Contribuição ao Entendimento do Fenômeno de Hidratação de Grãos: Caracterização, Mecanismos, Modelagem e Melhoria

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Contribuição ao Entendimento do Fenômeno de Hidratação de Grãos: Caracterização, Mecanismos, Modelagem e Melhoria

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“If I have seen further, it is by standing on the shoulders of giants”

Isaac Newton

(carta a Robert Hooke; 05 de fevereiro de 1675. Disponível em: http://digitallibrary.hsp.org/index.php/Detail/Object/Show/object_id/9285)
Aos meus amores,

Beatriz e Olívia,

Dedico.
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Preâmbulo

Este trabalho constitui minha Tese de Livre Docência, apresentado à Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), da Universidade de São Paulo (USP), para obtenção do título de Livre Docente em Ciência e Tecnologia de Alimentos, na área de Processamento de Alimentos.

Apresenta uma avaliação crítica de minha atuação Docente, através de uma área específica de pesquisa. Através de 12 artigos científicos, desenvolvidos durante 5 anos e conjuntamente com 19 coautores, descrevo minha contribuição até o momento para o entendimento do fenômeno de hidratação de grãos. Dentre outras possibilidades, escolhi este tópico para minha Tese de Livre Docência devido a diversos fatores: foi o primeiro tema que trabalhei de forma totalmente independente, logo após defesa do Doutorado; é um tema que ainda venho trabalhando e que pretendo trabalhar por mais um bom tempo; foi uma oportunidade única de aprender e descrever algo “novo” (comportamento sigmoidal na hidratação), partindo de experimentos cujos resultados pareciam “errados” (o que me permitiu aplicar perfeitamente o Método Científico); é um tema que possui interessante interface com outras áreas, se enquadrando muito bem no contexto da ESALQ; os trabalhos desenvolvidos me permitiram aprovar projetos de pesquisa, participar ativamente de importantes eventos científicos, realizar parcerias e, devido aos bons resultados, divulgar com impacto nossa pesquisa; entre outros. Tenho assim, um carinho especial por essa área de pesquisa.

Segundo normas da USP (Circ.SG/CLR/065, Resolução Nº 3745, de 19 de outubro de 1990), este documento é redigido em Português. Os trabalhos completos são apresentados nos apêndices.
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Resumo

O processo de hidratação (ou reidratação) é uma operação unitária importante em alimentos secos, pois descreve e define suas propriedades e usos durante diferentes etapas do processamento e consumo, como trituração, extração, germinação, fermentação, cozimento e digestão. Além disso, em especial no caso de grãos, a hidratação é uma operação unitária lenta e realizada de forma descontínua (isto é, em lotes). Consequentemente, é no processamento industrial, sendo necessário estudos para sua melhoria. O presente trabalho descreve minha contribuição até o momento para o entendimento do fenômeno de hidratação de grãos. O trabalho foi desenvolvido de forma a caracterizar o comportamento de hidratação de diferentes grãos, propor mecanismos para descrever o processo, descrever matematicamente os dados obtidos e melhorar o processo utilizando diferentes temperaturas e a tecnologia de ultrassom. Demonstrou-se que a hidratação de grãos é um fenômeno complexo, cujos mecanismos exatos ainda são desconhecidos. Embora progressos recentes tenham avançado o conhecimento de sua descrição, em especial macroscopicamente, ainda existem diversos desafios para descrição microscópica. A absorção de água pelo grão (e transporte através deste) ocorre não apenas por difusão, mas também por outros mecanismos de transferência de massa, como a capilaridade. Ainda, as propriedades das diferentes estruturas mudam com a atividade da água, fazendo com que a água escoe através de rotas específicas e levando a diferentes comportamentos de hidratação. O melhoramento da hidratação pode ser conseguido através de diferentes abordagens. Neste trabalho são descritos o aumento da temperatura do processo e o uso da tecnologia de ultrassom. Vantagens e limitações são discutidas. Em resumo, o presente trabalho auxilia a compreensão do fenômeno de hidratação do grão. Entretanto, estudos adicionais são necessários para elucidar, compreender e descrever melhor os mecanismos, bem como melhorar o processo.

Palavras chave: engenharia do processamento de alimentos, hidratação, reidratação, transferência de massa, novas tecnologias, tecnologias emergentes, ultrassom.
A Contribution to the Understanding of the Grain Hydration Phenomenon: Mechanisms, Modelling and Enhancement

Abstract

The hydration (or rehydration) process is an important unit operation for dried foods since it describes and defines their uses and properties during different processing and consumption steps, such as milling, extraction, fermentation, germination, cooking and digestion. Further, and especially for grains, the hydration is a slow and discontinuous unit operation (batch process). Therefore, its enhancement is desirable for the industrial processing. The present work describes my currently contribution for the understanding of the grains hydration phenomenon. The work was performed to characterize the hydration behaviour of different grains, proposing the mechanisms that describe the process, mathematically describing the obtained data and enhancing the process using different temperatures and the ultrasound technology. It was demonstrated that the hydration of grains is a complex process, whose exact mechanisms are still unknown. Although recent progress have offered new knowledge about the hydration description from the macroscopic point of view, there are still many challenges for microscopic description. The water absorption by the grain (and its transport through it) takes place not only by diffusion, but also by other mass transfer mechanisms, such as capillarity. Moreover, the properties of the different grain structures change with the water activity. Consequently, the water is transported through specific pathways, leading to different hydration behaviours. In addition, the hydration enhancement can be reached through different approaches. This work describes the use of heating (temperature increase) and the ultrasound technology to achieve it. In conclusion, the present work contributes to the understanding of the grain hydration process. However, additional studies are needed to elucidate, understand and better describe the involved mechanisms, as well as to improve the process.

Keywords: food process engineering, hydration, rehydration, mass transfer, new technologies, emerging technologies, ultrasound.
Una Contribución al Entendimiento del Fenómeno de Hidratación de Granos:
Mecanismos, Modelado y Mejora

Resumen

El proceso de hidratación (o rehidratación) es una operación unitaria importante en alimentos secos, pues describe y define sus propiedades y usos durante diferentes etapas del procesamiento y consumo, como en la molienda, extracción, germinación, fermentación, cocimiento y digestión. Además de esto, en especial en el caso de granos, la hidratación es una operación unitaria lenta y es realizada de manera discontinua (es decir, en lotes). Por lo tanto, son necesarios estudios para su mejora especialmente desde el punto de vista del procesamiento industrial. El presente trabajo describe mi contribución hasta el momento para un mejor entendimiento del fenómeno de hidratación de granos. El trabajo fue desarrollado con el objetivo de caracterizar el comportamiento de hidratación de diferentes granos, proponer mecanismos para describir el proceso, describir matemáticamente los datos obtenidos y mejorar el proceso utilizando diferentes temperaturas y la tecnología de ultrasonido. Se demostró que la hidratación de granos es un fenómeno complejo, cuyos mecanismos exactos son aún desconocidos. A pesar de recientes avances en la descripción del proceso (en especial macroscópicamente), aún existen diversos desafíos para su descripción microscópica. La absorción de agua por el grano (y transporte a través de este) ocurre no solo por difusión, sino también por otros mecanismos de transferencia de materia, como la capilaridad. Además, las propiedades de las diferentes estructuras se modifican con la actividad de agua, ocasionando que el agua fluya a través de rutas específicas lo que implica diferentes comportamientos de hidratación. La mejora de la hidratación puede ser obtenida a través de diferentes propuestas. En este trabajo son descritos el efecto del aumento de la temperatura de proceso y el uso de la tecnología de ultrasonido, cuyas ventajas y limitaciones son discutidas. En conclusión, el presente trabajo ayuda a la comprensión del fenómeno de hidratación de granos. Sin embargo, estudios adicionales son necesarios para aclarar, comprender y describir mejor los mecanismos, así como mejorar el proceso.

Palabras Clave: ingeniería del procesamiento de alimentos, hidratación, rehidratación, transferencia de materia, nuevas tecnologías, tecnologías emergentes, ultrasonido.
1. Contextualização

Esta tese começou em 2012, logo após terminar meu Doutorado.

Eu era Professor no Colégio Técnico da Universidade Estadual de Campinas (COTUCA/UNICAMP), e estávamos selecionando temas para a 4ª edição de um programa chamado "Jovens Talentos". Este programa tem como objetivos estimular os jovens estudantes para a Ciência, apoiando seu aprendizado. A participação é voluntária, e os alunos devem estar interessados em trocar suas férias de julho por passa-las no laboratório. Mesmo assim, nunca tive problemas em encontrar alunos interessados.

O período para o desenvolvimento do projeto era curto e a quantidade de dinheiro, muito escassa, pois o programa é financiado por contribuições espontâneas dos pais (APM). Além disso, os alunos participantes ainda estão no último ano do Ensino Técnico (que é realizada em conjunto com o Ensino Médio). Consequentemente, os projetos devem ser relativamente simples e baratos. Mesmo assim, no meu ponto de vista, devem ser relevantes.

Naquele ano, após algumas pesquisas, selecionei o processo de hidratação como um dos projetos a desenvolver. A verdade é que eu gostaria de trabalhar com o processo de secagem, mas não tínhamos nem uma estufa convectiva para fazer isso. Por outro lado, tínhamos compramos recentemente um banho maria com "bom" controle de temperatura - e assim eu percebi que poderíamos trabalhar com o "processo oposto". Depois de trabalhar com operações unitárias de transferência de energia/calor e de quantidade de movimento, seria minha primeira experiência com transferência de massa.

Com base na literatura, acreditei que seria um projeto simples, com "resultados previsíveis". Portanto, o projeto trataria da caracterização do processo de hidratação de um grão específico, avaliada em função da temperatura. De fato, haviam diversos trabalhos na literatura descrevendo o processo de hidratação de muitos alimentos. Na maioria deles, o comportamento observado é aquele predito pelas Leis de Fick de Difusão (Fick, 1855), que é representado na Figura 1A. Além disso, a maioria dos artigos (ainda) utilizam o Modelo de Peleg (Peleg, 1988) para descrever esse comportamento.

Portanto, hídrataríamos o grão selecionado em diferentes temperaturas e ajustariam o modelo de Peleg aos dados obtidos. Em seguida, correlacionaríamos os parâmetros deste modelo com a temperatura. Um projeto simples, mas interessante naquele contexto.
A primeira tarefa que passei às alunas (Amanda Leal Oliveira, Beatriz Gouveia Colnaghi, Emily Zucatti da Silva, Isabela Romão Gouvêa e Raquel Lopes Vieira) foi a seleção do grão. O grão não poderia ter sido estudado previamente, deveria ser barato e fácil de obter. Então eu as apresentei diversos artigos, e dei-lhes um tempo. Lembro-me que foi Amanda Leal Oliveira quem sugeriu o feijão Adzuki (*Vigna angularis*), que era preparado pela sua avó: era fácil de obter e não havia nenhum trabalho na literatura com o mesmo. Além disso, soubemos que este feijão é amplamente consumido na Ásia, diretamente cozido ou preparando pratos típicos doces e salgados. Dessa forma, tínhamos uma ideia e uma justificativa: a hidratação do grão é necessária durante o processamento industrial, e estudar esta operação unitária é importante para melhorar o processo.

Entretanto, uma vez que começamos a hidratar os grãos de feijão Adzuki, um comportamento diferente e (naquele momento) inesperado foi obtido (Figura 1B).

O comportamento de hidratação da maioria dos produtos alimentícios segue um padrão de absorção contínua de água, a uma taxa decrescente em relação ao tempo de processamento, descrevendo uma curva com forma côncava para baixo (Figura 1A; também denominada convexa ou hiperbólica, segundo a literatura, ou “tipo Peleg” como chamamos no Grupo). Neste comportamento, a taxa de absorção de água é máxima no início do processo, caindo para zero após tempo suficiente para atingir o teor de umidade de equilíbrio do produto. Esse comportamento é predito pelas Leis de Fick da Difusão (Fick, 1855), sendo também amplamente
descrito pelo Modelo de Peleg (Peleg, 1988) - embora muitos outros modelos são apresentados na literatura (Ibarz e Augusto, 2015a; Miano et al., 2017a).

Por outro lado, o comportamento observado para os grãos de Adzuki mostrou uma fase *lag* inicial, isto é, uma fase inicial com baixa taxa de absorção de água (Figura 1B). Neste caso, a taxa de absorção de água primeiro aumenta, até um ponto de inflexão, a partir do qual a taxa diminui até zero, após tempo suficiente para que o produto alcance a sua umidade de equilíbrio. Este comportamento de hidratação é descrito por uma curva de forma sigmoidal, sendo nesse momento inesperado e difícil de entender.

Então, de repente, o projeto “fácil e previsível” se tornou muito mais complexo. Na verdade, foi a melhor coisa que poderia nos acontecer, ao se tornar uma oportunidade: o projeto mudou de uma caracterização simples para uma descrição de um comportamento sub explorado. De fato, foi quando comecei a perceber que o fenômeno de hidratação de grãos é muito mais complexo do que o geralmente pensado - de fato, os exatos mecanismos envolvidos ainda são desconhecidos. Consequentemente, os resultados obtidos abriram novas possibilidades de pesquisa e a descrição do fenômeno de hidratação do grão ainda é uma das áreas mais importantes de minha pesquisa (imagine se tivéssemos escolhido um grão com o comportamento clássico da Figura 1A para esse primeiro projeto...).

Terminamos o projeto caracterizando a hidratação do feijão Adzuki em função da temperatura e, o mais importante, começando a descrever as possíveis razões para o comportamento sigmoidal observado. Este trabalho foi publicado no periódico *Journal of Food Engineering* (Oliveira et al., 2013).

Depois desse projeto, saí do COTUCA/UNICAMP (setembro/2013) e me mudei para a Universidade de São Paulo (USP), onde trabalho como Professor no Departamento de Agroindústria, Alimentação e Nutrição (LAN) da Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ). Devido aos interessantes resultados obtidos com os grãos de feijão Adzuki, e por falta de estudos descrevendo o comportamento de hidratação de grãos (em especial em relação ao comportamento sigmoidal), propus estudar esse processo como parte do meu primeiro projeto na USP.

Desde então, estudamos diferentes aspectos do processo de hidratação de grãos, envolvendo desde a caracterização, passando por descrição de mecanismos e modelagem matemática, até melhoria utilizando diferentes tecnologias.
Por todas essas razões, e entre todas as outras possibilidades, eu escolhi este tópico para minha Tese de Livre Docência. Portanto, esta Tese descreve minha contribuição, até o momento, para a compreensão do fenômeno de hidratação de grãos.

A Tese foi desenvolvida durante 5 anos e em diferentes etapas (objetivos, estudos), sendo composta por 12 artigos (publicados, aceitos para publicação ou em revisão). Embora as etapas não tenham sido realizadas necessariamente em ordem cronológica, a Figura 2 apresenta-as de acordo com sua publicação (linha do tempo). Mais informações são apresentadas nas seguintes seções e figuras.

Figura 2. Descrição dos 12 trabalhos que compõem esta Tese de Livre Docência, organizada de acordo com seu período de publicação. Interpretações, correlações e descrições são apresentadas nas próximas seções. Os textos completos são apresentados nos apêndices.
Como uma Tese de Livre Docência, o presente trabalho foi desenvolvido conjuntamente com 19 coautores (Alberto Claudio Miano, Albert Ibarz, Jessica da Costa Pereira, Meliza Lindsay Rojas, Viviane Deroldo Sabadoti, Tatiane Patero, Nanci Castanha, Manoel Divino da Matta Júnior, Amanda Leal Oliveira, Beatriz Gouveia Colnaghi, Emily Zucatti da Silva, Isabela Romão Gouvêa, Raquel Lopes Vieira, Jorge Armando García, Victor Augusto Forti, Haynna Fernandes Abud, Francisco Guilhien Gomes Junior, Silvio Moure Cicero), de 4 Universidades (Universidade de São Paulo - USP, Universidade Estadual de Campinas - UNICAMP, Universidad César Vallejo - UCV, Universitat de Lleida – UdL), de 3 países (Brasil, Peru e Espanha). Além disso, eu não considero o trabalho finalizado, sendo o assunto ainda uma área importante da minha pesquisa.
2. Introdução e objetivos

A redução da atividade de água ($a_w$) através da secagem é uma técnica antiga e amplamente utilizada para conservação de alimentos. Embora alguns produtos sejam consumidos diretamente secos, uma importante proporção deve ser hidratada antes do processamento e/ou consumo. Como exemplo, pode-se citar os grãos.

O processo de hidratação (ou reidratação) é, portanto, uma operação unitária importante em alimentos secos, pois descreve e define suas propriedades e usos durante a extração, fermentação, germinação, cozimento, consumo e digestão. Além disso, a hidratação é uma operação unitária descontínua (isto é, realizada em lotes) e lenta (especialmente no caso de grãos). Consequentemente, é uma etapa limitante no processamento industrial.

Portanto, destaca-se a importância em se compreender o fenômeno de hidratação de diferentes produtos alimentares, sendo este o primeiro passo para melhorá-lo.

De fato, existem diversos trabalhos na literatura descrevendo a hidratação de diferentes produtos. Entretanto, os exatos mecanismos de transferência de massa que ocorrem durante a hidratação ainda não são completamente compreendidos.

Por exemplo, o processo é geralmente descrito na literatura como puramente difusivo, seguindo as Leis de Fick da Difusão (Fick, 1855) - e as soluções fornecidas por Crank (1979). Entretanto, esta é uma simplificação que não descreve o comportamento de hidratação de alguns alimentos, em especial aqueles com estrutura complexa, como grãos.

De acordo com Aguilera et al. (2004), o estudo da transferência de massa através de outros mecanismos além da difusão tem sido negligenciado. De fato, Aguter et al. (2000) afirmam que "there are strong and widely acknowledged grounds for doubting the applicability of this theory [Diffusion] in biology". Por exemplo, Marabi e Saguy (2004) demonstraram que o comportamento de hidratação de alimentos se desvia da descrição difusiva de Fick quando a porosidade é alterada, e Goula e Adamopoulos (2009) destacaram o fluxo capilar durante a hidratação de alimentos secos. É interessante observar que o fluxo capilar ocorre nos poros e microcanais, cujas dimensões, estrutura e flexibilidade são função do teor de umidade do tecido (e, portanto, que os mecanismos envolvidos podem mudar durante o processo de hidratação).
Além disso, a hidratação de grãos é particularmente mais complexa do que de materiais homogêneos (por exemplo, a hidratação de fatias de cenoura secas é mais simples e fácil de descrever - Rice et al., 2016; Van der Sman et al., 2014). Devido às diferentes estruturas e composição, cada tecido do grão interage de forma diferente com a água em cada etapa do processo de hidratação, resultando em rotas de fluxo específicas. Por tal razão, é difícil descrever o fenômeno através de métodos como a abordagem de meios porosos (Van der Sman et al., 2014, Datta, 2007, Saguy et al., 2005), quando o grão inteiro é considerado.

Xiao et al. (2014) descreveram diferentes rotas para o fluxo de água dentro de materiais vegetais. Além disso, Tang et al. (1994) e Tang e Sokhansanj (1996) demonstraram que o mecanismo de transferência de vapor de água durante hidratação e secagem de grãos varia com o teor de umidade, devido ao comportamento diferente de cada tecido. Estes resultados sugerem que o processo de hidratação em materiais estruturados e complexos, tais como grãos, é também função do seu teor de umidade. De fato, demonstramos que o fluxo de água para os grãos segue caminhos específicos, que se alteram com a atividade de água do produto, e que envolve difusão e capilaridade através dos diferentes tecidos (Miano e Augusto, 2015; Miano et al., 2015b).

Embora muitos esforços vêm sendo realizados para se entender melhor o fenômeno de hidratação dos grãos, os exatos mecanismos envolvidos ainda são desconhecidos. Portanto, destaca-se a necessidade de estudos adicionais, tentando-se elucidar e melhorar este processo.

O objetivo deste trabalho foi compreender melhor o fenômeno de hidratação de grãos com fins a melhorar este processo. Os objetivos gerais foram caracterizar o comportamento de hidratação de diferentes grãos, propor mecanismos para descrever o processo, descrever matematicamente os dados obtidos e melhorar o processo utilizando diferentes temperaturas e a tecnologia de ultrassom.

Para tal, esta Tese foi desenvolvida em 12 estudos específicos, cada um resultando em um artigo científico (publicado, aceito para publicação ou em revisão), conforme apresentado na Figura 2. Como representado na Figura 3, cada estudo foi conduzido considerando um ou mais dos seguintes 4 objetivos principais: caracterização, compreensão/descrição do fenômeno, modelagem matemática e melhoria. Interpretações, correlações entre os estudos e descrições adicionais são apresentadas nas próximas seções. Os textos completos são apresentados nos apêndices.
Figure 3. Descrição dos 12 trabalhos que compõem esta Tese de Livre Docência, representados pelas caixas azuis (os detalhes bibliográficos são apresentados na Figura 2 e as descrições na seção 3; os textos completos são apresentados nos apêndices), organizados de acordo com os quatro objetivos principais da Tese (caixas verdes).
3. Desenvolvimento

3.1. O começo: a hidratação de feijão Adzuki e o comportamento sigmoidal

Este trabalho se iniciou em um projeto desenvolvido com o objetivo de caracterizar a hidratação do feijão Adzuki, avaliando-a em função da temperatura. Com base na literatura, esperávamos uma absorção contínua de água, a uma taxa decrescente em relação ao tempo de processamento, descrevendo uma curva com forma côncava para baixo (Figura 1A). Em outras palavras, esperávamos que a hidratação fosse um mero processo de difusão.

Entretanto, o comportamento observado para a hidratação dos grãos de feijão Adzuki apresentou uma fase *lag* inicial, isto é, uma fase com baixa taxa de absorção de água (Figura 1B). Neste caso, a taxa de absorção de água primeiro aumentou, até o ponto de inflexão (em *t*= *t*$_{inflexão}$). Esse comportamento não seria possível se a difusão fosse o único mecanismo de transferência de massa envolvido. Após o ponto de inflexão, a taxa de hidratação diminui, tendendo a zero após tempo suficiente (*t* → ∞) para que o produto atinja seu teor de umidade de equilíbrio (*M*$_{∞}$ ou *M*$_{e}$). Este comportamento sigmoidal foi completamente inesperado. Após a realização de novos experimentos para confirmação, nós começamos a procurar outros trabalhos na literatura para apoiar e interpretar nossos resultados.

Surpreendentemente, encontramos apenas um trabalho com a descrição adequada do comportamento sigmoidal na hidratação, realizado com feijão fradinho (assim como amendoim e bambara) por Kaptso et al. (2008). De fato, outros quatro trabalhos na literatura apresentavam leguminosas cuja hidratação segue o comportamento sigmoidal: feijão fradinho (Sefa-Dedeh e Stanley, 1979), feijão-lima (Piergiovanni et al., 2012), feijões Badda bianco e Badda nero (Piergiovanni, 2011) e tremoço branco (Solomon, 2009). Entretanto, esses trabalhos não consideram o comportamento como sigmoidal, nem em suas descrições, discussões ou modelagem.

Kaptso et al. (2008) sugeriram que o comportamento sigmoidal poderia “reflected the degree of hard shell in the grain”. O trabalho erroneamente associou esse comportamento com o fenômeno de endurecimento da casca, do ponto de vista de cocção, fenômeno que ocorre ao longo do tempo em muitos grãos. Mesmo assim, corroboramos a hipótese de que esse comportamento está relacionado com a casca das sementes, acrescentando que " once this external structure is hydrated,
it resistance to the water flow decrease, increasing the water uptake rate” (Oliveira et al., 2013). No entanto, ao contrário da minha vontade, não conseguimos realizar experimentos para medir a permeabilidade da casca dos grãos durante o processo (de fato é algo muito mais difícil de fazer do que aparenta), não conseguindo demonstrar nossa hipótese.

Mesmo assim, foi quando eu comecei a me interessar em entender e descrever corretamente este comportamento de hidratação, negligenciado e/ou omitido na literatura. De fato, tenho trabalhado com o assunto desde então, em vários aspectos. Meu grupo de pesquisa é um dos poucos no mundo que vem estudando sistematicamente o comportamento sigmoidal da hidratação dos grãos.

Kaptso et al. (2008) também sugeriu uma função sigmoidal para descrever seus dados experimentais, como um modelo empírico. O Modelo de Kaptso et al. (2008) é uma função de três parâmetros, relacionados com a fase lag, o parâmetro cinético e o teor de umidade de equilíbrio, se ajustando bem aos dados de hidratação de diferentes grãos. Na verdade, usamos esse modelo não somente neste trabalho com o feijão Adzuki, mas também em diversos outros trabalhos onde o grão estudado demonstrou comportamento sigmoidal.

Uma vez caracterizado o comportamento do feijão Adzuki como sigmoidal, avaliamos a hidratação em temperaturas entre 25 ºC e 70 ºC, obtendo outro resultado menos frequente: para esse feijão, a umidade de equilíbrio é reduzida com o aumento da temperatura. O comportamento oposto é muito mais frequente.

A redução na umidade de equilíbrio foi proposta por Abu-Ghannam e McKenna (1997) como relacionada à extração de componentes solúveis pela água em temperaturas mais altas. No entanto, observamos uma pequena extração de sólidos para a água de imersão, sempre desprezível (em temperaturas mais altas, no entanto, começa a ser importante). Portanto, nós propomos duas outras possibilidades para explicar esse resultado, relacionadas com a integridade celular e com o fenômeno de transferência de massa: “Firstly, it is expected that the higher temperatures damage the cell membranes, resulting in lower water holding capacity. Secondly, as the hydration rate is faster at higher temperatures, the seeds edges quickly absorb water, which, when the external layer is saturated, reduce the mass transfer from the soaking water to the grain surface” (Oliveira et al., 2013).

Em resumo, concluímos o projeto caracterizando a hidratação do feijão Adzuki em função da temperatura e, mais importante ainda, começando a descrever as possíveis razões para o comportamento sigmoidal observado. Este trabalho foi
publicado como "Modelling the effect of temperature on the hydration kinetic of Adzuki beans (Vigna angularis)" no periódico *Journal of Food Engineering* (Oliveira et al., 2013 - Apêndice 1).

Os resultados obtidos nesse estudo me levaram a outras questões, que iniciaram a sequência de estudos aqui apresentados - e representados na Figura 4, de acordo com a sequência de ideias.

![Diagrama de ideias](image)

Figura 4. Descrição dos 12 trabalhos que compõem esta Tese de Livre Docência, representados pelas caixas azuis (os detalhes bibliográficos são apresentados na Figura 2; os textos completos são apresentados nos apêndices), organizados de acordo com a sequência de ideias a partir do estudo original. As caixas vermelhas representam as principais conclusões obtidas após os trabalhos intermediários; as caixas verdes representam as perguntas resultantes de cada conclusão.

As duas principais ideias que conclui neste primeiro estudo foram: (1) a hidratação é um fenômeno complexo, com diferentes mecanismos de transferência de massa, levando ao comportamento sigmoidal; e (2) a hidratação é uma operação unitária limitante na indústria, uma vez que exige muito tempo, sendo realizada em lotes. A primeira conclusão me levou a duas perguntas: há outros grãos cuja
hidratação segue o comportamento sigmoidal? Quais são as causas, os mecanismos envolvidos nesse comportamento? A segunda conclusão levou naturalmente à seguinte questão: como o processo de hidratação pode ser melhorado?

Esse foi, portanto, o início de meu estudo sobre o processo de hidratação de grãos. Logo em seguida, eu saí do COTUCA/UNICAMP e fui para a ESALQ/USP, onde continuei na temática.

### 3.2. Caracterização do processo de hidratação de grãos

O primeiro passo na sequência de trabalho foi a caracterização de demais grãos. Mais do que simplesmente apresentar resultados para outros produtos, a caracterização de novos grãos teve por objetivo determinar quão frequente é o comportamento sigmoidal, bem como realizar prospecção de grãos com interesse para demais estudos. Trabalhamos com mais de 20 grãos diferentes, tanto da família Fabaceae (leguminosas) como da Poaceae (cereais).

Essa caracterização gerou um banco de dados que identificou outras 9 leguminosas com comportamento sigmoidal. Comparando todos os produtos, demonstrou-se que é difícil prever o comportamento de hidratação com base nas propriedades do grão (cor, tamanho, espécie, família, estrutura ou composição). Demonstramos que algumas das hipóteses apresentadas na literatura não são exatas. Por exemplo, demonstramos que grãos com casca de cor mais escura não apresentam necessariamente uma hidratação mais lenta do que os com cor mais clara; grãos com comportamento sigmoidal podem se hidratar mais rapidamente do que grãos com comportamento de forma côncava para baixo; o tamanho dos grãos não é uma propriedade que caracteriza a hidratação.

Este trabalho foi importante não apenas para ampliar os dados sobre o comportamento sigmoidal durante a hidratação de grãos, mas também para evitar má interpretações sobre algumas hipóteses amplamente descritas na literatura. Este estudo está atualmente em revisão no periódico *Journal of Food Process Engineering* como "Hydration kinetics of cereal and pulses: new data and hypothesis evaluation" (Miano et al., 2017c - Apêndice 2).

Os próximos estudos foram realizados com objetivo de descrever o processo de hidratação.
3.3. Descrição e entendimento do processo de hidratação de grãos: mecanismos e modelagem

O próximo passo foi relacionado com a descrição matemática.

Além das Leis de Fick da Difusão (Fick, 1855), em geral utilizadas através das soluções apresentadas por Crank (1979), a literatura apresenta diversos modelos empíricos ou semi-empíricos para descrever a hidratação de produtos com o comportamento de forma côncava (Figura 1A). Os mais utilizados são os modelos de Lewis (cinética de primeira ordem, Lewis, 1921), Page (1949), Henderson (1974), Peleg (1988) e Weibull (trata-se do uso do modelo estatístico de Weibull (1951), bastante utilizado na descrição da inativação microbiana e enzimática (ex: Rojas et al., 2017b), para descrever o processo de hidratação (ex: Saguy et al., 2005)). Ainda assim, outros modelos semi-empíricos interessantes são descritos na literatura, como por Paquet-Durand et al. (2015) e Ibarz et al. (2004) (bem como por nós - Miano et al., 2017a).

Entretanto, apenas um modelo era apresentado na literatura para descrever o comportamento sigmoidal de hidratação: o modelo empírico proposto por Kaptso et al. (2008). A disponibilidade de outros modelos matemáticos é sempre desejável, pois os dados reais não seguem modelos específicos, e cada conjunto de dados pode se ajustar melhor a um modelo específico (Ibarz e Augusto, 2015b). Ainda, sempre que possível, é desejável utilizar modelos constitutivos ou, na ausência destes, modelos semi-empíricos, em vez de empíricos. Consequentemente, propusemos um novo modelo semi-empírico para descrever o comportamento sigmoidal dos alimentos durante a hidratação.

Para tal, o processo de hidratação foi simplificado como uma reação cinética onde a matriz sólida absorve água seguindo um parâmetro cinético, resultando em um complexo contendo tanto a matriz sólida quanto a água. Assumimos que a água pode ser adsorvida tanto nos sítios secos ($M_\infty - M(t)$), interagindo com a matriz sólida, como na água ligada a esta matriz ($M(t)$), numa distribuição multicamada. Portanto, a variação da umidade do produto ao longo do tempo de processamento é função de ambos os termos ($M_\infty - M(t)$) e $M(t)$, sendo descrito por uma cinética autocatalítica de segunda ordem.

Resolvendo a equação diferencial obtida, obtém-se o novo modelo (Figura 5), composto por dois parâmetros: $k$, que é o parâmetro cinético, também relacionado à fase lag do produto, e $M_\infty$, que é a umidade no equilíbrio, relacionada com a máxima adsorção de água do produto. O modelo foi avaliado considerando o comportamento
sigmoidal de sete grãos durante a hidratação em diferentes temperaturas (dados apresentados na literatura), descrevendo bem todos eles. Portanto, foi considerado como válido e apropriado para descrever o processo de hidratação de alimentos com comportamento sigmoidal.

As principais vantagens do modelo estão relacionadas à sua natureza semi-empírica, já que seus parâmetros descrevem significados físicos específicos, e sua simplicidade, já que possui apenas dois parâmetros \((k e M_\infty)\). De fato, ele descreve bem a hidratação de grãos sigmoidais, sendo utilizado em demais trabalhos (por exemplo, nossos trabalhos com tremoços - Augusto e Miano, 2017, Miano et al., 2015b).

No entanto, como todo modelo, ele apresenta algumas limitações. Em primeiro lugar, não é um modelo constitutivo e não pode descrever o processo em escala microscópica. No entanto, é um modelo semi-empírico, que pode descrever com sucesso o processo a nível macroscópico, sendo relevante no contexto apropriado. Como em qualquer tentativa de descrição matemática, um número adequado de dados experimentais deve ser fornecido para obter uma regressão adequada - especialmente no início do processo (antes do ponto de inflexão) e por tempo suficiente para estimar adequadamente a umidade de equilíbrio \((M_\infty)\). Uma vez que o modelo proposto possui apenas dois parâmetros, ele precisa de mais dados do que o modelo de Kaptso et al. (2008 – com três parâmetros) para ajuste, o que pode limitar o uso. Essa foi, por exemplo, a principal razão pela qual não usamos nosso modelo para descrever a hidratação de feijão Moyashi (Miano et al., 2016b), já que sua rápida germinação nos impediu de obter número adequado de pontos experimentais próximos à umidade de equilíbrio.

Após avaliação, concluímos que o novo modelo de hidratação pode ser usado com sucesso para descrever o processo de hidratação de alimentos com comportamento sigmoidal. Este trabalho foi importante não apenas para fornecer um modelo alternativo para descrever o comportamento sigmoidal durante a hidratação de grãos, mas também fornecer discussão sobre os mecanismos envolvidos. Por exemplo, ressaltamos que “this result suggests that the hydration process in structured and complex materials, such as grains, is also function of its moisture content” – o que demonstramos em trabalhos futuros utilizando dois grãos (Miano e Augusto, 2015, Miano et al., 2015b), como descrito a seguir. Este estudo foi publicado no periódico *Drying Technology* como "Describing the food sigmoidal
behaviour during hydration based on a second-order autocatalytic kinetic" (Ibarz e Augusto, 2015a - Apêndice 3).

Figura 5. Hidratação de diferentes grãos a diferentes temperaturas (pontos) e sua descrição pelo novo modelo de hidratação proposto (curvas tracejadas). De Ibarz e Augusto (2015a).

Prosseguimos então com os estudos de caracterização e descrição dos mecanismos envolvidos no processo de hidratação.

Desenvolvemos estudo com trevo andino, leguminosa com alto teor de proteínas e cultura com grande potencial de exploração industrial, e cuja hidratação ainda não tinha sido estudada. Esse grão apresentou comportamento sigmoidal de hidratação, que foi descrito adequadamente pelos modelos propostos por Kaptso et al. (2008) e Ibarz e Augusto (2015a), avaliados em função da temperatura.

\[
M(t) = \frac{M_\infty}{1 + \frac{M_\infty - M_0}{M_0} \cdot \exp(-k \cdot M_\infty \cdot t)}
\]
Figura 6. Curva de hidratação de tremoço andino inteiro/com casca (■) e sem casca (○) (30 ºC). Descrição da rota de entrada de água em grãos de tremoço andino: capilaridade (setas azuis) e difusão (setas vermelhas). De Miano et al. (2015b).

Mais importante, o estudo com esse grão nos permitiu avançar na descrição do fenômeno. Ao realizar experimentos hidratando o grão com e sem a casca (Figura 6), comprovamos a hipótese levantada por Kaptso et al. (2008) e Oliveira et al. (2013) de que o comportamento sigmoidal é causado por essa estrutura. Nos perguntamos então como ocorre o fluxo de água do volume líquido para o grão e então através deste. Realizamos então experimentos hidratando o grão inteiro de forma convencional e forçando a água a passar somente pela casca – utilizando uma cola para impermeabilizar o hilo, estrutura porosa de leguminosas por onde a
água consegue entrar na semente com facilidade. Observamos que, quando o grão era hidratado apenas através da casca (i.e., com o hilo bloqueado), o processo era muito mais lento. Realizamos então o mesmo experimento, porém hidratando os grãos (inteiro e com o hilo recoberto) em uma solução de corante azul brilhante (Figura 6). Através desse experimento, observou-se que o corante penetrava no grão apenas através do hilo, enquanto apenas a água conseguia atravessar a casca. Microscopias eletrônicas das diferentes estruturas morfológicas confirmaram que o hilo é bastante poroso, como uma estrutura de microcanais, enquanto os poros da casca são de dimensão molecular (não podendo sequer serem vistos através dessa técnica). A partir desses resultados, entendemos que o fluxo de massa através dos microcanais do hilo ocorre por capilaridade, com fluxo volumétrico de solução (água e corante). Por outro lado, a transferência de massa pela casca ocorre em nível molecular, já que apenas as moléculas de água conseguem permean a estrutura, e assim consideramos que por difusão.

O primeiro passo da descrição do comportamento sigmoidal foi publicado como “Correlation between morphology, hydration kinetics and mathematical models on Andean lupin (Lupinus mutabilis Sweet) grains” no periódico científico Lebensmittel-Wissenschaft + Technologie / Food Science + Technology (Miano et al., 2015b – Apêndice 4).

Entretanto, ainda faltava melhorar a descrição do comportamento sigmoidal, e por isso voltamos ao feijão Adzuki – grão com forte comportamento sigmoidal e que, portanto, temos utilizado frequentemente como um modelo. Realizamos experimentos de hidratação com o feijão a diferentes umidades iniciais, distribuídas uniformemente em todas as suas estruturas. Demonstramos então que a umidade do grão afeta não somente a cinética de hidratação, como o próprio comportamento, passando de sigmoidal para côncavo (Figura 7A). Ao analisar a isoterma de sorção do grão, observamos que a faixa de umidade onde o comportamento tinha essa mudança corresponde à umidade no ponto de inflexão da curva sigmoidal. Ainda, corresponde à faixa de atividade de água onde há a mudança da zona II para a zona III da isoterma de sorção, onde a água começa a exercer efeito plastificante (Reid e Fennema, 2008).

Ao realizar então novos experimentos com grãos normais e com o hilo bloqueado, comprovamos que a permeabilidade da casca muda com a umidade (atividade de água; Figura 7B): a casca é impermeável à água quanto em atividade de água na zona II da isoterma de sorção, porém sendo permeável quando na zona
III. Essa mudança de permeabilidade faz com que o grão apresente comportamento sigmoidal durante a hidratação quando sua casca está com atividade de água na zona II, mudando para côncavo quando na zona III. Essa mudança pode ser associada à teoria de transição vítrea (Ross et al., 2013).

Assim, acreditamos que os componentes da casca estejam no estado vítreo nas condições de temperatura e atividade de água de equilíbrio com o ambiente para os grãos que apresentam comportamento sigmoidal de hidratação. Nessas condições, a passagem de água é (pelo menos) muito difícil. Entretanto, quando os mesmos se encontram em maior atividade de água (zona III), os componentes da casca passam para o estado gomoso, facilitando a permeação da água. Com base nesses resultados, propomos uma rota para a entrada de água no grão de feijão Adzuki, que pode ser expandida para outros grãos com comportamento semelhante. Inicialmente, em condições “normais”, a casca é impermeável e por isso a água entra no grão apenas pelo hilo. Ao entrar no grão, o espaço entre o cotilédone e a casca é preenchido por capilaridade, umidificando a casca e também penetrando no interior do grão. Uma vez que a casca chegue à atividade de água de transição vítrea, a mudança estrutural decorrente começa a permitir sua permeação pela água. Esse mecanismo é apresentado na Figura 7C.

Com esses resultados, aperfeiçoamos a descrição do comportamento sigmoidal de grãos, publicando o artigo “From the sigmoidal to the downward concave shape behavior during the hydration of grains: Effect of the initial moisture content on Adzuki beans (Vigna angularis)”, publicado no periódico Food and Bioproducts Processing (Miano e Augusto, 2015 – Apêndice 5). Ainda assim, não julgo que o assunto esteja completamente explicado, necessitando ainda detalhamento e comprovações. Por exemplo, ainda não conseguimos provar a teoria da transição vítrea da casca.

Com a teoria de hidratação descrita nos artigos com trevo andino (Miano et al., 2015b) e feijão Adzuki (Miano e Augusto, 2015), fizemos uma releitura de um artigo da literatura (Solomon, 2009). Nesse trabalho, o comportamento de hidratação de trevo é erroneamente modelado utilizando a Equação de Peleg (1988), mesmo a hidratação do grão sendo claramente sigmoidal. Assim, reinterpretamos os dados do autor e a descrição do processo.
Figura 7. Hidratação do feijão Adzuki. (A) Curva de hidratação a diferentes umidades iniciais. (B) Curva de hidratação do grão normal e com o hilo recoberto, em umidades acima e abaixo da mudança de comportamento. (C) Rota proposta para a entrada de água no grão. De Miano e Augusto (2015).

É importante ressaltar que se apenas uma pequena quantidade de dados experimentais é avaliada no início do processo de hidratação, o comportamento real sigmoidal do grão pode ser “camuflado”, sendo então os dados erroneamente interpretados. Deste modo, muitos comportamentos de hidratação de grãos são descritos na literatura como forma côncava, apesar do seu comportamento sigmoidal, devido a um erro de amostragem. Da mesma forma, alguns trabalhos na literatura mostram claramente a hidratação de grãos com comportamento sigmoidal; entretanto, avaliam os dados usando modelos como o de Peleg (1988). Dessa forma, a realização deste trabalho teve como objetivo levantar essa discussão e corrigir a interpretação dos dados de Solomon (2009). O trabalho resultante está atualmente in press no periódico Journal of Food Process Engineering com o título “Describing the sigmoidal behavior of roasted white lupin (Lupinus albus)” (Augusto e Miano, 2017–Apêndice 6).
3.4. Melhoria do processo de hidratação de grãos utilizando a tecnologia de ultrassom: descrição, mecanismos e efeitos nas propriedades

Resultados prévios (nossos e da literatura) comprovam que o aumento da temperatura em geral melhora o processo de hidratação, aumentando a taxa de absorção de água e umidade de equilíbrio (embora o aumento na temperatura possa reduzir a umidade de equilíbrio em alguns grãos, como observado para o feijão Adzuki – Oliveira et al., 2013). Entretanto, temperaturas mais elevadas podem resultar em consequências indesejáveis ao produto e processo, tais como consumo de energia, necessidade de isolamento térmico e alterações no produto (desnaturação de proteínas, gelatinização de amido e degradação molecular).

Por essa razão, o presente trabalho procurou garantir a melhoria do processo de hidratação através de uma tecnologia não térmica. A escolha da tecnologia foi baseada em diversos aspectos, tanto científicos, quanto práticos. Assim, analisei tanto os resultados positivos com processos de transferência de massa (trabalhos já realizados e apresentados na literatura e resultados potenciais, tendo em vista os princípios de atuação da tecnologia), quanto custo e complexidade de instalação (já que foi minha primeira proposta de projeto à USP e agências de fomento, quando nem laboratório eu tinha e já vivendo restrições orçamentárias). Após análise, selecionei a tecnologia de ultrassom.

A tecnologia de ultrassom se baseia na aplicação de ondas mecânicas de altas frequências (acima daquelas captadas pelo ouvido humano) que se propagam em sistemas gasosos, líquidos ou sólidos. A primeira aplicação em alimentos foi para realização de análises não destrutivas de alimentos processados (ultrassom de baixa potência) e, posteriormente, o ultrassom foi proposto como método não térmico para conservação e processamento de alimentos (ultrassom de alta potência). Informações detalhadas sobre a utilização da tecnologia de ultrassom no processamento de alimentos podem ser obtidas nos trabalhos de Cárcel et al. (2012), Awad et al. (2012), Rastogi (2011), Chemat et al. (2011), Soria e Villamiel (2010), Bhaskaracharya et al. (2009), Demirdöven e Baysal (2008) e Earnshaw et al. (1995), além de nossas contribuições em Augusto et al. (2017), Rojas et al. (2017a) e Miano et al. (2017d).

Quando um alimento é submetido a um processo de ultrassom de alta potência, a onda mecânica o percorre resultando em ciclos de compressão e expansão. Nos ciclos de expansão o alimento é submetido a uma condição de baixa pressão, capaz de superar as forças intermoleculares, resultando na formação de
microbolhas de água no alimento através de sua vaporização. Nos ciclos de compressão, por outro lado, o alimento é submetido a uma condição de alta pressão, resultando na condensação das microbolhas de vapor anteriormente formadas. O colapso dessas bolhas, ou cavitação, é um processo que envolve grande energia e cisalhamento, podendo resultar no rompimento de tecidos e células. A cavitação pode ocorrer tanto dentro do alimento quanto na interface alimento-líquido de imersão, podendo, assim, alterar tanto as propriedades internas quanto as de superfície dos alimentos. Ainda, efeitos diretos do uso dessa tecnologia, relacionados à movimentação do sólido e/ou líquido, fazem o uso do ultrassom interessante para processos de transferência de massa.

De fato, a tecnologia de ultrassom tem sido utilizada para melhoria de diversos processos de transferência de massa em alimentos, tais como em processos de extração sólido-líquido, salga, desidratação osmótica, filtração por membranas e secagem. Entretanto, apenas um trabalho (dividido em dois artigos) na literatura utilizava a tecnologia de ultrassom no processo de hidratação de grãos, porém sem explicação dos mecanismos de ação do ultrassom e considerando apenas produtos de insignificant impact industrial e comercial – destacando assim o desenvolvimento do projeto.

O meu primeiro trabalho com o uso da tecnologia de ultrassom foi com a hidratação de sorgo. A importância comercial e industrial desse grão está relacionada não somente com a alimentação animal e humana, mas também com processos industriais, como germinação/malteação, fermentação e obtenção de amido – processos que dependem de uma etapa inicial de hidratação. Além disso, o sorgo é um cereal, com comportamento côncavo de hidratação e formato quase esférico – características interessantes para a escolha em um primeiro estudo. Assim, nosso primeiro estudo comparou a hidratação de grãos de sorgo de forma convencional e assistida por ultrassom, ambos a 25°C ou com aquecimento a 53°C (temperatura escolhida por estar abaixo da faixa de gelatinização do amido e desnaturação de proteínas).

A Figura 8 apresenta os resultados obtidos, demonstrando que o ultrassom melhora o processo de hidratação do sorgo ao acelerar a incorporação de água e aumentar a umidade de equilíbrio. Entretanto, o efeito do aumento da temperatura (53°C) foi muito maior, inclusive se sobrepondo ao processo combinado (hidratação assistida por ultrassom a 53°C). Mesmo assim, consideramos os resultados bastante positivos, uma vez que as desvantagens do uso de altas temperaturas (gastos com
energia e isolamento térmico, alterações no produto) fazem com que o processo a alta temperatura seja raramente utilizado na indústria com o objetivo de aumentar a taxa de hidratação. Portanto, embora o efeito do aquecimento tenha sido maior do que o processamento por ultrassom na hidratação do sorgo, essa tecnologia ainda pode ser considerada uma alternativa.

Nesse trabalho também começamos a discutir os possíveis mecanismos envolvidos na melhoria do processo pela tecnologia de ultrassom.

O primeiro mecanismo está relacionado com a redução na resistência convectiva à transferência de massa na superfície do sólido, devido à intensa turbulência no fluido causada pelo ultrassom. Entretanto, sabe-se que a resistência interna à transferência de massa é o mecanismo limitante durante a hidratação dos alimentos. De fato, Simal et al. (1998) observaram taxas de transferência de massa mais altas durante a desidratação osmótica assistida por ultrassom de maçãs quando comparadas a um processo com agitação mecânica intensa. Assim, sugerimos que outros mecanismos podem ser mais importantes nesse processo.

O processamento de sólidos por ultrassom pode formar microcanais no produto devido às tensões mecânicas associadas à transmissão de onda e cavitação, facilitando a transferência de massa. A formação de microcanais foi previamente observada por Garcia-Nogueira (2010) e Fernandes et al. (2009, 2008) para alimentos úmidos e macios (morango, melão e abacaxi). Entretanto, até então não havia sido demonstrada para alimentos secos e rígidos como grãos, cuja baixa atividade de água pode limitar a ocorrência ou magnitude da cavitação. Se presente, a formação de microcanais seria um mecanismo indireto de melhoria do processo pelo ultrassom, já que esta tecnologia na verdade estaria “apenas” alterando a estrutura do produto.

Entretanto, mecanismos diretos também são passíveis de ocorrerem. Quando as ondas ultrassônicas percorrem o produto, provocam uma série rápida de compressões e expansões alternadas, que pode ser comparada a uma esponja espremida e liberada repetidamente e, por isso, sendo denominado “efeito esponja” (Yao et al., 2009; Fuente-Blanco et al., 2006; Mulet et al., 2003). Este fenômeno pode manter os microcanais e poros desobstruídos, facilitando a transferência de massa (Yao et al., 2009). Além disso, um fluxo inercial, decorrente da própria movimentação do fluido pelos canais devido à propagação da onda, pode ser esperado.
Com esse trabalho, demonstramos o potencial da tecnologia de ultrassom para a hidratação de grãos, assinalamos uma possível limitação e iniciamos as discussões sobre os mecanismos envolvidos nessa melhora. Esse trabalho foi publicado como “Ultrasound (US) enhances the hydration of sorghum (Sorghum bicolor) grains” no periódico Ultrasonics Sonochemistry (Patero e Augusto, 2015 – Apêndice 7). Ainda assim, faltava melhorar a descrição do processo e possíveis mecanismos, além de demonstrar para outros produtos de interesse, avaliando o efeito dessa tecnologia em demais propriedades.

Delineamos então um estudo para melhorar a descrição dos possíveis mecanismos através dos quais a tecnologia de ultrassom melhora processos de transferência de massa. Até aquele momento, os trabalhos da literatura (incluindo o nosso com sorgo) apenas citavam os possíveis mecanismos envolvidos nos processos, porém sem mensurar ou discutir quais são relevantes para cada caso (produto, processo) estudado. Assim, desenvolvemos estudo para demonstrar como os efeitos diretos (denominados “efeito esponja” e fluxo “inercial”) e indiretos (formação de microcanais, com consequente alteração de estrutura e, portanto, resistência à transferência de massa) melhoram o fenômeno de transferência de massa no processamento de alimentos, e em que parte do processo eles são mais efetivos.

O estudo foi desenvolvido com dois tipos de alimentos, representando produtos de baixa e alta atividade de água/umidade: grãos de sorgo e cilindros de melão. Tais alimentos foram escolhidos como modelo, com base em trabalhos anteriores e características como geometria e estrutura simples e uniforme.

Diferentes tratamentos, alternando pré-tratamentos e tratamentos e utilizando um banho de ultrassom e um banho-maria tradicional, foram realizados para avaliar e discriminar qualitativamente os efeitos diretos e indiretos. Avaliou-se a cinética de transferência de massa (hidratação dos grãos de sorgo e absorção de solução corante nos cilindros de melão) nas diferentes etapas dos diferentes processos, realizando-se também avaliações estruturais.

Comparando-se os resultados obtidos entre tratamentos, demonstrou-se que ambos efeitos da tecnologia de ultrassom são mais efetivos em alimentos com alta atividade de água, formando-se microcanais somente neste tipo de alimento. Além disso, a formação de microcanais pode ser observada usando cilindros de gel de ágar. Verificou-se que a cavitação acústica resulta na formação de cavidades dispersas pelo produto, de diferentes formas e que podem ou não se conectarem.
formando canais (Figura 9). Demonstrou-se que os efeitos diretos são mais importantes na melhoria da transferência de massa tanto em alimentos úmidos como em alimentos secos, tanto através dos microcanais formados quanto pela porosidade dos alimentos.

Figura 8. Hidratação de grãos de sorgo à temperatura ambiente (25°C), com aquecimento (53°C), utilizando a tecnologia de ultrassom à temperatura ambiente (US) e combinando aquecimento e ultrassom (US+53°C). De Patero e Augusto (2015).

Figura 9. Esquerda: géis de ágar tratados em solução corante de forma convencional (A) e com ultrassom (B). Direita: Diferentes tipos de cavidades e microcanais após tratamento dos géis com ultrassom: cavidades tortuosas (t), isoladas com falta de conectividade (i) e com conectividade ao meio externo (c). De Miano et al. (2016a).
Em conclusão, a melhora da transferência de massa devido aos efeitos diretos e indiretos foi primeiramente discriminada e descrita. Comprovamos que ambos mecanismos são importantes na transferência de massa em alimentos úmidos, enquanto somente os efeitos diretos são importantes para alimentos secos. Mesmo não conseguindo quantificar a contribuição relativa de cada efeito, e nem separar a contribuição de cada efeito direto, o trabalho apresentou descrição inédita que nos permitiu entender melhor os efeitos da tecnologia de ultrassom e sua aplicação no processamento de alimentos. Resultou no artigo científico “Mechanisms for improving mass transfer in food with ultrasound technology: describing the phenomena in two model cases”, publicado no periódico *Ultrasonics Sonochemistry* (Miano et al., 2016a – Apêndice 8). Ainda, e particularmente no presente contexto, a realização do estudo citado foi importante para os próximos estudos realizados com a hidratação assistida por ultrassom.

Após melhor entendimento dos mecanismos envolvidos no uso do ultrassom em processos de transferência de massa, e antes de voltar a estudar especificamente o processo de hidratação de grãos, exploramos o efeito da tecnologia de ultrassom nas características de germinação de sementes de cevada. Esse trabalho foi desenvolvido tendo em vista a possível aplicação dessa tecnologia na hidratação do grão para posterior produção de malte. Ainda, o estudo permitiu entender possíveis efeitos do uso do ultrassom nas propriedades e desempenho de grãos, fatores importantes para uso após hidratação. Nosso temor era que a cavitação decorrente do ultrassom afetasse negativamente a estrutura e propriedades dos grãos. Entretanto, após os resultados obtidos no trabalho anterior (Miano et al., 2016a) esperava-se que a formação de cavidades e microcanais fosse pequena em grãos. Dessa forma, estudou-se a aplicação do ultrassom em quatro condições de sementes de cevada, avaliando-se dez diferentes parâmetros relacionados ao potencial germinativo e propriedades da semente e plântula. As sementes foram tratadas com ultrassom embaladas à vácuo, abordagem realizada para separar os efeitos do ultrassom, além daqueles atribuídos à hidratação (que já sabíamos que melhora com a tecnologia).

Demonstrou-se que o ultrassom melhorou a taxa de germinação das sementes, sem afetar negativamente nenhum dos parâmetros avaliados – resultado de grande interesse para o uso dessa tecnologia para melhoria do processo de hidratação.
Discutimos possíveis razões para os resultados obtidos. A razão mais óbvia está relacionada com possíveis mudanças na microestrutura dos grãos, como o aumento da porosidade da semente, melhorando a transferência de água e entre os tecidos. Entretanto, como os grãos foram tratados ainda secos, com baixa atividade de água, esperava-se que a formação de cavidades e microcanais fosse pequena (Miano et al., 2016a). Outra hipótese está relacionada com possíveis efeitos biológicos. É bem conhecido que solicitações mecânicas, tais como vibração, corte e escorciação, podem aumentar a taxa de respiração e metabolismo de tecidos vegetais (no presente contexto é interessante observar que esse foi exatamente o meu primeiro envolvimento com a pesquisa científica, ao realizar Iniciação Científica no começo da graduação (2002-2003) com o projeto “Variação na Taxa Respiratória e Produção de Etileno em Figs Devido à Vibração” – Processo FAPESP 2002/01422-1) e tem sido utilizada, por exemplo, para acelerar o processo de germinação em sementes (Uchida e Yamamoto, 2002). Portanto, comportamento semelhante é esperado para o efeito do ultrasom sobre o metabolismo de materiais biológicos vivos, como sementes de cevada. O estudo dos mecanismos envolvidos, em especial o possível efeito biológico, é parte da minha pesquisa atual.

O presente estudo resultou no artigo científico “Effect of ultrasound technology on barley seed germination and vigour”, publicado no periódico Seed Science and Technology (Miano et al., 2015a – Apêndice 9). Nesse momento já sabíamos que a tecnologia de ultrassom melhora processos de transferência de massa, em especial o processo de hidratação de grãos, os principais mecanismos envolvidos e que a tecnologia não prejudica (podendo até melhorar) as propriedades de germinação das sementes. Entretanto, ainda faltava descrever o comportamento para outros grãos, em especial com o até então não estudado comportamento sigmoidal, comprovar o efeito na germinação quando o ultrassom é aplicado durante a hidratação (ou seja, quando há o aumento da atividade de água e, consequentemente, da cavitação acústica) e estudar o efeito dessa tecnologia em demais propriedades do produto e processo.

Assim, o próximo estudo avaliou o efeito da tecnologia de ultrassom no processo de hidratação de grãos de milho, através da cinética de absorção de água pelo grão e das propriedades do amido obtido.

O problema estudado não fora selecionado ao acaso.

O milho é uma das culturas mais importantes do mundo, sendo o amido o principal produto obtido desse grão. O amido de milho, nativo ou modificado, é
amplamente utilizado em diversas indústrias, como a alimentícia, química, petroquímica, farmacêutica, têxtil, agrícola e agropecuária, além de ser utilizado como substrato para a produção de etanol. A primeira operação unitária durante o processamento do milho é a hidratação dos grãos, que é realizada por imersão em água, seguida da moagem úmida para extração do amido. Entretanto, a hidratação do milho é um processo demorado que pode levar até 36 h (Singh e Eckhoff, 1996). Consequentemente, a melhoria do processo de hidratação é particularmente importante nesse caso, com grande apelo comercial e industrial.

Primeiramente, observou-se que o comportamento de hidratação dos grãos de milho é específico, diferindo da maioria dos grãos descritos na literatura. Embora a curva de hidratação apresente o comportamento côncavo característico da grande maioria dos produtos já estudados (Figura 1A), este é claramente caracterizado por duas etapas distintas (Figura 10). A hidratação do grão de milho ocorre seguindo dois processos paralelos, devido à morfologia do grão. No primeiro processo, a taxa de hidratação é muito rápida, sendo a etapa curta. Compreende principalmente a entrada de água no grão, preenchendo os espaços “vazios” (canais, espaços entre endosperma e pericarpo e endosperma e gérmen). No outro processo, o gérmen e o endosperma são hidratados a partir da película de água que ocupou os espaços “vazios”. Este processo é lento e longo em comparação com o primeiro.

Além disso, a água entra tanto por difusão como capilaridade no grão, e ambos os mecanismos ocorrem simultaneamente. Entretanto, o fenômeno de capilaridade é provavelmente predominante no primeiro processo de hidratação, devido à porosidade da “ponta” e “espacos vazios” do grão. Por outro lado, o fenômeno de difusão provavelmente domina o segundo processo, devido à resistência à hidratação do endosperma e gérmen.

Como resultado, os modelos de Lewis (cinética de primeira ordem; Lewis, 1921), Page (1949), Henderson (1974), Peleg (1988) e Ibarz et al. (2004) não se ajustaram adequadamente à curva de hidratação do milho, sendo necessária a proposição de novo modelo. Assim, propusemos um modelo matemático de dois termos para explicar o processo, o qual teve suas etapas relacionadas com os diferentes mecanismos de entrada de água no grão (Figura 10).

Procedeu-se então com a hidratação assistida por ultrassom.

Diferentes tratamentos foram realizados para se determinar os mecanismos de melhoria do processo de hidratação (efeitos diretos ou indiretos), estudando a cinética de hidratação e a microestrutura dos grãos (através de microscopia
eletrônica de varredura e radiografias). A tecnologia de ultrassom melhorou significativamente o processo de hidratação, aumentando a taxa de absorção de água e diminuindo o tempo de processo em ~35% (Figura 10). Demonstrou-se que a melhora do processo foi somente devido aos efeitos diretos (fluxo inercial e efeito esponja) e não devido aos efeitos indiretos (formação de micro canais) – o que pode ser compreendido a partir de nosso estudo prévio sobre os mecanismos (Miano et al., 2016a).

![ultrasound reduced 5 hours the processing time!](image)

$$M_t = M_0 + (M_\infty - M_0) \cdot [p \cdot (1 - \exp(-k_1 \cdot t)) + (1 - p) \cdot (1 - \exp(-k_2 \cdot t))]$$

Figura 10. Hidratação dos grãos de milho: rota de entrada de água, modelo proposto e melhoria utilizando a tecnologia de ultrassom. De Miano et al. (2017a).

Finalmente, as propriedades reológicas (através de RVA), térmicas (através de DSC) e estruturais (através de difração de raios-x e MEV) do amido do milho hidratado com e sem ultrassom foram avaliadas. Todos os parâmetros avaliados demonstraram que, felizmente, a tecnologia de ultrassom não afeta as propriedades do amido.

Dessa forma, provamos que os grãos de milho podem se hidratar mais rapidamente usando a tecnologia de ultrassom sem a modificação das propriedades do seu amido (principal produto industrial), sendo tal resultado bastante desejável industrialmente. Tal estudo resultou no artigo científico “Ultrasound technology enhances the hydration of corn kernels without affecting their starch properties”, publicado no periódico Journal of Food Engineering (Miano et al., 2017a – Apêndice 3).
Procedemos então com o estudo da hidratação assistida por ultrassom, porém utilizando um grão com comportamento sigmoidal (pela primeira vez), avaliando as alterações nos demais componentes do grão e explorando o efeito na germinação.

Após prospecção, selecionou-se o feijão Moyashi para esse estudo, especialmente devido ao fato de ser consumido tanto como grão, como broto germinado. Inicialmente, descreveu-se o comportamento de hidratação do grão, com ênfase na importância das diferentes estruturas e rota do fluxo de água, demonstrando-se as mudanças na permeabilidade da casca – de forma semelhante ao anteriormente demonstrado para o feijão Adzuki. Um resultado interessante é que a casca e o hilo apresentaram efeito sinérgico no processo de hidratação, uma vez que a soma da contribuição das duas estruturas (obtidas através de experimentos impermeabilizando-se cada uma) não atingiu a curva de hidratação do feijão sem interferências. Assim, ambas as estruturas trabalham juntas para hidratar o feijão: a água que entra pelo hilo ajuda a acelerar a hidratação da casca, provocando a alteração de sua permeabilidade, e consequentemente acelerando o processo de hidratação.

Durante o processo de germinação, a hidratação das sementes segue um padrão de absorção de água em três estágios (Marcos Filho, 2005), representado na Figura 11. O primeiro estágio consiste no próprio processo de hidratação, foco do presente trabalho, quando a semente absorve a água necessária para ativar seu metabolismo. O segundo estágio consiste na digestão de reservas e síntese de novas moléculas, sendo a hidratação desprezível (o teor de umidade é mantido na umidade de equilíbrio do processo de hidratação dos grãos). O estágio III ocorre quando a radícula começa a crescer e componentes estruturais são sintetizados. Como a água é necessária em muitos processos metabólicos, a absorção de água pelo grão volta a crescer nessa etapa. Enquanto os três estágios possuem importância do ponto de vista agronômico, apenas o estágio I é relevante para o processamento de alimentos – sendo o único estudado nas demais partes do presente trabalho. Entretanto, tendo em vista o presente estudo, onde um dos objetivos foi avaliar se o ultrassom afeta a germinação do feijão Moyashi, os três estágios foram avaliados.

A tecnologia de ultrassom reduziu em ~25% o tempo de hidratação do grão (Figura 11, estágio I), reduzindo drasticamente a duração do estágio II e acelerando a germinação (estágio III). Assim, demonstrou-se que o ultrassom acelera não
somente a hidratação, mas também a germinação das sementes. Como havíamos relatado anteriormente o possível aumento do metabolismo de sementes submetidas ao ultrassom (Miano et al., 2015a), propomos que o processo de hidratação pode ser melhorado pelo ultrassom não apenas por fenômenos físicos, mas também devido a fenômenos metabólicos / biológicos, também acelerando a germinação. De fato, embora ainda não tenha sido descrito, é uma possibilidade que pode explicar o comportamento observado. De fato, embora ainda não conseguimos demonstrar essa hipótese, o estudo dos mecanismos envolvidos no uso do ultrassom no processamento de alimentos, em especial o possível efeito biológico, é parte da minha pesquisa atual.

Por fim, demonstramos que a hidratação assistida por ultrassom não altera a estrutura (avaliada através de cromatografia de permeação em gel) e propriedades (viscoamilográficas e do gel obtido) do amido obtido. Entretanto, o ultrassom aumentou a consistência da farinha obtida. Como o amido não foi modificado, essa alteração foi atribuída às proteínas.

Observa-se, portanto, que os resultados obtidos não somente são importantes para melhor entendimento e descrição dos processos, mas também são desejáveis do ponto de vista industrial: a tecnologia de ultrassom melhora o processo de hidratação sem alterar as propriedades do amido, acelera o processo de germinação (que é importante para o processo de malteação e brotamento) e aumenta a viscosidade aparente da farinha, o que é desejável para produtos que precisam de maior consistência. Este estudo resultou no artigo “Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components”, publicado no periódico *Scientific Reports* (Miano et al., 2016b – Apêndice 11).

Por fim, realizamos estudo com o grão tremoço andino - grão escolhido por apresentar comportamento sigmoidal e necessitar de processo de extração de alcaloides antes do consumo. A hidratação do grão foi melhorada pelo uso do ultrassom, que reduziu o tempo de processamento em quase 40% (Figura 12). A fase lag foi reduzida em cerca de 13%, enquanto a umidade de equilíbrio aumentou cerca de 14%. Devido a quantidade de amostra obtida, não foi possível estudar o processo de extração “total” dos alcaloides presentes. Mesmo assim, demonstrou-se que a extracção de alcaloides durante o processo de hidratação também foi melhorada, extraindo-se 21% a mais em relação ao processo convencional (Figura 12). Este estudo resultou no artigo “Ultrasound enhances mass transfer in Andean
lupin grains: hydration and debittering*, atualmente em avaliação no periódico *Journal of Food Science and Technology* (Miano et al., 2017b – Apêndice 12).

**Figura 11.** Hidratação e germinação dos grãos de feijão Moyashi: descrição dos estágios (A) e melhoria utilizando a tecnologia de ultrassom (B). De Miano et al. (2016b).

**Figura 12.** Esquerda: Hidratação dos grãos de trevoço andino de forma convencional (control) e assistida por ultrassom (US). Direita: conteúdo total de alcaloides nos grãos inicialmente e após hidratação (com e sem ultrassom). De Miano et al. (2017b).
4. Conclusões

A hidratação de grãos é um fenômeno complexo, cujos mecanismos exatos ainda são desconhecidos. Embora progressos recentes tenham avançado o conhecimento de sua descrição, em especial macroscopicamente, ainda existem diversos desafios para descrição microscópica.

A absorção de água pelo grão (e transporte através deste) ocorre não apenas por difusão, mas também por outros mecanismos de transferência de massa, como a capilaridade. Devido às diferentes estruturas e composição, as propriedades mudam com a atividade da água, fazendo com que cada tecido do grão interaja de forma diferente com a água em cada etapa do processo de hidratação. Consequentemente, a água escoa através de rotas específicas.

Os grãos podem apresentar dois comportamentos de hidratação: o comportamento de forma côncava para baixo e o comportamento sigmoidal.

O comportamento sigmoidal é menos frequente, sendo observado apenas para algumas leguminosas. A casca da semente é a estrutura responsável por este comportamento, pois sua permeabilidade muda com a atividade da água. O comportamento sigmoidal ainda precisa de melhor descrição, incluindo a caracterização de outros grãos.

O comportamento de forma côncava é muito mais frequente, sendo observado tanto para cereais como para leguminosas (bem como outros materiais e alimentos). Mesmo assim, esse comportamento não é tão simples quanto a teoria difusional afirma, apresentando também rotas específicas de escoamento de água (isso é claramente demonstrado nos nossos resultados com grãos de milho).

O melhoramento da hidratação pode ser conseguido através de diferentes abordagens. Neste trabalho são descritos o aumento da temperatura do processo e o uso da tecnologia de ultrassom.

O aumento da temperatura acelera a absorção de água, melhorando o processo de hidratação. Na maioria dos casos, mas não em todos eles, a Equação de Arrhenius pode ser usada para descrever o efeito da temperatura. Entretanto, temperaturas mais elevadas podem resultar em consequências indesejáveis, como consumo de energia, isolamento e alterações no produto (desnaturação proteica, gelatinização de amido e degradação molecular). Além disso, o aumento da temperatura pode reduzir a umidade de equilíbrio, como observado para o feijão Adzuki.
O ultrasom é uma tecnologia promissora, sendo capaz de melhorar o processo de hidratação de diferentes grãos (bem como outras operações unitárias de transferência de massa). Os mecanismos de transferência de massa envolvidos podem ser divididos em efeitos diretos e indiretos, cuja importância é função da estrutura e propriedades do produto (em especial a atividade da água). São necessários mais estudos para melhor descrever os mecanismos, em especial se existem efeitos biológicos envolvidos.

O processo de hidratação por ultrasom não afeta o amido dos grãos, porém afeta as propriedades da proteína. Além disso, o ultrasom estimula a germinação dos grãos. Todos estes resultados podem ser desejáveis ou indesejáveis, dependendo dos objetivos.

Os trabalhos descritos nesta Tese contribuíram para a compreensão do fenômeno de hidratação do grão. Entretanto, estudos adicionais são necessários para elucidar, compreender e descrever melhor os mecanismos (em especial a relação microscópica e macroscópica), bem como melhorar o processo. Para tanto, tanto tecnologias tradicionais como não tradicionais devem ser estudadas, cujos mecanismos devem ser entendidos. Finalmente, o processo otimizado deve ser obtido, reunindo todas essas informações.
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Apêndice 1: “Modelling the effect of temperature on the hydration kinetic of Adzuki beans (Vigna angularis)”

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Research note

Modelling the effect of temperature on the hydration kinetic of adzuki beans (Vigna angularis)

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ABSTRACT

The Aw reduction is a widely used technique to preserve grains, which hydration kinetics needs to be studied in order to obtain optimized processes. This work evaluated the hydration kinetic of Adzuki beans, a widely consumed grain by the oriental culture, modelling its behaviour as function of temperature. The grains were soaked in water and the moisture over the time was evaluated at temperatures of 25–70 °C. The grain behaviour during water uptake showed an initial lag phase, with a low water absorption rate. Therefore, the hydration kinetic was evaluated using a sigmoidal model (R² > 0.99). When soaking temperature was increased the hydration rate increased and both the lag phase and the moisture at the equilibrium decreased. These behaviour were, then, modelled as function of the soaking temperature (R² > 0.99). The obtained results are potentially useful for future studies on product development, food properties and process design.

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1. Introduction

The activity of water (Aw) reduction due to drying process is a widely used technique to preserve grains and other foods, although, in general, the products are rehydrated before processing and consumption. The hydration process is, thus, an important unit operation in dried foods, as it describes and defines its properties and using during cooking, extraction, fermenting, germinating and eating. Therefore, it is important to understand the hydration kinetics of different food products, as well as the influence of process conditions (as temperature) on its rate.

In fact, there are many works relating the hydration kinetics of different grains, as some kidney beans (Piergiovanni, 2011; Sobukola and Abayomi, 2011; Kaptsö et al., 2008; Abu-Ghannam, 1998; Sopade and Obeka, 1990), chickpea (Gowen et al., 2007; Ibarz et al., 2004), rice (Fonseca et al., 2011; Cheevitsopon and Noom-Sopade and Obeka, 1990), corn (Fernández-Muñoz et al., 2010), soybean (Coutinho et al., 2010a; Coutinho et al., 2010b; Sopade and Obeka, 1990), bamba (Jideani and Mpotokwana, 2009; Kaptsö et al., 2008), lentil (Joshi et al., 2010), asha (Tunde-Akintunde, 2010), lupin (Solomon, 2009), basi (Demirhan and Özbek, 2010), peanuts (Sopade and Obeka, 1990), sesame (Khazaei and Mohammad, 2009) and Cicer arietinum (Prasad et al., 2010) seeds. Most of these works just evaluated the grain hydration using simple kinetic models, neglecting the initial lag phase.

The Peleg’s Equation (Peleg, 1988; Eq. (1)), is the most used model to describe the food products hydration phenomenon. It consists in a two-parameter model (k₁ and k₂), which function describes a continuous change from a first-order (at τ → 0) to a zero-order (at τ → ∞) kinetic. The Peleg’s equation is described on Eq. (1).

However, although being the most used model, and although suiting well some products hydration data, the Peleg’s equation cannot describe an initial lag phase, which is observed during the hydration of some dried grains. Therefore, other models must be evaluated, as that previous proposed by Kaptsö et al. (2008). It describes a sigmoidal behaviour with an initial lag phase followed by a high absorption rate phase and, finally, by a stationary phase, using three parameters: τ (that describes the function inflexion point, and relates the lag phase); k (the kinetic parameter) and Mₑq (the moisture at the equilibrium, that relates the maximum water absorption). The sigmoidal model is described on Eq. (2):

\[ M(t) = M_0 + \frac{t}{k_1 + k_2} \cdot t \]  

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

The Adzuki beans are small red seeds, widely consumed in Asia, directly cooked or prepared as many sweet and salty typical dishes (Mendes et al., 2011; Yousif et al., 2002). Therefore, its hydration is...
necessary during industrial processing, and its study is important in order to optimize those processes. However, there is not any work in the literature studying the hydration kinetics of Adzuki beans (\textit{Vigna angularis}).

The objective of the present work was to evaluate the hydration kinetic of Adzuki beans (\textit{Vigna angularis}) and modelling its behaviour as function of temperature.

2. Materials and methods

Adzuki beans (\textit{Vigna angularis}) with initial moisture of 13.04 ± 0.27 g water/100 g of dry basis were purchase in a local market. Prior to use, the samples were cleaned and selected, in order that only the intact grains were designated to be used. These grains were then sealed in plastic bags and storage in a dry place before using.

The hydration procedure was carried out in the same way as described in previous works (Cheevitsopon and Noomhorm, 2011; Fonseca et al., 2011; Piergiovanni, 2011; Sobukola and Abayomi, 2011; Botelho et al., 2010; Joshi et al., 2010; Prasad et al., 2010; Tunde-Akintunde, 2010; Jideani and Mpotokwana, 2009; Khazaei and Mohammadi, 2009; Solomon, 2009; Kaptso et al., 2008 and Ibarz et al., 2004).

A sample of 30 g, randomly selected, was used in each one of the four replicates. The samples were soaked in 200 mL of distilled water at the studied temperatures using a 600 mL beaker immersed in a water-bath with a temperature control system which guarantees a fluctuation between ±0.5 °C (Quimis Q215u2, PID controlled, Brazil). The studied temperatures were 25.0, 32.5, 40.0, 55.0, and 70.0 °C. At specific interval times (15 min until the first hour; 20 min until the second hour; 30 min until the third our an 40 min until stabilization), the grains were removed from the water, drained, superficially blotted with absorbent paper, weighed and returned to the water. The moisture content at each time step (\(M(t)\)) was, then, calculated by mass balance, considering the initial sample mass (\(m_0\)), the moisture (\(M_0\)), and the obtained mass at each time step. The sample soluble solids leakage to the hydration water was neglected, as its concentration was always less than 2 °Brix.

The Adzuki bean hydration kinetic was, then, evaluated for each temperature using two mathematical models: the Peleg’s equation (Eq. (1)) and the sigmoidal model (Eq. (2)). For the models that suitable described the experimental data, their parameters were, then, modelled as a function of the process temperature.

The parameters for each model were obtained by linear or non-linear regression using the CurveExpert Professional software (v.1.5, http://www.curveexpert.net/, USA) with a significant probability level of 95%. The regressions were carried out for each replicate. Thus, each model parameter could be evaluated by its mean value and standard deviation.

3. Results and discussion

Fig. 1 A shows the effect of temperature (25–70 °C) on the Adzuki beans hydration during time.

The grain behaviour during water uptake clearly showed an initial lag phase, i.e., a period with a low water absorption rate, in the evaluated temperatures range (25–70 °C). This behaviour is similar to those observed for some cowpeas (Kaptso et al., 2008) and common beans (Piergiovanni, 2011), but being different to those described by the Peleg’s equation. The observed behaviour is related to a hard seed coat in the grain (Kaptso et al., 2008), which
can also be affected by its previous drying and represents a high resistance to the mass transfer phenomenon (water flow). Once this external structure is hydrated, it resistance to the water flow decrease, increasing the water uptake rate. In fact, Piergiovanni (2011) divided the bean seeds in three groups in relation to its hydration rate. While the fast and intermediate hydration rate grains do not show an initial lag phase, the slow hydration grains are characterized by showing this phase. Consequently, the Peleg’s equation (Eq. (1)) could not be used to describe the Adzuki beans hydration kinetic, therefore, only the sigmoidal-behaviour model (Eq. (2)) was used. The sigmoidal model described well the experimental values, with R² always higher than 0.995 for each replicate. It can be clearly seen in Fig. 1A, where the dashed curves represent the values obtained by the model.

Figs. 1B and 1C show the effect of temperature on the hydration kinetic parameters of Adzuki beans (M_eq, k, τ; Eq. (2)). The moisture at the equilibrium (M_eq) decreased when the hydration temperature was increased (Figs. 1A and 1B). It is similar to the observed behaviour of lentils (Joshi et al., 2010), bambara (Jideani and Mpotokwana, 2009); chickpeas (Gowen et al., 2007) and red beans (Phaseolus vulgaris L. - Abu-Ghannam, 1998; Abu-Ghannam and McKenna, 1997). The M_eq behaviour in relation to the temperature could be modelled by a linear decreasing equation (Eq. (3)); R² = 0.989; Fig. 1B. It is similar to what was used by Gowen et al. (2007) in order to describe the chickpeas hydration.

\[ M_{eq}(T) = -0.535 \cdot T + 304.6 \] (3)

The reduction on the M_eq was proposed by Abu-Ghannam and McKenna (1997) to be related to the higher extraction of water-soluble components of the grain at higher temperatures. However, the observed concentration of the soaking water in the present work was always less than 2°Brix. Moreover, two other possibilities to explain the M_eq behaviour are related to the cell integrity and to the mass transfer phenomenon to the grain. Firstly, it is expected that the higher temperatures damage the cell membranes, resulting in lower water holding capacity. Secondly, as the hydration rate is faster at higher temperatures (Fig. 1A), the seeds edges quickly absorb water, which, when the external layer is saturated, reduce the mass transfer from the soaking water to the grain surface. The kinetic parameter (k) increased exponentially when the soaking temperature was increased (Fig. 1B), indicating higher water absorption rates. In fact, the water diffusivity through the product is expected to be higher at higher temperatures due to the lower fluid viscosity and higher grain pores. It explains the observed behaviour, which is similar to other grains, such as chickpea (Gowen et al., 2007), red bean (Abu-Ghannam, 1998), cowpea (Sobukola and Abayomi, 2011; Kaptsos et al., 2008), corn (Sobukola and Abayomi, 2011) and bambara (Kaptsos et al., 2008).

The Arrhenius relation is a widely used model to describe the effect of temperature on different physic-chemical properties. It is composed by an exponential function (Eq. (4)), where the activation energy (Ea) describes the property variation with temperature (T in K), and where R is the universal constant of the ideal gas (R = 8.314 J mol⁻¹ K⁻¹).

\[ P(T) = P_0 \cdot \exp \left( \frac{Ea}{RT} \right) \] (4)

Therefore, the Arrhenius equation was used to describe the k behaviour, with high agreement (Eq. (5); R² = 0.999; Fig. 1B). It was also previously successful for chickpeas (Gowen et al., 2007), red beans (Abu-Ghannam, 1998), cowpeas and bambaras (Kaptsos et al., 2008). Either the k values and the correspondent activation energy (Ea) were at the same magnitude of those results obtained for chickpea (Gowen et al., 2007), cowpea and bambara (Kaptsos et al., 2008) seeds.

\[ k(T) = 10110.9 \cdot \exp \left( \frac{-34815.3}{R - T} \right) \] (5)

Finally, the parameter τ, i.e., the time to the hydration curve inflexion point was evaluated. This parameter indirectly relates the lag phase as up to the inflexion point the hydration rate is increasing. The lag phase is a consequence of the seed coat structure, which is resistance to the water flow. The parameter τ decreased exponentially when the soaking temperature was increased (Fig. 1C), reflecting the same reduction on the lag phase. It is the similar behaviour to those observed for cowpea and bambara (Kaptsos et al., 2008) seeds. The τ reduction can be related to the explained k behaviour. As the temperature is increased, the seeds coat hydration is faster, reducing the resistance to the water intake flow, and, consequently, either the τ parameter and the lag phase.

Thus, the Arrhenius equation (Eq. (3)) was used to describe the τ parameter behaviour, with high agreement (Eq. (6); R² = 0.999; Fig. 1C).

\[ \tau(T) = 0.00041 \cdot \exp \left( \frac{33631.8}{R - T} \right) \] (6)

By combining the kinetic model of Eq. (2) with the temperature dependency of Eqs. (3), (5), and (6), the Adzuki beans hydration can be described by a single equation as function of the immersion time (t, in min) and temperature (T, in K, where 298.15 ≤ T ≤ 343.15), as described on Eq. (7).

\[ M(t, T) = \frac{-0.535 \cdot T + 304.6}{1 + \exp \left( \left[ -10110.9 \cdot \exp \left( \frac{-34815.3}{R - T} \right) \right] \cdot \left( t - 0.00041 \cdot \exp \left( \frac{33631.8}{R - T} \right) \right) \right) \} \] (7)

Therefore, the goodness of the obtained model (Eq. (7)) was evaluated by plotting the values of moisture obtained by the model (M_model) as function of the experimental values (M_experimental), as shown on Fig. 1D. The regression of those data to a linear function (Eq. (8)) results in three parameters that can be used to evaluate the description of the experimental values by the model, i.e. the linear slope (β; that must be as close as possible to the unit), the intercept (β; that must be as close as possible to zero) and the coefficient of determination (R²; that must be as close as possible to the unit). It is a simple and efficient approach to evaluate the model fit (Augusto et al., 2012a,b).

\[ M_{model} = \alpha \cdot M_{experimental} + \beta \] (8)

Fig. 1D shows the comparison between the experimental values and those obtained by the sigmoidal model with parameters as a function of the temperature (Eq. (7)). The regression to Eq. (7) resulted in α = 1.01, β = −0.34 with R² > 0.99, in the evaluated moisture range up to ∼140 g water/100 g of dry basis. It demonstrates that the experimental data were well described by the obtained model, which can be successfully used in order to describe the Adzuki beans hydration.

The obtained results are potentially useful for future studies on hydration process and grains technology. Moreover, it can contribute to the development of efficacious and optimized food processing design.

4. Conclusions

The present work evaluated the hydration kinetic of Adzuki beans as function of the process temperature. The grain behaviour during water uptake showed an initial lag phase, with a low water
absorption rate. Thus, the Peleg’s equation could not be used to describe the seeds hydration kinetic, which was evaluated using a sigmoidal model. As the soaking temperature was increased, the model parameter $k$ (hydration rate) increased and both the parameters $\tau$ (time to the hydration curve inflexion point, which relates the lag phase) and $M_{eq}$ (moisture at the equilibrium) decreased.

The parameters $k$, $\tau$ and $M_{eq}$ were, then, modelled as function of $s$ (hydration rate) and $M_{eq}$ (moisture at the equilibrium) decreased. The parameters $k$, $\tau$ and $M_{eq}$ were then, modelled as function of the soaking temperature, with high agreement. The obtained results are potentially useful for future studies on product development, food properties and process design.

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Apêndice 2: “Hydration kinetics of cereal and pulses: new data and hypothesis evaluation”
Hydration kinetics of cereal and pulses: new data and hypothesis evaluation*

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Abstract
This work described and modeled 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 and modeling the hydration kinetics of the studied grains, it was reinforced the idea that this process is very complex. 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 hypothesis about the hydration of grains are not completely exact: the grains with darker color seed coat do not always have a slower hydration than the lighter color; the grains with sigmoidal behavior could have a faster hydration 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 of each curve.

Keywords: hydration, cereal, pulses, grains, modeling

*By describing an important process for pulses industrialization, the author pays tribute to the International Year of Pulses (2016) of the Food and Agriculture Organization (FAO) of the United Nations.
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 an important source of carbohydrate as starch. Pulses, as many beans, peas, lentils and others are a good source of vegetable proteins, besides its carbohydrate contribution. After their harvest, both cereal and pulses are dried in order 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) (A. C. 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; A. C. 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 with the process 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. On the other hand, 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. Only two mathematical models are reported in the bibliography, the Kaptso et al. model (Kaptso et al., 2008) and the Ibarz-Augusto model (Ibarz & Augusto, 2015), which 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 grain hydration are not satisfactorily described as sigmoidal, being reported with DCS behavior (Augusto & Miano, 2016). 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 hypothesis 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 reused in several studies about hydration, even though 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.

Table 1. Grains used to study the hydration kinetics behavior

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

*average ± standard deviation
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±1 °C. The temperature was controlled using a water bath (Dubnoff MA 095 MARCONI, 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, Japan). The sampling was carried out every certain time (for example each 30 min or each 15 min depending on the behavior or the hydration rate) until constant mass was reached. The hydration process was performed in triplicate.

2.3 Mathematical model fitting

The grains hydration kinetics was modeled using the downward concave shape (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 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.

\[
M_t = M_0 + \frac{t}{k_1+k_2t} \tag{1}
\]

\[
M_t = \frac{M_\infty}{1+\exp[-k(t-t_0)]} \tag{2}
\]

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

\[
M_\infty = M_0 + \frac{1}{k_2} \tag{3}
\]

Finally, the goodness of fit of the models was evaluated by the determination coefficient \(R^2\), the root-mean-square deviation values (RMSD, Equation 3), the normalized RMSD (NRMSD, Equation 4) 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 5) 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).

\[
RMSD = \sqrt{\frac{\sum_{i=1}^{n}(M_{\text{experimental}}-M_{\text{model}})^2}{n}} \tag{3}
\]

\[
NRMSD = 100 \cdot \frac{RMSD}{(M_{\text{experimental}})_{\text{maximum}}-(M_{\text{experimental}})_{\text{minimum}}} \tag{4}
\]
\[ M_{model} = a \cdot M_{experimental} + b \]  

3. Results and discussion

Table 2, Figure 1 and Figure 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 hydration behaviors, there is a great variety of curves types: some grains absorb more quantity of water and/or at different rates. The relation between the equilibrium moisture content and the initial moisture is interesting to observe. There are grains that can absorb almost 20 times of their initial moisture and others only 4 times. This characteristic could be attributed to the composition of grains; however, by observing the hydration kinetics of the studied grains, the composition would not be the only factor that would affect, the structure could also have an important influence.

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, specially by the seed coat (A. C. Miano & Augusto, 2015; A. C. Miano et al., 2015). Figure 1 shows that this behavior is observed only during the hydration of grains from the *phabaceae* family (considering only the *phabaceae* and *poaceae* family, corresponding to the majority of edible grains). In contrast to the *poaceae* family grains, the grains from the *phabaceae* family have a seed coat with a more complex structure (Lush & Evans, 1980; A. C. Miano et al., 2015; Perissé & Planchuelo, 2004). The *poaceae* family grains has 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; A. Miano, 2015; Singh & Eckhoff, 1996) while the seed coat from *phabaceae* family grains can be completely impermeable depending of the variety, composition and moisture content (A. C. Miano & Augusto, 2015; Sefa-Dedeh & Stanley, 1979). On the other hand, the velocity of the hydration process and the maximum water holding capacity probably is caused by the internal structure and composition of the grain (Prasad, Vairagar, & Bera, 2010; Sobukola & Abayomi, 2011). Therefore, the effect in combination of the different intrinsic properties of the beans (seed coat or pericarp structure and composition and cotyledon or endosperm structure and composition) are the cause of the different hydration kinetics shapes, which are shown in Figure 1 and Figure 2.
| Grain                        | \(M_0\) (% d.b) | \(M_{\infty}\) (% d.b) | \(k\) (min\(^{-1}\)) | \(\tau\) (min) | \(R^2\)  | RMSD   | NMSD   | a   | b   |
|-----------------------------|-----------------|------------------------|-----------------------|----------------|---------|--------|--------|-----|-----|
| Adzuki bean                 | 15.0 ± 0.1      | 134 ± 1                | 0.0100 ± 0.0003       | 284 ± 6        | 0.993   | 0.780  | 0.733  | 1.02| 2.14|
| Mung bean                   | 13.3 ± 0.1      | 140 ± 1                | 0.0103 ± 0.0001       | 249 ± 2        | 0.997   | 0.563  | 0.499  | 1.01| 0.84|
| Caballero bean              | 15.9 ± 0.2      | 113 ± 3                | 0.0097 ± 0.0009       | 193 ± 28       | 0.999   | 0.207  | 0.228  | 1.00| -0.03|
| White kidney bean           | 13.1 ± 0.1      | 114 ± 1                | 0.0112 ± 0.0006       | 189 ± 12       | 0.999   | 0.245  | 0.260  | 1.00| -0.23|
| Fava bean                   | 13.4 ± 0.1      | 127 ± 1                | 0.0142 ± 0.0016       | 163 ± 14       | 0.992   | 0.732  | 0.620  | 1.00| -0.16|
| Dark red kidney bean        | 14.2 ± 0.2      | 111 ± 4                | 0.0132 ± 0.0011       | 130 ± 12       | 0.998   | 0.418  | 0.436  | 1.01| -0.35|
| Egyptian kidney bean        | 13.4 ± 0.2      | 124 ± 2                | 0.0175 ± 0.0012       | 107 ± 7        | 0.991   | 1.080  | 0.939  | 1.02| -1.65|
| Canary bean                 | 15.7 ± 0.2      | 108 ± 1                | 0.0180 ± 0.0013       | 81 ± 4         | 0.994   | 0.808  | 0.845  | 1.01| -0.98|
| Carioca kidney bean         | 16.0 ± 0.1      | 112 ± 1                | 0.0224 ± 0.0009       | 76 ± 1         | 0.997   | 0.644  | 0.646  | 1.00| -0.46|
| Green Lentils               | 10.4 ± 0.1      | 117 ± 1                | 0.0432 ± 0.0023       | 39 ± 1         | 0.990   | 1.556  | 1.439  | 1.03| -3.01|
### Downward concave shape behavior - Peleg Model

| Grain             | \(M_0\) (% d.b) | \(M_\infty\) (% d.b) | \(k_1\) (min \cdot % d.b. \(^{-1}\)) | \(k_2\) (% d.b. \(^{-1}\)) | \(R^2\) | RMSD | NMSD | a     | b     |
|-------------------|------------------|-----------------------|--------------------------------------|--------------------------|--------|------|------|-------|-------|
| White lupin       | 10.7 ± 0.1       | 237 ± 7               | 1.2575 ± 0.0401                      | 0.0044 ± 0.0001          | 0.999  | 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          | 0.996  | 0.696| 0.627| 0.98  | -1.24 |
| Lentils           | 10.3 ± 0.1       | 170 ± 6               | 0.4391 ± 0.0400                      | 0.0063 ± 0.0002          | 0.992  | 2.258| 1.955| 0.97  | -2.30 |
| Cowpea            | 11.8 ± 0.1       | 165 ± 2               | 0.3768 ± 0.0311                      | 0.0065 ± 0.0001          | 0.995  | 0.750| 0.566| 1.00  | 0.94  |
| Chick pea         | 10.9 ± 0.2       | 148 ± 1               | 0.5913 ± 0.0454                      | 0.0073 ± 0.0001          | 0.997  | 0.645| 0.559| 0.98  | 1.55  |
| Ñuña bean         | 11.7 ± 0.1       | 142 ± 2               | 0.9171 ± 0.0276                      | 0.0077 ± 0.0001          | 0.995  | 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          | 0.996  | 1.032| 0.984| 1.00  | 0.41  |
| Panamito bean     | 15.1 ± 0.2       | 141 ± 1               | 0.6975 ± 0.0510                      | 0.0080 ± 0.0001          | 0.995  | 0.739| 0.711| 1.02  | -2.37 |
| Barley kernels    | 10.0 ± 0.1       | 77 ± 1                | 1.6274 ± 0.0262                      | 0.0148 ± 0.0001          | 0.993  | 0.378| 0.753| 1.01  | 1.00  |
| Wheat kernels     | 11.3 ± 0.1       | 67 ± 1                | 1.9406 ± 0.0375                      | 0.0181 ± 0.0001          | 0.981  | 0.547| 1.213| 1.03  | 2.08  |
| Corn kernels      | 12.6 ± 0.1       | 57 ± 1                | 4.6615 ± 0.1412                      | 0.0224 ± 0.0006          | 0.990  | 0.271| 0.744| 1.03  | 1.51  |
| Sorghum kernels   | 11.8 ± 0.1       | 40 ± 2                | 1.6227 ± 0.3261                      | 0.0354 ± 0.0020          | 0.970  | 0.362| 1.459| 0.97  | 0.94  |
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 Downward Concave Shape 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.
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 attributed 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 Figure 1 and 2 more examples can be seen). As stated before, the hydration behavior is caused by the seed coat structure and the hydration velocity and the maximum water holding capacity is caused by the internal structure and composition. Therefore, grains with sigmoidal behavior not always have slow water uptake rate during processing, and 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 with 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 one. Therefore, the individual effect of the seed 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, due to the surface area per unit mass is higher for the water transfer (Montanuci et al., 2015). In Figure 3c two beans from the same species (Phaseolus 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. However, more comparisons can be performed using Figure 1 and Figure 2, unsatisfying this hypothesis. For instance: mung bean hydrates slower than canary bean, despite the first is much smaller than the second one. 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 great number of factor that acts simultaneously and is difficult to generalized hypothesis.

Figure 3d shows the hydration behavior of different cultivars of the same species of grains (Phaseolus vulgaris). All those grains are different in size, color, composition and
structure, which causes different hydration behavior. It can be seen that Panamito beans and Black kidney beans have the same hydration kinetics despite 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 has 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 *phabaceae* family. The *phabaceae* family grains have higher quantity of protein (16 – 25%) and shorter quantity of starch (16 – 45%) (Siddiq, Butt, & Sultan, 2011), which could allow to reach higher equilibrium moisture content than *poaceae* family grains (8 – 12 % of protein and 55 – 70 % of starch (Koehler & Wieser, 2013)). This is due to the higher affinity to water that proteins have.

**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 *Phaseolus 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).
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, cultivars, since those intrinsic properties are very difficult to control.

In fact, there are works in the literature focusing on established how structure (Lush & Evans, 1980; A. C. Miano et al., 2015; Sefa-Dedeh & Stanley, 1979) and composition (for example the the initial moisture content (A. C. Miano & Augusto, 2015)) affect the hydration process of grains. However, many doubts still exist, and the exactly mechanisms involved in this phenomenon 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 mechanisms take place at each processing period. Therefore, it is important to perform more studies about the relation of the composition, structure and hydration process in order to establish 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 behaviors: the downward concave shape, 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. Further, by comparing the hydration kinetics of twenty-two grains, some hypothesis about the hydration process were evaluated, finding that they are not always satisfied. Despite the difficulty of evaluating each individual factor isolated from the others, future studies are recommended to establish 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]
\[ k = \text{Kaptso et al. kinetic parameter related to water absorption rate (Equation 2) [% d.b.]} \]
\[ M_\infty = \text{Equilibrium moisture content (Equation 2) [% d.b.]} \]
\[ M_{\text{Experimental}} = \text{Experimental moisture content value (Equation 3 and 4) [% d.b.]} \]
\[ M_{\text{Model}} = \text{Model moisture content value (Equation 3 and 4) [% d.b.]} \]
\[ M_0 = \text{Initial moisture content (Equation 1) [% d.b.]} \]
\[ M_t = \text{Moisture content over the time } t \text{ (Equation 1 and 2) [% d.b.]} \]
\[ NRMSD = \text{normalized root-mean-square deviation values (Equation 4) [%exp]} \]
\[ RMSD = \text{root-mean-square deviation values (Equation 3) [% d.b.]} \]
\[ t = \text{Time (Equation 1 and 2) [min]} \]
\[ \tau = \text{Kaptso et al. kinetic parameter related to lag phase (Equation 2) [min}^{\text{-1}}\text{]} \]

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Apêndice 3: “Describing the food sigmoidal behaviour during hydration based on a second-order autocatalytic kinetic”
Describing the Food Sigmoidal Behavior During Hydration Based on a Second-Order Autocatalytic Kinetic

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Although many mathematical models have been proposed to describe the hydration process, there is only one empirical model in the literature describing the sigmoidal behavior. This work proposed a new semi-empirical model to describe the sigmoidal behavior of food during hydration. The new model was based on a second-order autocatalytic kinetic, composed of only two parameters, whose physical meaning was discussed. The model was successfully validated using data from different beans and temperatures. Its main advantages and disadvantages are also discussed, while showing its possible use in describing the hydration process of foods with sigmoidal behavior.

Keywords Food properties; Hydration; Kinetic; Modeling; Physical properties; Rehydration

INTRODUCTION

Reducing water activity (a_w) through drying is a widely used technique to preserve food products, which are frequently further rehydrated before processing and consumption. The hydration (or rehydration) process is thus an important unit operation in dried foods, because it describes and defines their properties and use during cooking, extraction, fermenting, germinating, and eating. Therefore, it is important to understand the hydration kinetics of different food products, as well as to appropriately model the process.

There are many works presenting the hydration processes of different products in the literature, and many mathematical models have been proposed to describe this process. However, although extensively studied, the exact mechanism of mass transfer during hydration is still not completely understood.

For example, the process is generally described in the literature as purely diffusive, following Fick’s laws. However, this is a simplification that does not describe the hydration behavior of some foods.

According to Aguilera et al., the study of mass transfer through other mechanisms besides diffusion has been neglected. For example, Marabi and Saguy showed that the food hydration behavior deviates from the Fickian description when its porosity is changed, and Goula and Adamopoulos highlighted the capillary flow during the hydration of dried foods. It is interesting to note that the capillary flow takes place in the pores and microchannels, whose dimensions, structure, and flexibility are functions of the tissue moisture content.

Xiao et al. described the different routes for water flow inside plant materials. Further, Tang et al. and Tang and Sokhansanj demonstrated that the water uptake mechanism during the hydration of grains changes with moisture content, due to the different behavior of each tissue in the seed. This result suggests that the hydration process in structured and complex materials, such as grains, is also a function of its moisture content.

Therefore, it highlights the need for a better understanding of the hydration kinetics of food products and appropriately modeling the process.

The hydration behavior of most food products follows a continuous water uptake, at a decreasing rate in relation to processing time, describing a curve with a downward concave shape (Fig. 1). In this behavior, the water influx rate is maximum at the beginning of the process (at t → 0), falling to zero after time enough (at t → ∞) to reach the product equilibrium moisture content (M_∞). Table 1 shows the mathematical models most often used to describe this behavior, including the Lewis (first-order kinetics), Page, Henderson, Peleg, Weibull, and Ibarz-Gonzalez-Barbosa-Cánovas models. The two-term model proposed by Peleg is, without doubt, the most widely used in the literature.
However, these models only describe the hydration of products with the downward concave shape behavior, without an initial lag phase.

An initial lag phase—that is, an initial phase with a low water uptake rate (Fig. 1)—is observed during the hydration of some dried grains, such as Adzuki beans,[8] different varieties of cowpeas,[9,10] lentils,[11] lima beans,[12] or Badda bianco and nero beans.[13] In this case, the water influx rate first increases until an inflexion point (at \( t = t_{\text{inflexion}} \)), after which the rate decreases until zero after enough time (at \( t \to \infty \)) for the product to reach its equilibrium moisture content (\( M_\infty \)). This hydration behavior of products is described by a sigmoidal shaped curve, as shown in Figs. 1–3.

Therefore, other models must be evaluated, like the one previously proposed by Kaptso et al.[9] that describe the sigmoidal behavior with an initial lag phase followed by a high absorption rate phase and, finally, a stationary phase, using three parameters: \( \tau \) (which describes the function inflexion point and is related to the lag phase), \( k_K \) (the kinetic parameter), and \( M_\infty \) (the moisture at equilibrium, which is related to the maximum water absorption). The sigmoidal model is described in Table 2.

However, only the model by Kaptso et al.[9] is described in the literature for food products with sigmoidal hydration behavior, so it is interesting to evaluate other possible models. Further, the Kaptso et al.[9] model is described by three parameters (\( \tau \), \( k_K \), and \( M_\infty \)). It is well known that it is interesting to use mathematical models with the least possible number of parameters. Finally, the Kaptso et al.[9] model is an empirical model, though it is desirable to use semi-empirical or constitutive models.

### TABLE 1

The most used mathematical models to describe the food hydration with downward concave shape behavior

| Model                        | Equation                                                                 | Reference          |
|------------------------------|--------------------------------------------------------------------------|--------------------|
| Lewis (first-order kinetics) | \( \frac{M(t) - M_0}{M_\infty - M_0} = \exp(-k_L \cdot t) \)             | Lewis[14]          |
| Page                         | \( \frac{M(t) - M_0}{M_\infty - M_0} = \exp(-k_{P1} \cdot t^{n}) \)     | Page[15]          |
| Henderson                    | \( \frac{M(t) - M_0}{M_\infty - M_0} = P_1 \cdot \exp(-k_{H1} \cdot t) + P_2 \cdot \exp(-k_{H2} \cdot t) \) | Henderson[16]     |
| Peleg                        | \( M(t) = M_0 + \frac{k_{I1}}{k_{I2}} \cdot \left( 1 - \exp\left( -\left( \frac{t}{k_{I2}} \right)^{\theta} \right) \right) \) | Peleg[7]          |
| Weibull                      | \( M(t) = \frac{k_{I0}}{k_{I1}} \cdot \left( \frac{k_{I1}}{k_{I2}} - M_0 \right) \exp(-k_{I1} \cdot t) \) | Saguy et al.[17]  |
| Ibarz-González-Barbosa-Cánovas | \( M(t) = \frac{k_{I0}}{k_{I1}} \cdot \left( \frac{k_{I1}}{k_{I2}} - M_0 \right) \exp(-k_{I1} \cdot t) \) | Ibarz et al.[18]  |
The aim of the present work was to propose a new semi-empirical model to describe the sigmoidal behavior of certain food materials during hydration.

**MODEL DEVELOPMENT**

When a dried product is immersed in enough water, it hydrates until saturation, when its moisture content is called the equilibrium moisture ($M_1$, g water/100 g dry basis = % db). Then, its moisture content ($M(t)$) increases from its initial value ($M_0$, at $t = 0$) until its equilibrium value ($M_1$, when $t \rightarrow \infty$) during the hydration process.

The hydration process can be simplified as the kinetic reaction described on Eq. (1), where the solid matrix (SM) absorbs water (H$_2$O) following a kinetic parameter ($k$), resulting in a complex containing both the solid matrix and water (SM—H$_2$O):

$$\text{SM} + \text{H}_2\text{O} \rightarrow \text{SM} - \text{H}_2\text{O}. \quad (1)$$

During each moment of the hydration process, the $M(t)$ value represents the actual water content, and the product sites available to absorb water are represented by the difference between the actual moisture content and the equilibrium value ($M_\infty - M(t)$).

The water influx, then, can be adsorbed in both the dried sites (represented by ($M_\infty - M(t)$), interacting with the solid matrix, and the product that already holds water (represented by $M(t)$), interacting by hydrogen bonds in the latter case. Consequently, the water retained in the product can interact with both the solid matrix and the water linked to this matrix, in a multilayer retention. Therefore, the product moisture variation over the time of processing is a function of both ($M_\infty - M(t)$) and $M(t)$, which is described by a second-order autocatalytic kinetic (Eq. (2)):

$$\frac{dM}{dt} = k \cdot (M_\infty - M) \cdot M \quad (2)$$

To solve Eq. (2), after isolating the variables (Eq. (3)), the expression on the left side must be integrated by the partial

| Model                                | Equation                             | Reference           |
|--------------------------------------|--------------------------------------|---------------------|
| Kaptso-Njintang-Komnek-Hounhouigan-Scher-Mbolung | $M(t) = \frac{M_\infty}{1 + \exp(-k (t-t_1))}$ | Kaptso et al. [9]   |
| Ibarz-Augusto                        | $M(t) = \frac{M_\infty}{1 + \frac{M_\infty - M_0}{M_0} \exp(-k M_\infty t)}$ | This work (Eq. (10)) |

**TABLE 2**

Mathematical models to describe the food hydration with sigmoidal shape behavior

**FIG. 3.** Hydration of different beans: (A) Adzuki beans at 25–70°C; (B) West cowpeas at 25, 35, and 45°C; (C) Badda biando and Badda nero beans at 45 and 55°C; and (D) Adua ayera and new era cowpeas and Fagiolo a Formella lima beans at 25°C. The dots are the experimental values; the curves are the new hydration model (Eq. (10)).
fractions method. Thus, this rational function can be rewritten as Eq. (4):

$$\frac{dM}{(M_\infty - M) \cdot M} = k \cdot dt$$

(3)

$$\frac{1}{(M_\infty - M) \cdot M} = \frac{A}{(M_\infty - M)} + \frac{B}{M}.$$  

(4)

The values of $A$ and $B$ (Eq. (5)) are obtained by solving Eq. (4). Then, by substituting it in Eq. (3), Eq. (6) is obtained:

$$A = B = \frac{1}{M_\infty}$$

(5)

$$k \cdot dt = \frac{dM}{(M_\infty - M) \cdot M} = \frac{1}{M_\infty} \left[ \frac{dM}{(M_\infty - M)} \right] + \frac{1}{M_\infty} \left[ \frac{dM}{M} \right]$$

(6)

Equation (6) is thus integrated, with the following appropriate boundary conditions, resulting in Eq. (9):

- When $t = 0$, $M(t) = M_0$;
- When $t \neq 0$, $M(t) = M(t)$.

$$\int_0^t k \cdot dt = \frac{1}{M_\infty} \left[ \int_{M_0}^M \frac{dM}{(M_\infty - M)} + \int_{M_0}^M \frac{dM}{M} \right]$$

(7)

$$k \cdot t = \frac{1}{M_\infty} \left[ - \ln(M_\infty - M) + \ln(M) \right]_{M_0}^M$$

(8)

$$k \cdot M_\infty \cdot t = \ln \left[ \frac{(M_\infty - M_0) \cdot M}{(M_\infty - M) \cdot M_0} \right]$$

(9)

Finally, by isolating $M(t)$ in Eq. (9), the proposed new model is obtained (Eq. (10)):

$$M(t) = \frac{M_\infty}{1 + \frac{M_\infty - M_0}{M_0} \cdot \exp(-k \cdot M_\infty \cdot t)}$$

(10)

The proposed model, described in Eq. (10), is a sigmoidal mathematical function with two parameters (because the initial value of the moisture ($M_0$) is, in fact, not a variable or a property of the studied material but a known parameter, which is different in each storage condition): $k$, which is the kinetic parameter, also related to the product lag phase, and $M_\infty$, which is the moisture at equilibrium and is related to the maximum water adsorption of the product.

A graphic description of these parameters is shown in Fig. 2, where a theoretical hydration of a grain with 15% (db) of initial moisture ($M_0$) is described for different values of $k$ (from $5 \cdot 10^{-5}$ to $8 \cdot 10^{-4} [\% \text{ db} \cdot \text{min}]^{-1}$) and $M_\infty$ (from 60 to 140% db). These values ($M_0$, $k$, and $M_\infty$) were based on those obtained in the following section (see Table 3), thus representing real values.

It can be clearly seen that the kinetic parameter $k$ describes the rate of water uptake. Thus, higher $k$ values show faster water uptake. Further, the higher the hydration process is, the less pronounced its lag phase is. At higher $k$ values, the curve shape moves closer to the downward concave shape behavior (Figs. 1 and 2). The parameter $M_\infty$ describes the maximum moisture content of the product, with a small influence on the curve shape.

The applicability of the proposed new model was then evaluated considering different grain hydration processes.

**Model Evaluation**

Once the model was theoretically obtained (Eq. (10)), we evaluated whether it could be used to appropriately describe real experimental data.

The model evaluation was conducted considering the sigmoidal behavior of seven different beans during hydration, based on the previously published data for Adzuki beans[8] at five temperatures (25–70°C), West cowpeas[9] at three temperatures (25–45°C), Badda biando and Badda nero beans[13] at 45 and 55°C, Adua ayera and new era cowpeas[10] at 25°C, and ‘Fagiolo a Formella’ lima beans[12] at 25°C.

The model parameters were obtained by nonlinear regression using CurveExpert Professional software (v. 2.0.3, http://www.curveexpert.net/) with a significant probability level of 95%. According to its documentation, the software uses the Levenberg-Marquardt method to solve the nonlinear regressions, minimizing the chi-square value. Therefore, for each data set, an initial value for both $k$ and $M_\infty$ were estimated, and the software then applied the algorithm in order to obtain the appropriate values for each parameter. The obtained values were then evaluated by plotting the achieved curve with the experimental data and, objectively, by different approaches.

The goodness of the obtained model was evaluated by the $R^2$ regression value, the root mean square error values (RMSE, Eq. (11)), and the normalized RMSE (NRMSE, Eq. (12)) and by plotting the moisture values obtained by the model ($M_{\text{model}}$) as a function of the experimental values ($M_{\text{experimental}}$). The regression of those data to a linear function (Eq. (13)) 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 ($R^2$, which must be as close as possible to one). It is a simple and efficient approach to evaluating the model fit.
The results obtained are shown in Table 3 and Figs. 3 and 4. Table 3 also shows the values of $k$ and $M_\infty$ for the different grains and temperatures evaluated. It can clearly be seen that the proposed model described the experimental data well, with high values of $R^2$ (obtained both by the direct regression of Eq. (10) and by the evaluation using Eq. (13)) and small values of $a$, $b$, RMSE, and NRMSE. This can also be seen by evaluating the plotted model values in Fig. 3 (dashed curves) and the comparison among the experimental values and those obtained by the proposed model (Fig. 4).

By evaluating that, it can be affirmed that the proposed model well describes the sigmoidal behavior of the seven evaluated grains at different temperatures, even considering conditions with the sigmoidal behavior more or less pronounced (i.e., higher or shorter lag phases).

Therefore, the proposed model is considered valid and appropriate to describe the hydration process of foods with sigmoidal behavior.

The best model description was the regressions of the Adzuki beans, due to the appropriate number of experimental data available, especially at the beginning of the process (i.e., before the inflexion point) and at the end (i.e., close to equilibrium).

\[
M(t) = \frac{M_\infty}{1 + \frac{M_\infty}{M_0} \exp(-k \cdot M_\infty \cdot t)}
\]

\[
M_{\text{model}} = a \cdot M_{\text{experimental}} + b.
\]

The results obtained are shown in Table 3 and Figs. 3 and 4. Table 3 also shows the values of $k$ and $M_\infty$ for the different grains and temperatures evaluated.

It can clearly be seen that the proposed model described the experimental data well, with high values of $R^2$ (obtained both by the direct regression of Eq. (10) and by the evaluation using Eq. (13)) and small values of $a$, $b$, RMSE, and NRMSE. This can also be seen by evaluating the plotted model values in Fig. 3 (dashed curves) and the comparison among the experimental values and those obtained by the proposed model (Fig. 4).

By evaluating that, it can be affirmed that the proposed model well describes the sigmoidal behavior of the seven evaluated grains at different temperatures, even considering conditions with the sigmoidal behavior more or less pronounced (i.e., higher or shorter lag phases).

\[
\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (M_{\text{experimental}} - M_{\text{model}})_i^2}
\]

\[
\text{NRMSE} = 100 \cdot \frac{\text{RMSE}}{(M_{\text{experimental}})_{\text{maximum}} - (M_{\text{experimental}})_{\text{minimum}}}
\]

\[
M_{\text{model}} = a \cdot M_{\text{experimental}} + b.
\]
The worst model description was the regression of the West cowpea at 45°C, although it described the data well at the other temperatures. This is related to the length of the lag phase and the number of available experimental data points before the inflexion point. At higher temperatures, the water absorption rate (related to the parameter \( k \) in the proposed model) is higher, the lag phase is shorter, and the sigmoidal behavior is more subtle. It can be seen in Fig. 3B that the water uptake rate greatly increased from 25 to 45°C, which results in a very short lag phase. Consequently, there is a need for more data before the inflexion point in order to clearly confirm and evaluate the sigmoidal behavior. Unfortunately, it was not possible due to the amount of experimental data and, consequently, the regression was poor.

This highlights the need to obtain a large amount of experimental data at the beginning of the process, especially before the inflexion point, as carried out for Adzuki beans (Fig. 3A). It has a very high impact on the estimation of \( k \) during nonlinear regression. Similarly, for an adequate \( M_\infty \) estimation during nonlinear regression, the hydration experiment needs to be conducted for sufficient time (as close as possible to equilibrium).

### Advantages and Limitations of the Model

The proposed mathematical model is a semi-empirical model that well describes the sigmoidal behavior of food during hydration. It was based on a second-order autocatalytic kinetic, and it was successfully validated using data from seven different bean hydrations.

The main advantages of the model are related to its semi-empirical nature, because its parameters describe specific physical meanings, and its simplicity, because it is described by only two parameters (\( k \) and \( M_\infty \)).

However, like every model, it has some limitations, which must be known in order to be employed adequately.

Firstly, the data modeling must be carried out using nonlinear regression, thus requiring appropriate software. Nevertheless, there is a large range of appropriate software (including freeware), which reduces the importance of this issue.

Secondly, as in any mathematical modeling, an appropriate amount of experimental data must be provided in order to obtain a suitable regression. Then, an appropriate amount of experimental data must be provided from the beginning of hydration, before the inflexion point, to characterize the sigmoidal behavior and, consequently, to estimate \( k \). Further, the hydration experiments must be carried out for long enough to estimate the equilibrium moisture (\( M_\infty \)) of the product.

Thirdly, the proposed model describes sigmoidal hydration behavior, whereas many food products show the downward concave shape behavior, according to the literature. However, as previously discussed, it is important to highlight that a small amount of experimental data at the beginning of the process may hide the actual sigmoidal behavior, which appears as a downward concave shape behavior. Thus, many food products in the literature may show sigmoidal behavior, although this behavior is described by a concave shape as a result of the small amount of data available. In fact, the only way to know the real behavior of the product is to obtain a large amount of data from the beginning of the process.

Finally, in contrast with the model by Kaptso et al.\textsuperscript{[9]} (Table 2), the inflexion point is not directly provided. It is interesting information because it describes the time when the water uptake rate stops rising and starts decreasing, which is directly related to the lag phase of the product. However, this information can be obtained and evaluated as follows.

If the function obtained (Eq. (10)) is symmetric, the inflexion point time (\( t_{inflexion} \)) is the one where:

\[
M(t_{inflexion}) = M_0 + \frac{M_\infty - M_0}{2} = \frac{M_\infty + M_0}{2}.
\]

(14)

Thus, substituting Eq. (10) into Eq. (14) and solving it, an expression for \( t_{inflexion} \) is obtained (Eq. (16)):

\[
M(t_{inflexion}) = \frac{M_\infty}{1 + \frac{M_\infty - M_0}{M_0}} \cdot \exp(-k \cdot M_\infty \cdot t_{inflexion}),
\]

(15)

\[
t_{inflexion} = \frac{1}{k \cdot M_\infty} \ln\left(\frac{M_\infty + M_0}{M_0}\right)
\]

(16)
Table 4 shows the time of the inflexion point \( t_{\text{inflexion}} \) for the seven evaluated grains, which can be compared with the data in Fig. 3. Thus, the lag phase estimation, through the time of the inflexion point, can be evaluated by using Eq. (16). Further, the general behavior for the grain hydration can be evaluated using the same equation, because it tends toward downward concave behavior when \( t_{\text{inflexion}} \to 0 \) and deviates from that when the \( t_{\text{inflexion}} \) is higher.

In conclusion, the new hydration model proposed here can be used to describe the hydration process of foods with sigmoidal behavior and is potentially useful for future studies of food properties and process design.

CONCLUSIONS

A new semi-empirical mathematical model, based on a second-order autocatalytic kinetic, was developed and proposed to describe the sigmoidal behavior of food during hydration. The model was theoretically described and successfully validated using the data from seven different bean hydrations at different temperatures. Its main advantages and disadvantages were discussed. After evaluation, it can be concluded that the new hydration model proposed here can be used to describe the hydration process of foods with sigmoidal behavior. Consequently, the obtained model is potentially useful for future studies on product development, food properties, and process design, for both industry and academia, because it accurately describes the sigmoidal hydration behavior using only two parameters with adequate physical meaning.

NOMENCLATURE

\( A, B \)  
Parameters for the partial fractions integration (Eq. (4)) (different units)

\( a, b \)  
Parameters of model fit evaluation (Eq. (13)) (different units)

\( k \)  
Kinetic parameter of the proposed model (Eq. (9)) \([\% \text{db} \cdot \text{min}^{-1}]\)

\( k_L, k_{P1}, k_{P2}, P_1, P_2 \)  
Kinetic parameters of the most used mathematical models to describe the food hydration with downward concave shape behavior (Table 1) (different units)

\( k_K, \tau \)  
Kinetic parameters of the Kaptso et al.\(^{[9]} \) model (Table 2, food hydration with sigmoidal shape behavior) (different units)

\( M \)  
Product moisture content on a dry basis (g water/100 g solids = \% db)

\( M_0 \)  
Initial moisture content (g water/100 g db)

\( M_{\infty} \)  
Equilibrium moisture content (g water/100 g db)

\( T, T_K \)  
Temperature (°C) Absolute temperature (K)

\( t, t_{\text{inflexion}} \)  
Time (min) Time at the inflexion point (min)

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Apêndice 4: “Correlation between morphology, hydration kinetics and mathematical models on Andean lupin (Lupinus mutabilis Sweet) grains”

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Correlation between morphology, hydration kinetics and mathematical models on Andean lupin (Lupinus mutabilis Sweet) grains

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A B S T R A C T

This work describes the hydration kinetics of Andean lupin (Lupinus mutabilis Sweet) grains, correlating its morphology with mathematical models in order to explain the process. Microstructural analysis of the grains was performed using a scanning electron microscope and the hydration kinetics was determined between 23 °C and 60 °C. The hydration kinetics showed the sigmoidal behavior, which was fitted with two sigmoidal models. Further, this behavior was explained by the grain morphology, demonstrating that the seed coat was the cause of this behavior, related to the slow initial water intake. It was demonstrated that the water entered to the grain by diffusion through the seed coat, and by capillarity through the hilar fissure. Besides, an increase in the process temperature resulted in higher water absorption rate, smaller hydration time and higher final moisture content, enhancing the process. Each model’s parameter (equilibrium moisture, water absorption rate and lag phase time) was then modeled as function of the temperature. Finally, two general models were obtained with good agreement, which can be used to predict the moisture of the grain as function of both time and temperature.

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1. Introduction

The Andean lupin, also called Chocho or Tarwi (NRC-US, 1989) is a legume from the Andean region, of South America, which is widely used by the local population as food and natural medicine (Jacobsen & Mujica, 2006). It is known by its high nutritional value, with a high content of proteins (44.3 g/100 g) and unsaturated fatty acids (40.4 g/100 g of omega-9, 37.1 g/100 g of omega-6 and 2.9 g/100 g of omega-3, with respect to the total fat content — 16.5 g/100 g) (Jacobsen & Mujica, 2006). It is used mainly as a protein source in human and animal nutrition in various parts of the world, and its consumption has increased in recent years (Guémes-Vera, Peña-Bautista, Jimenez-Martinez, Davila-Ortiz, & Calderón-Dominguez, 2008). This grain is being considered an internationally promising crop, especially in Peru, where its production is growing since it started to be incentivized (Brigas Céspedes, 2014; Mohme Seminario, 2014).

The hydration process in grains is a prior step to different processes such as cooking, extraction, germination and wet milling, since it prepares the grains for processing. In most cases, this stage is a batch unit operation, with a long duration (between 4 and 18 h on average). Many studies have already been conducted about the hydration of different grains, such as adzuki beans (Oliveira et al., 2013), chickpeas (Gowen, Abu-Ghannam, Frias, & Oliveira, 2007; Ibarz, González, & Barbosa-Cánovas, 2004; Yildirim, Öner, & Bayram, 2011), white lupin (Solomon, 2009), red kidney beans (Abu-Ghannam & McKenna, 1997a, 1997b), sesame seeds (Khazaet & Mohammedi, 2009), and so on. However, most of these works just evaluated the grain hydration using simple kinetics models, neglecting the initial lag phase that some grains have. The few studies that considered sigmoidal hydration kinetics for grains neither ensure the cause of lag phase nor explain the process morphologically giving only suppositions. Further, there is not any work in the literature studying the hydration kinetics of Andean lupin (Lupinus mutabilis Sweet), despite that it is an important stage because it increases the water content of the grain and enhances the alkaloids extraction in the subsequent stages (Carvajal-Larenas, Nout, van Boekel, Koziol, & Linnemann, 2013).
The present work correlated the morphology, hydration behavior and mathematical models in order to explain and predict the hydration kinetics of Andean lupin (L. mutabilis Sweet) grains.

2. Materials and methods

2.1. Water uptake behavior

Andean lupin grains (L. mutabilis Sweet) (9.08 ± 1.44 g/100 g d.b moisture, 9.98 ± 0.64 mm length, 8.39 ± 0.41 mm width and 6.02 ± 0.35 mm thick) were purchased in a local market of Trujillo – Perú. The Andean lupin used was breed in the north Andean region of Perú (La Libertad –3200 masl (meters above the sea level)). After harvest, the grains were stored for 2 months before being sold. The grains were selected by eliminating those that were not intact and stained. The grains were stored in a bag of low-density polyethylene (-15.6 μm thick) at average room temperature of 20 ± 2 °C and 60% RH before processing.

For structural analysis, samples of grains were cut using a scalpel blade in order to see the different tissues (seed coat, cotyledon, and external surface) and dehydrated using silica gel for 3 days in a closed container. Then, they were sputtered with a 30 nm gold layer. Finally, the samples were observed in a scanning electron microscope operated at an acceleration voltage of 15 kV (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England).

In order to establish the principal mechanism of water entrance into the grains, they were hydrated in a solution of brilliant blue 0.01 g/100 mL (food grade, gently donated by SanLeon, Brazil, www.sanleon.com.br). Ten grains were normally hydrated and other ten grains were covered in the hilar fissure (Figs. 3) with a cyanacrylate ester glue before hydration process. Images were obtained at 30, 60, 90, 120, 150, 180, 210 and 330 min of the grains previously rinsed with distilled water and superficially dried with toilet paper.

2.2. Modeling hydration kinetics as function of temperature

The hydration procedure was carried out in the same way as described in previous works (for example, (Yildirim, Oner, & Bayram, 2010)). In each replicate, 40 g of Andean lupin grains were immersed in 800 mL of distilled water (proportion of 1:20, in order to ensure the excess of water). Then, they were immersed in a water bath to control the temperature of processing (23, 30, 40, 50 °C). At each 30 min interval, approximately 2 g of grains and 40 mL of water was taken out to maintain the grains-water ratio constant. Then, the grains were superficially dried with absorbent paper in order to determine the moisture content by oven method (AOAC, 1990). The procedure was conducted until stabilization and replicated four times.

The Andean lupin hydration kinetics was modeled using appropriate mathematical functions, as described further. For that, the amount of water absorbed in grams per 100 g of dry matter (g/100 g) versus time of the hydration process was tabulated for each evaluated temperature. The data were fitted to the mathematical model with a confidence level of 95% using the Levemberg–Marquardt algorithm in Statistica 12.0 (StatSoft) software. Then, the model parameters were evaluated and modeled as function of the temperature using appropriate mathematical functions. For them, the same procedure using the software Statistica 12.0 was used.

Finally, the goodness of the models fitting was evaluated by the coefficient of determination (R²) of the regression value, the root-mean-square deviation values (RMSD, Equation (1)), and by plotting the moisture values obtained by the model (Mmodel) as a function of the experimental values (Mexperimental). The fitting of those data to a linear function (Equation (3)) 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; that must be as close as possible to one), the intercept (b; that must be as close as possible to zero) and the coefficient of determination (R²; that must be as close as possible to one). It is a simple and efficient approach to evaluating the model fit.

\[
\text{RMSD} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (M_{\text{experimental}} - M_{\text{model}})^2}
\]

(1)

\[
\text{NRMSD} = \frac{100}{\frac{M_{\text{maximum}}}{M_{\text{minimum}}}} \cdot \frac{\text{RMSD}}{\text{R}_{\text{model}}}
\]

(2)

\[
M_{\text{model}} = a \cdot M_{\text{experimental}} + b
\]

(3)

3. Results and discussion

3.1. Andean lupin hydration kinetics

The effect of temperature on the moisture content of Andean lupin grains is shown in Fig. 1. The moisture increased with the duration of soaking and a lag phase at the first part of the curve can be clearly seen, where the water uptake rate was low at all evaluated temperatures. Thus, the grain hydration can be described by a sigmoidal behavior, with an initial lag phase followed by a higher absorption rate phase and, finally, by a stationary phase.

This behavior is similar to different grains such as adzuki beans (Oliveira et al., 2013), cowpeas (Kaptsos et al., 2008; Sefa-Dedeh & Stanley, 1979), lima beans (Piergiovanni, Sparvoli, & Zaccardelli, 2012) and badda bianco and badda nero beans (Piergirovanni, 2011), although most of the cited works have not appropriately described this behavior. This behavior is attributed, in the literature (Kaptsos et al., 2008; Oliveira et al., 2013), to the seed coat resistance to the mass transfer phenomenon (water flow), although it was still not demonstrated. In addition, most of the grain hydration behavior were described in the literature by simple downward concave shape curves, mostly described by the Peleg Model (Peleg, 1988). In that behavior, the lag phase does not exist, probably because the seed coat exerts a very small resistance to the water flow (Abu-Ghannam, 1998; Abu-Ghannam & McKenna, 1997a, 1997b; Jideani & Mpotokwana, 2009; Piergirovanni, 2011; Solomon, 2009). It is interesting to observe that there is a work in the literature where the hydration kinetics of roasted lupin (Lupinus albus) was fitted to the Peleg Model despite that the data showed sigmoidal behavior (Solomon, 2009).

Therefore, the mathematical modeling of the Andean lupin grains hydration process was conducted using the two sigmoidal functions described in the literature: Equation (4) (Kaptsos et al., 2008) and Equation (5) (Ibarz & Augusto, 2014); where \( M_t \) is the sample moisture content at each time \( t \), \( \tau \) describes the inflection point, being thus related to the lag phase, \( k_0 \) is the water absorption rate kinetics parameter, \( M_{eq} \) is the equilibrium moisture content (maximum absorbed moisture), \( M_0 \) is the initial moisture content and \( k_{20} \) is a kinetics parameter related to the lag phase and water absorption rate. The goodness of both models fitting was good, describing well the experimental values and obtaining R² always higher than 0.96 for each replicate. It can also be clearly seen in
As both hydration models described well the Andean lupin behavior, the following evaluation was carried out using them.

$$M_t = \frac{M_{eq}}{1 + \exp \left[-k_K \cdot (t - \tau)\right]}$$  \hspace{1cm} (4)

$$M_t = \frac{M_{eq}}{1 + \left(\frac{M_{eq}}{M_c}\right) \exp \left(-k_{IA} \cdot M_{eq} \cdot t\right)}$$  \hspace{1cm} (5)

3.2. Explanation of Andean lupin water intake behavior

Although many works have presented the hydration processes of different products in the literature, the exact mechanism of mass transfer during hydration is still not completely understood. For example, there are just a few works in the literature partially describing the sigmoidal behavior during hydration (Kaptso et al., 2008; Oliveira et al., 2013). Many others do not describe the sigmoidal behavior, even though the presented data clearly showed it (Piergiovanni, 2011; Solomon, 2009). In consequence, the reason for the sigmoidal behavior has not been proven in the literature yet.

The water absorption during soaking of Andean lupin grains with and without its seed coat is presented in Fig. 2. It can be observed that seed coat exerts a high resistance to the hydration process not only by reducing the water absorption rate, but also by changing the shape of the curve. While the whole grains show a sigmoidal behavior during hydration, the uncoated grains clearly show a downward concave shape behavior (as described by the Peleg Model – (Peleg, 1988)). Consequently, it clearly demonstrates that the seed coat is the main cause of the sigmoidal shape hydration kinetics, and confirms the previous statements of Kaptso et al. (2008) and Oliveira et al. (2013).

The external morphology of the Andean lupin grain (Fig. 3) has a structure called hilum and hilar fissure where it was demonstrated as the principal water entrance into the grain. Fig. 4 shows the water and colorant solution pathway through the grain in two conditions (normal grain and hilar fissure covered grain). In the first condition, it was noticed that colorant solution entered first by the hilar fissure routed by a structure (directional structure or radicle pocket (Fig. 5)) which ensured the radicle hydration. Then, the water filled the seed coat-cotyledon space for later distributing the water into the cotyledon in a homogeneous way. It can be ensured that water also get into the grain through the seed coat since the second condition helped to confirm that. When the hilar fissure was covered, the grain hydrated anyway in the colorant solution; however, the colorant did not enter to the grain, concluding that water enters to the grain by diffusion through the seed coat while by capillarity through the hilar fissure. It should be mentioned that it is very probable that water enters by hilum fissure more quickly than by the seed coat. The water pathway in a non-covered grain was similarly described by Perisse and Planchuelo (2004) in *L. albus* and *Lupinus angustifolius*. Nevertheless, they concluded that the seed coat might participate in the grain hydration after they completely hydrate from inside and that it does not have participation in the beginning of the process. It could be possible in some grains, depending on seed coat anatomy and composition, but in Andean lupin it does not happens. Besides, the hilar fissure is considered as a water entrance regulator which opening depends on the relative humidity (Lush & Evans, 1980), thus, it permits the water entrance during soaking process.

The seed coat's surface is covered by cuticle compound by wax that gives impermeability to different plant tissues (Graven, de Koster, Boon, & Bouman, 1997). In Fig. 5A, it is shown that the surface of seed coat of Andean lupin is apparently not porous. Consequently, the water intake by capillary flow should be very small or negligible, being preferably transferred by diffusion to the inner tissues.

![Fig. 1. Water uptake behavior in Andean lupin grains at different temperatures (T (°C): 23, 30, 40, 50 and 60). The dots are the experimental values; the vertical bars the standard deviations.](image1)

![Fig. 2. Hydration kinetics of Andean lupin with seed coat (•) and without seed coat (○) at 30 °C. The dots are the experimental values; the vertical bars the standard deviations.](image2)
The Andean lupin’s seed coat has three principal cell layers, which can be seen by a transversal cut (Fig. 5B).

The external layer is formed by macrosclereids cells (palisade tissue). These cells are died and, in Phabaceae family, they can have different kind of hydrophobic substance like lignin polysaccharides, pectin, calose, quinones, suberin, cutin and phenols (Bewley, Black, & Halmer, 2006; Castillo & Guenni, 2001). This layer also gives the impermeability to the Andean lupin grain. It can be the main reason for the resistance to the water intake and, it is very probable that water transferring through that is by diffusion, according to its microstructure.

The second layer is formed by osteosclereids cells that have bone shape and a wide intercellular space in which water can cross principally by capillarity.

The third layer is a sclerified parenchyma formed by many layers of died crushed cells. In this layer, water is probably transferred by diffusion to enter to the dyed cell, by capillarity to cross the cell interspace and by diffusion again to leave the cell to enter to the next layer of parenchyma.

Once the seed coat-cotyledon space is practically filled of water, the water absorption rate increased and the lag phase finished. In this stage, water had to cross the cotyledon of the grain. According to Fig. 5C, between the seed coat and cotyledon, there is a layer known, as endosperm remains formed by aleurone cells and parenchyma. This part is compound by residual nutrients (lipids and proteins) that were given to the principal reserve tissue (cotyledon) (Shewry & Casey, 1999). This layer has intercellular spaces that can measure approximately 9 μm as maximum (Fig. 5D) where water can be transferred by capillarity. Besides, the principal component of these cells are proteins (~44.3 g/100 g (Jacobsen & Mujica, 2006)) which can give polarity to the media and hold water in their structure. For that reason, the water intake in this part of the grain was quickly until raising the equilibrium moisture.

One of the main functions of seed coats is to regulate the water intake rate (Borguetti, 2008). Entire lupin grain approximately needs six hours to reach the maximum moisture; in contrast, lupin’s cotyledon only needs a little over an hour to reach the maximum moisture content. Moreover, the seed coat helped lupin grains to maintain more water inside itself. L. mutabilis Sweet is the specie with less amount of seed coat of all species of Lupinus (~12.7 g/100 g of the grain weight as mean) (Clements, Dracup, Buirchell, & Smith, 2005). Even so, it has more than other legumes like soybean and red kidney bean (~7 g/100 g and ~8 g/100 g, respectively) (Lush & Evans, 1980). In addition, the water absorption is avoided by macrosclereids cells in palisade tissue presented on the seed coat of Leguminosae family (Borguetti, 2008) and regulated by the hilar fissure in the hilum.

Therefore, it can be concluded that the seed coat, due to both its structure and composition, is the main reason for the sigmoidal behavior during the hydration of Andean lupin grains.

3.3. Andean lupin hydration kinetics modeling as function of temperature

The temperature of the experiments was between 23 °C and 60 °C, because lower temperatures imply additional cost in refrigeration (refrigeration costs are higher than heating) and a longer process (which is highly undesirable) and higher temperatures can...
cause undesirable changes on this grain like proteins denaturation. It is well known that the increase in process temperature improves the hydration process of grains, highlighting the importance for modeling the effect of temperature on the hydration parameters of Andean lupin. Consequently, the sigmoidal model parameters were modeled as function of temperature. Figs. 6–9 show the effect of temperature on the model parameters $M_{eq}$ (both models), $k_b$, $r$ and $k_i$ respectively.

Regarding the equilibrium moisture content ($M_{eq}$), in both studied models, the higher the soaking water temperature, the higher was the equilibrium moisture. This behavior was similar to most of the studied grains, such as sesame seeds (Khazaei & Mohammadi, 2009), botswana bambara bean (Jideani & Mpotokwana, 2009), wheat (Maskan, 2001), roasted white lupin (Solomon, 2009) and sorghum (Kashiriz, Kashaninejad, & Aghajani, 2010), being also highly desirable. The increment of temperature causes, in the majority of cases, changes on cell wall structure, composition and compactness of the grain, so grains can hold more water in its structure (Sabapathy, 2005; Siddiq, Butt, & Sultan, 2011). Further, at higher temperatures, the solutes solubility is higher and the product and its pores are expanded, allowing a higher water holding. The equilibrium moisture content was then modeled as function of the temperature. Due to its behavior, it was modeled using linear equations (Eq. (3) and Eq. (4)) with a $R^2$ of 0.945 for Katpso et al. model and 0.963 for Ibarz-Augusto model (Fig. 6).

$$M_{eq}(T) = 2.6461 \cdot T - 657.6$$  \hspace{1cm} (6)

$$M_{eq}(T) = 2.6125 \cdot T - 651.8$$  \hspace{1cm} (7)
The hydration rate increased exponentially in relation to the increased temperature. It means that the water flow through the product is expected to be faster at higher temperatures due to the lower fluid viscosity and higher grain pores dimension (especially in cotyledon), facilitating both diffusion and capillary flow mass transfer phenomena. Further, structural changes on the cells and tissues can enhance the water absorption rate, such as the partial degradation of pectin and solubility of cell wall polysaccharides (Oliveira et al., 2013; Siddiq et al., 2011). It explains the observed behavior, which was similar to other grains, such as adzuki beans (Oliveira et al., 2013), cowpea (Kaptso et al., 2008), rice (Bello, Tolaba, & Suarez, 2004), chickpeas (Gowen et al., 2007), bambara beans (Jideani & Mpotokwana, 2009) and roasted white lupin (Solomon, 2009). The hydration rate is represented by the parameter $k_K$ on the Kaptso et al. model, and $k_{IA}$ on the Ibarz–Augusto model. Therefore, both parameters were evaluated by the Arrhenius equation, a widely used model to describe the effect of temperature on different physic-chemical properties. It is composed by an exponential function, where the activation energy ($E_a$) describes the property variation with temperature ($T$ in K), and where $R$ is the universal constant of the ideal gas ($R = 8.314$ J mol$^{-1}$ K$^{-1}$).

The kinetics parameter $k_K$ (water absorption rate) was successfully modeled using the Arrhenius model (Eq. (8)) with a $R^2$ of 0.99 (Fig. 7).

$$k_K(T) = 381.668 \cdot e^{-\frac{3280.7}{R} T}$$  \hspace{1cm} (8)

In addition, the parameter $k_{IA}$ of Ibarz–Augusto model also exponentially increases with the increment of the temperature (Fig. 8). This parameter is related to both the lag phase of the curve and the water absorption rate, as, for sigmoidal behavior grains, as higher the hydration rate, lower is the lag phase (Ibarz & Augusto). As the temperature increases, the water absorption rate increases and the lag phase become shorter, reducing the hydration time. Since the seed coat-cotyledon space has to be first filled with water, the decrease of water viscosity can cause the rapid distribution of it reducing the lag phase. Further, the thermal dilatation can also increase the seed coat-cotyledon space. Besides, the higher
temperatures can cause the faster opening of the hilar fissure, entering more water in the beginning of the process and reducing the lag time. The Arrhenius equation was also used to explain this behavior ($R^2 = 0.96$, [Eq. (9)]), with high agreement.

$$k_{\text{IA}}(T) = 0.1485 \cdot e^{-\frac{18144}{2841T}}$$  \hspace{1cm} (9)

Furthermore, the value of the parameter $\tau$ on Kaptso et al. model, which is related to the lag phase length (as it is the time of the inflexion point), exponentially decreases with the increment of temperature (Fig. 9). It means that at higher temperatures, the lag phase decreases, although the hydration still fits the sigmoidal behavior. The $\tau$ reduction can be related to the same explanation of $k_K$ behavior. As the temperature is increased, the seed coat-cotyledon spaces is faster filled with water, reducing the value of $\tau$. The Arrhenius equation was successfully used to explain this behavior ($R^2 = 0.99$, [Eq (10)]. Similarly, other authors have observed the same behavior for adzuki and cowpea beans (Kaptso et al., 2008; Oliveira et al., 2013).

$$\tau(T) = 0.003099 \cdot e^{\frac{3100}{T}}$$  \hspace{1cm} (10)

Finally, all the obtained parameters could be combined in only one equation, for each model, describing the Andean lupin grain moisture content as a function of both time (min) and temperature ($296 \leq T(K) \leq 333$) of soaking. These models are shown in Equations (11) and (12) for Kaptso et al. and Ibarz–Augusto models, respectively, with $R^2 > 0.98$. Besides, the models were plotted in a 3D surface, being shown on Fig. 10. Both surfaces describe that the higher the soaking temperature is, the faster the water uptake is which involves a shorter lag phase time, a more inclined slope (water absorption rate) and a higher equilibrium absorbed water. Further, it can clearly be seen that even at the higher temperatures, the grain hydration behavior is still sigmoidal.

$$M(t, T) = \frac{2.6461 \cdot T - 657.6}{1 + \exp \left( -381.668 \cdot \exp \left( \frac{3101}{T} \right) \cdot \left( t - 0.003099 \cdot \exp \left( \frac{3324.64}{T} \right) \right) \right)}$$  \hspace{1cm} (11)

$$M(t, T) = \frac{2.6125 \cdot T - 651.8}{1 + 2.6125 \cdot \frac{T - 651.8}{10.771} \exp \left( -0.1485 \cdot \exp \left( -\frac{2182.4}{T} \right) \right) \left( 2.6125 \cdot T - 651.79 \right) \cdot t}$$  \hspace{1cm} (12)

The values obtained by the models (Equations (11) and (12)) were then plotted with the experimental ones in order to assure the goodness of it. It was obtained a well-fitted linear equation ($R^2 = 0.991$, Eq (13) for Kaptso et al. model and $R^2 = 0.989$, Eq (14) for Ibarz–Augusto model) that demonstrated they can be successfully used to predict the moisture of Andean lupin. Therefore, the parameter a and b from Equation (4) are very close to one and zero, respectively, in Equations (13) and (14). The Ibarz–Augusto model fitted better the experimental data according to these parameters (Table 1). In addition, the root-mean-square deviation

![Fig. 10. Generated surface describing the Andean lupin grains moisture content as function of the temperature and time of soaking (A) Kaptso et al. model (Equation (11)), (B) Ibarz–Augusto Model (Equation (12)).](image-url)
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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_A: Ibarz–Augusto’s kinetics parameter related to the lag phase and water absorption rate (Equations (5) and (9)) [g/100 g d.b. min⁻¹]
k_K: Kaptso et al. kinetics parameter related to water absorption rate (Equations (4) and (8)) [g/100 g d.b.⁻¹]
M_eq: Equilibrium moisture content (Equations (4)–(7)) [g/100 g d.b.]
M_exp: Experimental moisture content value (Equations (13) and (14)) [g/100 g d.b.]
M_model: Model moisture content value (Equations (13) and (14)) [g/100 g d.b.]
M_0: Initial moisture content (Equation(5)) [g/100 g d.b.]
M_t: Moisture content in time t (Equations(4) and (5)) [g/100 g d.b.]
M(t,T): Moisture content in time t and temperature T (Equations (11) and (12)) [g/100 g d.b.]
NRMSD: Normalized root-mean-square deviation values (Equation (2)) [% exp]
R: Constant of the ideal gases (Equations (8)–(10)) [= 8.314 J mol⁻¹ K⁻¹]
RMSD: Root-mean-square deviation values (Equation (1)) [g/100 g d.b.]
T: Temperature (Equations (6)–(12)) [K]
t: Time (Equations (4), (5), (11) and (12)) [min]
t_K: Kaptso et al. kinetics parameter related to lag phase (Equations (4) and (10)) [min⁻¹]
W_0: Initial weight [g]
W_t: Weight in time t [g]
Apêndice 5: “From the sigmoidal to the downward concave shape behavior during the hydration of grains: Effect of the initial moisture content on Adzuki beans (Vigna angularis)”

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From the sigmoidal to the downward concave shape behavior during the hydration of grains: Effect of the initial moisture content on Adzuki beans (Vigna angularis)

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This work studied the change of the hydration behavior of Adzuki beans (Vigna angularis) as a function of their initial moisture content. By studying the hydration kinetics at different initial moisture contents, it was demonstrated that the hydration behavior of this grain changes from the sigmoidal to the downward concave shape (DCS) when the initial moisture content is above ∼20% d.b. This change happens when the moisture content passes from zone II to zone III of the grain’s adsorption isotherm. This was attributed to the transition from the glassy state to the rubbery state, especially in seed coat components. The seed coat is impermeable when the moisture content of the grain is low, so the water ingress is only by way of the hilum. However, when the seed coat is above ∼20% d.b. the water enters not only via the hilum, but also by way of the seed coat. The hydration behavior of the grain was modeled using a sigmoidal mathematical model, whose parameters were evaluated as a function of the initial moisture content; the water absorption rate parameter demonstrated a sigmoidal increasing behavior. The time to reach the inflexion point of the curve, related to the lag phase, showed an exponential decay. The equilibrium moisture content was not affected. Finally, a general model, which was able to predict the moisture content as a function of the initial moisture content of the grain and the process time, was obtained.

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1. Introduction

The grain soaking process is an important step during industrialization since it is one of the first steps in many grain processes. For instance, this process is performed to reduce cooking time, to facilitate starch gelatinization and protein denaturation, and to facilitate the extraction of some components (starch, protein, phenols, tannins, etc.) (Siddiq et al., 2011; Abu-Channam, 1998; Deshpande and Bal, 2001). Moreover, this process is used before germination (Singh and Eckhoff, 1996) and fermentation (Egounlety and Aworh, 2003).

The water uptake of the grains can show two forms of behavior, which are differentiated by the mass transfer rate at the beginning of the process (Fig. 1). In the downward concave shape (DCS) behavior, the water influx rate is a maximum at the beginning of the process (at \( t \to 0 \)), falling to zero after enough time has elapsed (at \( t \to \infty \)) to reach the product equilibrium moisture content (\( M_{eq} \)). Among many models, the Peleg Model (Peleg, 1988) is the most widely used equation to describe this behavior. The sigmoidal behavior is described by an initial lag phase, i.e., an initial phase with a low water uptake rate. In this case, the water influx rate firstly increases,
Nomenclature

\( a \) linear model parameter (slope) (Eq. (5)) [different unit]

A and B parameters of Oswin Model (Eq. (3)) [different unit]

\( b \) linear model parameter (ordinate axis intercept) (Eq. (5)) [different unit]

\( k_1 \) and \( k_2 \) Peleg’s Model parameters (Eq. (2)) [different unit]

\( k_X \) Kaptso et al. kinetic parameter related to water absorption rate (Eqs. (1) and (8), and 8 [% d.b.\(^{-1}\)]

\( M \) moisture content (Eqs. (6) and (7)) [% d.b.]

\( M_{eq} \) equilibrium moisture content (Eq. (1)) [% d.b.]

\( M_{\text{experimental}} \) experimental moisture content value (Eqs. (3)–(5) and (11)) [% d.b.]

\( M_{\text{model}} \) model moisture content value (Eqs. (3), (5) and (11)) [% d.b.]

\( M_0 \) initial moisture content (Eqs. (2), (8)–(10)) [% d.b.]

\( M_t \) moisture content in time \( t \) (Eqs. (1) and (2)) [% d.b.]

\( M_{0(t,T)} \) moisture content in time \( t \) and temperature \( T \) (Eq. (10)) [% d.b.]

NRMSD normalized root-mean-square deviation values (Eq. (4)) [\%exp]

RMSD root-mean-square deviation values (Eq. (3)) [% d.b.]

\( t \) time (Eqs. (1), (2) and (10)) [min]

\( \tau \) Kaptso et al. kinetic parameter related to lag phase (Eqs. (1) and (9)) [min\(^{-1}\)]

Fig. 1 – General behavior of grains during hydration: downward concave (DCS) and sigmoidal shapes.

until an inflexion point (at \( t = t_{\text{inflection}} \)) is reached. After that, the rate decreases to zero (at \( t \to \infty \)), when the product reaches its equilibrium moisture content (\( M_{eq} \)). This behavior can be described by the Kaptso et al. model (Kaptso et al., 2008) or by the Ibarz-Augusto model (Ibarz and Augusto, 2015).

The sigmoidal behavior is of higher interest, since it is the lag phase that slows the process. All the grains that presented this behavior are from Leguminosae or Fabaceae family, like cowpea (Kaptso et al., 2008), common bean (Piergiovanni, 2011), lima bean (Piergiovanni et al., 2012), Adzuki beans (Oliveira et al., 2013) and andean lupin beans (Miano et al., 2015). In order to increase the knowledge of the sigmoidal behavior, the objective of this study was to evaluate the effect of the initial moisture content on the hydration kinetics of Adzuki beans (Vigna angularis), a grain with clear sigmoidal hydration kinetics. Therefore, this work combined and related the grain structure, initial moisture content and hydration rates, in order to understand and describe the phenomena.

2. Material and methods

2.1. Sample preparation

The Adzuki beans were obtained at a local market of Piracicaba (SP, Brazil), with a moisture content of 14.96 ± 0.16% d.b. (g water/100 g of dry matter). In order to prepare samples with a higher initial moisture content (19.66, 22.44, 26.76, 29.25 and 75.00% d.b.) the grains were hydrated for a specific time at 32.5 °C using the mathematical model obtained in a previous study (Oliveira et al., 2013). Then, these grains were put into sealed containers for a week at 5 °C in order to homogenize the moisture into the grains. The lower initial moisture content sample (4.23% 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.

2.2. Hydration process and mathematical modeling

For the hydration process, 10 g of pre-selected grains were soaked in 250 mL of distilled water at 35 °C using a water bath at different conditions of initial moisture content (\( M_0 \)). During the hydration process, the samples were periodically drained, superficially dried and its moisture content was obtained by a mass balance. The sampling was carried out each 20 min for the first hour and each 30 min for the later hours, until a constant mass was obtained. The hydration process was performed in triplicate.

The Adzuki bean hydration kinetics was modeled using the Kaptso et al. Model (Eq. (1); Kaptso et al., 2008) and the Peleg Model (Eq. (2); Peleg, 1988) depending on the hydration behavior (sigmoidal or DCS behavior) (Fig. 1). For that purpose, the dry basis moisture content of the grains (M% d.b.) versus 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.

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

(1)

\[
M_t = M_0 + \frac{t}{k_1 + k_2 \cdot t}
\]

(2)

where \( M_t \) is the sample moisture content (% d.b.) at each time \( t \) and \( M_{eq} \) is the equilibrium moisture content. In the Kaptso et al. Model (Eq. (1)) \( \tau \) describes necessary time to reach the inflection point of the curve, being thus related to the lag phase, \( k_X \) is the water absorption rate kinetics parameter. In the Peleg Model (Eq. (2)), \( M_0 \) is the initial moisture content, \( k_1 \) is a parameter related to the water absorption rate and \( k_2 \) is a parameter related to the equilibrium moisture content.

Finally, the goodness of fit of the models was evaluated by the \( R^2 \) regression value, the root-mean-square deviation...
values (RMSD, Eq. (3)), the normalized RMSD (NRMSD, Eq. (4)) and by plotting the moisture content values obtained by the model ($M_{\text{model}}$) as a function of the experimental values ($M_{\text{experimental}}$). The regression of those data to a linear function (Eq. (5)) 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). It is a simple and efficient approach to evaluating the model fitting.

$$
\text{RMSD} = \sqrt{\frac{\sum_{i=1}^{n} (M_{\text{experimental}} - M_{\text{model}})^2}{n}} \quad (3)
$$

$$
\text{NRMSD} = 100 \cdot \frac{\text{RMSD}}{M_{\text{experimental}}^{\text{maximum}} - M_{\text{experimental}}^{\text{minimum}}} \quad (4)
$$

$$
M_{\text{model}} = a \cdot M_{\text{experimental}} + b \quad (5)
$$

### 2.3. Adsorption isotherms

Grains with different moisture contents (prepared using the procedure described above) were ground using a hammer 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 a function of the water activity. The obtained curve was modeled using the Oswin Equation (Eq. (6)) since it is recommended for starchy food (Al-Muhtaseb et al., 2002). 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.

$$
M = A \left( \frac{a_w}{1 - a_w} \right)^B \quad (6)
$$

### 2.4. Explanation of the Adzuki beans hydration process

Three different treatments were performed in order to explain the Adzuki beans hydration phenomenon, comparing and relating the obtained hydration rates with the grain morphology and initial moisture content. Especially, the function of two tissues of the grain during the hydration process was studied: the seed coat and the hilum (Fig. 2).

In the first treatment, the Adzuki beans were hydrated at $M_0$ (14.96% d.b.) without the seed coat, which was manually and carefully removed. In the second treatment, the hydration of the beans was carried out at a low initial moisture content (4.23% d.b.), with their hilum covered with a contact glue (cyanoacrylate ester glue-Loctite Super Bonder®, Henkel Brazil). Finally, in the third treatment, the hydration of the beans was carried out at a high initial moisture content (29.24% d.b.), also covering their hilum.

Further, the grain structure evaluation was performed by a microstructural analysis (using a scanning electronic microscope – SEM) and X-ray analysis, in order to explain the hydration process.

For the microstructural analysis, the samples were cut with a scalpel blade in order 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 15 kV (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England).

For X-ray analysis, twelve grains were X-rayed using a model MX-20 DC-12 digital Faxitron X-ray connected to a computer, exposing them to the radiation at 20 kV for 10 s. The grain (at $M_0$) was X-rayed at 0, 1, 2, 4, and 6 h of hydration at 35°C.

### 3. Results and discussion

#### 3.1. Hydration process

Adzuki beans showed a sigmoidal behavior hydration at the moisture content in equilibrium with the environment ($M_e$) (14.96% d.b.; Fig. 3) and needed more than eight hours to reach the equilibrium moisture content at 35°C. Therefore, it is highlighted that the hydration process is mainly a discontinuous and slow unit operation in the industrial process. Consequently, a structure evaluation was carried out in order to explain this process.

Fig. 4A shows the hydration of Adzuki beans with and without its seed coat. It can be seen that the seed coat is the main cause of the slow water intake and the low water holding capacity, defining the sigmoidal behavior during hydration.

Further, the seed coat was not demonstrated as the only pathway for the water ingress during the hydration process. The hilum structure is also an important route. Fig. 4B shows the results of two special treatments that helped to explain the principal function of the seed coat and hilum during the hydration process.

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**Fig. 2** – External morphology (XZ plane) and internal morphology (YX plane) of Adzuki grain (Vigna angularis).
water uptake in Adzuki beans. When the hilum was covered in the grains at $M_0$ (14.96% d.b.), the grains practically did not hydrate, indicating the high impermeability of the seed coat, and also highlighting that the hilum was the main route to the water intake in this condition. It means that the seed coat was impermeable when the grains had a moisture content below the critical one (lower than 20% d.b.). In fact, those grains were soaked for two more days and they still did not hydrate.

On the other hand, when the hilum was covered at a moisture content higher than the critical one (at 29.94% d.b.), the water entered into the grain, but in a slower rate than the normal (uncovered) grains (Fig. 5B). It demonstrates that the seed coat permeability is function of the grain moisture, and that the hilum still exerts an important function even though the higher moisture content of the grains.

Fig. 5 shows the microstructure of the principal tissues of Adzuki beans. It can be seen that the seed coat is little or almost not porous (Fig. 5B and E), in contrast to the hilum which is very porous (Fig. 5C). Consequently, at $M_0$ (14.96% d.b.), the water enters principally by the hilum, and then, a directional structure (Fig. 5D) guides it to firstly hydrate the radicle. Once the water reaches the radicle, the water starts to be distributed to the seed coat-cotyledon space. This is the slowest step since the seed coat-cotyledon space is very small (Fig. 5F) and the water influx is practically only through the hilum. When the seed coat reaches a particular moisture content, it starts to let water to pass into the grain, contributing to the hydration process. The structure of the seed coat is very compact and it is only formed by a macrosclereids layer and a thin layer of parenchyma (Fig. 5E) controlling the entrance of the water (Borguetti, 2008). The cotyledon of Adzuki beans is more easily hydrated since it has many intercellular spaces where the water can be easily distributed by capillarity (Fig. 5F).

Fig. 6 shows the radiographs of Adzuki beans at different hydration times at 35 °C. It can be observed that the seed coat-cotyledon space near the hilum increased during the six first hours of hydration, demonstrating that the water entered by the hilum in the first phase of the hydration process. According to the hydration curve at $M_0$ (Fig. 3), at 6 h of hydration, the grains raised the inflexion point. This is in agreement with the radiographs results. After this time, the water was distributed to the cotyledon until reaching the equilibrium moisture content, and the hydration behavior turned into a DCS shape. It should be mentioned that the volume of the grains did not increase during the first six hours of the process; however,
3.2. Effect of the initial moisture content

When the initial moisture content of the grain was increased, the lag phase of the sigmoidal behavior decreased until it disappeared at 22.44% d.b. In this condition, the grain behavior changed from the sigmoidal to the DCS behavior. It means that the hydration behavior depends on the initial moisture content of the grain. Further, when the grains, at lower initial moisture content (4.23% d.b.), were soaked, the hydration was too slow that they did not reach even the 20% of the equilibrium moisture content after 8 h.

By evaluating these results, there probably exists a “critical” initial moisture content where the hydration behavior changes. In fact, it may be related with the corresponding water activity (aw), and the consequent grain morphological changes. For example, the seed coat permeability change.

The reasons for the seed coat permeability increase with increasing moisture content can be described as follows. The
permeability of the seed coat depends on its physical chemical characteristics since its component’s organization and properties can change with the different moisture contents.

The adsorption isotherm of Adzuki beans is shown in Fig. 7 and represented by Eq. (7) \( R^2 = 0.985 \). It is interesting to note that the initial moisture content, where the hydration behavior changes is between 19.66% d.b. and 22.44% d.b., the same region where the adsorption isotherm shows the change of the water from the zone II to the zone III (Reid and Fennema, 2008).

\[
M = 9.748 \cdot \left( \frac{a_w}{1 - a_w} \right)^{0.461}
\]

The application of the glassy state theory in grains can partially describe this relation (Ross et al., 2013). This theory stated that there exists a condition (temperature or water activity-consequently, the moisture content) where the grain (especially the seed coat’s components) passes from a glassy state to a rubbery state through a phase transition. During this phase transition, there are changes of the physical properties of foods during contact between water and hydrophilic ingredients (Belitz et al., 2009). Near the junction of zones II and III of the adsorption isotherm, the amount of water is sufficient to lower the glass transition temperature of the hydrated macromolecules so the sample temperature and the sample glassy transition temperature are equal. In consequence, the addition of water (zone III) causes a glass–rubber transition in samples containing glassy regions (Reid and Fennema, 2008). These changes on the seed coat components can affect the permeability of the structure.

The plasticizing effect takes place when the moisture content is increased at constant temperature or when the temperature is increased at constant moisture content (Al-Muhidae et al., 2002). In this study, the plasticizing of the seed coat took place through increasing of the initial moisture content. Therefore, when the initial moisture content passes ~20% d.b., the seed coat permeability and the hydration behavior changed.

The lag phase appears, during the grain hydration. It is probably due to need for the seed coat to be saturated with the solvent (water) in order to pass from the glassy state to the rubbery state. When it happens, the lag phase ends and the hydration behavior changes from the sigmoidal to the DCS, as shown in Fig. 3. It means that it is necessary to reach a certain quantity of energy to cause the phase change, for example, provided by a higher water soaking temperature (reducing lag phase, as in Oliveira et al., 2013) or by increasing the water activity which involves the moisture content increase. As consequence, the grain behavior depends on the initial moisture content. Therefore, higher initial moisture content decreases the lag phase that disappears in certain moisture content when the seed coat is enough hydrated to continue the hydration following the DCS behavior.

In conclusion, the hydration mechanism of a grain with sigmoidal behavior has been firstly explained according to its initial moisture content and its morphology. Fig. 8 shows a summary of the entire hydration process explained above. It was demonstrating that the mass transfer (water intake) is not a mere diffusional phenomenon in the grain’s hydration; it is a complex process that depends on the physicochemical and morphology properties.

### 3.3. Mathematical modeling

The hydration kinetics of Adzuki beans at different initial moisture contents exhibited different behaviors. Below ~20% d.b., the hydration kinetics had sigmoidal behavior and above that moisture content, it had DCS behavior. Therefore, it was used the Kaptso et al. Model (Kaptso et al., 2008) to fit the data of sigmoidal behavior and the Peleg Model (Peleg, 1988) to fit the DCS data. The parameters of both models are shown in Table 1.

On the other hand, in order to obtain only one model to describe the grain hydration at all values of \( M_0 \), all the data were modeled using Kaptso et al. model since it can approximately fit the DCS data. It should be mentioned that the hydration kinetics at 4.23% d.b. was not evaluated, as the grains took much longer than the other conditions to hydrate.

The Kaptso et al. Model has three parameters: the equilibrium moisture content \((M_{eq})\), the water absorption rate parameter \(k_3\), and the parameter that describe the inflection point in the curve \((i)\). Therefore, the effect of the initial moisture content on those parameters was studied.
Table 1 – Calculated parameters of Kaptsø et al. Model (Eq. (1); Kaptsø et al., 2008) and Peleg Model (Eq. (2); Peleg, 1988) for each evaluated initial moisture content.

| $M_0$ (% d.b.) | Kaptsø et al. Model | Peleg Model |
|----------------|---------------------|-------------|
|                | $M_{eq}$ (% d.b.)   | $r$ (min)  | $k_b$ (min$^{-1}$) | $R^2$ | $k_1$ (min (% d.b.$^{-1}$) | $k_2$ (% d.b.$^{-1}$) | $R^2$ |
| 4.23           | a                   | a           | a               | a     | a | a | a |
| 14.96          | 143.19 ± 3.18       | 301.52 ± 9.91 | 0.0093 ± 0.0004 | 0.99 | a | a | a |
| 19.66          | 128.17 ± 3.61       | 157.84 ± 17.92 | 0.0114 ± 0.0011 | 0.99 | a | a | a |
| 22.44          | 124.11 ± 2.22       | 79.96 ± 11.35 | 0.0138 ± 0.0008 | 0.99 | 1.308 ± 0.216 | 0.0062 ± 0.0007 | 0.99 |
| 26.76          | 123.38 ± 1.69       | 39.69 ± 4.20 | 0.0200 ± 0.0034 | 0.98 | 0.657 ± 0.100 | 0.0081 ± 0.0002 | 0.99 |
| 29.25          | 121.77 ± 2.00       | 23.7 ± 4.20  | 0.0304 ± 0.0052 | 0.97 | 0.414 ± 0.084 | 0.0088 ± 0.0004 | 0.99 |
| 75.00          | 125.52 ± 1.91       | 6.38 ± 0.36  | 0.0642 ± 0.0076 | 0.99 | 0.257 ± 0.043 | 0.0173 ± 0.0010 | 0.99 |

* Undetermined parameters.

The value of the parameter $k_b$ exhibited sigmoidal behavior as a function of initial moisture content (Fig. 9). This parameter is constant until the initial moisture content reaches ~20% d.b. Then, it rapidly increases until a certain initial moisture content where the maximum is reached and this parameter becomes constant again. Consequently, when the grains initial moisture content is higher than ~20% d.b., the water absorption rate is higher, reflecting the change on the seed coat permeability. Further, it is necessary to complete the hydration of the seed coat by filling the seed coat-cotyledon with water, which is a very slow process reducing the general water absorption rate. Consequently, the first plateau of the parameter $k_b$ behavior is related to the water flow through the hilum, while the rest of the curve is related to the water flow through the hilum and the seed coat at the same time.

For modeling this parameter, and due to the observed behavior (Fig. 9), a mathematical function, which describes a sigmoidal change between the known minimum and maximum values (Eq. (8)), was used. This model fitted very well ($R^2 = 0.99$).

$$k_b = \frac{0.008663 + \left[\frac{0.05633}{1 + 53587 \cdot 10^5 \cdot M_0^{6.4981}}\right]}{1}$$

(8)

The value of the parameter $r$, which is related to the length of the lag phase, exhibited an exponential decay in relation to the initial moisture content of the grain (Fig. 9). The value of this parameter tends to zero when the initial moisture content of the grains is above ~20% d.b. since the lag phase of the hydration process disappears. In contrast, below this initial moisture content, the $r$ value exponentially increases,
which means that the seed coat permeability is greatly reduced. In fact, when the grain at 4.23% d.b. was soaked, the hydration was so slow that it did not reach even the 20% equilibrium moisture content after 8 h. Due to the observed behavior, an exponential function was used to model it (Eq. (9), \( R^2 = 0.99 \)).

\[
\tau = 3424.67 \cdot e^{-0.1617 M_0}
\]  

(9)

The equilibrium moisture content (\( M_{eq} \)) of all experiments was practically the same, and it was considered that the initial moisture content of the grains did not affect the equilibrium moisture content, but only the water uptake kinetics (which, in fact, is to be expected). Consequently, it was considered the average equilibrium moisture (128.5 ± 2.43% d.b.) for the general model.

Finally, a general model was sought to obtain the moisture content of the Adzuki beans as function of both initial moisture content (14 < \( M_0 \) (% d.b.) < 70) and time of soaking (in min). This model is shown in Eq. (10) with \( R^2 = 0.97 \). Moreover, the model was plotted in a 3D surface, being shown in Fig. 9. The surface highlights the initial moisture content when the hydration behavior changes from the sigmoidal shape to the DCS.

\[
M(t, M_0) = \frac{128.5}{1 + \exp\left(-\left(0.008663 + \left(\frac{0.0563}{1+5387 \cdot 10^{0.4943 \cdot M_0}}\right)\right) \cdot (t - 3424.67 \cdot \exp(-0.1617 \cdot M_0))\right)}
\]  

(10)

The values obtained by the model (Eq. (10)) were then plotted with the experimental ones in order to verify the goodness of fit. A well-fitted linear equation (Eq. (11), \( R^2 = 0.974 \)) was obtained that demonstrated it can be successfully used to predict the moisture content of Adzuki beans. Therefore, the parameters \( a \) and \( b \) from Eq. (5) were statistically equal to one and zero, respectively (\( p \)-values are 0.064 and 0.120, respectively) (Eq. (11) and Fig. 9), reinforcing that the proposed model can be successfully used to predict the moisture content as function of the initial moisture content and the time. In addition, the root-mean-square deviation (RMSD) and the normalized RMSD (NRMSD) values were calculates being 6.49% d.b. and 5.37% respectively, also highlighting the goodness of fitting.

\[ M_{\text{model}} = 1.0351 \cdot M_{\text{experimental}} + 2.7138 \]  

(11)

Consequently, for process engineering purposes, the Adzuki beans hydration behavior can be described by Eq. (10).

4. Conclusions

By combining and relating the grain’s morphology, initial moisture content and hydration rates, the Adzuki bean (\( V. \ angulares \)) hydration phenomenon could be properly described. It was demonstrated that the initial moisture content affects the grain’s hydration behavior. The process showed a sigmoidal behavior at initial moisture contents below ~20% d.b. and a DCS behavior above that initial moisture content. This behavior was attributed to changes in the seed coat permeability. The water mainly enters into the grain by the hilum at moisture contents below the critical one, since the seed coat is impermeable. The seed coat becomes permeable when the initial moisture content is above ~20% d.b. The Kaptsos et al. Model (Eq. (3)) fitted the data very well at the different initial moisture contents, obtaining the relation of each parameter as function of the initial moisture content. The water absorption rate (\( k_0 \)) exhibited a sigmoidal increasing behavior; the time to rise to the inflexion point was related to the lag phase of the hydration (\( \tau \)). It showed an exponential decay; the equilibrium moisture constant (\( M_{eq} \)) was considered constant at all initial moisture contents. Finally, a general model was derived, which can be used to predict the moisture content of Adzuki beans as function of the initial moisture content of the grains and time. Consequently, this work can help to understand and to enhance the hydration process of the grains.

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Apêndice 6: “Describing the sigmoidal behavior of roasted white lupin
(Lupinus albus)"
DESCRIBING THE SIGMOIDAL BEHAVIOR OF ROASTED WHITE LUPIN (LUPINUS ALBUS) DURING HYDRATION*

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ABSTRACT

The hydration process is an important unit operation, being one of the first steps in dry foods processing. The hydration of grains is a complex phenomenon, whose exactly mechanisms are still unknown. In special, the sigmoidal hydration behavior of grains needs further studies. In fact, the behavior of many food products is inappropriately described in the literature due to an unsuitable evaluation. The present work evaluated the hydration of roasted white lupin (Lupinus albus) by reinterpreting previously published data. The new results shows that the white lupin grains hydration clearly showed a sigmoidal behavior, which was adequately modeled. As the temperature was increased, the model kinetic parameter increased, the lag phase decreased and the equilibrium moisture did not change. The model parameters were then modeled as a function of the temperature. The obtained results highlight the sigmoidal hydration behavior of the grains, as well as the need for further studies regarding the hydration of foods.

PRACTICAL APPLICATIONS

The hydration is one of the first unit operations in dry products processing, such as grains. It is a discontinuous and time-consuming step, thus limiting the industrial process. Furthermore, the hydration is a complex phenomenon, whose exactly mechanisms are still under study. Therefore, it is important to understand the hydration kinetics of different food products, as well as appropriately modeling the process. The present work described and modeled the sigmoidal behavior of roasted white lupin (Lupinus albus) grains, a product with a complex sigmoidal behavior. Therefore, the obtained results are useful for process design and optimization.

INTRODUCTION

The hydration process is an important unit operation in dried foods, such as grains. It describes and defines the final product properties and it is used before many processes such as cooking, extractions, fermenting, germinating and eating. Regrettably, it is a discontinuous and time-consuming step limiting the industrial process. Therefore, it is important to understand the hydration kinetics of different food products, as well as to model the process appropriately.

In fact, there are many works presenting the hydration processes of different products in the literature. It is highly documented that most of the food products hydrate at a decreasing rate in relation to the processing time, whose hydration behavior follows a curve with a downward concave shape (Fig. 1). Because of that, many mathematical models are available to describe this behavior, being the most important the Lewis (first-order kinetics), Page, Henderson, Peleg, Weibull and Ibarz-Gonzalez-Barbosa-Cánovas (Table 1).

However, these models only describe the hydration of products with the downward concave shape behavior, while some grains show a different behavior. In fact, an initial lag phase, i.e., an initial phase with low water uptake rate, is observed during the hydration of some grains, such as Andean lupin (Miano et al. 2015), Adzuki beans (Oliveira et al., 2013; Miano and Augusto 2015), cowpeas (Sefa-Dedeh
and Stanley 1979; Kaptso et al. 2008), lentils (Joshi et al. 2010), lima beans (Piergiovanni et al. 2012), Badda bianco and Badda nero beans (Piergiovanni 2011). In this case, the water influx rate first increases, until an inflexion point, after which this rate decreases until zero after enough time for the product to reach its equilibrium moisture content ($M_{\infty}$). This hydration behavior of products is described by a sigmoidal shaped curve, as shown in Fig. 1.

The sigmoidal behavior during the hydration of grains is still slightly understood and investigated. For example, only two mathematical models are defined in the literature in order to describe it (Table 1), proposed by Kaptso-Njintang-Komnek-KMeter (2010) and Ibarz-Augusto (2015). Further, it is important to highlight that if a small amount of experimental data at the beginning of the process is evaluated, the actual sigmoidal behavior of the grain might be hidden, interpreting the data as a downward concave shape behavior. Therefore, many grains hydration behavior are described as downward concave shape despite their sigmoidal behavior, because of a small number of data available. Similarly, some works in the literature clearly show the hydration of grains with sigmoidal behavior; however, evaluating the data using downward concave shape behavior models. This is, for example, the case of roasted white lupin (Lupinus albus), which was studied by Solomon (2009).

Consequently, the aim of the present work was to reinterpret the (Solomon 2009) results, explaining the actual sigmoidal behavior of roasted white lupin (Lupinus albus).

**MATERIAL AND METHODS**

The roasted white lupin (Lupinus albus) hydration was studied by Solomon (2009). As stated before, despite the grains clearly showed a sigmoidal behavior during hydration, that work only evaluated models that describe the downward concave shape behavior. Consequently, the present work reinterpreted those results using its data to explain the sigmoidal behavior, thus expanding and complementing that article.

According to the author (Solomon 2009), the grains were first cleaned and selected and then roasted at 125°C for 12 min, reaching a moisture content of 0.45%db. (g water/100 g of dry solids). Then, the grains were hydrated at different soaking temperatures (25, 35, 45 and 55°C) in four replicates. The data was evaluated using the models of Peleg (which can be considered as a pseudo-second-order kinetic model, according to Kumar et al. (2011)) and Lewis (which describe the first-order kinetics), both described in Table 1.

In the present work, the data from Solomon (2009) was obtained using the software xyExtract Graph Digitizer (v5.1, http://www.df.ufcg.edu.br/, Brazil), and then evaluated using the two models that describe the sigmoidal shape behavior during hydration: the Kaptso et al. (Eq. 1) and the Ibarz-Augusto (Eq. 2) models (Table 2). Therefore, only the mean values at each temperature reported by Solomon (2009) were evaluated.

**TABLE 1. THE MOST USED MATHEMATICAL MODELS TO DESCRIBE THE FOOD HYDRATION BEHAVIORS**

| Hydration                        | Model                                      | Equation                        | Reference                        |
|----------------------------------|--------------------------------------------|---------------------------------|----------------------------------|
| Downward concave shape behavior  | Lewis (first-order kinetics)               | $M(t) = M_\infty - M_0 \exp \left( -k_1 \cdot t \right)$ | Lewis (1921)                     |
|                                  | Page                                        | $M(t) = M_0 \exp \left( -k_p \cdot t \right)$ | Page (1949)                      |
|                                  | Henderson                                   | $M(t) = M_0 \exp \left( -k_1 \cdot t \right) + M_1 \exp \left( -k_2 \cdot t \right)$ | Henderson (1974)                 |
|                                  | Peleg                                       | $M(t) = M_0 + \frac{1}{M_{\infty}} \left( 1 - \exp \left( -\left( \frac{t}{\tau} \right)^2 \right) \right)$ | Peleg (1988)                     |
|                                  | Weibull                                     | $M(t) = k_2 M_0 \exp \left( -k_1 \cdot t \right) + k_1 M_0 \exp \left( -k_1 \cdot t \right)$ | Sam Saguy et al. (2005)          |
|                                  | Ibarz-González-Barbosa-Cánovas             | $M(t) = M_0 \exp \left( -k_1 \cdot t \right)$ | Ibarz et al. (2004)              |
| Sigoidal shape behavior          | Kaptso-Njintang-Komneck-Hounhouigan-Scher-Mbolung | $M(t) = \frac{M_0}{1 + \exp \left( -k_1 M_0 t \right)}$ | Kaptso et al. (2008)             |
|                                  | Ibarz-Augusto                               | $M(t) = \frac{M_0}{1 + \exp \left( -k_1 M_0 t \right)}$ | Ibarz and Augusto (2015)         |
The model parameters were obtained by nonlinear regression using the CurveExpert Professional software (v.2.0.3, http://www.curveexpert.net/) with a significant probability level of 95%. The software uses the Levenberg-Marquardt algorithm to solve the nonlinear regressions by minimizing the chi-square value.

The goodness of the obtained model was evaluated by the value of the $R^2$ (that must be as close as possible to the unit) of the regression, the root-mean-square deviation values (RMSD, Eq. 3, that must be as close as possible to zero) and by plotting the moisture values obtained by the model ($M_{\text{model}}$) as a function of the experimental values ($M_{\text{experimental}}$).

The regression of the hydration process (Eq. 4) results in three parameters that can also be used to evaluate the model adjustment, i.e., the linear slope ($a$; that must be as close as possible to the unit), the intercept ($b$; that must be as close as possible to zero) and the coefficient of determination ($R^2$; that must be as close as possible to the unit).

\[
RMSD = \frac{\sqrt{\frac{\sum_{i=1}^{n} (M_{\text{experimental}} - M_{\text{model}})^2}{n}}}{M_{\text{model}} = a \cdot M_{\text{experimental}} + b.}
\]

**RESULTS AND DISCUSSION**

Figures 2 and 3 show the hydration behavior of roasted white lupin (Lupinus albus) grains from 25–55°C. The grain clearly showed an initial lag phase (i.e., a period with a low water absorption rate) during water uptake, thus characterizing the sigmoidal behavior. Consequently, the sigmoidal models were used to describe it.

Figure 2 shows the description of the hydration process by the Kaptso *et al* model, while Fig. 3 shows it by the Ibarz-Augusto model. Although the Ibarz-Augusto model showed acceptable values of $R^2$ for the regressions ($>0.91$), Fig. 3 clearly shows that it does not fit adequately the experimental data.

The Ibarz-Augusto model is a semi-empirical model that was based on a second-order autocatalytic kinetic, being described by only two parameters ($k_{\text{IA}}$ and $M_\infty$), while the Kaptso *et al* model is described by three parameters ($\tau$, $k_\kappa$ and $M_\infty$). Consequently, the main drawback of the Ibarz-Augusto model is that it needs a “high” number of experimental data in order to obtain a suitable regression. In special, an appropriate amount of experimental data must be provided from the beginning of hydration process, before the inflexion point, to characterize the sigmoidal behavior.
As the data provided by Solomon (2009) was restricted, the Ibarz-Augusto model was not further evaluated.

On the other hand, the Kaptso et al. model showed good fit of the experimental data, with high values for the regression coefficient of determination ($R^2 > 0.98$) and good values for the parameters of the model fit evaluation using Eqs. (1) and (2) (Table 2). For example, the root-mean-square deviation values (RMSD, Eq. 2) were smaller to 6.5% d.b. in a moisture range up to 120% d.b. However, most important than these statistics, Fig. 2 clearly shows that the Kaptso et al. model describe a sigmoidal curve that closely passes through the experimental data, highlighting the actual hydration behavior of the roasted white lupin (Lupinus albus) grains and the need for conducting the evaluations using sigmoidal functions.

Although apparently simple, the hydration of grains is a complex phenomenon, whose exactly mechanisms are still under study. For example, recent works showed that the water entrance into grains takes place not only by diffusional mechanisms, but also by capillarity ones. In addition, water entrance follows specific pathways (Meyer et al. 2007; Miano et al. 2015; Miano and Augusto 2015). The different grain tissues have specific permeability to water, which can also changes with the water activity (Miano and Augusto 2015). In fact, these specificities turn the idea that the hydration is a simple diffusional process (described by the Fick’s law and, consequently, by the models that describe the downward concave shape behavior [Table 1]), into a complex process, that is explained by the sigmoidal behavior.

Miano et al. (2015) studied the hydration of Andean lupin (Lupinus mutabilis), as a function of temperature. The Andean lupin is a grain similar to the white lupin (Lupinus albus), evaluated in the present work; both grains are pulses from genus Lupinus, with high content of proteins (Ernest 2009). Therefore, despite their differences, the general behavior of water uptake should be the same.

The Andean lupin hydration kinetics showed the sigmoidal behavior, which was explained by the grain morphology and mass transfer mechanisms (Miano et al. 2015). According to the authors, the seed coat was the cause of the sigmoidal behavior due to both its structure and composition, triggering the slow initial water intake. It was demonstrated that the water entered into the grain by diffusion through the seed coat and by capillarity through the hilar fissure. The water flow though these structures changes during the hydration process, leading to the sigmoidal behavior.

In fact, as described by Miano and Augusto (2015) for Adzuki beans, the seed coat permeability of a grain is function of the water activity, which was attributed to the glass transition of the seed coat components. It can be explained by the results of Perissé and Planchuelo (2004) that studied the water influx in white lupin (Lupinus albus) and blue lupin (Lupinus angustifolius) seeds, concluding that the seed coat might only participate in the grain hydration after it is completely hydrated and that it does not have participation in the beginning of the process. Moreover, the seed coat permeability is function of its composition. For example, the seed coats with more lignine in their composition are less permeable than other seed coats (Morrison et al. 1995), which can lead to the sigmoidal behavior during the hydration process.

In addition, the Lupinus albus used by Solomon (2009) was toasted before the hydration, which could also result in changes in the structure of those grains. First the high temperatures dehydrated the grains until a very low moisture content (0.45% d.b.), which could have caused the change on the hydration behavior (Miano and Augusto 2015). Further, the severe dehydration during toasting could have caused the seed coat to crack, changing the hydration process — even though the hydration behavior was still sigmoidal. Therefore, this hydration behavior can be caused by the extremely dehydration not only of the seed coat, but also the cotyledon, which caused the decreasing of their permeability due to the cell membrane inversion and/or by passing its components to the glassy state (Bewley and Black 1978; Perissé and Planchuelo 2004).

Once the sigmoidal behavior during the hydration of white lupin (Lupinus albus) was described, the effect of the temperature was evaluated. Figure 4 shows the parameters of Kaptso et al. model ($M_{\infty}, k_K, \tau$; Table 1) as a function of temperature between 25 and 55°C.

As expected, the water absorption kinetic parameter ($k_K$) increased with the temperature increment, while the parameter $\tau$, which describes the time of the inflexion point, related to the lag phase, was reduced. Both behaviors are the general ones since the higher temperatures reduces the water viscosity and can dilate pores and other structures. In fact, this was
also reported for Andean lupin (Miano et al. 2015), Adzuki beans (Oliveira et al. 2013) and cowpeas (Kaptso et al. 2008). Further, both behaviors could be described by the Arrhenius model (Eqs. 3 and 4), whose activation energies were at the same order of magnitude of Andean lupin (Miano et al. 2015) and Adzuki beans (Oliveira et al. 2013).

\[ k_T(T_k) = 3704 \cdot \exp \left( \frac{-29431}{R \cdot T_k} \right), \quad (3) \]

\[ \tau(T_k) = 2.23 \cdot 10^{-4} \cdot \exp \left( \frac{32925}{R \cdot T_k} \right). \quad (4) \]

On the other hand, the equilibrium moisture content \( (M_\infty) \) showed a stationary behavior in relation to the temperature in the evaluated range. Consequently, the main value (113.98%d.b.) could be used to describe it. In fact, the equilibrium moisture content \( (M_\infty) \) can increase, decrease or does not change when the temperature is increased (Miano et al. 2015), which is function of both product structure/composition and the evaluated temperature range. For example, the equilibrium moisture content \( (M_\infty) \) decreased for Adzuki beans (Oliveira et al. 2013), increased for Andean lupin (Miano et al. 2015) and did not change for cowpeas (Kaptso et al. 2008). Despite white lupin is similar to Andean lupin, the equilibrium moisture content as function of the temperature showed a different behavior. This was probably caused by the physicochemical changes related with the roasting process, such as the denaturation of the proteins and seed coat cracks, decreasing the water holding capacity of the grains.

\[ M_\infty(T_k) = \bar{M}_\infty = 113.98 \text{d.b.}. \quad (5) \]

Equations (3–5) can be combined, obtaining Eq. (6), which can be used to estimate the roasted white lupin moisture as function of both temperature and time.

\[ M(t, T_k) = 113.98 \frac{1}{1 + \exp \left[ -3704 \cdot \exp \left( \frac{-29431}{R \cdot T_k} \right) \cdot \left( t - 2.23 \cdot 10^{-4} \cdot \exp \left( \frac{32925}{R \cdot T_k} \right) \right) \right]} \quad (6) \]

The obtained results highlight the actual sigmoidal hydration behavior of the roasted white lupin \( (Lupinus albus) \) grains, as well as the need for further studies regarding the hydration of grains – in special those with sigmoidal behavior. In fact, the sigmoidal behavior still needs to be better understood, and further prospection must be conducted in order to demonstrate grains that follows this behavior (specially as many food products in the literature may show sigmoidal behavior, although this behavior is described by a concave shape as a result of the small amount of data available and/or an unsuitable evaluation).

**CONCLUSIONS**

The present work reinterpreted the results previously published by Solomon (2009) regarding the hydration of roasted white lupin \( (Lupinus albus) \). The grains hydration followed a sigmoidal behavior, which was explained and modeled using appropriated models. As the soaking temperature was increased, the water absorption rate kinetic parameter \( (k_k) \) increased, the lag phase \( (\tau) \) decreased and the equilibrium moisture content \( (M_\infty) \) did not change. The model parameters were then modeled as a function of the soaking water temperature. The obtained results highlight the need for further studies regarding the hydration of grains, in special those with sigmoidal behavior.

**NOMENCLATURE**

\[ a, b \]  
parameters of model fit evaluation (Eq. 2) (different units)

\[ k_{IA}, k_{IB}, k_{IP1}, k_{IP2}, P_1, P_2, k_{HI1}, k_{HI2}, k_{II}, k_{II}, \alpha, \beta, k_{IO}, k_{II} \]  
kinetic parameters of the mathematical models used to describe the food hydration behavior (Table 1) (different units)

\[ k_k \]  
kinetic parameter of the Kaptso et al. (2008) model (Table 1) (min)

\[ M \]  
product moisture content on dry basis (g water/100 g solids = %d.b.)

\[ M_0 \]  
initial moisture content (g water/100 g d.b.)

\[ M_\infty \]  
equilibrium moisture content (g water/100 g d.b.)

\[ R \]  
constant of the ideal gases (\( = 8.314 \text{ J/mol/K} \))

\[ RMSD \]  
root-mean-square deviation values (Eq. (1)) (g water/100 g d.b.)

\[ t \]  
time (min)

\[ T \]  
absolute temperature (K)

\[ \tau \]  
parameter that describes the time of the inflexion point, related to the lag phase, in the Kaptso et al. (2008) model (Table 1) (min)

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Apêndice 7: “Ultrasound (US) enhances the hydration of sorghum (Sorghum bicolor) grains”
Short Communication

Ultrasound (US) enhances the hydration of sorghum (\textit{Sorghum bicolor}) grains

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**Abstract**

The water activity (Aw) reduction technique is widely used to preserve different food products, which are further rehydrated in order to be processed or consumed. The food hydration is time-consuming and, thus, a limiting unit operation during process. Therefore, there is an ongoing need to enhance the mass transfer phenomena during processing. The ultrasound technology (US) has been widely studied to improve different mass transfer processes of food. However, there is a lack of knowledge in relation to its application in the hydration process. This work evaluated the hydration process of sorghum seeds, comparing the effect of heating and ultrasound application in order to improve the hydration rate. The sorghum hydration kinetic was described by Peleg Model, whose parameters were evaluated for both processes. The US increased both water uptake rate (related to Peleg $k_1$ parameter) and equilibrium moisture content (related to Peleg $k_2$ parameter). The time for reach 90% of the control process equilibrium moisture content was 40% lower when the US was applied. The effect of processing at 53°C was higher than applying US at the evaluated power, and its limitations were discussed. The effect of combining both ultrasound and heating application was negligible when it was compared to the heated process. The obtained results highlighted that the US technology can be successfully used to optimize the hydration process of grains with directly industrial application.

**Keywords:** Hydration, Kinetics, Modeling, Ultrasound

1. Introduction

Many food products are preserved by the low activity of water (Aw), which can be obtained by different procedures. In general, these products are further hydrated or rehydrated before processing and consumption. The grains are typical examples. The hydration (or rehydration) process is an important unit operation in dried foods, as this process describes and defines the product properties and using during further steps, such as cooking, extraction, fermenting, germinating and eating. Also, the hydration process is usually a discontinuous and time-expensive unit operation. Therefore, it is important to understand the hydration kinetics of different food products, as well as the influence of process conditions on its rate.

The ultrasound technology (US) has been used to improve different mass transfer processes in food, such as solid–liquid extraction [1], salting [2], osmotic dehydration [3,4], membrane filtration [5] and drying [6–8].

However, the hydration process under US was only recently studied for chickpeas [9,10] and navy beans [11], improving their rate of mass transfer. Further than the need of evaluating the US technology for other products, both chickpeas and navy beans importance is only related to the grain directly consumption, with small economic and industrial impact. Therefore, there is a need for the evaluation of the effect of this technology on the hydration process of grains with other industrial applications.

\textit{Sorghum} (\textit{Sorghum bicolor}) is considered one of the most important cereal crops in the world [12]. Its economic importance is related to the ability to grow where the agricultural productivity is poor, to be an excellent energy source for both human and animal consumption, to the malting industry and to the different industrial uses of its starch and derivatives, including the ethanol production [12–19].

The sorghum grains must be hydrated before many industrial processes, such as steam flaking or brewing. Further, in some other industrial applications, the grains can be grinded by the wet milling process [13,15,16,20], which is conducted after the hydration process. The starch extraction processing is, for example, a typical example of wet milling application. Therefore, it is highly desirable...
to study possible optimization processes for the sorghum grains hydration.

The aim of the present work was to evaluate the possible use of the ultrasound technology (US) for optimization of the sorghum grains hydration process.

2. Material and methods

The effect of two technologies (treatments) on the hydration process of sorghum grains was evaluated: heating and ultrasound application. The process at room temperature (25 °C), without the US technology, was considered as control.

As temperature improves the hydration process, this unit operation was also conducted at a higher temperature condition. The upper level for the temperature treatment was fixed at 53 °C, below the sorghum starch gelatinization temperature [15,21] and most of the food proteins denaturation temperature [22]. The US condition was set considering the equipment specification (see the following Sections).

Therefore, the experiments were performed in four different conditions: room temperature (25 °C; considered the standard process and lower efficiency), with heating (53 °C, to evaluate the effect of temperature alone), ultrasound bath (US, at room temperature, evaluating the effect of ultrasound technology alone) and the combination of both technologies (US + 53 °C, evaluating the combined effect of the two technologies). The experimental design is shown on Fig. 1.

2.1. Raw material

The sorghum (S. bicolor) grains were gently donated by Guabi Group (http://www.guabi.com.br; Brazil). They were cleaned and selected before processing, ensuring that only intact grains were used. The grains moisture content was determined by the AOAC [23] method, oven-drying at 105 °C, in five replicates. The grains initial moisture was (average ± standard deviation) 15.4 ± 0.2 g water/100 g dry basis (%d.b.).

2.2. Ultrasound technology (US)

The ultrasound technology (US) was applied using an ultrasound bath (Spencer USC1400A, Unique, Brazil), whose piezoelectric elements were arranged below the tub. It generates the mechanical waves that are transmitted through the water, addressing the product. Both, the operating frequency (40 kHz) and the ultrasonic volumetric power transmitted to the fluid (PUS; W/mL) were fixed. The equipment had an independent heating system, allowing the experiments at higher temperatures.

The ultrasonic volumetric power (PUS; W/mL) was determined following the method described by Cárcel et al. [2]. A known volume of distilled water (2 L) at room temperature was placed in the bath, which was insulated in order to minimize the heat transfer through the surroundings. The equipment was then turned on for 30 min, while the water temperature was monitored. Thus, the water heating was the result of the energy delivered by the US waves, and the ultrasonic volumetric power (PUS; W/mL) could be obtained using Eq. (1):

\[ P_{US} = \frac{m \cdot Cp \cdot \frac{dT}{dt}}{V} \]  

(1)

The obtained PUS was 0.026 W/mL, similar to the values reported by Yildirim et al. [9,10] (up to 0.025 W/mL).

2.3. Hydration process

The hydration procedure was carried out in the same way as described in previous works [9–11,24–28].

The sorghum grains were placed in perforated plastic cups and then immersed in distilled water in the equipment previously described. The product:water ratio (1:50) was fixed to assure the excess of water, avoiding a possible limitation in the mass transfer phenomenon due to the lack of water.

At specific interval times (10 min until 30 min; 15 min until the first hour; 20 min until the second hour; and 30 min until stabilization) the grains were removed from the water, drained, superficially blotted with absorbent paper, weighed and returned to the water. The moisture content at each time step (m(t)) was then, calculated by mass balance, considering the initial sample mass (m0) and moisture (M0), and the obtained mass at each time step (m(t)). By using this procedure, the real grain kinetic was evaluated, as the same grains were evaluated through the whole process.

After confirmation, the sample soluble solids leakage to the hydration water could be neglected, enabling the moisture estimation by the mass balance [10,11,25]. Further, the sampling interval was different during processing to ensure a larger number of points in the initial part of the process. The experiments were performed using four replicates.

2.4. Mathematical modeling and statistical evaluation

Due to the obtained sorghum hydration behavior (Fig. 1), the empirical model proposed by Peleg ([29]; Eq. (2)) was used to describe the process.

The parameters of Peleg Model were obtained by non-linear regression using the software CurveExpert Professional (v.1.5, http://www.curvecxpert.net/, USA), with a confidence level of 95%. The regressions were carried out for each replicate in order to obtain the mean value and the associated standard deviation of each of the model parameters (k1, k2). Thus, the effect of both treatments (US and heating) was evaluated using the analysis of variance (ANOVA) and the Tukey test at 95% of confidence level. The Statistica 5.5 (StatSoft, Inc., USA) software was used for this purpose.

\[ M(t) = M_0 + \frac{t}{k_1 + k_2 \cdot t} \]

(2)

3. Results and discussion

The sorghum hydration behavior is shown on Fig. 1. The hydration behavior had a continuous water uptake, at a decreasing rate
in relation to processing time, which was described by a curve with a downward concave shape. In this behavior, the water influx rate is maximum in the first part of the process (at \( t = 0 \)), falling to zero after time enough (at \( t \to \infty \)) to reach the product equilibrium moisture content (\( M_{\infty} \)). The Peleg Model (Peleg, [29]; Eq. (2)), thus, was used to describe this process with high agreement (\( R^2 > 0.98 \)). The values obtained by the Peleg Model were also plotted on Fig. 1 showing a high confidence with the experimental values.

The Peleg Model is a two-term \((k_1, k_2)\) mathematical function.

For solving Eq. (2), at the beginning of the process \((t = 0)\), the moisture of the product is the initial moisture content (Eq. (3)). After sufficiently long periods of time (i.e., when \( t \to \infty \)), the product moisture tends to the initial value plus the amount of water absorbed, described by \( 1/k_2 \) (Eq. (4)). Therefore, the parameter \( k_2 \) is related to the product equilibrium moisture content (maximum moisture, or the moisture obtained after sufficiently long process time), as described on Eq. (5):

\[
M(t = 0) = M_0 
\]  
(3)

\[
M(t \to \infty) = M_0 + \frac{1}{k_2} 
\]  
(4)

\[
M_{\infty} \propto \frac{1}{k_2} 
\]  
(5)

The rate of water absorption is described by the product moisture change during time \((dM(t)/dt)\). Deriving Eq. (2) with respect to time, it is obtained:

\[
\frac{dM(t)}{dt} = \frac{d}{dt} \left( M_0 + \frac{t}{k_1 + k_2 \cdot t} \right) = \frac{k_1}{(k_1 + k_2 \cdot t)} 
\]  
(6)

Thus, it is observed that the water absorption rate in the process beginning \((t = 0)\) is described by \( 1/k_1 \) (Eq. (7)), being the maximum value during the process. This rate decreases with hydration time, getting zero (Eq. (8)) when the product reaches the equilibrium. Therefore, the parameter \( k_1 \) is related to the maximum absorption rate, as described on Eq. (9):

\[
\frac{dM(t)}{dt} = \frac{1}{k_1} 
\]  
(7)

\[
\frac{dM(t \to \infty)}{dt} = 0 
\]  
(8)

\[
\frac{dM}{dt} \text{ maximum} = \frac{1}{k_1} 
\]  
(9)

Fig. 2 shows the Peleg Model parameters \( k_1 \) and \( k_2 \) for the different evaluated process conditions. The standard deviation values were always lower than 5% of the average, indicating data consistency. The values of \( k_1 \) and \( k_2 \) in the process at room temperature were \( 1.73 \pm 0.06 \) min/%d.b and \( 0.0286 \pm 0.0003 \) 1/%d.b, respectively. These values are in accordance with those reported in the literature for other grains, such as chickpeas [9,10], navy beans [11], bambara [28], lentil [27] and rice [24,26].

Both US and heating technologies (53 °C) reduced the parameters \( k_1 \) and \( k_2 \) (Fig. 2; \( p < 0.05 \)), indicating an increase in both water uptake rate (proportional to \( 1/k_1 \) – Eq. (7)) and equilibrium moisture content (proportional to \( 1/k_2 \) – Eq. (5)). On the other hand, the combined process (US + 53 °C) had no significant difference when compared to the 53 °C process (Fig. 2; \( p > 0.05 \)), neither for \( k_1 \) nor \( k_2 \).

The \( k_1 \) was reduced to 88% and 42% of the control value (25 °C) by the US and heating (53 °C) technologies, respectively. It shows that the effect of increasing the soaking temperature to 53 °C on the sorghum hydration kinetic is higher than those of using ultrasound at 0.026 W/mL and 40 kHz.

The \( k_2 \) was reduced to 90% and 78% of the control value (25 °C) by the US and heating (53 °C) technologies, respectively. It shows that both US and heating technologies increased the sorghum maximum water absorption, although the effect of heating (53 °C) was also higher than that of US.

Nevertheless, it is important to highlight that some reactions can occur during processing at high temperature, such as protein denaturation, starch gelatinization and molecular degradation. These modifications can be desirable, acceptable or undesirable, depending of the process purpose. Even so, additional equipment

\[ \text{Fig. 1. Hydration behavior of sorghum grains at room temperature (25 °C), with heating (53 °C), using the ultrasound technology (US) and combining both technologies (US + 53 °C). The dots are the experimental values; the bars the standard deviation; the curves are the Peleg Model.} \]
and energy amount are needed to increase the process temperature in order to heat the whole grain and the water amount during hydration. At high temperatures, the costs of insulation or energy dissipation to the boundaries during the long period of processing (in this case, 5–6 h) can also be important. By those reasons, the high temperature process is rarely used in the industry with the objective of increasing the hydration rate, even so resulting in longer processes (although it can be used considering other purposes, as for steam flaking). Therefore, although the effect of heating was higher than the ultrasound processing in the sorghum hydration, the ultrasound technology can be considered a reasonable alternative to the grain hydration processing.

In fact, the ultrasound assisted hydration of sorghum showed an initial water intake rate ~12% higher than the control process, with a maximum water intake close to 10%, which highly impacts the product’s quality. The time for reaching 90% of the control process equilibrium moisture content ($M_{90\%,25^\circ C} \sim 50%d.b.$) is close to 320 min for the conventional process. By using the ultrasound technology, this time drops to 190 min, a reduction of ~40%. It highlights once more the value of using the ultrasound technology on grain hydration processes.

Ghafoor et al. [11] modeled the navy beans hydration during ultrasound assisted process at 47 kHz and room temperature using the Peleg Model. The authors have not described the ultrasonic volumetric power ($P_{us}$), but it can be considered in the same order of magnitude of the present work. The ultrasound technology reduced the $k_1$ parameter to 55% of the control value, with any effect on $k_2$.

Yıldırım et al. [9] evaluated the chickpeas hydration during ultrasound assisted process at 40 kHz and 25 kHz and 0.020 W/mL using the Peleg Model. The ultrasound processing at 40 kHz did not statistically change both $k_1$ and $k_2$. On the other hand, when the chickpea grains were processed using ultrasound at 25 kHz, a reduction on $k_1$ (~22% of reduction) and a slightly increasing on $k_2$ (~6% of increasing) were observed. When the processes with and without using ultrasound were compared at 20 °C and 50 °C (i.e., similar values of temperature and ultrasonic volumetric power of the present work), any important change was observed when both technologies (heating and ultrasound) were combined, as here observed during the sorghum hydration. The same behavior was observed when the data was evaluated using the Fick Model [10].

In fact, it is interesting to observe that similar results were obtained by Rodriguez et al. [30], even though at different processes and conditions. The authors studied the drying kinetics of thyme leaves using ultrasound. Although, increasing air temperature or using ultrasound increased the drying rate, the combination of both techniques showed negligible effect, with the results very close to those that only heated the air.

When the grains are immersed into the water, the difference in the water activity ($A_w$) leads to the hydration phenomenon. The water is firstly transferred from the liquid to the solid surface by convection. Then, due to the gradient within the product, the water is transferred by diffusion and capillary through the solid. Thus, the increasing in the mass transfer rate can be obtained by both reducing the resistance to the mass transfer on the solid surface and/or reducing the resistance to the mass transfer through the solid.

Some possible reasons can explain the increase in the grains hydration rate, although it is difficult to confirm the relative importance of each one.

The first reason is related to the reduction on the convective resistance in the solid surface, due to the intense turbulence in the fluid do to the ultrasound waves. However, it is expected and well documented that the internal resistance to the mass transfer during hydration of food is much higher than the boundary resistance. Thus, the food hydration is generally considered a diffusion-limited reaction. In fact, Simal et al. [4] observed higher mass transfer rates during ultrasound assisted osmotic dehydration of apples when compared to a process with fluid agitation, suggesting other effects of US.

The US processing of solids can form micro-channels in the product due to the mechanical stresses associated to the wave transmission, facilitating the mass transfer. The micro-channels formation due to US processing was observed by Garcia-Nogueira [3] and Fernandes et al. [31,32] for soft foods (strawberry, melon and pineapple). In fact, the ultrasound processing has been used as a pre-treatment to drying, highlighting the structural importance during US processing.

When the ultrasonic waves travels through the product, it causes a rapid series of alternative compressions and expansions, which is compared to a sponge squeezed and released repeatedly [7,8,33]. This phenomenon can keep the micro-channels and pores unobstructed, facilitating the mass transfer [33]. Further, an inertial flow can be expected.

Mulet et al. [8] highlighted that the effect of ultrasound is different for each food material, as it is affected by properties such as the amount of air present in the cells/tissue and attenuation due to mechanical properties. It explains the similarities and differences observed during the hydration of sorghum (this work), navy beans [11] and chickpeas [9], which were here evaluated with similar process conditions (temperature, ultrasonic volumetric power, ultrasonic frequency) and approach (mathematical modeling by the Peleg Model). Further, it highlights the need to conduct more studies relating this important and limiting unit operation in food processing.

The results here obtained demonstrate that the ultrasound technology (US) can be successfully used to optimize the hydration process.

Fig. 2. Parameters $k_1$ and $k_2$ of the Peleg Model (Eq. (2)) describing the sorghum grains hydration at room temperature (25 °C), with heating (53 °C), using the ultrasound technology (US) and combining both technologies (US + 53 °C). The bars are the standard deviation. For each parameter, different letters represent significantly different values ($p<0.05$).
process of grains with directly industrial application. However, it also highlights the need for further studies explaining the reasons for the hydration improvement, evaluating other grains and optimizing the process conditions.

4. Conclusions

The present work evaluated the hydration process of sorghum grains, selected due to its economic and industrial importance. The effect of applying heating and ultrasound to improve the mass transfer was evaluated. The sorghum hydration behavior showed a curve with a downward concave shape, and then it was evaluated by the Peleg Model. The US increased both water uptake rate (related to the Peleg $k_1$ parameter) and equilibrium moisture content (related to the Peleg $k_2$ parameter). The time for reaching 90% of the control process equilibrium moisture content were 40% lower when the US was applied. The effect of heating was evaluated by performing the process at 53°C. This effect was higher than US applied (at the evaluated volumetric power). Even so, the limitations of heating were discussed. The effect of combining both ultrasound and heating was negligible when compared to the heated process (for both $k_1$ and $k_2$ parameters). The obtained results highlighted that the US technology can be successfully used to optimize the hydration process of grains with directly industrial application.

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Apêndice 8: “Mechanisms for improving mass transfer in food with ultrasound technology: Describing the phenomena in two model cases”
Mechanisms for improving mass transfer in food with ultrasound technology: Describing the phenomena in two model cases

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Abstract
The aim of this work was to demonstrate how ultrasound mechanisms (direct and indirect effects) improve the mass transfer phenomena in food processing, and which part of the process they are more effective in. Two model cases were evaluated: the hydration of sorghum grain (with two water activities) and the influx of a pigment into melon cylinders. Different treatments enabled us to evaluate and discriminate both direct (inertial flow and “sponge effect”) and indirect effects (micro channel formation), alternating pre-treatments and treatments using an ultrasonic bath (20 kHz of frequency and 28 W/L of volumetric power) and a traditional water-bath. It was demonstrated that both the effects of ultrasound technology are more effective in food with higher water activity, the micro channels only forming in moist food. Moreover, micro channel formation could also be observed using agar gel cylinders, verifying the random formation of these due to cavitation. The direct effects were shown to be important in mass transfer enhancement not only in moist food, but also in dry food, this being improved by the micro channels formed and the porosity of the food. In conclusion, the improvement in mass transfer due to direct and indirect effects was firstly discriminated and described. It was proven that both phenomena are important for mass transfer in moist foods, while only the direct effects are important for dry foods. Based on these results, better processing using ultrasound technology can be obtained.

1. Introduction
Ultrasound technology has been widely studied as an alternative for improving food processing in such operations as defoaming, freezing, extraction, emulsification, hydration, drying and others [1]. In mass transfer unit operations, the ultrasound technology has been successfully used in different processes, such as extraction [2], drying [3–5], osmotic dehydration [6], hydration [7,8], and desalting [9].

The enhancement of the mass transfer unit operation by ultrasound technology has been attributed to different mechanisms. These are considered to be direct and indirect effects of ultrasound. The direct effects are related to the “sponge effect” and inertial flux. When ultrasonic waves travel through the product, they cause a rapid alternating compression and expansion of the tissue matrix, which is compared to a sponge squeezed and released repeatedly [10–12]. This phenomenon can keep micro channels and pores unobstructed, facilitating mass transfer [12]. Further, it can promote mass flow due to pumping. However, although it is frequently attributed with these direct effects on the mass flow [4,9,13–15], these have not been demonstrated during food processing yet.

The indirect effect is related to micro channel formation due to the acoustic cavitation [16]. When ultrasound waves travel through the product, the phenomenon of cavitation takes place in the water inside or outside the product cells, resulting in cell and tissue disruption and the consequent formation of cavities and micro channels. In fact, micro-channel formation due to ultrasound has been shown in various moist foods, such as melons, potatoes, strawberries, apples and cod [3,9,14,17,18]. In addition, the presence of these micro-channels is believed to be the main effect of the ultrasound technology in enhancing the mass transfer phenomena in food processing [6,8,9,19]. However, the formation of micro channels and its importance for mass flow has not been studied for dry foods, such as grain. As the water activity of these products is low, the lower water vapor pressure can limit cavitation, reinforcing the need for evaluation.

Consequently, this work aimed to demonstrate how these mechanisms improve the mass transfer phenomena in food pro-
cessing, and which part of the process they are more effective in. Therefore, two model cases were evaluated: the hydration of sorghum grain and the influx of a pigment into melon cylinders.

2. Materials and methods

The mechanisms of ultrasound enhancement during mass transfer processes were studied in two kinds of food: sorghum grain (representing dry foods, with low water activity) and yellow melon cylinders (representing moist foods, with high water activity). Moreover, these two foods have already shown good results when treated with ultrasound during osmotic dehydration [14] and hydration [8].

During the experiments, an ultrasonic bath with a frequency of 40 kHz and a volumetric power of 28 W/L (USC-1400, Unique Brazil) was used. This bath had its piezoelectric elements arranged below the tub. These generated the mechanical waves that are transmitted through the water (or solution), reaching the product. The volumetric power was determined following the method described by Tiwari, Muthukumarappan, O’Donnell and Cullen [20], and it was the same or very close to that used in previous works [8,15]. The temperature of the water was controlled using a stainless steel heat exchanger coupled to an external water bath, which was placed at the top of the solution inside the ultrasonic water bath.

2.1. Ultrasound mass transfer enhancement on grain

For sorghum grain, the hydration process was chosen as the evaluated mass transfer processing. Sorghum grains (Sorghum bicolor) with water activity of 0.653 ± 0.001 and a moisture content of 12.46 ± 0.17% d.b. (g water/100 g of dry matter) were used. Furthermore, in order to prepare a sample with higher water activity (0.985 ± 0.003) the grains were hydrated for 3 h at 25°C. Then, these grains were superficially dried and put into sealed containers for two days at 5°C in order to homogenize the moisture. Consequently, the evaluation was carried out using two different conditions of water activity.

Different treatments were performed in order to identify the mechanism of mass transfer enhancement caused by the ultrasound technology (Fig. 1). These treatments helped to differentiate the indirect effects (micro-channel formation) with the direct effects (the sponge effect, inertial flow), as well as the moment these acted during processing.

Three treatments were performed for the low water activity grains:

- Treatment 1 (TS1: H) consisted of hydrating the grains (15 g of grains in a beaker with 2 L of distilled water) without the application of ultrasound at 25°C throughout the process (2 h).
- Treatment 2 (TS2: PUS/H) consisted of vacuum packing one layer of sorghum grains in order to treat the grain with ultrasound without it becoming hydrated. This pack was placed at the bottom of the water bath to receive the sound waves better. After 3 h of pretreatment, the grains were unpacked and hydrated (beaker with 2 L of distilled water) without ultrasound application at 25°C for 2 h.
- Treatment 3 (TS3: H + US) consisted of hydrating the grains (15 g of grains in the ultrasonic bath with 2 L of distilled water) with the application of ultrasound at 25°C throughout the process (2 h).

Four treatments were performed for the high water activity grains. Treatments 1, 2 and 3 were the same as those applied to the low water activity grains. The other was:

- Treatment 4 (TS4: PUS/H + U) consisted of pretreating the grains, as in treatment 2, but, after that, hydrating them (15 g of grains in the ultrasonic bath with 2 L of distilled water) with the application of ultrasound at 25°C for 2 h.

During the hydration process, the samples were periodically drained, superficially dried and their moisture content was obtained by mass balance. The sampling was carried out each 15 min for 2 h. All the treatments described above were performed in triplicate. The results were presented as the mean and the standard deviation.

Fig. 1. Treatments to differentiate the ultrasound mechanisms (direct and indirect effects) that enhance mass transfer on the sorghum grains.
2.2. Ultrasound mass transfer enhancement on melon cylinders

The effect of ultrasound processing on the mass transfer phenomena in foods was studied by considering pigment transfer into canary melon (*Cucumis melo inodorus*) cylinders (89.6 ± 0.6% w.b. of moisture, 9.4 ± 0.5 °Brix and 5.74 ± 0.08 of pH). The melon was cut into cylinders in order to obtain homogeneous and uniform specimens.

By evaluating pigment flow into the cylinders in processes under ultrasound, without ultrasound and with a pre-treatment using ultrasound, it was possible to evaluate the direct effects of ultrasound (sponge effect, inertial flow) and those related to the changes in the product microstructure caused by the acoustic cavitation (micro-channel formation; indirect effects).

Melon cylinders of 4 cm long and 1.5 cm in diameter were obtained using a fruit corer. For some treatments (Fig. 2; explanation as follows), the cylinders were perforated using a 0.3 mm-diameter needle. 50 perforations were done randomly along the cylinder, and in the direction of its diameter. The cylinders were perforated in order to simulate higher porosity generated by the micro channels formation and to discriminate the direct effects (i.e., to guarantee samples with micro channels and then evaluate their behavior during processing, in order to compare it with the other treatments).

Ten cylinders were immersed in a beaker or in the ultrasonic bath (depending on the treatment) with 2 L of brilliant blue (food grade, kindly donated by SanLeon, Brazil, www.sanleon.com.br) solution (0.0625 g/L) at 25 °C for the mass transfer evaluation. Six different treatments were considered in order to discriminate the ultrasound effects on the mass transfer process. The treatments are shown in Fig. 2:

- **Treatment 1** (TM1: PS) consisted of immersing the cylinders in the pigment solution without ultrasound application throughout the process (2.5 h).
- **Treatment 2** (TM2: PS + US) consisted of immersing the cylinders in the pigment solution with ultrasound application throughout the process (2.5 h).
- **Treatment 3** (TM3: W/PS) consisted of immersing the cylinders in distilled water without ultrasound application for 60 min as a pretreatment. Then, the process continued in the pigment solution without ultrasound for 1 h.
- **Treatment 4** (TM4: W + US/PS) consisted of immersing the cylinders in distilled water with ultrasound application for 60 min as a pretreatment. The process then continued in the pigment solution without ultrasound for 1 h.
- **Treatment 5** (TM5: P/PS) consisted of immersing the perforated cylinders in the pigment solution without ultrasound application throughout the process (1 h).
- **Treatment 6** (TM6: P/PS + US) consisted of immersing the perforated cylinders in the pigment solution with ultrasound application throughout the process (1 h).

The mass transfer process was evaluated for 2.5 h, removing one cylinder each 30 min for treatments 1–4. For treatments 5 and 6, the mass transfer process was evaluated for 1 h removing one cylinder each 15 min. The removed cylinders were quickly washed with distilled water, drained and superficially blotted with absorbent paper before evaluation. Then, 2 g of the cylinder (after discarding its edges) was triturated with 8 mL of distilled water using a Ultra Turrax homogenizer (IKA® T25D, Brazil) for 30 s at 104 RPM, and then filtered with Whatman grade 2 filter paper. The filtered absorbance at 630 nm of wavelength (maximum absorbance of the pigment solution) was obtained for each cylinder using a spectrophotometer (Femto 600S, Brazil). Following the Beer–Lambert Law, the higher absorbance was considered as the higher pigment solution concentration in the cylinders. All the treatments described above were performed in triplicate. The results were presented as the mean and the standard deviation.

2.3. Micro channels formation observation

In order to evaluate the formation of micro channels due to ultrasound technology, cylinders of agar gel (2%; Oxoid – Thermo Fisher Scientific, Inc. USA) 4 cm long and 1.5 cm in diameter) were considered as model foods. They were treated with ultrasound in 2 L of brilliant blue solution (0.0625 g/L) at 25 °C for 2.5 h (the same time as the melon cylinder treatments). This was carried out to obtain images for better visualization. The cylinders were placed in a black background illuminated from the side with a LED Flashlight and the images were obtained using a regular digital camera.

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![Fig. 2. Treatments for differentiate the ultrasound mechanisms (direct and indirect effects) that enhance mass transfer on the melon cylinders.](image-url)
3. Results and discussion

3.1. Ultrasound mass transfer enhancement in grains

Fig. 3 shows the application of the ultrasound technology as a treatment and pretreatment to the sorghum grains, representing a food with low water activity. As expected, when the grains were hydrated in the ultrasonic bath throughout the process (TS3), the water intake was improved compared with the conventional hydration process (TS1) \[8\]. In contrast, when the packaged grains were treated with 3 h of ultrasound and then hydrated without it (TS2), they did not hydrate faster than with the conventional process (TS1). These three treatments demonstrated that ultrasound technology does not generate micro channels in sorghum grains under these conditions of water activity (0.6533 ± 0.0004), or at least, this generation is negligible. The low vapor pressure of the water in the grains hinders cavitation because the cavitation bubbles contain less vapor from the solvent \[21\]. That is why it is difficult for the micro channels to form inside the product by cavitation. It means that, at this water activity, ultrasound enhanced sorghum grains hydration due to direct effects that caused water to enter by inertial flow and/or the sponge effect.

On the other hand, when the same treatments were performed in sorghum grains with a higher water activity (0.9851 ± 0.0029), different results were obtained. Fig. 4 shows that when ultrasound is applied as a pretreatment (TS2) and the grains are then conventionally hydrated, there is an improvement in the hydration process. Micro-channel formation during the pretreatment was demonstrated, and this improved the mass transfer by reducing internal resistance. In contrast to the low water activity grains, the higher vapor pressure of the water facilitated cavitation and the formation of micro-channels, probably by cell disruption and matrix rupture.

Furthermore, when the hydration of the high water activity sorghum grains was assisted with the ultrasound technology (TS3), there was no improvement in the early stages, but the water intake began to increase with the process time. Although these grains had the right conditions to be affected by cavitation (high water activity) and to form micro channels, a certain process time was necessary for this to be effective. Apparently, acoustic cavitation occurs randomly inside the food matrix, causing the formation of micro cavities, which grow in size with the passing of time. They start to form cavities with different tortuosity and permeability. Many of these cavities can lack connection between each other or with the external medium, thus not improving or only slightly improving the mass transfer. Finally, the mass transfer phenomena are improved when a reasonable number of cavities are formed and/or when there are connections between those and the external medium, forming channels.

Cylinders of agar gel were used to try to see the micro-channel formation, and these were treated with and without ultrasound technology (Fig. 5). The formation of different kind of cavities by ultrasound technology was observed, confirming the statement above (Fig. 6). The different kind of cavities and micro channels formed can have varied tortuosity, permeability and diffusion, improving or not the mass transfer in different ways \[22\].

Finally, treatment 4 (TS4), which consisted of hydrating the sorghum grains using the ultrasound technology after the pretreatment with ultrasound, showed an even better hydration rate than treatment 2 (TS2), which consisted of hydrating the grains without using the ultrasound technology after the pretreatment with ultrasound. It not only proves that micro channels form, but also the direct effects (inertial flow and “sponge effect”) were important for enhancing mass transfer. Moreover, it means that
the higher porosity of the grain, caused by micro-channel formation, enhance the direct effects of the ultrasound.

Based on these results, ultrasound-assisted hydration of grains can be explained.

When a grain is hydrated, firstly, the enhancement caused by the ultrasound technology is due to the direct effects (inertial flow and “sponge effect”). Then, from a certain water activity, the formation of micro channels starts to take place, further improving mass transfer. This explains why the enhancement of the hydration process is higher after a certain processing time than in the early stages. The few studies of ultrasound assisted grain hydration confirm this [7,8,15], and it can be seen that the differences between the hydration curves grow wider as the process time passes.

Furthermore, these results highlight that the effect of applying the ultrasound technology is probably higher in products with higher water activity. Consequently, the use of this technology in food processing could be designed to maximize its effects, for example, by applying it only at some stages of the process.

Finally, it is important to highlight that the obtained results can only be directly applied for sorghum grains at this level of ultrasonic volumetric power. Each grain has its own structure and composition, which can be differently affected by the ultrasound technology. Further, different volumetric powers can change the relative importance of each mechanism during the ultrasonic processing. Despite this, the main qualitative finds of the present work can be generally interpreted.

3.2. Ultrasound mass transfer enhancement in melon cylinders

Fig. 7 shows the results of the treatments 1 (TM1), which consisted of immersing the melon cylinders in the pigment solution without ultrasound, and 2 (TM2), which consisted of immersing the melon cylinders in the pigment solution with ultrasound. These treatments showed that the ultrasound technology improves pigment intake. It can be clearly noted that the ultrasound almost doubles the pigment retention. Nevertheless, they do not indicate which ultrasound mechanism leads to this improvement.

Fig. 8 shows treatments 3 (TM3), which consisted of pretreating the melon cylinders in water (as a control treatment) before they were immersed in the pigment solution, and 4 (TM4), which consisted of pretreating the melon cylinders in the ultrasonic bath before they were immersed in the pigment solution. They showed that pigment transfer was improved by applying the ultrasound as a pretreatment, demonstrating the formation of micro channels in the melon cylinders. These channels promoted the pigment influx by capillarity, increasing the total concentration of pigment in the melon cylinders. The micro-channel formation was caused by acoustic cavitation, which could cause cell disruption and/or matrix rupture inside the food, generating microscopic channels that reduce the internal resistance to the mass flow [16]. In fact, this mechanism was demonstrated when ultrasound was applied as a pretreatment in melon before its dehydration [19]. Furthermore, it should be mentioned that the formation of micro channels in melon cylinders was probably faster than in the sorghum grains due to the softer matrix and higher water activity of the melon.

Fig. 9 shows treatments 5 (TM5), which consisted of immersing the melon cylinders in the pigment solution without ultrasound, and 6 (TM6), which consisted of immersing the melon cylinders in the pigment solution with ultrasound, both using perforated melon cylinders. Consequently, these cylinders already contained many micro channels before contact with the pigment solution. It can be seen that pigment retention was higher even in the early stages of contact with the solution, where the micro-channel formation by the ultrasound is expected to be low in comparison with those previously generated by the needle. Therefore, this result...
demonstrated that the ultrasound enhancement of the mass transfer also occurs due to direct effects (inertial flow and "sponge effect"). Since the formation of micro-channel by cavitation needs time (due to the random distribution of cavitation until the formation of channels), these treatments were evaluated in only one hour in order to despise subsequent micro-channel formation and thus, evaluate only the direct effects. It can clearly be seen (Fig. 9) that after 15 min, there was an improvement in pigment solution transfer, which means that the direct effect had taken place. Using ultrasound, the pigment flowed into the cylinders faster by the pre formed channels and all the natural cavities. Once they were full with the pigment solution, it was later transferred by diffusion, increasing the total concentration of the pigment inside the cylinders. The ultrasound waves also produced a rapid series of contractions and expansions ("sponge effect") in the melon cylinders, causing the pigment solution to flow through the preformed micro channels, enhancing the mass transfer by reducing internal resistance [11]. It means that the direct effects of ultrasound are enhanced by the porosity of the medium.

Finally, it is important to highlight that the obtained results can only be directly applied for the evaluated pigment influx into melon cylinders at this level of ultrasonic volumetric power. Each component that is transferred (mass transfer) through a food matrix has different behavior since they have different sizes, charge, molecular weight, etc. Therefore, the effect of the ultrasound technology could be different for different components. In addition, since the acoustic cavitation depends on the water vapor pressure, the ultrasound effect (especially the indirect effects) could change due to the water vapor pressure change with the presence of solutes. Further, different volumetric powers can change the relative importance of each mechanism during the ultrasonic processing. Despite this, the main qualitative finds of the present work can be generally interpreted.

3.3. Final considerations

According to the results, the ultrasound-assisted processes could be optimized by taking the water activity and porosity of the food into count. If the aim is to take advantage of the indirect effects of ultrasound technology, it would have to be used on porous food.

It should be mentioned that these results were obtained with a specific ultrasonic volumetric power and frequency (28 W/L; 40 kHz), similar to other works were the ultrasound technology was proven to enhance mass transfer processes in food [8,15,23]. Different results could be obtained with different conditions of power and food products.

The direct effects ("sponge effect" and inertial flow) can be enhanced by the porosity and cavities of the food. The ultrasound waves generate the contraction and relaxation of the tissues causing the fluids be displaced since it acts as a pump. Therefore, spaces in the food are required to the flow of the fluid. The indirect effects (micro channels formation due to cavitation) can be affected by the structure. If the structure is very compact and rigid, the cavitation can take more time to generate cavities, or a higher ultrasound energy would be needed.

However, although the obtained results can only be directly applied for the two model cases here evaluated, the main qualitative finds of the present work can be generally interpreted.

In conclusion, this work can contribute to both academic knowledge and industrial application, since its results can help to optimize the different processes where mass transfer is involved, deciding at which point in the process it is better to use the ultrasound technology and reducing the cost and energy used.

4. Conclusions

This work demonstrated that the ultrasound mechanisms (indirect effect related to micro-channel formation by acoustic cavitation and the direct effects related to the inertial flow and the "sponge effect") enhance the mass transfer in food. Further, those mechanisms can occur in food processing, and their importance is in function of the water activity in the food. Acoustic cavitation takes place more easily in food with higher water activity, resulting in micro-channel formation, while the direct ultrasound effects take place in both low and high water activity food. Moreover, the direct effects are enhanced by the porosity of the food and the micro channels formed. It was also demonstrated that cavitation forms cavities randomly. They are differentiated by their tortuosity and lack of connection with the external medium. Consequently, the micro channels need time to form well, resulting in an improvement in the mass transfer. As a conclusion, based on the results of this work, the application of ultrasound in food processing can be revised in order to maximize its effects, for example only applying it in some periods of the whole process.

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Apêndice 9: “Effect of ultrasound technology on barley seed germination and vigour”
Research Note

Effect of ultrasound technology on barley seed germination and vigour

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Abstract

Ultrasound technology has been used to enhance the hydration process of grains and seeds, obtaining positive results. However, the effect of ultrasound treatment on seed quality parameters such as germination and vigour has not been sufficiently studied. This work investigated whether ultrasound technology affected the germination and vigour of four lots of barley seeds. It was demonstrated that ultrasound did not negatively affect germination and vigour and improved germination rate. Several possible explanations for this are given. Therefore, ultrasound technology can be used to enhance the hydration process without negatively affecting seed quality. However, further studies are required to determine the main cause (physical and/or physiological) of the germination rate enhancement. Finally, it is clear that ultrasound is a promising technology in the area of seed science.

Experimental and discussion

Ultrasound is a form of energy generated by acoustic waves at frequencies higher than 20 kHz (Mason and Peters, 2004). The technology is often used in the agroindustry in order to enhance processes such as drying, extraction, emulsification and defoaming (Mason et al., 2005). The application of ultrasound in processing, analysis and quality control is described as low or high power ultrasound. Low power ultrasound (LPU) is generated with frequencies higher than 100 kHz and intensities lower than $10^4$ W m$^{-2}$, while high power ultrasound (HPU) is generated with frequencies between 20 and 500 kHz and intensities higher than $10^4$ W m$^{-2}$ (Awad et al., 2012).

Ultrasound treatment has been shown to reduce the soaking time for chickpeas (Yildirim et al., 2010; Ranjbari et al., 2013), sorghum (Patero and Augusto, 2014) and navy beans (Ghafoor et al., 2014). This improvement has been attributed to a greater...
reduction of internal resistance than external resistance (Cunningham et al., 2008), as well as possible changes in microstructure by cavitation (micro-channel formation) and/or the so called “sponge effect”, causing inertial flow (Patero and Augusto, 2014).

As ultrasound technology enhances the hydration process of seeds and grains, seed quality must be studied in order to verify whether the treatment has affected properties such as germination capacity and vigour. Moreover, due to the importance of germination of barley seeds for cultivation or for malting (one of the first steps in the production of drinks such as beer and whisky), the objective of this work was to verify whether ultrasound technology affects the germination and vigour of barley seeds.

Four lots of barley seeds were used. Lot 1 was seeds of cv. Scarlet; lot 2 was a mixture of 50% of lot 1 and 50% of lot 3; lot 3 was lot 1 seed that had been subject to accelerated ageing (24 hours at 42°C, 100% relative humidity); and lot 4 was barley grains grains provided by a malting company. The moisture content of each lot, determined in an oven at 105 ± 3°C for 24 hours (Brasil, 2009), was between 10.0 and 10.7% (fresh weight basis).

An ultrasonic bath with 20 kHz frequency and 0.028 W m⁻³ volumetric power (USC-1400, Unique, Brazil) was used. This bath has its piezoelectric elements arranged below the tub. It generates mechanical waves that are transmitted through the water. Volumetric power was determined according to the method described by Cárcel et al. (2012).

Barley seeds were vacuum-packed as single layers in low-density polyethylene bags of approximately 62.5 µm thickness. Bags were placed at the bottom of the ultrasonic bath to improve delivery of the sound waves. Each lot was treated for four hours. Both ultrasonicated and non-ultrasonicated lots were evaluated by germination and vigour tests after 24 hours pre-treatment.

**Germination test:** four replicates of 50 seeds each were placed into rolled paper towels moistened with water at a proportion of 2.5-times the dry weight of the paper towels and placed at 20°C with a photoperiod of 16 hours light per day (Brasil, 2009). Number of normal seedlings were recorded after 4 and 7 days.

**Seedling emergence:** four replicates of 50 seeds were sown in sand in plastic boxes (470 × 300 × 110 mm). After sowing, sand was watered to 60% of its water-holding capacity. Normal seedlings were recorded after 10 days (Brasil, 2009). The boxes were kept in non-controlled environmental conditions.

**Accelerated ageing:** one layer of 200 seeds were placed on a metal mesh into plastic boxes (110 × 110 × 30 mm) containing 40 mL distilled water. The boxes were closed and placed in a chamber at 42 ± 1°C. Hence, within the boxes, the seeds were exposed to 100% relative humidity. After 72 hours, a germination test was performed and the number of normal seedlings recorded after four days.

**Seedling automated computed analysis:** four replicates of 25 seeds were used for each lot. The seeds were placed in two horizontal rows on the upper third of the surface of moistened paper towels so that the primary root could grow downwards. The towels were rolled and transferred to a dark germination chamber, following the same conditions described for germination test. After three days, seedlings were arranged on a sheet of blue pasteboard, set on the surface of an aluminium box (600 × 500 × 120 mm), inside of which was placed an HP Scanjet 2410 scanner, placed upside down and operated in a 100
dpi resolution. The digitalized images of the seedlings were analysed by the SVIS® (Seed Vigour Imaging System) software (Sako et al., 2001), which calculated the vigour index and other seedling growth and uniformity parameters. The vigour index was generated by a combination of growth parameters (70% contribution) and seedling uniformity (30% contribution). The uniformity index was calculated on the basis of deviations from the length of each seedling sample in relation to the estimated maximum value for seedlings with pre-established age, defined in the software settings (index: values 0-1000). The growth index was calculated based on the size of each sample seedlings in relation to the estimated maximum value for seedlings with pre-established age, defined in the software settings (index: values 0-1000).

The experiment was carried out in a completely randomised factorial design with two treatments (with and without ultrasonication) and four lots. ANOVA was performed on the results and the means were compared by Tukey's test ($P \leq 0.05$) using the SAS (Statistical Analysis Software).

To test for possible physical injury to the seeds caused by ultrasound treatment, 200 vacuum-packaged seeds were radiographed before and after ultrasonication. Packets of seeds were placed 0.143 m from the source of X-ray emission and were radiographed with the aid of digital equipment Faxitron X-ray brand, model MX-20 DC12, connected to a computer.

Germination of the four lots was significantly different, lot 1 had the highest germination (89%), followed by lot 2 (79%), lot 3 (68%) and lot 4 (43%; table 1). Application of ultrasound did not reduce germination capacity nor vigour. Moreover, the radiographs show that ultrasound technology did not cause any macroscopic physical damage such as cracks or morphological damage (figure 1). Ultrasound application can therefore be used to increase the water uptake of seeds and grains during hydration (Yildirim et al., 2010; Ranjbari et al., 2013; Ghafoor et al., 2014; Patero and Augusto, 2014) without negatively affecting germination capacity.

Some of the vigour tests showed a slight enhancement of vigour following ultrasound treatment, especially for lot 1. It should be highlighted that the enhancement of vigour was apparent during the first four days of germination, which means that the ultrasound technology improves the germination speed represented by the first count evaluation and SVIS®. Previous work has found similar results for other cultivars of barley (Yaldagard et al., 2008), switchgrass (Chen et al., 2012) and pea (Chiu and Sung, 2013). Unfortunately, these studies did not report the volumetric power of the ultrasound used, giving only the frequency. Therefore, it is difficult to compare these improvements with the results of the present work. Previous studies have attributed the improvement to various causes:

- Ultrasound increases the porosity of the seed by acoustic cavitation improving water uptake and oxygen availability.
- Ultrasound intensifies mass transfer; the extra-absorbed water reacts freely and readily with the cell embryo, in a manner that releases gibberellic acid and causes an increase in the rate of metabolic processes in aleurone cells.
- Ultrasound helps the mobilisation of endosperm nutrients by cell membrane disruption.
In contrast to applying ultrasound during the hydration process, the present work applied ultrasound treatment to dry seeds (10.0-10.7% moisture content) avoiding hydration by vacuum-packing. Consequently, the cause of vigour enhancement cannot be due to water intake, but must be related to direct changes in seed micro-structure, such as increasing seed porosity (and improving water and oxygen transfer), and improving mass transfers among the seed tissues. Another possible explanation could be a reduction of the microbial load, improving physiological potential. Further, it is well known that mechanical events, such as vibration, cutting and brushing, can increase the respiration rate and metabolism of plant tissues and has been shown for example, to accelerate the germination process in *Arabidopsis thaliana* L. seeds (Uchida and Yamamoto, 2002). Therefore, similar behaviour is expected for the effect of ultrasound on the metabolism of barley seeds.

Table 1. Results of germination and vigour of barley seed: control (C) and ultrasonicated (U). Lot 1 - barley seed (cv. Scarlet); lot 2 - mixture of 50% of lot 1 and 50% of lot 3; lot 3 - accelerated aged lot 1; lot 4 - barley seeds provided by a malting company.

| Lot   | Germination first count(%) | Germination at seven days (%) | Seedling emergence (%) |
|-------|----------------------------|------------------------------|------------------------|
|       | C  | U  | C  | U  | C  | U  | C  | U  |
| 1     | 81 Ba | 89 Aa | 89 Aa | 93 Aa | 89 Aa | 86 Aa |
| 2     | 68 Ab | 71 Ab | 79 Aab | 77 Ab | 70 Ab | 64 Ab |
| 3     | 58 Ac | 60 Ac | 68 Ab | 66 Ab | 53 Ac | 55 Abc |
| 4     | 34 Bd | 48 Ad | 43 Ac | 54 Ac | 56 Ac | 46 Ac |
| CV    | 5.5 |    | 7.4 |    | 7.4 |    |

| Lot   | Accelerated ageing (%) | Seedling dry matter (mg) | Vigour index |
|-------|------------------------|--------------------------|--------------|
|       | C  | U  | C  | U  | C  | U  | C  | U  |
| 1     | 50 Ba | 60 Aa | 107 Aa | 109 Aa | 560.7 Ba | 653.5 Aa |
| 2     | 39 Ab | 45 Ab | 80 Aab | 82 Aab | 505.0 Aab | 569.0 Ab |
| 3     | 31 Bb | 44 Ab | 58 Ab | 71 Ab | 457.7 Ab | 517.8 Abc |
| 4     | 18 Ac | 24 Ac | 61 Ab | 77 Ab | 460.2 Ab | 478.5 Ac |
| CV    | 10.9 |    | 16.5 |    | 7.4 |    |

| Lot   | Growth index | Uniformity index | Seedling length (mm) |
|-------|--------------|------------------|----------------------|
|       | C  | U  | C  | U  | C  | U  | C  | U  |
| 1     | 437.5 Ba | 552.2 Aa | 875.2 Aa | 891.0 Aa | 52.3 Ba | 69.0 Aa |
| 2     | 431.5 Aab | 495.2 Aab | 772.7 Aab | 801.0 Aab | 44.3 Aab | 48.1 Ab |
| 3     | 348.5 Aab | 432.2 Abc | 716.7 Ab | 718.7 Ab | 29.3 Ac | 38.4 Ab |
| 4     | 326.2 Ab | 345.0 Ac | 775.0 Aab | 792.5 Aab | 35.5 Abc | 39.8 Ab |
| CV    | 12.6 |    | 6.9 |    | 16.1 |    |

* Mean comparison within each column (lower case letter) and within each row (capital letter) (Tukey’s test, \( P < 0.05 \)). CV are the coefficients of variation.
Ultrasound-treated lot 1 seeds had better germination after ageing than non-treated lot 1 seeds (table 1). This may be due to improved capacity to repair damaged proteins and enzymes. It is also probable that ultrasound improved mass transfer of nutrients from the endosperm to the embryo, allowing it to become better prepared to repair any damage. These ideas could also explain the enhancement of germination speed. Lots 2 and 3 did not show any improvement in germination or vigour following ultrasound treatment. Since these lots were prepared with pre-aged seeds, their metabolic capacity may have been damaged too much to permit repair. In contrast, the effects of seed ageing after the application of ultrasound (lot 1) was less severe than the ageing without ultrasound application.

Ultrasound technology does not negatively affect the germination nor vigour of barley seeds, which means that this novel technology can be used for enhancing the hydration process. Ultrasound could cause many physiological changes that improve seed vigour. For that reason, it is necessary to perform more studies in order to establish the true effect of ultrasound on seed germination and vigour and optimise the process. This technology could be very useful for studying the mechanisms involved in improving seed vigour, even of lots with high germination capacity.

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Apêndice 10: “Ultrasound technology enhances the hydration of corn kernels without affecting their starch properties”
Ultrasound technology enhances the hydration of corn kernels without affecting their starch properties

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\textbf{A B S T R A C T}

This work studied the effects of ultrasound treatment on the process of hydration of corn kernels, evaluating both the water uptake and its starch properties. For that, an ultrasound bath at a frequency of 25 kHz and volumetric power of 41 W/L was used. Furthermore, different treatments were applied in order to determine the mechanisms of enhancement of the hydration process (direct or indirect effects), by studying the hydration kinetics and the microstructure of the kernels. Finally, the rheological, thermal and structural properties of the starch extracted from the corn kernels (hydrated with and without ultrasound) were evaluated. Due to the particular behavior of the corn kernels during hydration, a two terms semi-empirical equation was proposed to explain the process, which contains two simultaneous ways related to the different mechanisms of water influx. Ultrasound significantly improved the hydration process, increasing water uptake and decreasing the process time by ~35%. In contrast to other grains, it was demonstrated that the enhancement of the process was only due to the direct effects (inertial flow and sponge effect) and not the indirect effects (micro-channels formation). Finally, it was demonstrated that the ultrasound treatment did not alter the properties of the starch. As a conclusion, it was shown that the corn kernels can be quickly hydrated using ultrasound treatment without modifying any of the properties of the starch, this being highly desirable for the starch industry.

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1. Introduction

Corn is one of the most important crops in the world, being widely used in the food, chemical, pharmaceutical and agricultural industries, due to its high starch content. Cornstarch, native, modified or cleaved, is used in food preparation, drug production, paper making, the textile industry, petroleum refining, among others uses (Bertolini, 2010). Moreover, it is used as a substrate for ethanol production. The first unit operation during the corn processing is the hydration of the corn kernels, which is carried out by immersion in water, as it is necessary for wet milling of the grain. The hydration of corn kernels is a lengthy batch process that may take up to 36 h (Singh and Eckhoff, 1996). Therefore, optimization of the hydration process is very desirable for the starch industry.

The ultrasound technology seems to be a promising option to improve the hydration process. Ultrasound technology has been widely studied as an alternative for improving food processing, in such operations as on defoaming, freezing, extraction, emulsification, hydration, drying (Awad et al., 2012) and germination (Miano et al., 2015a). The application of ultrasound showed good results in the hydration process of chickpeas (Yildirim et al., 2013), navy beans (Ghafoor et al., 2014), sorghum grains (Patero and Augusto, 2015) and common beans (Ulloa et al., 2015), reducing the process time and increasing the equilibrium moisture. However, the hydration process of a grain with high economic importance, such as corn, has yet to be studied, likewise the impact of this process on the starch properties. It should be a very important evaluation from an industrial point of view.

Furthermore, from a scientific point of view, much more than proving the effect of the ultrasound treatment, it should be important to identify the mechanisms that allow improvements in the process. As described by Miano et al. (2016), most of the published works only mention all the possible effects of the ultrasound...
treatment on the mass transfer processes, although each specific process and condition has its own mechanisms (direct and indirect effects). This then highlights the importance of evaluating the precise mechanisms that take place during corn hydration.

Finally, it is highly desirable to enhance the hydration process of grain without impairing its quality, especially its starch properties. In consequence, this work studied and described the effect of ultrasound treatment on the hydration process of corn kernels, verifying the possible effects on the starch properties by comparing the pasting properties, thermal properties and microstructure.

2. Materials and methods

2.1. Raw material and hydration process

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. (2008)) was used. This bath has its piezoelectric elements arranged below the tub. It generates 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 (Mason, 1991; Vinatouru, 2015). Further, the other good practices described by Mason (1991) and Vinatouru (2015) were also verified. Thus, the samples were placed in the parts where the waves had the highest and more homogeneous intensity.

Corn kernels (Zea mays var. amylacea; 12.68 ± 0.82 mm length, 8.45 ± 0.46 mm width and 4.27 ± 0.46 mm thick; kindly provided by Ingredion Brazil) with a moisture content of 12.55 ± 0.55% d.b. (g water/100 g of dry matter) were used. For the hydration process, 15 g of pre-selected (only intact grains were used) kernels were soaked in 4 L of distilled water at 25 °C with and without using ultrasound. During the hydration process, the samples were periodically drained, superficially dried and their moisture content was obtained by mass balance. The sampling was carried out every 20 min for the first hour and every 30 min from then on, until constant mass was reached. The hydration process was performed at constant temperature and in triplicate.

2.2. Hydration mechanisms and effect of the ultrasound treatment

Ultrasound enhances the mass transfer due to two possible mechanisms: direct and indirect effects produced by the alternate expansion and rarefaction of the sound wave. Therefore, experiments were carried out in order to evaluate the mechanisms that take place during the hydration of corn kernels with the ultrasound treatment.

To verify whether the micro-channels were formed during the process (the so-called indirect effects), the kernels were pretreated with ultrasound, then hydrated conventionally. One layer of corn kernels was vacuum packed in order to treat the kernels with ultrasound without hydration. This pack was placed at the bottom of the water bath. To verify if the direct effects (“sponge effect” and inertial flow) took place during the process, the kernels were hydrated with different treatments with or without the ultrasound treatment. The first treatment (US + N) consisted of hydrating the kernels using ultrasound for the three hours, then hydrating them without ultrasound. The second treatment (N + US + N) consisted of hydrating the kernels without ultrasound for the first three hours. Over next three hours, they were hydrated with ultrasound, and finally they were hydrated without ultrasound (the conditions were determined after pretests and taking into account the time magnitudes to hydrate the grains). In addition, as described by Ramos et al. (2004), a varnish (nail polish; Risqué – Cosmed Industry Brazil) was used as a sealant to cover the pericarp or the tip cap in order to determine the effect of ultrasound on the hydration rate of these structures.

Finally, the microstructure of the corn kernels was analyzed by SEM analysis and X-ray diffraction. For SEM, the samples were cut in order to see the different tissues (pericarp, endosperm and tip cap) and dehydrated 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 operating at an acceleration voltage of 15 kV (LEO 435VP; Leo Electron Microscopy Ltd., Cambridge, England). SEM analysis was performed for corn kernels hydrated with and without ultrasound. For the X-ray analysis, the kernels were X-rayed using a model MX-20 DC-12 digital Faxitron X-ray, exposing them to the radiation at 20 kV for 10 s. The grains were subjected to X-ray at different positions in order to evaluate their internal structure.

2.3. Modeling of the hydration process

The corn kernels hydration kinetics were fitted using different equations, which describe a downward concave shape (DCS) behavior: the Peleg equation (Peleg, 1988), the Page equation (Page, 1949), the First order kinetics equation (Lewis, 1921), the Ibarz-González-Barbosa-Cánovas equation (Ibarz et al., 2004) and the equation proposed in this work (Table 1). The proposed model is an equation with two terms, similarly to that used to explain the drying process (Verma et al., 1985).

For that, the kernels moisture content at dry basis (M; g water/100 g of dry matter) versus time (min) of the hydration process was evaluated for each initial moisture. The data were fitted to the equations with a confidence level of 95% using the Levenberg-Marquardt algorithm in Statistica 12.0 (StatSoft, USA) software. The goodness of the fit of the models was evaluated by the coefficient of determination (R²) of the regression value, the root-mean-square deviation values (RMSD, Eq. (1)), and the normalized RMSD (NRMSD, Eq. (2)).

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

\[
\text{NRMSD} = 100 \left(\frac{\text{RMSD}}{M_{\text{experimental}}^{\text{maximum}} - M_{\text{experimental}}^{\text{minimum}}}\right) \tag{2}
\]

2.4. Starch properties

The starch from the corn kernels (hydrated with and without ultrasound) was extracted as follow (Ji et al., 2004): The kernels were soaked in a sodium metabisulfite solution (0.1% w/v) for...
72 h at 60 °C, followed by manual removal of the pericarp and germ with a spatula. The endosperms were then wet milled for 20 s (with distilled water) using a blender and sieved (60 and 325 mesh). The supernatant was washed twice with distilled water and filtered again. The filtrate was centrifuged at 3200 g for 5 min to separate the starch from the rest of the components (water, proteins and lipids). Finally, the starch was dried at 35 °C for 6 h in a flat tray and was lightly ground with a mortar and pestle.

In order to understand whether the starch of the hydrated corn kernels was affected by ultrasound, the following evaluation was performed. The pasting properties (rheological) were evaluated in a Rapid Visco Analyzer (RVA-S4A; Newport Scientific, Warriewood, NSW, Australia) using 3 g of sample (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 min⁻¹. The sample was then kept at 95 °C for 5 min, followed by cooling to 50 °C at a rate of 6 °C min⁻¹, and finally holding it at 50 °C for 2 min (Polesi et al., 2011).

The thermal properties of the starch were evaluated using a differential scanning calorimeter (DSC-60 Shimadzu, Japan). A corresponding sample of 3 mg of dry starch was placed in an aluminum pan with 7 μL of deionized water. The samples in the hermetically sealed pans were equilibrated at room temperature for 60 min before measurement. The scanning temperature was from 30 °C to 95 °C and the heating rate was 10 °C min⁻¹. An empty pan was used as reference (Soulaka and Morrison, 1985).

To obtain the X-ray diffraction profiles, the starch moisture was equilibrated in a desiccator containing saturated BaCl₂ solution (25 °C, a_w = 0.900) for 10 days. The patterns of X-ray diffraction were determined in an X-ray diffractometer (Miniflex II, Rigaku, Tokyo, Japan) using copper radiation, at a scanning speed of 2° per min, angle 2 (2θ) ranging from 4° to 50°, 40 kV, and 40 mA. The relative crystallinity of starch was quantitatively determined following the method proposed by Nara and Komiya (1983) with the Origin 7.5 software (Microcal Inc., Northampton, MA, USA). The data were smoothed with the Adjacent Averaging tool and plotted on graphs between 2 angles (2θ) ranging from 4° to 30°.

The structure of the starch was analyzed using Scanning Electronic Microscopy (SEM). A thin layer of dry starch (which was obtained using a brush) was sputtered with a 30-nm gold layer and observed in a scanning electronic microscope operating at an acceleration voltage of 20 kV (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England) (Polesi et al., 2011).

3. Results and discussion

3.1. Description of the hydration process

As expected, the hydration of the corn kernels showed a downward concave shape (DCS) behavior (Fig. 1) (Fernández-Muñoz et al., 2011; Marques et al., 2014; Ramos et al., 2004; Verma and Prasad, 1999). This behavior is described by a higher hydration rate at the beginning of the process, which drops continuously during the process. In contrast with other grains, the corn kernel was particularly characterized by very fast hydration in...
the first part of the process until a certain time when the hydration process starts to slow down. In fact, it seems that the corn kernels hydrate following two parallel processes. Therefore, it is very important to describe this phenomenon properly (Fig. 1).

The equilibrium moisture \((M_{e})\) was reached after approximately 840 min (14 h) of soaking, and is thus a very slow process in comparison with other grains. Further, the quantity of water absorbed was approximately 4.5 times the initial moisture content. This is similar to other starchy grains, like the ~3 times for wheat (Iglinathinath and Chattopadhyay, 1997), ~3.5 for rice (Bello et al., 2004) and ~3.3 times for sorghum (Paterno and Augusto, 2015); and very different to legumes (higher protein content), for instance, ~21 times for soy bean (Sopade and Obekpa, 1990), ~30 times for chickpeas (Gowen et al., 2007), ~11 times for Andean lupin (Miano and Augusto, 2015). It confirms that the composition affects the water holding capacity of the grains.

The corn kernels have different tissues, structures and cavities (Fig. 2a and b). The first structures that have a contact with water are the tip cap and the pericarp. Since the tip cap is formed by tube cells of ~11 μm of diameter (Fig. 2c and d), the water enters by capillarity while the pericarp is formed by layers of dead cells with high porosity (Fig. 2e) where the water enters by diffusion. Despite that the water enters faster by the tip cap, most of the hydration occurs through the pericarp since the pericarp area is much larger than the tip cap area, (Ramos et al., 2004; Ruan et al., 1992). In addition, there is a space between the endosperm and germ (Fig. 2), which is initially "empty" and then fills with water due to capillary flow from the tip cap. This explains the existence of two parallel processes during the hydration of the corn kernels. In one process \((M_1)\), the hydration is very fast and short. In the other process \((M_2)\), the germ and endosperm are probably hydrated from the bulk water, the film of water between the endosperm and pericarp and the water in the space between the endosperm and the germ (Fig. 1). This process is slow and long in comparison with the first process, probably due to the compactness of the endosperm, especially in the vitreous region (Fig. 2g).

Due to this hydration behavior (a very high hydration rate at the beginning of the process and a slower hydration rate in the rest of the process), many common semi-empirical equations do not successfully fit the hydration of corn kernels. Therefore, a suitable equation had to be found.

### 3.2. Curve fitting of the hydration process

The data of the corn kernel hydration kinetics (25 °C) were plotted and fitted using the most commonly used hydration kinetics model (Table 1). However, many of them did not fit well the experimental data (Fig. 3), even presenting a suitable statistical fit. Moreover, some models have parameters that are difficult to explain in a physical way. Therefore, a new model was proposed and evaluated here.

The process of hydration of the corn kernel takes place following two parallel processes due to the morphology of the grain, as described above. In addition, the water enters by diffusion and capillarity because of the complex structure of the kernels, with different tissues and cavities. Both mechanisms take place simultaneously. However, the capillarity phenomenon is probably predominant in one process of the hydration due to the porosity of the tip cap and the "empty spaces" in the grain and the diffusion phenomenon probably dominates the other process due to the compactness of the endosperm and germ. Therefore, this suggests that the mechanisms of water influx into the corn grains during the hydration process are more complex, the evaluation of further equations and mathematical models that can explain the water flow route being necessary.

It is interesting to mention that a composite model that combines two terms of the Peleg equation (summing the terms) in series was used to describe wheat-grain hydration (Paquet-Durand et al., 2015). This model considered the first term for the hydration of the bran layer, and the second term for the hydration of the endosperm. Therefore, these explained the model by the hydration of each tissue separately. In contrast, it was shown that the corn-kernels hydration takes place following two parallel processes, and simultaneous hydration of the tissues. As a result, the model proposed by those authors (Paquet-Durand et al., 2015) was not evaluated, and the following one was proposed.

For those reasons, based on this work, we are proposing the following semi-empirical model: A two-term equation is proposed to describe this behavior (Table 1, Fig. 3, and Eq. (10)). This equation derives from the following reasoning.

The gained moisture as function of time is represented by Eq. (3), where \(k\) is the global coefficient of mass transfer, related to the diffusion and capillary mechanisms combined. The value of \((M_e - M)\) is the driving force:

\[
\frac{dM}{dt} = k \cdot (M_e - M)
\]

This equation can be integrated with the appropriate boundary limit (Eq. (4)), thus obtaining Eq. (5), which can be rearranged as Eq. (6):

\[
\int_{M_0}^{M} \frac{dM}{(M_e - M)} = \int_0^t k \cdot dt
\]

\[
\frac{M_e - M_t}{M_e - M_0} = \exp(-k \cdot t)
\]

\[
M_t = M_e - (M_e - M_0) \cdot \exp(-k \cdot t)
\]

The hydration of corn kernels takes place by two parallel processes. Thus, the grain moisture \((M_t)\) can be represented by the initial moisture content plus the contribution of the two processes of hydration \((\Delta M_1)\) (Eqs. (7) and (8)):

\[
M_t = M_0 + \Delta M_1
\]

\[
\Delta M_t = \Delta M_{1t} + \Delta M_{2t}
\]

The contribution of one process can be represented by "p", which would be the ratio between the water absorbed by one process and the total absorbed water during time (Eq. (9)). The other process would contribute "1-p", since the sum of both parallel processes would represent the total absorbed water by the kernel (Eq. (10)).

\[
p = \frac{\Delta M_{1t}}{M_t - M_0}
\]

\[
1 - p = \frac{\Delta M_{2t}}{M_t - M_0}
\]

Therefore, each process \((\Delta M_{1t} \text{ and } \Delta M_{2t})\) contributes with a complementary percentage \((p \text{ and } 1-p)\) of the total gained moisture \((M_t - M_0)\) (Eqs. (11) and (12)).

\[
\Delta M_{1t} = p \cdot (M_t - M_0)
\]

\[
\Delta M_{2t} = (1-p) \cdot (M_t - M_0)
\]
\[ \Delta M_{2i} = (1 - p) \cdot (M_{i} - M_0) \quad (12) \]

Then, replacing these equations into Eq. (8), Eq. (13) is obtained:

\[ \Delta M_i = p \cdot (M_i - M_0) + (1 - p) \cdot (M_{i} - M_0) \quad (13) \]

Incorporating Eq. (6) into the values of \( M_{1i} \) and \( M_{2i} \) in Eq. (13) and rearranging it, Eq. (14) is obtained:

\[ \Delta M_i = (M_{\infty} - M_0) \cdot \left[ p \cdot (1 - \exp(-k_9 \cdot t)) + (1 - p) \cdot (1 - \exp(-k_9 \cdot t)) \right] \quad (14) \]

Finally, by incorporating Eq. (14) into Eq. (7), the proposed model (Eq. (15)) is obtained:

\[ M_i = M_0 + (M_{\infty} - M_0) \cdot \left[ p \cdot (1 - \exp(-k_9 \cdot t)) + (1 - p) \cdot (1 - \exp(-k_9 \cdot t)) \right] \quad (15) \]

This equation has a suitable fit to the experimental data.
(R² = 0.999; RMSD = 0.15% d.b.; NRSD = 0.42%; Table 1; Figs. 1 and 3). Furthermore, and most importantly, it explains the process, estimating the equilibrium moisture well and having explainable parameters.

This equation has four parameters: the equilibrium moisture content (\(M_\infty\)); the parameter “p” which means the fraction of water that is absorbed by the kernel in the fast/short process; and the two global mass transfer parameters (\(k_8\) and \(k_9\)) (Table 1 and Fig. 3).

The \(p\) value was 0.191 (Table 1). It means that approximately 19% of the total water is absorbed in one process (where the predominant mechanism is capillarity) and the other 81% is absorbed in the other process (where the predominant mechanism is diffusion). In fact, this can also be graphically confirmed in Fig. 1.

By using the parameter “p”, the fast/short process duration can be estimated. The value of the maximum absorbed water \((M_\infty - M_0)\) was 44.62 g water/100 g d.b. The 19.1% of this absorbed water was 8.52 g water/100 g db. Knowing that the initial moisture in the corn kernels was 12.55 g water/100 g d.b, then, the kernels would have reached 21.07 g water/100 g d.b of moisture content during the fast/short process. Consequently, the necessary time to reach that moisture level was approximately 29.5 min (Fig. 1).

The value of \(k_9\) is ~20 times higher than the \(k_9\) value, reinforcing that the rate of water absorption during the fast/short process is much higher than during the slow/long process. It is interesting to observe that although the fast/short process has a very high water absorption rate, it has a short duration (~30 min, comparing with the ~840 min (14 h) of hydration). Therefore, the proposed semi-empirical model successfully explained the hydration of the corn kernel following its morphology and having explainable parameters, which is very desirable.

### 3.3. Effect of the ultrasound treatment on the corn-kernel hydration process and description of the mechanisms of enhancement

Fig. 4 shows that the ultrasound treatment greatly enhanced the hydration process of the corn kernels. This treatment significantly reduced the process time by approximately 35%, which represents a reduction of almost 300 min. Since the hydration process is a discontinuous and time consuming step, this reduction in time is very desirable for the industries, as it improves the efficiency of the process.

The ultrasound technology increased the hydration rate and the equilibrium moisture content of the corn kernels. These desirable results were also reported for chickpeas (Yıldırım et al., 2013), navy beans (Ghafoor et al., 2014), sorghum grains (Patero and Augusto, 2015) and common beans (Ulloa et al., 2015). The principal mechanisms of enhancement attributed by these works were indirect (micro-channel formation by acoustic cavitation) and direct effects (inertial flow and sponge effect). The existence of both was demonstrated by Miano et al. (2016) for sorghum grains, although the existence or absence, as well as the relative importance of each one, was demonstrated to be a function of the food water activity. Furthermore, the fact that each grain behaves differently due to its morphology and composition highlights the need for further evaluation.

The direct effects take place due to the contraction and expansion of the tissues and cell by the compression and rarefaction of the ultrasonic waves causing the fluid pumping or an inertial flux. This is called as sponge effect due to the similarity to a sponge squeezed and released repeatedly. On the other hand, the ultrasonic wave traveling causes the acoustic cavitation too. Due to the pressure differences in the fluid (water), bubbles of gas start to increase their size until exploding with a high energy, causing cell disruption or matrix rupture. This indirect effect causes the formation of micro cavities and micro channels that, for mass transfer processes, is very important (Miano et al., 2016). Many works of hydration have attributed these phenomena as a principal cause of process enhancement (Ghafoor et al., 2014; Ulloa et al., 2015; Yıldırım et al., 2014). However, many of them did not analyze if they take place at the same time or only one of them. Therefore, different special treatments were performed in order to evaluate which mechanism improved the hydration of corn kernels with the
ultrasound treatment (Fig. 5).

When the corn kernels were hydrated with ultrasound for periods of 3 h, they only showed an enhancement during that time (treatment US + N and N + US + N, Fig. 5a). Nevertheless, when they were again hydrated conventionally, the hydration rate slowed down, reaching the rate of the conventionally hydrated corn kernels. This means that the corn-kernel hydration process needs to be assisted by ultrasound in order to be improved, demonstrating that the direct effects took place and that there were not indirect effects (micro-channel formation).

For instance, the pressure differences in the water and inside the kernels due to the wave propagation and the “sponge effect” could cause water pumping inside the grain (the so-called inertial flow), thus accelerating the hydration process. It was demonstrated that for sorghum grains (Miano et al., 2016) with low water activity, the micro channels were not formed by ultrasound treatment, because the low water vapor pressure prevented acoustic cavitation.

The vacuum-packed corn kernels with high water activity (water activity of 0.988 ± 0.005; moisture of 40 g water/100 g d.b), which were treated with ultrasound (at different times), were then

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**Fig. 4.** Effect of ultrasound on the hydration kinetics of corn kernels. The dots are the experimental values. The vertical bars are the standard deviation and the lines are the estimation of the proposed model (Eq. (11)).

**Fig. 5.** Evaluating the mechanism of water absorption improvement due to the ultrasound technology: a. Treatments to evaluate the direct effects. b. Treatments to evaluate the indirect effects (micro-channels formation). c. Comparison between the hydration kinetics without and with ultrasound through the tip cap. d. Comparison between the hydration kinetics without and with ultrasound through the pericarp. The dots are the experimental values and the vertical bars are the standard deviation.
hydrated conventionally, without obtaining improvement in the hydration process being obtained (treatment 3 h and 5 h, Fig. 5b). It means that there was no formation of micro channels, even at a high water activity. The absence of micro-channel formation in corn kernels can thus be related to their microstructure.

Micro-channel formation due to the ultrasound treatment was demonstrated for sorghum grains (Miano et al., 2016) when they had high water activity (0.985 ± 0.002). However, these channels were not formed in corn kernels. This could be due to the higher compactness of the vitreous endosperm in this grain (Fig. 2). In corn, the endosperm cells are packed with starch granules embedded in a continuous matrix of amorphous proteins (Kulp, 2000), which probably avoid the formation of micro channels by cavitation (at least at the current power conditions of 41 W/L). In fact, Fig. 6 shows that any micro channels were formed in the different tissues of the corn kernel (pericarp, tip cap and endosperm). Therefore, the enhancement of the hydration process for corn kernels could only be due to the direct effects. Furthermore, due to the rigid structure of the corn kernel, it is likely that the enhancement is preferably due to the inertial flow, as the “sponge effect” needs a series of contractions and expansions of the existing channels to take place.

Fig. 5c and d shows that ultrasound enhances the water flow through both the pericarp and the tip cap. When the pericarp was sealed (Fig. 5c), hydration only occurred through the tip cap. Ultrasound improved the hydration process through the tip cap. The tip cap is very porous, with structures similar to tubes (Figs. 2 and 6). Therefore, the water is probably pumped through these “tubes” because of the ultrasonic wave traveling.

Fig. 5d shows that, when the tip cap was sealed (Fig. 5d), hydration only occurred through the pericarp. Ultrasound also improved the hydration rate in this case, the improvement being very significant since the hydration kinetics of these covered kernels was the same as the hydration kinetics of the uncovered kernels, both with ultrasound (i.e., the mass transfer improvement due to the ultrasound was greater than the suppression of mass transfer through the tip cap).

The microstructure analysis (Fig. 6) shows that, as expected due to the hydration results (Fig. 5), there was no structural change on the kernel tissues. In addition, it demonstrates that there was no micro channel formation in the seed coat (pericarp), tip cap and endosperm. The compactness and lack of intercellular spaces in the endosperm, and the lack of porosity of the pericarp can also be seen, indicating that the water transfer in these tissues is mainly by diffusion. In contrast, the porosity of the tip cap permits the water to enter by capillarity.

Finally, Fig. 7 shows the effect of ultrasound on the parameters of the proposed model. The equilibrium moisture (M∞) significantly increased by 5%. Since it was demonstrated that micro channels were not formed in the corn kernels, the explanation for this improvement can be that the ultrasound keeps the porous and internal cavities unobstructed, permitting the increase in the water holding capacity.

The “k” parameter did not change significantly. This means that despite the ultrasound treatment, the fraction of water absorbed in each process (fast/short and slow/long) was the same (Fig. 4). This parameter was called the “shape factor” by Verma et al. (1985) and they stated that this parameter did not change during the drying process at different temperatures. Therefore, it is a characteristic parameter of the grain, related to its structure and composition. As this parameter did not change, it means that ultrasound did not change the structure of the corn grains. In fact, this is in accordance with the non-formation of micro channels during the ultrasound-assisted hydration of corn (agreement between the results of the hydration processes, mathematical modeling and microstructure).

The k9 parameter is related to the fast/short process, when the capillary flow is probably predominant. This did not change significantly with the application of ultrasound. The hydration of the kernels in this process is too fast due to the capillarity for the possible enhancement caused by the ultrasound to be significant. On the other hand, the k9 parameter, which is related to slow/long process, where diffusion is predominant, increased significantly (30%). The area for water diffusion is too large in the slow/long process since the water is transferred from the bulk water, the spaces between the germ and endosperm and between the endosperm and pericarp. Ultrasound maintained the water supply for

![Fig. 6. Effect of ultrasound on corn kernel microstructure (SEM, 20 kV; the magnifications are shown in the figures). The captions C and US indicate the treatments without and with ultrasound respectively. The lowercase letters a, b, c indicates the observed tissue: pericarp, tip cap and endosperm respectively.]

![Fig. 7. Effect of ultrasound on the parameters of the proposed model (C: without ultrasound; US: with ultrasound) (Eq. (11)). The letters above the bars represent the mean comparison test (p < 0.05).]
the diffusional phenomena since the porosity and spaces inside the kernels remained full of water through the direct effects, explaining the increase in $k_0$.

In conclusion, it was demonstrated that ultrasound treatment (41 W/L of volumetric power and 25 kHz of frequency) enhances the hydration of corn kernels, accelerating the process and increasing the equilibrium moisture. However, it is important to evaluate the impact of this technology on the properties of the grain starch.

3.4. The effect of ultrasound treatment on the starch properties

The starch extracted from the corn kernels hydrated with and without ultrasound was analyzed in order to verify any changes on its properties. Table 2 and Fig. 8 show no significant differences among the results of RVA, DSC and X-ray diffraction. In addition, as the SEM images show, the structure of the starch was not changed (Fig. 9). This means that the ultrasound-assisted hydration did not affect the starch properties of the corn compared with the conventional process.

In contrast, previous works demonstrated that ultrasound treatment affects the starch granules when they are free in suspension and ultrasound is applied directly, modifying the pasting and thermal properties and damaging their structure (Amini et al., 2015; Sujka and Jamroz, 2013; Zbu et al., 2012). However, when the starch is packed into the cells of the endosperm of the corn kernels, it is probably protected from the acoustic cavitation due to its compactness, which explains the results observed here.

No studies have evaluated the effect of ultrasound treatment on the properties of starch during hydration of grains. In fact, only the pasting properties of navy-bean flour hydrated with ultrasound have been studied (Chaofoor et al., 2014). In that work, the apparent viscosity was increased by ultrasound. However, the navy-bean flour is made up not only of starch, but also proteins and other components. In addition, the quantity of starch is ~23% (Hoover and Ratnayake, 2002) compared to ~73% in corn kernels. Therefore, that apparent increase in viscosity could be due to the modification of other components, such as proteins, and not the modification of the starch. Therefore, it can be concluded that the corn kernels can be hydrated using ultrasound treatment (41 W/L of volumetric power and 55 kHz of frequency) without affecting the quality of the starch. In fact, it is a highly relevant and desirable result from an industrial point of view.

4. Conclusions

Ultrasound treatment (41 W/L of volumetric power and 25 kHz of frequency) enhances the process of hydrating corn kernels, reducing the time required by ~35%. It was demonstrated that the improvement in the process was due to direct effects caused by the ultrasound and not to the formation of micro channels. Moreover, since the corn kernels hydrate by capillarity and diffusion following two parallels process of hydration, a suitable semi-empirical model (two terms equation) was proposed to explain the hydration process of the corn kernels in two parts. Finally, the pasting, thermal and structural properties of the starch obtained were compared, no significant differences being found between the hydrated corn kernels (with and without ultrasound). Therefore, the hydration process of corn kernels can be improved using ultrasound treatment without changing their starch quality. It is highlighted that the results obtained are highly relevant from both the scientific and industrial point of view.

Table 2

Analysis of the starch extracted from corn kernels hydrated conventionally and with ultrasound (mean values ± standard deviation). The letters next to the values represent the mean comparison test ($p < 0.05$).

| Analysis               | Result                  | Starch sample                           |
|------------------------|-------------------------|-----------------------------------------|
|                        | RVA                     | Conventional hydration                 | With ultrasound                           |
|                        |                        |                                          |                                          |
| Peak 1 (mPa.s)         | 2618.25 ± 33.54a        | 2629.25 ± 13.12a                        |
| Trough 1 (mPa.s)       | 1619 ± 46.15a           | 1665 ± 39.35a                          |
| Breakdown (mPa.s)      | 999.25 ± 48.42a         | 964.25 ± 40.93a                        |
| Final apparent viscosity (mPa.s) | 3305.5 ± 41.4a         | 3407.25 ± 21.7a                        |
| Setback (mPa.s)        | 1686.5 ± 34.72a         | 1742.25 ± 39.25a                       |
| Peak time (min)        | 8.58 ± 0.04a            | 8.63 ± 0.09a                           |
| Pasting temperature (°C) | 71.14 ± 0.06a           | 71.5 ± 0.45a                           |
| Pasting enthalpy (kJ/kg) | 72.73 ± 0.01a          | 73.46 ± 0.08a                          |
|                        | Pasting (kJ/kg)         | -13.1 ± 0.92a                          | -11.95 ± 0.69a                          |
|                        | Relative crystallinity  | 20.4 ± 0.4a                            | 20.2 ± 0.6a                             |

Fig. 8. Evaluation of the cornstarch properties extracted from corn kernels hydrated conventionally (C) and with ultrasound (US). a. X-ray diffraction profile, b. DSC profile and c. RVA profile.

Fig. 9. Evaluation of the cornstarch microstructure extracted from corn kernels hydrated conventionally (C) and with ultrasound (US). (SEM, 20 kV; the magnifications are shown in the figure).
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Apêndice 11: “Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components”
Enhancing mung bean hydration using the ultrasound technology: description of mechanisms and impact on its germination and main components

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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 spendper 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. 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). 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.

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, navy beans, chickpeas, common
beans\textsuperscript{15} and corn kernels\textsuperscript{16}, as well as on the rehydration of other kind of food such as sea cucumber\textsuperscript{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\textsuperscript{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 (\textit{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\textsuperscript{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\textsuperscript{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\textsuperscript{4}, Adzuki beans\textsuperscript{3,21,22}, Cowpea\textsuperscript{8} and Italian Lima beans\textsuperscript{23}. Further, its hydration behavior changes to the DCS when the initial moisture is increased.

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\textsuperscript{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)\textsuperscript{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

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{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) \( \tau \) (Equation 3) (e) \( k \) (Equation 4) and (f) \( M_\infty \) (Equation 5).}
\end{figure}
cells, reducing the space between the seed coat and the cotyledon, and the closure of the hilum, avoiding the water entrance\(^2\). Secondly, the low moisture content may cause that the seed coat components pass from the rubbery state to the glassy state reducing its permeability\(^7\).

The state transition of the grain components is related to the grain’s water activity. Based on recent works\(^2\),\(^3\)\(^7\), there is a critical moisture content (due to a critical water activity value) when the hydration changes its behavior. According to Reid and Fennema\(^8\), 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)\(^9\) were similar to the obtained for Adzuky beans (A = 9.75 and B = 0.46)\(^3\), which means that the values could be similar for aleuro-amylaceous grains.

\[
M = \frac{a_w^{0.49}}{1 - a_w}
\]  

(1)

**Water pathway during mung bean hydration.** As all beans from the \(\textit{fabaceae}\) 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\(^3\). Further, osteoesclereids cells are presented in the seed coat, which have large intercellular spaces probably contributing to the water lateral distribution\(^2\). Figure 2e shows the hilum, microple and raphé 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\(^1\), while for Carioca beans and black beans, the entrance of water is by the microple, the hilum and the raphé\(^5\) 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\(^3\) and for Andean lupin\(^4\). Although there is a probability that the water enters through the microple or raphé, 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 microple and raphé.

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. Similar to previous works with soybean\(^1\), Andean lupins\(^4\) and adzuki beans\(^2\), 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\(^7\). 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 \textit{et al.} model (Equation 2; ref. 8) was used at each moisture content obtaining a successful fit (Table 1). This
The model has explainable parameters with physical meaning. Therefore, they were useful to explain the behavior change of the hydration process.

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

where \( M_t \) is the sample moisture content (% d.b.) at each time \( t \); \( M_\infty \) is the equilibrium moisture content; \( \tau \) 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 \( \tau \) 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 disappears and the sigmoidal

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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.
increased. However, this result was not presented for Adzuki beans21 nor lentils26. This can be explained according the following hypothesis. Mung bean is characterized by a fast germination 35, and due to the germination equation (Equation 5; R2 of 0.99; Fig. 1f).

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 3; R2 of 0.99; Fig. 1d).

Netial equation was used to model the effect of the initial moisture content on this parameters obtaining the parameter (Equation 4; R2 of 0.97; Fig. 1e).

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; R2 of 0.97; Fig. 1e).

Finally, the equilibrium moisture content parameter \( M_\infty \) value decreased as the initial moisture content increased. However, this result was not presented for Adzuki beans21 nor lentils26. This can be explained according the following hypothesis. Mung bean is characterized by a fast germination35, 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 activated36), 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; R2 of 0.97; Fig. 1f).

### 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).

| Initial Moisture Content (% d.b.) | \( \tau \) (min) | \( k \) (min\(^{-1}\)) | \( M_\infty \) (% 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                             | 7.8 ± 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     |

### 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 grain37.

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 reactivation37. 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 \( M_\infty \) 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 absorption35. 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.
mung bean has a short phase II, of approximately 2 h, 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, navy beans, sorghum grains, common beans, and corn kernels. Most of these works attributed the improvement to the direct and indirect effects of ultrasound on mass transfer processes – strictly physical mechanisms of mass transfer improvement.

However, it was demonstrated that the ultrasound technology enhances the seeds vigor, probably by enhancing its metabolism. 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. 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. Consequently, both the ultrasound 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, 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, 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 decreased 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, switchgrass, pea and grass seeds. 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. Therefore, ultrasound could have improved these 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. 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.

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 Kaptsa et al. model 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 ($M_e$) was fixed and considered the same to the control treatment. Despite this consideration, the Kaptsa et al. model successfully fitted the experimental data (R² of 0.99 for both treatments). The parameters $k$ and $\tau$ 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 ± 0.0004 min⁻¹ to 0.0150 ± 0.0016 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 $\tau$, 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. 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 osteosclerids cells of the seed coat.

There is not any work in the literature relating the ultrasound-assisted hydration of sigmoidal behavior hydration process. 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...
without and with ultrasound, respectively. Both shows an oval to spherical shaped with smooth surface without fissures as verified by Rupollo et al. 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. 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. 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.

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 ultrasound assisted hydrated beans.
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. 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. 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. 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. 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. 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...
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.

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**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.
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 being 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.

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 foods. 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:

\[
M = A \left( \frac{a_w}{1 - a_w} \right)^B
\]  

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. 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. 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. Further, the other good practices described by 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 (NRMbSD, 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).

\[
\text{RMSD} = \sqrt{\frac{\sum_{i=1}^{n} (M_{experimental} - M_{model})^2}{n}}
\]
Starch and flour evaluation. The starch of the mung beans (hydrated with and without ultrasound) was extracted as follows: 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 · 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 · 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 starch⁶¹ and 23.8% of protein²⁰ 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 10 N. The gelatinized starch was placed on the plate 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 30 min, 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 min⁵². An aliquot of 4 mL was then eluted in the chromatographic column upwardly. A solution containing 25 mmol·L⁻¹ of NaCl and NaOH 1 mmol·L⁻¹ was used as eluent at a rate of 60 mL·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, Biochrom, England).

Statistical evaluation. When relevant, statistical analysis was performed to the treatments through analysis of variance (ANOVA) and Tukey's test (P < 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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Apêndice 12: “Ultrasound enhances mass transfer in Andean lupin grains: hydration and debittering”
Ultrasound enhances mass transfer in Andean lupin grains: hydration and debittering *

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Abstract
This work aimed to study the effect of ultrasound technology in the Andean lupin grains (Lupinus albus Sweet.) hydration process, as well to verify if this technology can improve their alkaloids extraction. The hydration process was conducted using an ultrasonic water bath with 41 W/L of volumetric power and 25 kHz of frequency was used. The hydration kinetics of this grain showed a sigmoidal behavior, which was improved by reducing the processing time almost 40 %. The lag phase was reduced about 13 %, and the equilibrium moisture content was increased about 14 %. Finally, the alkaloid extraction during the hydration process was also improved, extracting 21 % more compared to the normally extracted in the conventional hydration process.

Keywords: hydration, Lupinus mutabilis, debittering, grains

*By describing an important process for pulses industrialization, the author pays tribute to the International Year of Pulses (2016) of the Food and Agriculture Organization (FAO) of the United Nations.
1. Introduction

Andean lupin (*Lupinus mutabilis* Sweet) is a grain that grows in the Andean part 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 (each 100 g of lipid contain 40.4 g of omega-9, 37.1 g of omega-6 and 2.9 g of omega-3 fatty acids) (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 by facilitating the alkaloid extraction in the following steps (Carvajal-Larenas et al., 2013). However, the hydration is a time spender 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) and corn kernels (Miano et al., 2017). Ultrasound consists of using the sound energy, with frequencies between 20 kHz and 500 kHz with high power, which causes structure and physical chemical changes on food (Awad et al., 2012). Further, it also affects the transport phenomena, by both direct and indirect effects. The direct effects of using this technology are related to the acoustic wave traveling, which causes pressure differences in the food, contracting and expanding the tissues as a sponge. It causes pumping of fluids and pores unblocking. Those mechanisms are known as “sponge effect” (Floros and Liang, 1994) and “inertial flow” (Patero and Augusto, 2015). The indirect effects are caused by the acoustic cavitation, which changes the food structure by tissue and cell disruption, with the consequent formation of micro cavities and micro channels, helping the entrance or exit of substances (Miano et al., 2016, Rodrigues et al., 2009).

Although there are some works improving the hydration process using the ultrasound technology, there is still a lack of studies in grains with sigmoidal behavior. Further, there is not any work using this technology in the Andean lupin, nor about the extraction of alkaloids using this promising technology. Therefore, this work aimed to study the effect of the ultrasound technology on the Andean lupin hydration kinetics, as well as to evaluate if the ultrasound also improves the alkaloid extraction during this process.
2. Materials and Methods

2.1 Raw Material

Andean lupin grains (*Lupinus mutabilis* Sweet) (3.65 ± 0.01 % d.b. of moisture, 9.98 ± 0.64 mm length, 8.39 ± 0.41 mm width and 6.02 ± 0.35 mm thick) were purchased in a local market of Trujillo – Perú. Before being hydrated, the grains were selected by eliminating those that were not intact.

2.2 Hydration Process

2.2.1 Conventional hydration

To perform the hydration process, approximately 10 g of grains were placed in a net bag and soaked inside a Beaker with 250 mL of distilled water at 25±1 °C (water bath Dubnoff MA 095 MARCONI, 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, Japan). 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 until constant mass was reached. The hydration process was performed in triplicate.

2.2.2 Ultrasound assisted hydration

During the experiments, an ultrasonic bath (Q13/25, Ultronique Brazil) with a frequency of 25 kHz and a volumetric power of 41 W/L (determined following the method described by Tiwari et al. (2008)) 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 (Vinatoru, 2015, Mason, 1991). Further, the other good practices described by (Vinatoru, 2015, Mason, 1991) were also verified. Thus, the samples were placed in the parts where the waves had the highest and more homogeneous intensity.

The ultrasound-assisted hydration was performed in the ultrasonic water bath with 4 L of water at 25±1 °C using 160 g of grains to maintain the same grains/water relation to the conventional processing. The grains were placed into net bags and placed on the bottom of the ultrasonic water bath. The data were collected as the conventional hydration process explained above (section 2.2.1).

2.4 Mathematical description

The Andean lupin grains hydration kinetics was modeled using the sigmoidal equation of Kaptso et al. (Equation 1; (Kaptso et al., 2008)). For that purpose, the grain moisture content at dry basis (M % d.b.) was tabulated versus the hydration time (min). 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.

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

(1)

Where \( M_t \) is the sample moisture content (% d.b.) at each time \( t \); \( M_{eq} \) is the equilibrium moisture content; \( \tau \) 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.

Finally, the goodness of fit was evaluated by the \( R^2 \) regression value 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 2) results in three parameters that were used to evaluate 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).

\[ M_{model} = a \cdot M_{experimental} + b \]  

(2)

2.5 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 organic solvents, which are then volumetrically quantified by titrating with Sulphur acid.

3. Results and discussion

As expected, the Andean lupin grains showed a sigmoidal hydration behavior (Miano et al., 2015), which is attributed to the seed coat. It means that this grain has an initial lag phase followed by an increment of the hydration rate and finally a decrease of the hydration rate until reaching the equilibrium moisture content (Figure 1a). In addition, Figure 1a shows that the Kaptso et. al. model successfully fit the hydration kinetics data (Conventional hydration fit: \( R^2 = 0.995; a = 0.976; b = -1.92 \); Ultrasound assisted hydration fit: \( R^2 = 0.995; a = 0.974; b = -2.66 \)).

Further, Figure 1 shows that the ultrasound technology significantly enhanced the hydration process of Andean lupin. This technology reduces the hydration process time almost 40% (25 °C). This value is similar to the work of Miano et al. (2017), that reduced 35% of the processing time (25 °C) for corn kernels (Zea mays); Patero and Augusto (2015), which reduced 40% of the processing time (25 °C) for sorghum kernels (Sorghum sp); and to the
work of Ghafoor et al. (2014) that reduced 45 % of the processing time \((16 \, ^{\circ}{C})\) for navy beans \((\textit{Phaseolus vulgaris})\) – all the works using volumetric power of the same magnitude. It should be mentioned, that this result depends on the applied volumetric power of ultrasound, as well as the structure-composition characteristics of the grain. Further, it is important to highlight that there are only works in the literature studying the use of this technology for the hydration of grains with downward concave shape behavior of hydration. Consequently, there is the necessity to study grains with sigmoidal behavior of hydration, since they have a particular way of hydration due to the presence of a lag phase.

The lag phase time \((\tau)\) of the hydration process was reduced \((p<0.05)\) from \(193 \pm 11\) min to \(169 \pm 8\) min (Figure 1b). 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 mechanisms called “sponge effect” (Floros and Liang, 1994, Mulet et al., 2003, Miano et al., 2016) and “inertial flow” (Patero and Augusto, 2015). As the ultrasound wave passes through the grain, its pores and spaces (for example between the cotyledon and seed coat) expand and compress due to the pressure differences, pumping the water to the inside. This caused a rapid filling of the cotyledon – seed coat space with water, thus reducing the lag phase. Another ultrasound mechanism that improves the mass transfer phenomenon is the micro channel formation due to the acoustic cavitation. However, as this mechanisms is more likely to happen in food with high water activities (Miano et al., 2016), and as the grain hydration already showed improvement at the beginning of the process (when it has low water activity – Figure 1a), the micro channel formation is probably negligible for the lag phase time reduction.
Figure 1. (a) Hydration of Andean lupin grains with (US) and without (CONTROL) ultrasound (25 °C, 41 W/L and 45 kHz). (b, c, and d) Effect of ultrasound technology on the Kaptso et al model parameters (Equation 1). The dots are the experimental values; the bars are the standard deviation and the curves are the model values.

In addition, the equilibrium moisture content ($M_{eq}$) of the Andean lupin grain was significantly ($p<0.05$) increased by ultrasound, from $140 \pm 3 \%$ d.b. to $160 \pm 3 \%$ d.b (Figure 1c). This result was similar for corn kernels (Miano et al., 2017), chick peas (Yildirim et al., 2011) and sorghum kernels (Patero and Augusto, 2015). As the grains absorb water, their water activity increases, increasing the probability of the acoustic cavitation takes place and forming micro channels. The $M_{eq}$ increase 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., 2017) 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 Figure 1d shows this parameter tends to increase. As the increase in the water influx rate due to the
ultrasound technology was demonstrated for other grains, such as corn kernels (Miano et al., 2017), 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). 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, related to the its formation, 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. which is an enhancement of almost 21 %. This is an interesting result since it means that if the ultrasound process is used in the following extraction stages, the debittering process time could be reduced, as well as the use of water.

![Figure 2](image_url)

**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).

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 the hydration process time 40 %, as well as the alkaloid content almost 21 %, when compared to the conventional process. These results demonstrate the potential use of this technology in many processes of grains industrialization.
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Resumo Simples

O processo de hidratação é uma etapa demorada e complicada no processamento de grãos, sendo necessários estudos para sua melhoria. O presente trabalho descreve estudos para melhorar o entendimento do fenômeno de hidratação de grãos. O trabalho foi desenvolvido de forma a caracterizar o comportamento de hidratação de diferentes grãos, propor mecanismos para descrever o processo, descrever matematicamente os dados obtidos e melhorar o processo utilizando diferentes temperaturas e a tecnologia de ultrassom.

Simple Abstract

The hydration is a slow and complicated step of the grain processing. Consequently, studies are necessary in order to enhance it. The present work describes different studies for the understanding of the grains hydration phenomenon. The work was performed to characterize the hydration behaviour of different grains, proposing the mechanisms that describe the process, mathematically describing the obtained data and enhancing the process using different temperatures and the ultrasound technology.

Resumen Sencillo

El proceso de hidratación es una operación unitaria lenta y complicada en el procesamiento de granos. Por lo tanto, son necesarios estudios para su mejora. El presente trabajo describe diferentes estudios para un mejor entendimiento del fenómeno de hidratación de granos. El trabajo fue desarrollado con el objetivo de caracterizar el comportamiento de hidratación de diferentes granos, proponer mecanismos para describir el proceso, describir matemáticamente los datos obtenidos y mejorar el proceso utilizando diferentes temperaturas y la tecnología de ultrasonido.