantages in further processes.

In fact, this work is the first step for future research.

Not only iron, but also other nutrients and functional components can be incorporated into different kinds of grain, directly in solution or maybe protected by microcapsules. In addition, ultrasound can be used to accelerate this process.

Therefore, an easier and a cheaper way of fortification could be developed. However, future studies must be performed to determine if the incorporated nutrients are bioavailable and bioaccessible, as well as how relevant this approach is as a nutritional policy.

4. Conclusions

The present work demonstrated that it is possible to incorporate iron into beans during the hydration process and that ultrasound technology enhances this process. The iron influx kinetics and the entrance pathway were suggested as well as the data was fitted using suitable mathematical models. Furthermore, the incorporation of iron accelerated the softening of the beans during cooking, thus improving this process. However, germination of the beans was hindered, not only by the incorporation of iron, but also through the use ultrasound during hydration. Finally, the application of this work is as an alternative way to obtain fortified beans and the possibility remains open for future studies that attempt to incorporate other nutrients. However, future studies must be performed to determine if the incorporated nutrients are bioavailable and bioaccessible, to optimize the process and also to evaluate the relevance of this approach as a nutritional policy.

Nomenclature

F_0 initial penetration force before cooking and after hydration (Eq. (2)) [N]
F_t penetration force as a function of cooking time (Eq. (2)) [N]
F_∞ minimum penetration force after cooking (Eq. (2)) [N]
G_t percentage of germinated seed as a function of time (Eq. (3)) [%]
G_{max} maximum percentage of germination (Eq. (3)) [%]
G_r germination rate (Eq. (3)) [h^{-1}]
G_{50} required time for germinating 50% of the seeds (Eq. (3)) [h]
k Kaptso et al. kinetic parameter related to the water absorption rate (Eq. (1)) [min^{-1}]
k_F softening kinetics rate [min^{-1}]
M_t moisture content as a function of the hydration time (Eq. (1)) [% d.b.]
M_∞ equilibrium moisture content (Eq. (1)) [% d.b.]
t process time: hydration, cooking and germination (Eqs. (1), (2) and (3)) [min or h]
τ lag phase time of hydration (Eq. (1)) [min]
W/L volumetric power provided by the ultrasonic bath [W/L (Watts per liter)]

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APPENDIX I: SIMPLE ABSTRACTS (ENGLISH, PORTUGUESE, SPANISH)

SIMPLE ABSTRACT

The grains hydration is important for further processes as cooking, gemination or malting. In fact, this process takes too much time to be complete, which is a drawback in industrialization. For that reason, the present thesis focused on explaining the hydration of grains with sigmoidal behavior (a less studied behavior) and improving this processing by using the ultrasound technology (technology that uses high power acoustic waves with frequencies that cannot be heard by humans). Several grains were characterized, observing that only legume grains as beans, lens and peas have this sigmoidal behavior. In addition, it was demonstrated that, by using ultrasound, the hydration of grains can be reduced almost 5 hours, with a high impact on industrial processing. Further, it was demonstrated a nutrient can be incorporated into the grains during hydration, which is accelerated if ultrasound is used. Moreover, by using ultrasound, the germination of some grains can be accelerated, the extraction of some undesirable component can be accelerated, but the cooking process was not affected. In conclusion, the use of ultrasound would be very useful not only for accelerating the hydration process, but also for fortification of the grains.

RESUMO SIMPLES

A hidratação de grãos é importante para processos como cozimento, germinação ou malteação. Este processo leva muito tempo para ser completado, o que é uma desvantagem durante a industrialização. Por essa razão, a presente Tese foi focada na explicação da hidratação de grãos com comportamento sigmoidal (um comportamento pouco estudado) e melhorar este processo usando a tecnologia de ultrassom (tecnologia que usa ondas acústicas de alta frequência e potência). Diversos grãos foram caracterizados, observando que somente leguminosas como os feijões, lentilhas e ervilhas possuem o comportamento sigmoidal. Ainda, foi demostrado que quando o ultrassom é usado, o tempo de hidratação de grãos pode ser reduzido em até 5 horas, representando um impacto positivo para a indústria. Além disso, demostrou-se que nutrientes podem ser incorporados nos grãos durante o processo de hidratação, e que este processo também pode ser acelerado usando ultrassom. Ainda, o uso do ultrassom acelerou a germinação de alguns grãos e a extração de compostos indesejáveis, sem afetar o cozimento. Em conclusão, o ultrassom é uma tecnologia muito promissora para acelerar a hidratação e fortificação de grãos.

RESUMEN SIMPLE

La hidratación de granos es una importante etapa antes de otros procesos como la cocción, germinación o malteado. Este proceso tiene como gran desventaja que demora para ser completada, siendo indeseable para la industria. Por tal motivo la presentes Tesis se enfocó en explicar la hidratación de granos con comportamientos sigmoidal (un comportamiento poco estudiado) e mejorarlo usando la tecnología de ultrasonido de alta potencia (ondas acústicas de frecuencias altas las cuales no son escuchadas por el oído humano). Muchos granos fueron caracterizados, observando que sólo las leguminosas como los frejoles, lentejas, garbanzos arvejas, etc., presentan el comportamiento sigmoidal. Además, se demostró que la tecnología de ultrasonido puede acelerar la hidratación de granos por casi 5 horas, lo cual es muy deseable para la industria. Además, se demostró que se puede aprovechar el tiempo de hidratación para incorporar algún nutriente en los granos y que el ultrasonido puede acelerar también este proceso. Por último, se verificó el uso de ultrasonido en los granos puede acelerar su germinación y la extracción de compuesto indeseables. Sin embargo, esta tecnología no afectó el proceso de cocción. En conclusión, el ultrasonido es una tecnología muy prometedora para acelerar la hidratación y fortificación de granos.

APPENDIX K: PHOTOS

Ultrasonic bath of 91 W/L of volumetric power and 25 kHz of frequency. **Left:** frontal view. **Right:** top view – notice the heat interchanger.

Ultrasonic bath of 28 W/L of volumetric power and 40 kHz of frequency. **Left:** frontal view. **Right:** top view – notice the heat interchanger.

Hydrated carioca beans. **Left:** conventional hydration. **Right:** hydration in ferrous sulphate solution.