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Caracterização de curdlana produzida por *Agrobacterium* sp. IFO 13140 e seus géis por meio de FT-IR, FT-Raman e análises térmicas. Avaliação da morfologia, propriedades físico-químicas, tecnológicas, reológicas e aplicação em alimentos

CAMILA SAMPAIO MANGOLIM

Maringá

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Tese apresentada ao programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutor em Ciência de Alimentos.

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"CARACTERIZAÇÃO DE CURDLANA PRODUZIDA POR *AGROBACTERIUM* SP. IFO 13140 E SEUS GÉIS POR MEIO DE FT-IR, FT-RAMAN E ANÁLISES TÉRMICAS. AVALIAÇÃO DA MORFOLOGIA, PROPRIEDADES FÍSICO-QUÍMICAS, TECNOLÓGICAS, REOLÓGICAS E APLICAÇÃO EM ALIMENTOS".

Tese apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Doutor em Ciência de Alimentos.

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Orientadora

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BIOGRAFIA

Camila Sampaio Mangolim nasceu em 8 de fevereiro de 1988 na cidade de Paranavaí, Paraná. Possui graduação em Engenharia de Alimentos pela Universidade Estadual de Maringá e mestrado em Ciência de Alimentos pela mesma universidade. Tem experiência na área de Biotecnologia, atuando principalmente nos seguintes temas: produção de ciclodextrinas e suas aplicações em alimentos; produção e caracterização de biopolímeros microbianos para aplicação em alimentos.

Dedico

À minha família, meu alicerce, meu porto seguro. Que sempre me fez acreditar que era possível...

"Porque eu sou do tamanho do que vejo
E não, do tamanho da minha altura."
(Fernando Pessoa)

"Não coloque limites nos seus sonhos,
coloque fé."
(Autor desconhecido)

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A todos aqueles que de alguma forma doaram um pouco de si para que a conclusão deste trabalho se tornasse possível.

APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de dois artigos científicos:

1. **AUTORES:** Camila Sampaio Mangolim, Thamara Thaiane da Silva, Vanderson Carvalho Fenelon, Luciana Numata Koga, Sabrina Barbosa de Souza Ferreira, Marcos Luciano Bruschi e Graciette Matioli.
   TÍTULO: Description of recovery method used for curdlan produced by *Agrobacterium* sp. IFO 13140 and its relation to the morphology and physicochemical and technological properties of the polysaccharide.
   REVISTA: Plos One.
   Artigo publicado.

2. **AUTORES:** Camila Sampaio Mangolim, Thamara Thaiane da Silva, Vanderson Carvalho Fenelon, Adriane do Nascimento, Francielle Sato e Graciette Matioli.
   TÍTULO: Use of FT-IR, FT-Raman and thermal analysis to evaluate the gel formation of curdlan produced by *Agrobacterium* sp. IFO 13140 and determination of its rheological properties with food applicability.
   REVISTA: Food Chemistry.
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RESUMO GERAL

INTRODUÇÃO. Curdlana é um polissacarídeo linear, neutro, composto por repetidas unidades de glicose unidas por ligações β-(1,3), sendo considerada uma fibra alimentar. Possui especial interesse devido as suas propriedades de formação de gel, as quais permitem seu uso como agente gelificante para melhorar a textura, capacidade de retenção de água e estabilidade térmica de vários produtos alimentícios. Quando aquecida, sua suspensão aquosa forma dois tipos de gel: o gel *low-set*, o qual é termorreversível e obtido pelo aquecimento em temperaturas entre 55 e 60 °C, seguido de resfriamento, e o gel *high-set*, que é termo-irreversível e obtido pelo aquecimento acima de 80 °C. Devido a sua elevada firmeza e estabilidade, os géis de curdlana são utilizados na elaboração de massas, molhos, alimentos congelados e enlatados, além de serem biodegradáveis, comestíveis e não tóxicos para humanos e para o ambiente.

Apesar de a curdlana ser um polissacarídeo linear, sua estrutura conformacional exerce grande influência nas suas propriedades de dispersão e gelificação. Uma vez que a forma de recuperação do meio e a purificação do polissacarídeo podem influenciar na sua estrutura conformacional, o estudo estrutural da curdlana, bem como das suas propriedades físico-químicas e tecnológicas, são essenciais para melhor adequar seu emprego na indústria de alimentos.

OBJETIVOS. Considerando o interesse na curdlana devido às suas propriedades de dispersão, viscosidade e gelificação, o presente trabalho objetivou avaliar a estrutura, morfologia e propriedades físico-químicas e tecnológicas da curdlana produzida por *Agrobacterium* sp. IFO 13140, comparativamente com a curdlana comercial. Também foi objetivo desta pesquisa a aplicação da curdlana em alimentos, como massa alimentícia caseira e iogurte.

MATERIAL E MÉTODOS. A cepa de *Agrobacterium* sp. IFO 13140 adquirida na forma liofilizada foi reativada e adicionada ao meio de produção de curdlana utilizando glicose como fonte de carbono. Duas metodologias foram utilizadas para a recuperação do polissacarídeo: a de pré-gelificação e a de precipitação. Em ambas as metodologias a curdlana foi solubilizada com solução de NaOH 3 mol/L. Posteriormente foi neutralizada com HCl 3 mol/L (pré-gelificação) ou com água (precipitação). A curdlana comercial também foi submetida ao processo de pré-gelificação e, então, denominada comercial pré-gelificada.

As metodologias empregadas para recuperação da curdlana foram relacionadas com suas propriedades funcionais. As curdlanas comercial, comercial pré-gelificada e produzidas por *Agrobacterium* sp. IFO 13140 (recuperadas pelos dois métodos) foram avaliadas quanto a sua estrutura, morfologia, propriedades físico-químicas e capacidade de dispersão e gelificação. Após esta etapa, as duas curdlanas submetidas ao processo de pré-gelificação foram utilizadas para o preparo de dispersões e géis. Foram estudados os mecanismos de formação de géis de curdlana por espectroscopias de FT-IR e FT-Raman, bem como suas temperaturas de formação por análises térmicas de DSC e TGA.

As curdlanas submetidas ao processo de pre-gelificação, assim como a curdlana comercial, foram avaliadas quanto as suas propriedades tecnológicas de absorção de água, óleo e solubilidade em água. Posteriormente, foram aplicadas em massa alimentícia caseira e iogurte nas proporções de 1% e 1,5%, em relação às quantidades de farinha e leite, respectivamente. As características de cozimento e o perfil de textura das massas foram avaliados. Dos iogurtes, foram avaliadas reologia, textura e sinérese.

RESULTADOS E DISCUSSÃO. A análise de FT-Raman das curdlanas comercial, comercial pré-gelificada e produzida por *Agrobacterium* sp. IFO 13140 (recuperada pelos métodos de pré-gelificação e precipitação) revelou similaridade estrutural entre todas as amostras, o que significa que a curdlana produzida pelo micro-organismo tem estrutura similar à comercial e, também, que o método empregado para recuperação do polissacarídeo não influencia na sua estrutura. Entretanto, dados da microscopia eletrônica de varredura revelaram que tanto o método de secagem quanto o método de recuperação implicam significativamente na forma e no tamanho dos grânulos da curdlana. A amostra comercial (atomizada) apresentou grânulos grandes e com concavidades. As demais amostras (liofilizadas) exibiram partículas menores. Ainda, as duas curdlanas submetidas ao processo de pré-gelificação apresentaram o menor tamanho de partícula, o que pode ter influenciado na sua melhor dispersão em água.

Os resultados obtidos nas análises físico-químicas revelaram que a curdlana comercial apresentou o maior teor de carboidratos, seguida da produzida pelo micro-organismo e recuperada por precipitação, demonstrando o maior grau de pureza dessas amostras. As curdlanas submetidas ao processo de pré-gelificação apresentaram menor teor de carboidratos devido ao sal incorporado no processo (estimado pelo teor de sódio). Quando avaliadas as características de dispersão e gelificação das curdlanas, as amostras pré-gelificadas formaram facilmente uma dispersão homogênea em água, além de desenvolverem gel firme e homogêneo. A curdlana comercial não formou dispersão homogênea com facilidade e nem gel homogêneo, assim como a curdlana produzida por *Agrobacterium* sp. IFO 13140 recuperada por precipitação, que não formou gel pela ausência de sódio. Desta forma, as análises térmicas, reológicas e de força de gel foram realizadas apenas com as curdlanas pré-gelificadas. A curdlana comercial foi comparada com as pré-gelificadas em relação às propriedades tecnológicas de absorção de água, de óleo e solubilidade em água, além da aplicação em alimentos.

As análises de FT-IR e FT-Raman revelaram similaridade estrutural entre a curdlana comercial e a produzida por *Agrobacterium* sp. IFO 13140. Não foi possível identificar variações estruturais decorrentes da formação de gel pela técnica de FT-IR. Entretanto, a técnica de FT-Raman evidenciou essas variações, sendo possível observar variações relacionadas às ligações de hidrogênio e interações hidrofóbicas, que ocorrem com a formação dos géis *low-set* e *high-set*, respectivamente. As temperaturas de formação de ambos os géis foram determinadas por DSC. As amostras apresentaram um pico endotérmico entre 40 e 55 °C, decorrente do entumescimento da curdlana, e um pico entre 70 e 80 °C devido a interações hidrofóbicas entre as moléculas. Ainda, as análises de reologia e força do gel revelaram que a curdlana produzida por *Agrobacterium* sp. IFO 13140 mostrou capacidade espessante superior à curdlana comercial pré-gelificada. Porém, a última mostrou capacidade gelificante mais elevada, com gel de força 17% superior e módulo elástico seis vezes maior, a 95 °C. Esta grande diferença entre as capacidades gelificantes das curdlanas é devido aos seus distintos graus de polimerização (DP_n) ou massa molecular, pois enquanto a amostra comercial apresentou DP_n de 334 ± 8, a amostra produzida pelo micro-organismo apresentou DP_n de 232 ± 10, os quais correspondem a massas moleculares de 54000 e 38000, respectivamente.

Quando avaliadas as propriedades de absorção de água e óleo e solubilidade em água das curdlanas comercial e das submetidas ao processo de pré-gelificação, confirmou-se a baixa solubilidade e baixa absorção de água de todas as amostras. Entretanto, as curdlanas submetidas ao processo de pré-gelificação apresentaram valores de absorção de óleo elevados e superiores aos de absorção de água, dado este que torna estes ingredientes úteis nas interações estruturais de diversos alimentos.

O emprego das curdlanas submetidas ao processo de pré-gelificação em massa alimentícia e iogurte melhorou as características de textura dos produtos. Na massa, as curdlanas pré-gelificadas tanto aumentaram seu peso cozido (cerca de 10%) quanto aumentaram significativamente sua dureza, adesividade e gomusidade. A curdlana comercial não provocou melhora significativa em nenhum dos parâmetros da massa porque, devido a sua dificuldade de homogeneização, não gelifica de forma homogênea após o cozimento do produto. De forma semelhante, quando aplicadas no iogurte e submetidas a tratamento térmico, as curdlanas pré-gelificadas aumentaram significativamente os parâmetros de firmeza e adesividade e diminuíram a coesividade dos produtos, devido à estruturação provocada pela formação do gel de curdlana. A sinérese foi reduzida nos iogurtes com curdlana, sendo mais eficiente para a curdlana comercial pré-gelificada que, apesar de ter menor influência na firmeza que a produzida por *Agrobacterium* sp. IFO 13140, formou um gel mais estável ao armazenamento. Os dados reológicos corroboraram com os dados do perfil de textura dos iogurtes, pois as amostras submetidas ao tratamento térmico apresentaram maiores valores de viscosidade em toda a faixa de taxa de deformação avaliada.

CONCLUSÕES. As características de dispersão, de gelificação e reológicas da curdlana dependem demasiadamente dos métodos de recuperação empregados após a sua produção, pois as curdlanas comercial e produzida por *Agrobacterium* sp. IFO 13140 submetidas ao processo de pré-gelificação dispersaram melhor em água e atuaram como espessantes, além de formarem géis homogêneos. Os dados obtidos nas análises estruturais mostraram similaridade entre a curdlana produzida por *Agrobacterium* sp. IFO 13140 e a comercial. Dentre as análises empregadas, a técnica de FT-Raman mostrou-se especialmente valiosa no estudo das variações estruturais decorrentes da formação dos géis de curdlana, evidenciando as ligações de hidrogênio e as interações hidrofóbicas que ocorrem com a formação dos géis *low-set* e *high-set*, respectivamente. Apesar da similaridade estrutural, as propriedades tecnológicas das curdlanas apresentaram diferenças significativas, as quais estão relacionadas com seus diferentes graus de polimerização. A curdlana produzida por *Agrobacterium* sp. IFO 13140 (método de pré-gelificação), que apresentou o menor grau de polimerização, mostrou maior capacidade espessante e de retenção de água e óleo que a comercial pré-gelificada, porém a última revelou capacidade gelificante superior. Como consequência, apesar de ambas terem aumentado a dureza e absorção de água das massas alimentícias e terem aumentado a firmeza e a viscosidade dos iogurtes e diminuído sua sinérese, a curdlana produzida por *Agrobacterium* sp. IFO 13140 (método de pré-gelificação) provocou aumento maior nos parâmetros de textura dos produtos, mas com formação de gel menos estável que a comercial pré-gelificada. Conclui-se que os géis de curdlana avaliados mostraram estrutura rígida e estável, com potencial de aplicabilidade no aprimoramento da textura de produtos alimentícios. Desta forma, o estudo estrutural dos géis de curdlana e das suas propriedades físico-químicas e tecnológicas realizados nesta pesquisa são fundamentais para ajustar seu emprego na indústria de alimentos.

Palavras-chave: β-glucana, polissacarídeo microbiano, melhorador de textura, espessante, gelificante.

GENERAL ABSTRACT

INTRODUCTION. Curdlan is a linear, neutral polysaccharide composed of repeated β-(1,3)-linked glucose residues, and is considered a food fiber. It is of special interest due to its gel formation properties, which allow it to be used as a gelling agent to improve the texture, water retention capacity and thermal stability of various food products. When heated, its aqueous suspension forms two types of gel: low-set gel, which is thermo-reversible and obtained by heating at temperatures between 55 and 60 °C, followed by cooling, and high-set gel, which is thermo-irreversible and obtained by heating at over 80 °C. Because of their high firmness and stability, curdlan gels are used in the manufacture of pasta, sauces, frozen and canned foods, and are biodegradable, edible and non-toxic to humans and the environment.

Although curdlan is a linear polysaccharide, its conformational structure has a great influence on its dispersion and gelling properties. As the recovery of the medium and the purification of the polysaccharide can influence its conformational structure, the structural study of curdlan, as well as its physicochemical and technological properties, is essential for its more effective use in the food industry.

AIMS. Considering the interest in curdlan due to its dispersion, viscosity and gelling properties, the present study aimed to evaluate the structure, morphology and physical-chemical and technological properties of curdlan produced by *Agrobacterium* sp. IFO 13140, compared to commercial curdlan. A further objective of this study was the application of curdlan in foods, such as homemade pasta and yogurt.

MATERIAL AND METHODS. The strain of *Agrobacterium* sp. IFO 13140 acquired in lyophilized form was reactivated and added to the curdlan production medium using glucose as a carbon source. Two methodologies were used to recover the polysaccharide: pre-gelation and precipitation. In both methodologies the curdlan was solubilized with NaOH 3 mol/L solution. Subsequently it was neutralized with HCl 3 mol/L (pre-gelation) or with water (precipitation). The commercial curdlan was also subjected to the pre-gelation process and entitled pre-gelled commercial curdlan.

The methodologies used to recover the curdlan were related to their functional properties. The commercial and pre-gelled commercial curdlans and the curdlan produced by *Agrobacterium* sp. IFO 13140 (recovered by both methods) were evaluated for their structure, morphology, physicochemical properties and dispersion and gelation capacity. After this step, the two curdlans submitted to the pre-gelation process were used for the preparation of the dispersions and gels. The mechanisms of formation of the curdlan gels were examined by FT-IR and FT-Raman spectroscopy, and their formation temperatures were evaluated by DSC and TGA analyzes.

The curdlans submitted to the pre-gelation process, as well as the commercial curdlan, were evaluated for their technological properties of water absorption and oil and water solubility. Subsequently, they were applied in homemade pasta and yogurt in proportions of 1% and 1.5%, in relation to the quantities of flour and milk, respectively. The cooking characteristics and the texture profile of the pasta were evaluated. Rheology, texture and syneresis were evaluated from the yoghurt.

RESULTS AND DISCUSSION. FT-Raman analysis of the commercial and pre-gelled commercial curdlans and the curdlan produced by *Agrobacterium* sp. IFO 13140 (recovered by the pre-gelation and precipitation methods) revealed a structural similarity between all the samples, meaning that the curdlan produced by the microorganism has a

similar structure to the commercial curdlan, and that the method used for the recovery of the polysaccharide does not influence its structure. However, scanning electron microscopy data revealed that both the drying method and the recovery method significantly affected the shape and size of the curdlan granules. The commercial (atomized) sample had large granules with concavities. The other samples (lyophilized) exhibited smaller particles. Also, the two curdlans submitted to the pre-gelation process had the smallest particle size, which may have influenced their improved dispersion in water.

The results obtained in the physical-chemical analyzes revealed that the commercial curdlan had the highest carbohydrate content, followed by that produced by the microorganism and recovered by precipitation, demonstrating the greater purity of these samples. The curdlans submitted to the pre-gelation process presented a lower carbohydrate content, due to the salt incorporated in this process (calculated by the sodium content). When the characteristics of the dispersion and gelation of the curdlans were evaluated, the pre-gelled samples dispersed easily and homogenously in water, and developed a firm and homogenous gel. The commercial curdlan did not easily form a homogeneous dispersion or gel. The same was true for the curdlan produced by *Agrobacterium* sp. IFO 13140 recovered by precipitation, which did not gel due to the absence of sodium. Therefore, the thermal, rheological and gel strength analyzes were performed using the pre-gelled curdlans only. The commercial curdlan was compared with the pre-gelled curdlans in terms of the technological properties of absorption of water and oil and solubility in water, as well as application in foods.

The FT-IR and FT-Raman analyses revealed a structural similarity between the commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140. It was not possible to identify the structural variations resulting from the gel formation using the FT-IR technique. However, the FT-Raman technique did identify these variations, allowing variations related to the hydrogen bonding and hydrophobic interactions to be observed. The formation temperatures of both gels were determined by DSC. The samples revealed an endothermic peak between 40 and 55 °C, due to the swelling of the curdlan, and another peak between 70 and 80 °C due to hydrophobic interactions between the molecules. Additionally, rheology and gel strength analysis revealed that the curdlan produced by *Agrobacterium* sp. IFO 13140 had a greater thickening capacity than the pre-gelled commercial curdlan. However, the latter exhibited a greater gelling capacity, resulting in a gel with a 17% greater strength and a six times greater elastic modulus at 95 °C. This difference between the gelling properties of the curdlans is due to their different degrees of polymerization (DP_n) or molecular mass, as while the commercial sample had a DP_n of 334 ± 8, the sample produced by the microorganism had a DP_n of 232 ± 10, corresponding to molecular masses of 54000 and 38000, respectively.

When the water and oil absorption and water solubility properties of the commercial curdlan and the curdlans from the pre-gelation process were evaluated, the low water solubility and absorption of all the samples were confirmed. However, the curdlans submitted to the pre-gelation process presented high oil absorption values that were greater than those of water absorption, making these ingredients useful in the structural interactions of various foods.

The use of curdlans submitted to the pre-gelation process in pasta and yoghurt improved the texture characteristics of these products. In the pasta, pre-gelled curdlans increased the cooked weight (about 10%) and significantly increased hardness, adhesiveness and gumminess. The commercial curdlan did not result in a significant improvement in any of the parameters of mass as, due to its difficulty with

homogenization, it did not gel homogeneously after cooking. Similarly, when applied to the yogurt and subjected to heat treatment, the pre-gelled curdlans significantly increased the firmness and adhesiveness parameters and decreased the cohesiveness of the products, due to the structuring caused by the formation of the curdlan gel. Syneresis was reduced in the yoghurts with curdlan, and was more efficient with the pre-gelled commercial curdlan which, although having less influence on firmness than the curdlan produced by *Agrobacterium* sp. IFO 13140, formed a more stable gel for storage. The rheological data corroborated with the texture profile data of the yoghurts, as the samples that underwent heat treatment showed higher values of viscosity across the entire deformation rate range evaluated.

CONCLUSIONS. The dispersion, gelling and rheological characteristics of curdlan are highly dependent on the recovery method employed after its production, as the commercial curdlan and the one produced by *Agrobacterium* sp. IFO 13140, submitted to the pre-gelation process, dispersed more effectively in water and acted as a thickener, in addition to forming homogeneous gels. The data obtained in the structural analyzes showed a similarity between curdlan produced by *Agrobacterium* sp. IFO 13140 and commercial curdlan. Among the analyzes employed, the FT-Raman technique proved to be especially valuable in the study of the structural variations resulting from the formation of curdlan gels, identifying the hydrogen bonds and the hydrophobic interactions that occur with the formation of the low-set and high-set gels, respectively. Despite the structural similarity, the technological properties of curdlans presented significant differences, related to their different degrees of polymerization. The curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method), which had the lowest degree of polymerization, exhibited a greater thickening and water and oil retention capacity than the pre-gelled commercial curdlan, although the latter demonstrated a superior gelling capacity. Therefore, although both increased the hardness and water absorption of the pasta and increased the firmness and viscosity of the yoghurts and decreased syneresis, the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) resulted in a greater increase in the texture parameters of the products, but with a less stable gel formation than the pre-gelled commercial curdlan. It can be concluded that the curdlan gels evaluated exhibited a rigid and stable structure, with potential application in the improvement of the texture of food products. In this way, the structural study of the curdlan gels and their physical-chemical and technological properties undertaken in this research is fundamental for refining their use in the food industry.

Keywords: β-glucan, microbial polysaccharide, texture improver, thickener, gelling agent.

ARTICLE 1

Description of Recovery Method Used for Curdlan Produced by *Agrobacterium* sp. IFO 13140 and its Relation to the Morphology and Physicochemical and Technological Properties of the Polysaccharide

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Abstract

Curdlan is a linear polysaccharide considered a dietary fiber and with gelation properties. This study evaluated the structure, morphology and the physicochemical and technological properties of curdlan produced by *Agrobacterium* sp. IFO 13140 recovered by pre-gelation and precipitation methods. Commercial curdlan submitted or

otherwise to the pre-gelation process was also evaluated. The data obtained from structural analysis revealed a similarity between the curdlan produced by *Agrobacterium* sp. IFO 13140 (recovered by both methods) and the commercial curdlans. The results showed that the curdlans evaluated differed significantly in terms of dispersibility and gelation, and only the pre-gelled ones had significant potential for food application, because this method influence on the size of the particles and in the presence of NaCl. In terms of technological properties, the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) had a greater water and oil holding capacity (64% and 98% greater, respectively) and a greater thickening capacity than the pre-gelled commercial curdlan. The pre-gelled commercial curdlan displayed a greater gelling capacity at 95 °C than the others. When applied to food, only the pre-gelled curdlans significantly improved the texture parameters of yogurts and reduced syneresis. The curdlan gels, which are rigid and stable in structure, demonstrated potential for improving the texture of food products, with potential industrial use.

Introduction

Polysaccharides are an extremely diverse family of natural biopolymers, which are industrially used as thickeners, stabilizers and gelling agents in foodstuffs [1]. There is currently growing interest in their biological functions, such as their antioxidant and prebiotic activity. Although polysaccharides are derived from various sources such as microorganisms, algae and higher plants, the market is dominated by polysaccharides obtained from algae (such as carrageenans, alginates and agar) and higher plants (such as starch, cellulose and pectin) [2].

The synthesis of microbial polysaccharides has emerged as an important source of new biopolymers for industrial use [3]. It is an attractive alternative as

microorganisms can grow under controlled conditions and produce a large variety of polysaccharides with unique properties. Production can be carried out in large quantities by biotechnological routes and from renewable and low-cost raw materials, which are easily recovered [4]. One of the microbial polysaccharides of industrial interest is curdlan, a neutral exopolysaccharide composed exclusively of glucose residues joined by β-(1→3) bonds, commercially produced by bacterial species of *Agrobacterium* [2,5-7].

After being discovered by Harada in 1966, curdlan has received considerable attention in both the food and non-food industry due to its physicochemical properties, which are unique when compared to other polysaccharides commonly used, such as starch. When in aqueous suspension, curdlan may form a thermoreversible gel (low-set gel) when heated to temperatures close to 55 °C with subsequent cooling. This gel has similar behavior to agar and gelatin gels. In addition, curdlan can form a thermoirreversible gel (high-set gel) when its aqueous suspension is heated to temperatures above 80 °C, which is very stable. Furthermore, gels with different strengths can be formed by varying the temperature, the heating time and the concentration of curdlan [1,6].

Curdlan was approved for use in foodstuffs in Korea, Taiwan and Japan in 1989 and registered in 1996 by the FDA (*Food and Drug Administration*) in the USA as a food additive with the following functions: a formulation and processing aid, and a stabilizer, thickener and texturizer [8]. It is widely used due to its ability to form the thermoirreversible gel, which exhibits great stability during the industrial processes of autoclaving, frying and freezing-thawing cycles. It has no taste, color or odor, can form gels at a wide pH range (from 2 to 10) and can mimic the palatability of foods containing fat [9]. It is also considered a dietary fiber, thus improving the functionality

of various food products. The versatility of its applications associated with its health benefits make curdlan a valuable tool in the development of innovative food systems [1,3].

The dispersion and gelation characteristics of curdlan in an aqueous system, as well as its mechanical properties, are associated with several factors such as concentration, temperature, heating time, dispersal method, and the presence of ions, salts and low molecular weight sugars [10]. Therefore, the form of recovery from the culture medium and the purification of the polysaccharide are steps which decisively influence the physical and technological properties of curdlan, which directly affect the texture characteristics of foods in which the polysaccharide is inserted. So, it is necessary to study these properties to define the most appropriate form of the implementation of the polysaccharide in the industry [11].

Considering the interest in the properties of dispersion, viscosity, gelation and the health benefits of curdlan, the present study aimed to characterize the physicochemical and technological properties of curdlan produced by *Agrobacterium* sp. IFO 13140 and recovered using the pre-gelation and precipitation methods, as well as commercial curdlan subjected or otherwise to pre-gelation treatment. The methodologies employed for the recovery of the curdlans were studied. The structures of all curdlans were compared by FT-Raman spectroscopy. In addition, the application of curdlans in yogurts was evaluated.

Materials and methods

Materials

The bacterial strain *Agrobacterium* sp. IFO 13140 was purchased in lyophilized form from the Institute for Fermentation of Osaka (Japan). Commercial curdlan was acquired from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All solvents were of analytical grade.

Curdlan production by *Agrobacterium* sp. IFO 13140

The culture medium used to reactivate the microorganism was proposed by the supplier (g L^{-1}), pH 7: polypeptone (10), yeast extract (2), $MgSO_4.7H_2O$ (1). 30 mg of the lyophilized bacteria were incubated in 100 mL of the medium at 30 °C and 120 rpm for 48 h. The cells were recovered through centrifugation (6000 ×g, 10 min), washed with 9 g L^{-1} NaCl and transferred to the production medium. For curdlan production, the liquid medium described by Martinez et al. [12], pH 7, was used (g L^{-1}): glucose (50), KH_2PO_4 (2.7), NH_4Cl (1.6), $MgSO_4$ (0.5) and trace elements (10 mL L^{-1}). The composition of the trace elements (g L^{-1}) in HCl 0.1 mol L^{-1} was: $FeCl_3.6H_2O$ (1), $MnCl_2.4H_2O$ (1), ZnCl (1), $CaCl_2$ (1) and $CaCO_3$ (0.03). The reactivated microorganisms were transferred to Erlenmeyer flasks containing 100 mL of the production medium and maintained at 30 °C and 150 rpm for 5 days.

Recovery of curdlan from production medium

Two methodologies were used to recover curdlan from the medium. In pre-gelation method [12,13], NaOH 3 mol L^{-1} was added to the Erlenmeyer flasks

containing the production medium at a ratio of 1.8:1 (NaOH:medium) for curdlan solubilization. This mixture was centrifuged (18000 ×g, 15 min, 4 °C) to separate the cells. HCl 3 mol L^{-1} was added to the supernatant until pH 6-7 to obtain the curdlan gel, which was recovered by centrifugation (18000 ×g, 15 min, 4 °C). Subsequently, it was washed three times with distilled water and lyophilized. The commercial curdlan was subjected to the same treatment and it was named pre-gelled commercial curdlan.

The precipitation method is an adaptation of the industrial purification of curdlan. Industrially produced curdlan is purified by dissolution in a strong alkaline solution and dried in a spray-dryer, then washed with water until neutralization [14]. NaOH 3 mol L^{-1} was added to the Erlenmeyer flasks containing the production medium at a ratio of 1.8:1 (NaOH: medium) to solubilize the curdlan and the mixture was centrifuged at 18000 ×g for 15 min at 4 °C to separate the cells, before being lyophilized. The dried mixture was resuspended in water, filtered to remove larger impurities and then ultra-filtered using the system described by Fenelon et al. [15] with a 30 kDa membrane and pressure of 1 kgf cm^{-2}. During ultrafiltration, the material was washed with water until the pH was neutralized, causing the precipitation of the curdlan, that was thereafter lyophilized.

Structural analysis of curdlans by FT-Raman

Samples of the commercial and commercial pre-gelled curdlans and those produced by *Agrobacterium* sp. IFO 13140 (pre-gelation and precipitation methods) were analyzed by Fourier transform Raman spectroscopy (FT-Raman) using a Fourier Transform infrared spectrometer (Vertex 70v model with Ram II module, Bruker, Germany) equipped with a Germanium detector cooled with liquid nitrogen. A Nd:YAG

laser was used for excitation at 1064 nm. The spectra were based an average of 200 scans with a resolution of 4 cm^{-1}.

Morphology of curdlans

The morphology of the commercial and commercial pre-gelled curdlans, and the curdlans produced by *Agrobacterium* sp. IFO 13140 (pre-gelation and precipitation methods) was analyzed in a scanning electron microscope (SS-550 model, Superscan, Shimadzu, Japan), at an accelerating voltage of 15 kV.

Physicochemical characterization of curdlans

The commercial and commercial pre-gelled curdlans and those produced by *Agrobacterium* sp. IFO 13140 (pre-gelation and precipitation methods) were characterized for their carbohydrate, moisture and sodium content. The carbohydrate content was determined through the phenol-sulfuric method [16]. The moisture content was determined by the gravimetric method [17] and sodium was identified using atomic absorption spectrometry [18].

Technological properties of curdlans

Water dispersion and gel formation capacities of curdlans

To verify the dispersal capacity in water of the commercial and commercial pre-gelled curdlans and those produced by *Agrobacterium* sp. IFO 13140 (pre-gelation and precipitation methods), they were subjected to stirring in a mixer and/or in a magnetic

stirrer until homogeneous dispersion was achieved. The dispersions were heated at 95 °C/1 h to assess the gel formation capacity.

Rheological characteristics and gel strength of curdlans

Dispersions of the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) were prepared at three concentrations: 20 g L^{-1}, 40 g L^{-1} and 80 g L^{-1} in water. The curdlans were dispersed in water using a mixer at room temperature for 5 min, sonicated for 10 min and then stirred in a magnetic stirrer for 12 h at 40 °C. The samples were analyzed in a controlled stress rotational rheometer (HAAKE MARS II model, Thermo Fisher Scientific Inc., Newington, Germany), with steel cone/plate geometry (60 mm, gap 0.052 mm). The elastic (G') and viscous (G'') modulus, and the dynamic viscosity were measured depending on temperature (20-60 °C) at a frequency of 10 Hz. For gel strength evaluation, the dispersions of both samples at the concentration of 20 g L^{-1} were kept or not in a water bath at 61 °C/1 h to prepare the low-set gel and at 95 °C/1 h for preparation of the high-set gel [19]. After heating, they were cooled at room temperature. The strength of the suspensions and gels was evaluated in triplicate in a Stable Micro Systems texturometer (TA-XT Plus model, Texture Technologies Corp., UK), using a 36 mm probe for compression analysis.

Water holding capacity (WHC), Oil holding capacity (OHC) and Water solubility index (WSI)

Samples of 0.25 g of the commercial and pre-gelled commercial curdlans and curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) were diluted in 10 mL of distilled water or soya oil at 30 °C, homogenized in a magnetic stirrer for

30 min and centrifuged (1500 ×g, 10 min). The WHC was expressed as g of water absorbed per g of curdlan sample and the OHC was described as g of oil absorbed per g of curdlan sample. To determine the WSI, the supernatant of the WHC analysis was oven dried at 105 °C and the ratio between the weight of the solid residue present in the supernatant after drying and the weight of the curdlan sample was calculated [11].

Application of curdlan in yogurt

Preparation of yogurts

Eight yogurt samples were prepared: A1) without curdlan or heat treatment; A2) without curdlan and with heat treatment; B1) with commercial curdlan and without heat treatment; B2) with commercial curdlan and heat treatment; C1) with pre-gelled commercial curdlan and without heat treatment; C2) with pre-gelled commercial curdlan and heat treatment; D1) with curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) without heat treatment; D2) with curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) with heat treatment.

The curdlan was mixed in UHT (ultra-high temperature) whole milk at a concentration of 15 g L^{-1} using a mixer. Subsequently, the milk of the samples subjected to heat treatment was heated at 90 °C for 120 seconds and cooled to 42 °C. For samples without heat treatment, the milk was directly heated to 42 °C. Next, all samples were inoculated with 2 g L^{-1} of mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, and maintained at 42 °C for 6 h. The yogurts were cooled and refrigerated for 24 h for rheological, texture and syneresis analysis [3,20].

Texture profile analysis (TPA)

The TPA of the yogurts was carried out in a TA-XT Plus texturometer (Stable Micro Systems, Godalming, UK), equipped with Texture Expert software (Stable Micro Systems, Godalming, UK). A 10 mm cylindrical probe was used (ref. P/0.5R, Stable Micro Systems). Two cycles were applied, at a velocity of 2 mm s^{-1} and a depth of 15 mm, producing force-time curves which were used to determine the firmness, cohesiveness, adhesiveness, springiness, gumminess and chewiness of the samples. The analysis was performed in triplicate, at 8 °C.

Syneresis

The syneresis analysis of each preparation was performed after 28 days of storage of the yogurt at 8 °C in triplicate. Yogurt samples (30-40 g) were centrifuged (222 ×g, 10 min, 4 °C) and the supernatant was separated and centrifuged again (222 ×g, 10 min, 4 °C), weighed and the syneresis (%) was calculated from the ratio between the mass of the supernatant and the initial mass of the yogurt [20].

Rheological analysis

The rheological properties of flow of the yogurts were analyzed in triplicate, in a controlled stress rotational rheometer (HAAKE MARS II model, Thermo Fisher Scientific Inc., Newington, Germany), with steel cone/plate geometry (60 mm, gap 0.052 mm). The measurements were performed at 8 °C. The scan of the deformation rate was performed from 0 to 116 s^{-1}, obtaining the outward and return data. The flow and viscosity curves were obtained by determining the stress and viscosity versus the shear rate [20]. The rheological parameters K (consistency index) and n (flow behavior index) were calculated using the Ostwald de Waele model, while the parameter τ_0 (yield

247 stress) was calculated by the Herschel-Bulkley model. The hysteresis was calculated
248 from the area of the flow curves of the yogurts using the RheoWin 4.10.000 software
249 program (HAAKE software, Thermo Fisher Scientific Inc., Newington, Germany).
250

251 Statistical analysis

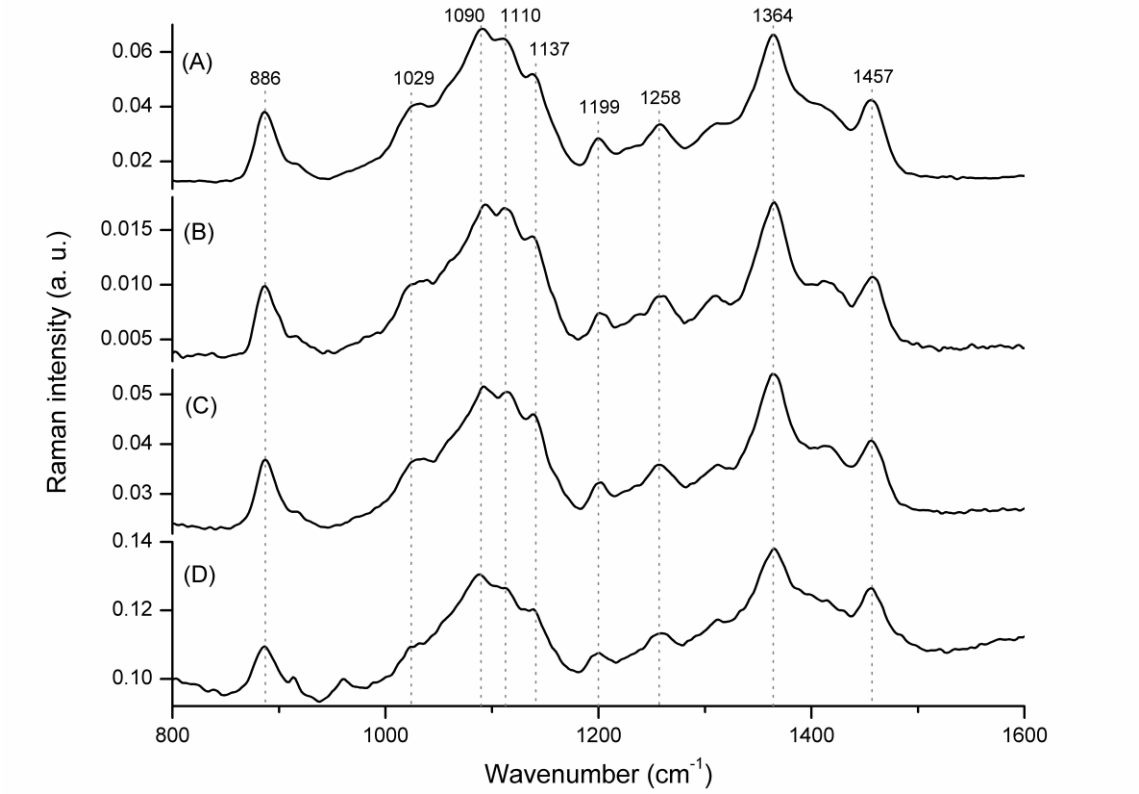
252 Data were analyzed by analysis of variance (ANOVA), and means were
253 compared with the Tukey Test ($p<0.05$) using the Statistica 8.0/2008 software package
254 (Stat Soft, Inc., Tulsa, USA).
255

256 Results and Discussion

257

258 Structural analysis of curdlans by FT-Raman

259 Fig 1 shows FT-Raman spectra of commercial and pre-gelled commercial
260 curdlans and those produced by *Agrobacterium* sp. IFO 13140 (pre-gelation and
261 precipitation methods). From the data, it is verified a structural similarity between all
262 samples, which means that the curdlan produced by the microorganism has a similar
263 structure to commercial curdlan and it also means that the method employed to recover
264 curdlan from the medium did not modify the structure of the polysaccharide.



265

266 **Fig 1. FT-Raman spectra of: (A) commercial curdlan, (B) pre-gelled commercial**
267 **curdlan, (C) curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation**
268 **method), (D) curdlan produced by *Agrobacterium* sp. IFO 13140 (precipitation**
269 **method).** The dotted lines show the characteristic peaks of the samples.

270

271 Several authors described the chemical bonds present in the structure of curdlan
272 molecule based in FT-IR analysis [3,14,21]. They stated that, from the FT-IR data for
273 curdlan, the bands around 890, 1080 and 1160 cm^{-1} correspond to the β-(1,3)-glucan
274 linkages. Despite the difference in band assignments for FT-IR and FT-Raman
275 spectroscopies, the spectra of carbohydrates in both techniques show a characteristic
276 absorption band of β-anomeric configuration at ~890 cm^{-1} (886 cm^{-1} in this work) [22].
277 The Raman bands and shoulders at 1090 and 1137 cm^{-1} are typical for β-glucans.
278 Intense highly overlapped Raman bands between 990 and 1200 cm^{-1} are attributed to
279 COC and CC stretching vibrations of polysaccharides. The features between 1200 and

1440 cm^{-1} are mainly assigned to in-plane ring deformation including CH and OH bending modes. Finally, the band at 1457 cm^{-1} is assigned to CH_2 in-plane bending in CH_2OH of the molecule [23].

Morphology of curdlans

Fig 2 shows scanning electron microscopy images of the commercial curdlan (Figs 2A and 2B), the pre-gelled commercial curdlan (Figs 2C and 2D) and the curdlans produced by *Agrobacterium* sp. IFO 13140 recovered by pre-gelation and precipitation methods (Figs 2E-2H) with a range of magnifications.

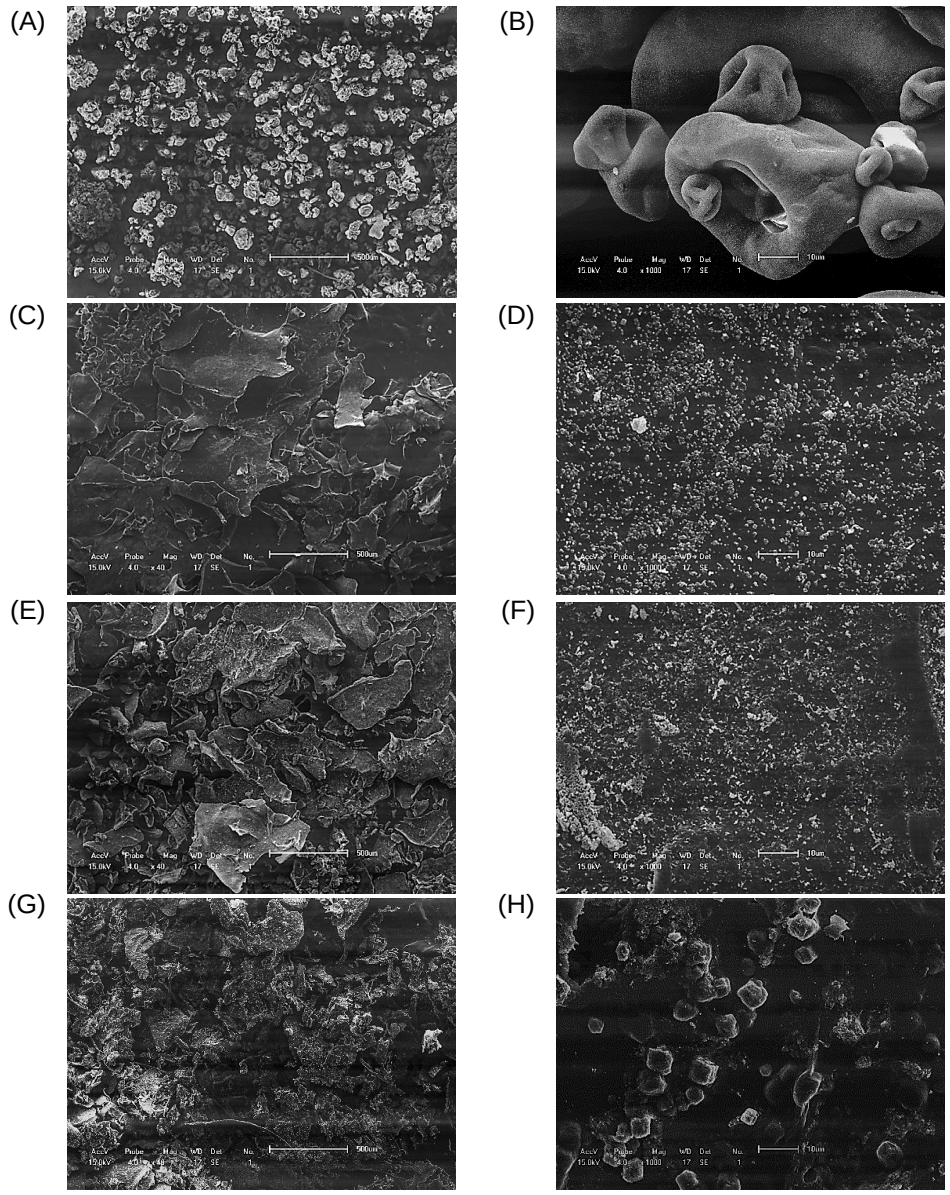


Fig 2. Scanning electron microscopy of: commercial curdlan – (A) 40x and (B) 1000x magnification; pre-gelled commercial curdlan – (C) 40x and (D) 1000x magnification; curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) – (E) 40x and (F) 1000x magnification; curdlan produced by *Agrobacterium* sp. IFO 13140 (precipitation method) – (G) 40x and (H) 1000x magnification.

Marchessault and Deslandes [24] define the shape of the granules of commercial curdlan as collapsed or invaginated, which coincides with the commercial curdlan shape illustrated in Figs 2A and 2B. The granules have widely differing sizes, varying from around 10 to 100 μm in diameter. Industrially produced curdlan is dried by spray-drying [14] and the type of drying has a major influence on the particle bead structure [25]. The expected range of particle size of polymers dried by ordinary spray dryers is 5-150 μm. Furthermore, spherical microspheres or those with pores/concavities, as shown in Figs 2A and 2B, are characteristic of the drying of products by a spray-dryer. The formation of pores or concavities is associated with the rapid evaporation of the liquid particles of this process [26].

The pre-gelled commercial curdlan and both the curdlans produced by the microorganism, when viewed at low magnification (40x), were in flake form and displayed irregularities. These characteristics are expected for lyophilized products, since the working conditions of the lyophilizer exert great pressure on the particles to be dried, meaning that a product dried by lyophilization remains amorphous in comparison with a spray dried product [25]. Furthermore, at a higher magnification (1000x), it can be seen that the particles obtained for the three samples that underwent the lyophilization process (Figs 2D, 2F and 2H), were considerably smaller than the commercial curdlan (obtained by spray-dryer). Therefore, it can be inferred that the lyophilization process can facilitate the dispersion of curdlan molecules in water, especially the pre-gelled commercial curdlan and the one produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method), which have smaller particle sizes (Figs 2D and 2F). The smaller diameter increases the accessibility of water molecules to the inside of the particles, facilitating dispersion.

The structure displayed by the commercial curdlan is maintained by a large amount of hydrogen bonds. But, when added to an alkaline solution, these bonds are broken due to their ionization, and the granule loses its structure. The neutralization of the curdlan suspension causes a reshaping of the hydrogen bonds that depends on the neutralizing agent used. With the addition of HCl (pre-gelation method), the reassociation causes the formation of a firm gel, with small structures of less than 1 μm in diameter (Figs 2D and 2F). With water (precipitation method), reassociation causes the precipitation of the curdlan into small particles with diameters greater than those obtained by neutralization with HCl, ranging from 2-10 μm in diameter (Fig 2H). Therefore, the difference between the sizes of the curdlan particles produced by the microorganism using the two methods is due to the different forms of reassociation of the hydrogen bonds, which are dependent on the recovery method employed, and can also influences the characteristics of water dispersion of the polysaccharide.

Physicochemical characterization of curdlans

As an exopolysaccharide, curdlan is secreted to the extracellular medium in the form of biofilms, and has the advantage of being easy to recover. As a result, the polysaccharide should present a low degree of impurities, and consequently the carbohydrate content represents an indirect measure of the purity of the samples. The levels of carbohydrates and moisture found in the curdlan samples are shown in Table 1.

Table 1. Carbohydrate, moisture and sodium content (%) of different samples of curdlan. Values indicate mean ± standard-deviation.

Curdlan sample	Carbohydrate	Moisture	Sodium
Commercial	94.5 ± 0.2	4.1 ± 0.4	1.2 ± 0.3
Commercial pre-gelled	82 ± 1	4.0 ± 0.3	4.4 ± 0.8
Microbial* (pre-gelation method)	70.6 ± 0.6	5.4 ± 0.9	0.024 ± 0.001
Microbial* (precipitation method)	85.4 ± 0.7	4.8 ± 0.6	< 0.001

*Produced by *Agrobacterium* sp. IFO 13140.

The highest carbohydrate content was found for the commercial sample. The pre-gelled commercial sample had a carbohydrate content lower than the commercial curdlan due to the salt (NaCl) incorporated in the pre-gelling process. The two samples produced by *Agrobacterium* sp. IFO 13140 had different carbohydrate contents, with that produced by precipitation method having greater purity due to the successive washings performed. The impurities in the last samples consist of remnants of the production medium and the salt formed in the recovery step.

The sodium content of curdlans was determined because curdlan is a glucose polymer with a low amount of inorganic salts, mainly sodium chloride [27]. Thus, sodium content is an indirect estimate of the salt content of the material. The sodium concentrations disposed in Table 1 directly influence the dispersion and gelling characteristics of the polysaccharide, which will be further discussed in the next section.

Technological properties

Water dispersibility and gelling capacities

Curdlan can be used as a thickener, stabilizer and texturizer in food industry [8,27]. However, as curdlan is insoluble in water, it is necessary to use an efficient homogenizer to obtain a homogeneous dispersion and apply a further stirring of the dispersion prior to the carrying out of analyses requiring uniformity [21].

The pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) dispersed easily in water when subjected to stirring in a mixer for less than 5 min, and also acted as thickening agents. Both dispersions remained visibly homogeneous for days and, when heated at 95 °C/1 h, formed a firm and homogeneous gel. The commercial curdlan did not easily disperse in water using the mixer. After 24 hours of stirring in a magnetic stirrer, a homogeneous dispersion formed for a brief period, displaying a phase separation after 10 min of rest. After 48 h of stirring, phase separation began to be observed at 30 min; after 72 h, at 1 hour; and after 96 h, phase separation began at 1.5 h. The gelling of commercial curdlan also occurred after heating at 95 °C/1 h, but there was phase separation in the gel formed, which became more pronounced as the stirring period for formation of the dispersion was reduced. Marchessault and Deslandes [24] have previously identified the formation of a non-homogenous gel from curdlan in its native form. Probably, the difficulty of dispersion of commercial curdlan compared with those submitted to pre-gelation process is related to the particle size of the sample (as noted in morphological analysis), which makes difficult the accessibility of water molecules to the inside of the particles.

Another important factor related to the dispersion and gelling of curdlan is the presence of salts. In the gelling of curdlan, the swelling phase of the molecule is

407 essential and may be influenced by salts, as they affect the mobility of the water
408 molecules during hydration [10]. The swelling of curdlan is facilitated with the salts
409 present because it increases intermolecular association and consequently increases the
410 viscosity of curdlan water dispersions. The curdlan produced by *Agrobacterium* sp. IFO
411 13140 (precipitation method) displayed greater difficulty in forming a homogenous
412 dispersion in water and, in contrast to the other curdlans, did not form a gel when
413 heated. This can be explained by its considerably lower sodium content, due to the
414 countless washings with water used to achieve neutralization. Samples of the other
415 curdlans were also washed in the ultrafiltration device and, following such process, no
416 longer had the same dispersion and gelling capacities. This proves therefore, that as with
417 other biopolymers, for curdlan, the mechanical characteristics depend excessively on the
418 methods of recovery employed following production. Due to the characteristics
419 displayed, the technological characteristics of the curdlan produced by *Agrobacterium*
420 sp. IFO 13140 (precipitation method) were not evaluated, and this sample was not
421 applied in yogurt. Additionally, dispersions of commercial curdlan were not evaluated
422 by rheological and gel strength analysis because the commercial curdlan did not act as a
423 thickener with the methodology employed for preparation of the dispersions.
424

425 ## Rheological characteristics and gel strength of curdlans

426 Thermal scanning rheological measurements were made to evaluate both the
427 dynamic viscosity and the gelation behavior of curdlan dispersions. The temperature
428 dependence of dynamic viscosity and of G' and G" modulus for aqueous dispersions of
429 pre-gelled commercial curdlan and of the curdlan produced by *Agrobacterium* sp. IFO
430 13140 (pre-gelation method) at 20, 40 and 80 g L^{-1} are shown in Figs 3A and 3B.
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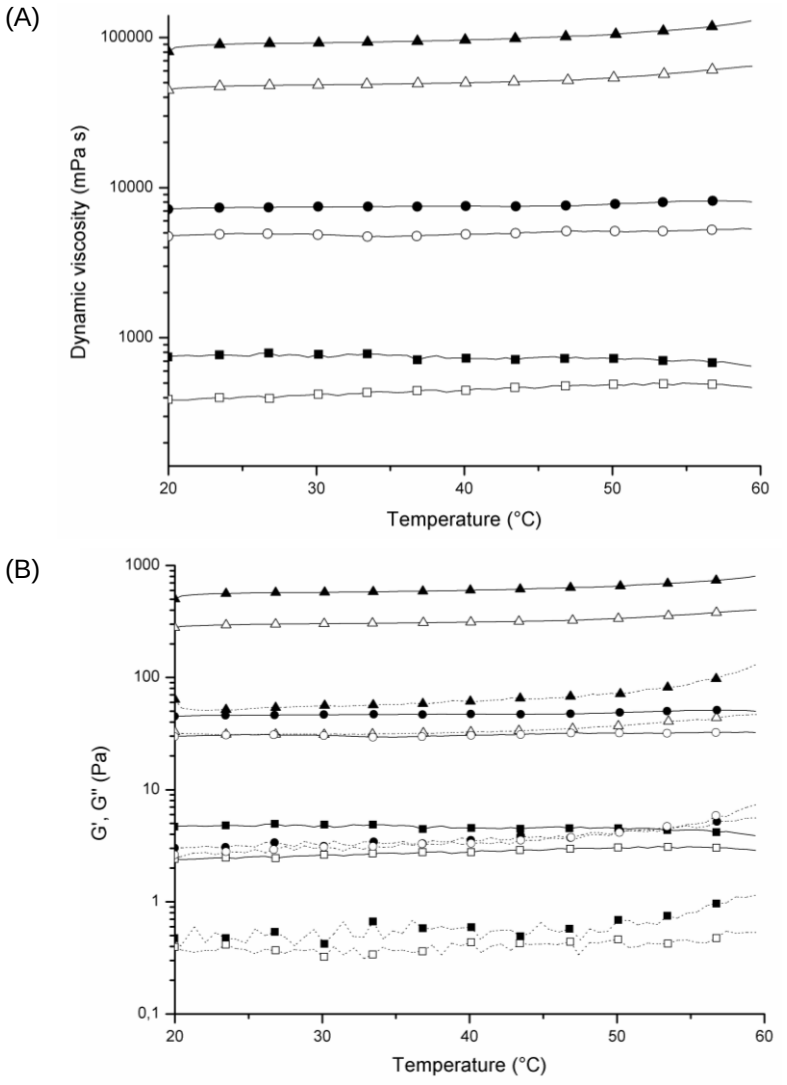

Fig 3. Temperature dependency of: (A) dynamic viscosity and (B) G' (continuous line) and G" (dotted line) modulus of the aqueous dispersions of curdlan. Pre-gelled commercial curdlan (empty symbol) and curdlan produced by *Agrobacterium* sp. IFO 13140 recovered by the pre-gelation method (full symbol) at (■) 20, (●) 40 and (▲) 80 g L^{-1}.

The curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) exhibited considerably higher viscosity than pre-gelled commercial curdlan (Fig 3A). For example, at 20 °C, the dynamic viscosity of curdlan produced by the microorganism was almost 100% higher than pre-gelled commercial curdlan at 20 and 80 g L^{-1}.

443 Equally, G' and G" modulus (Fig 3B) were also higher for the curdlan produced by the
444 microorganism, revealing the highest thickening potential of this curdlan when
445 compared to the pre-gelled commercial one.

446 Both the dynamic viscosity and the G' module of both curdlans exhibited a
447 different behavior as a function of temperature for each concentration employed. At 20
448 g L^{-1}, both parameters increased with temperature for pre-gelled comercial curdlan,
449 while for curdlan produced by the microorganism they decreased. This behavior is
450 related to the swelling of curdlan because of the breakage of hydrogen bonds during
451 heating. The difference between the curdlanas is because, for pre-gelled commercial
452 curdlan, inter-molecular entanglements between the particles that were swollen formed
453 pseudo-links, which contributed to the little increase in G'. This phenomenon did not
454 occur for curdlan produced by the microorganism. However, for higher concentrations
455 (40 and 80 g L^{-1}), the decrease in the viscosity and in G' no longer occurred with
456 temperature for any sample, because the high concentration favors the inter-molecular
457 entanglements between the particles, which contribute to the increase of both
458 parameters. Additionally, the increase in G' and G" modulus and in dynamic viscosity of
459 curdlan dispersions at about 50-55 °C, especially at 80 g L^{-1}, suggests the beginning of
460 the formation of the thermo-irreversible gels due to hydrophobic interactions.

461 Jin et al. [21] stated that G' decreased until 50 °C for curdlan suspensions at 20 g
462 L^{-1}, which is related to the swelling of curdlan because of the breakage of hydrogen
463 bonds, as observed for curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation
464 method) in this study. However, Funami et al. [28] stated only increasing in G'
465 parameter for dispersions of curdlans at 20, 40 and 100 g L^{-1} with increasing
466 temperature. The authors attributed this behavior to inter-molecular entanglements
467 between the particles that were swollen forming pseudo-links, being this result similar

to the one obtained for pre-gelled comercial curdlan in all concentrations evaluated and for curdlan produced by the microorganism for concentrations from 40 to 80 g L^{-1} in this work.

The strength data of suspensions and gels of the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) at 20 g L^{-1} are presented in Table 2. According to the strength values of the suspensions and gels, the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) has a better thickening capacity than the pre-gelled curdlan, as the suspension of the same without heat treatment was approximately 10% stronger. This result agrees with the results of rheological analysis. However, the gelling capacity of the pre-gelled commercial curdlan is considerably higher.

Table 2. Strength (×10^{-3} N) of pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) samples after undergoing different heat treatments. Values indicate mean ± standard-deviation.

Heat treatment of the dispersion in a concentration of 20 g L^{-1}	Strength (× 10^{-3} N)	
	Pre-gelled commercial curdlan	Curdlan from microorganism (pre-gelation method)
Without heat treatment	69 ± 2[d]	76 ± 2[c]
Low-set gel (61 °C/1 h)	79 ± 1[c]	77 ± 2[c]
High-set gel (95 °C/1 h)	96.8 ± 0.8[a]	83 ± 1[b]

[a–d] Means with different letters are significantly different ($p < 0.05$).

The strength of the low-set gel of the pre-gelled commercial curdlan was around 15% greater than that of the suspension without heat treatment, and statistically equal to that of the low-set gel of the curdlan produced by the microorganism. The high-set gel

of the pre-gelled commercial curdlan had strength 40% greater than the suspension without heat treatment, and 17% greater than the high-set gel produced by the microorganism.

When an aqueous dispersion of curdlan is heated around 55 °C, the low-set gel formed is maintained by intramolecular hydrogen bonds and the curdlan chains adopt a predominantly single helix conformation. But when heated above 80 °C, the high-set gel formed is maintained by intermolecular hydrophobic interactions and the curdlan chains adopt a predominantly triple helix conformation [14,29], forming an organized and rigid gel configuration. As such, the increase in the strength of the high-set curdlan gel (95 ºC/1 h) is due to the greater presence of the triple helix conformation.

The fact that the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) have formed a considerably weaker gel than the pre-gelled commercial curdlan, as well as the fact that it does not exhibit the same behavior that pre-gelled commercial sample in rheological analysis at low concentrations (increase of G' and dynamic viscosity with temperature), are probably due to a difference of molecular weight/degree of polymerization of the polymers. A variety of physical properties of β-(1→3)-glucans, including gel strength (or gel-forming ability) is related to the molecular weight/degree of polymerization of the biopolymer. The higher the degree of polymerization of the polysaccharide, the greater is its gel forming ability with heating [30,31].

Nakanishi et al. [32] studied the formation of a complex of curdlan with aniline blue dye and noted a relation between the rate of interaction of the polymer with the dye and the concentration, degree of polymerization, and gel-forming ability of the polymer. The variation in absorbance at 590 nm is proportional to the concentration of curdlan and to its gel-forming ability (and consequently to its degree of polymerization). S1

Appendix contains the methodology used by the authors described to evaluate curdlan interaction with aniline blue dye and the results of the relationship between absorbance variation and concentration of the pre-gelled commercial curdlan and curdlan produced by the microorganism. By comparing the results of this study to those obtained by Nakanishi et al. [32] and using the relation obtained by these authors between absorbance variation and degree of polymerization, it is concluded that the degree of polymerization of the pre-gelled commercial curdlan is about 45% higher than of the curdlan produced by the microorganism, which explains the lowest gel strength and the decrease in dynamic viscosity and G' in low concentration dispersions of the polymer.

Water holding capacity (WHC), Oil holding capacity (OHC) and Water solubility index (WSI)

Determining the technological properties of polysaccharides is of great importance when predicting their possible industrial applications. The values for the properties of water solubility index (WSI) water holding capacity (WHC) and oil holding capacity (OHC) of the curdlan samples are described in Table 3.

Table 3. Water holding capacity (WHC), oil holding capacity (OHC) and water solubility index (WSI) (g g^{-1}) of the commercial, pre-gelled commercial curdlans and the one produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method). Values indicate mean ± standard-deviation.

Curdlan sample	WHC	OHC	WSI
Commercial	4.6 ± 0.4^{a}	0.62 ± 0.08^{c}	0.006 ± 0.002^{a}
Commercial pre-gelled	2.20 ± 0.08^{c}	4.4 ± 0.2^{b}	$0.00923 \pm 7E\text{-}5^{a}$
Microbial (pre-gelation method)	3.6 ± 0.3^{b}	8.7 ± 0.1^{a}	0.0068 ± 0.003^{a}

[a–c] Means in the same column with different letters are significantly different ($p < 0.05$).

The three curdlans had very low and statistically equal WSIs, which was expected as curdlan is insoluble in water. Despite this, however, the curdlans presented some water absorption, with the commercial curdlan achieving the highest value, followed by the curdlan produced by the microorganism and the pre-gelled commercial curdlan. Seguchi and Kusunose [33] found water absorption rates for curdlan between 5.244 g g^{-1} and 7.724 g g^{-1}.

Compared to other polysaccharides employed in food industry as gums, curdlan has low water holding capacity; the guar and xanthan gums have a WHC of 25.77 g g^{-1} and 27.33 g g^{-1}, respectively [34]. However, both are soluble in water. The low solubility and water holding capacity of curdlans is explained by the existence of a large amount of intra/intermolecular hydrogen bonds within the polymer. This also explains the fact that commercial curdlan has a higher water absorption index than the other types as the recovery by the pre-gelation process employed in the latter two types favors the formation of large quantities of hydrogen bonds in the polymer. Thus, the polysaccharide interacts more strongly with itself than with water [21,34].

The three evaluated curdlans also differed with respect to oil holding capacity. With the exception of the commercial curdlan, they presented greater OHC values than WHC, indicating that the samples have a higher lipolytic than hydrophilic capacity. Oil absorption values greater than three make curdlan a potentially useful ingredient in structural interactions in foods, especially in aroma retention, improved palatability and maintenance of the stability of the product during storage [11]. This result justifies the use of curdlan as a fat replacer or mimicker of fat in the food industry [9,35].

Application of curdlan in yogurt

The main characteristics that define the quality of yogurts are its texture and propensity for serum separation (syneresis). Typically, polysaccharides such as xanthan, guar, gellan, pectin, carrageenan are used to give the product a firmer texture, increase its stability and hence make it more acceptable to the consumer [3,20]. Table 4 displays the parameters of texture and syneresis after 28 days of storage of yogurt samples with and without curdlan, submitted or not to heat treatment with the aim of gelling the curdlan in the milk prior to the fermentation process.

Table 4. Texture parameters and syneresis of yogurts with and without curdlan and submitted or not to heat treatment. Values indicate mean ± standard-deviation.

Sample	Firmness ($\times 10^{-3}$ N)	Cohesiveness	Adhesiveness (N × mm)	Springiness (mm)	Gumminess (N)	Chewiness (N)	Syneresis (%)
A1	77 ± 2^{c}	0.77 ± 0.02^{a}	0.02 ± 0.01^{c}	1.13 ± 0.01^{ab}	5.9 ± 0.3^{c}	6.7 ± 0.4^{bcd}	49.3 ± 0.8^{a}
A2	84 ± 8^{c}	0.71 ± 0.07^{ab}	0.04 ± 0.03^{c}	1.00 ± 0.04^{bc}	5.9 ± 0.1^{c}	5.9 ± 0.3^{cde}	48.7 ± 0.4^{a}
B1	83 ± 8^{c}	0.66 ± 0.02^{bc}	0.08 ± 0.03^{c}	0.94 ± 0.04^{c}	5.5 ± 0.7^{c}	5.1 ± 0.7^{e}	45.6 ± 0.9^{b}
B2	129 ± 7^{b}	0.47 ± 0.04^{d}	0.4 ± 0.1^{b}	0.97 ± 0.05^{bc}	6.1 ± 0.2^{bc}	5.9 ± 0.4^{cde}	38.2 ± 0.3^{c}
C1	94 ± 9^{c}	0.65 ± 0.02^{bc}	0.16 ± 0.04^{c}	1.2 ± 0.1^{a}	6.0 ± 0.6^{bc}	7.1 ± 0.3^{bc}	26.4 ± 0.8^{d}
C2	125.1 ± 0.6^{b}	0.58 ± 0.02^{c}	0.58 ± 0.02^{ab}	1.01 ± 0.01^{bc}	7.2 ± 0.2^{b}	7.3 ± 0.2^{b}	29.2 ± 0.7^{d}
D1	128 ± 6^{b}	0.46 ± 0.02^{d}	0.68 ± 0.04^{a}	0.96 ± 0.03^{c}	5.8 ± 0.3^{c}	5.6 ± 0.5^{de}	28.8 ± 0.2^{d}
D2	160 ± 7^{a}	0.59 ± 0.01^{c}	0.5 ± 0.1^{ab}	0.99 ± 0.06^{bc}	9.5 ± 0.6^{a}	9.3 ± 0.3^{a}	35.8 ± 0.9^{c}

$^{a–e}$ Means within the same column with different letters are significantly different ($p < 0.05$).

Yogurts: A1) without curdlan or heat treatment; A2) without curdlan and with heat treatment; B1) with commercial curdlan and without heat treatment; B2) with commercial curdlan and heat treatment; C1) with pre-gelled commercial curdlan and without heat treatment; C2) with pre-gelled commercial curdlan and heat treatment; D1) with curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) without heat treatment; D2) with curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) with heat treatment.

It can be seen that the use of curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) produced significant alterations in the parameters of firmness, cohesiveness and adhesiveness of the yogurt, even without being subjected to heat treatment. This is due to the thickening potential of the material. Heat treatment caused major changes in these parameters, both for the yogurts with commercial curdlan and pre-gelled commercial curdlan, although the highest values for firmness, gumminess and chewiness were obtained for the yogurt with curdlan produced by the microorganism. Heat treatment promotes the formation of a firm gel due to the intermolecular hydrophobic interactions that structure the system, making the yogurt

more consistent, resulting in greater difficulty to separate in the mouth and making it denser during chewing, which therefore takes longer.

It was notable that the pre-gelled commercial curdlan formed a stronger gel in water, but had less effect on the texture parameters than that produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) when applied to the yogurt, which was not expected. Thus, it is likely that the latter has a greater ability to interact with water and the other components of milk, especially proteins, more efficiently stiffening the protein network formed after the fermentation process. As described before, the curdlan produced by the microorganism has the capacity to absorb 64% more water and 98% more oil than the pre-gelled commercial curdlan, with these parameters being important for products such as yogurts prepared with whole milk (3% fat).

The use of commercial curdlan without heat treatment did not produce many changes in the texture parameters or in the syneresis of the yogurts. This is because it was not effectively homogenized in the milk as it dispersed with greater difficulty, interfering little in the formation of the protein network during fermentation.

It was found that syneresis was reduced for the yogurt samples with curdlan, particularly the pre-gelled commercial variety and the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method). The latter displayed higher syneresis after the first 28 days of storage because its gel is less stable than the pre-gelled commercial curdlan. As such, the pre-gelled commercial curdlan demonstrated a greater ability to preserve the structure of the yogurt, avoiding rearrangements in the casein network due to the time of storage and avoiding whey expulsion. Martinez et al. [3] produced yogurts with added curdlan and achieved a significant reduction in the syneresis of the products compared to the control (without curdlan). The authors found that the high stability obtained by the yogurt with curdlan is attributed to interactions

626 between the curdlan molecules, either between one another or with the proteins, which
627 cause the formation of a more compact and continuous three-dimensional protein
628 network, which effectively entraps the protein molecules and water in its structure.

629 Importantly, there was no difference between any of the texture parameters and
630 the syneresis of yogurts without curdlan submitted or not to heat treatment. This was
631 expected as the aim of treatment was to gel the curdlan and, therefore, the A2 sample
632 was not submitted to rheological analysis. Fig 4 shows the flow curves (Figs 4A and
633 4B) and the viscosity curves (Figs 4C and 4D) for the yogurts produced with curdlan
634 with and without heat treatment of the milk prior to fermentation.

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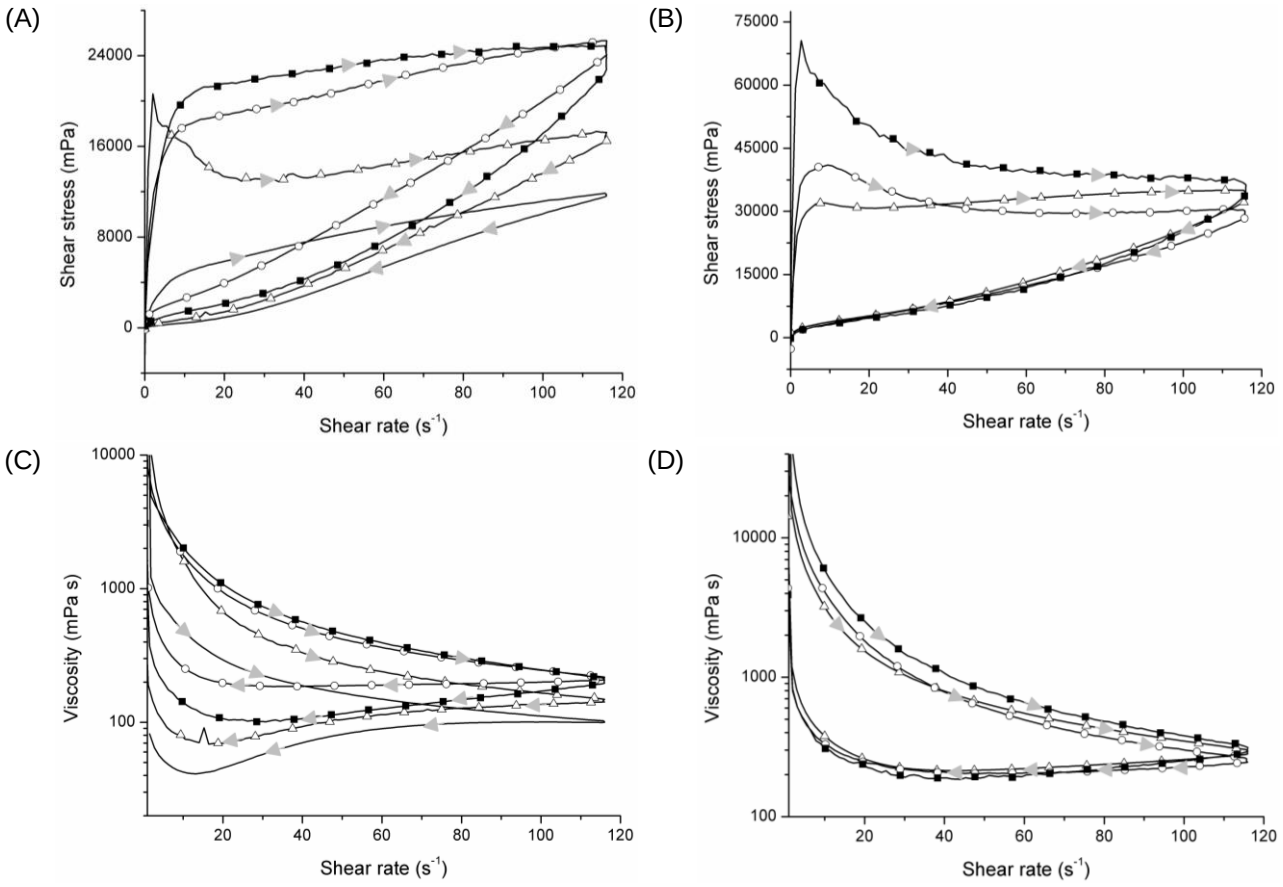

636

637 **Fig 4. Flow curves of yogurt samples without heat treatment (A) with heat**
638 **treatment (B); viscosity curves of yogurt samples without heat treatment (C) and**

with heat treatment (D). Yogurt without curdlan (–), with commercial curdlan (Δ), with pre-gelled commercial curdlan (○) and with curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) (■). The direction of the gray arrows indicates the ascendant and descendant curves.

All the yogurts behaved as non-Newtonian pseudo-plastic fluids, as their viscosity decreased due to the shear rate applied. It can be seen that the samples subjected to heat treatment of the milk had higher viscosity values across the entire shear rate analyzed due to the gelling of the curdlan. However, the decrease in viscosity versus the shear rate in the return data was much more significant for these samples. All the samples also exhibited thixotropic characteristics, due to the difference of tension and viscosity between the ascending and descending shear rate curves. The hysteresis observed is due to the breakdown of the gel structure formed by coagulation of the protein during fermentation, in the presence and absence of curdlan. Hysteresis is measured as the area between the ascending and descending curves, where the greater the area (when positive), the greater the thixotropic effect. Hysteresis, together with the other rheological parameters, are described in Table 5.

Table 5. Rheological parameters of yogurt samples with and without curdlan and submitted or not to heat treatment. Hysteresis, consistency index (K), flow behavior index (n) and yield stress (τ_0). Values indicate mean ± standard-deviation.

Sample	*K (mPa s^n)*	*n*	*τ_0 (Pa)*	*Hysteresis (Pa s^{-1})*
A	98 ± 3^{d}	0.997 ± 0.006^{a}	2 ± 1^{e}	362 ± 43^{e}
B1	223 ± 26^{d}	0.938 ± 0.001^{b}	12 ± 1^{d}	1088 ± 75^{d}
B2	1685 ± 18^{a}	0.662 ± 0.004^{e}	30 ± 4^{c}	2241 ± 39^{b}
C1	922 ± 35^{b}	0.73 ± 0.04^{d}	12 ± 1^{d}	1134 ± 92^{d}
C2	--*	--*	36 ± 2^{b}	2200 ± 49^{b}
D1	467 ± 56^{c}	0.87 ± 0.02^{c}	10.9 ± 0.5^{d}	1623 ± 36^{c}
D2	--*	--*	75 ± 3^{a}	3461 ± 10^{a}

[a–e] Means within the same column with different letters are significantly different ($p < 0.05$).

*It was not possible to calculate the parameters by the Oswald de Waele model.

In Fig 4B, the heat-treated samples of yogurt with pre-gelled commercial curdlan and curdlan produced by the microorganism displayed a peak in shear strain at the beginning of the growth of the deformation rate. This peak is related to the lack of homogeneity of the product, because during the gelling process of curdlan small lumps are formed, which are not necessarily noticeable to the palate, but which alter rheology. With the presence of these lumps, the tension needed for a small deformation is high, and as a result, it was not possible to calculate the K and n parameters for samples C2 and D2 by the Oswald de Waele model.

From the data of Table 5 it is clear that the higher viscosity samples in Figs 4C and 4D, which are those subjected to heat treatment, had more evident thixotropic characteristics (higher hysteresis), as they underwent a major reduction in dynamic viscosity with time, in a rate constant with shearing. This is due to the breakdown of the organized yogurt structure when submitted to a determined strain. After remained stationary, the samples returned to their original state more quickly, but with lower

viscosity during the return, being this recovery dependent on time. Therefore, the gel formed with heat treatment presented low stability, in particular the gel of the curdlan produced by the microorganism, corroborating with the syneresis data of the yogurt.

Curiously, it was observed that of the samples without heat treatment, that which had the greater viscosity in the increasing shear rate curve did not present higher K and n values, or in other words, the sample with pre-gelled commercial curdlan was greater than the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) in the consistency index and flow behavior. This was due to the sharp drop in viscosity in the decreasing curve of the latter, revealing again the lower stability of the gel formed.

The results of the yield strength parameter (τ_0) were consistent with the firmness of the yogurt, as the yogurt with curdlan produced by *Agrobacterium* sp. IFO 13140 with thermic treatment withstood the greatest tension before suffering deformation, followed by the yogurts with pre-gelled commercial curdlan and commercial curdlan with heat treatment. It is important to note that yield stress refers to the maximum stress that the material can bear before yielding (in the elastic deformation regime) and that the greatest τ_0 values are those of the firmer samples. These samples displayed a minimum stress for deformation that was more difficult to break due to the increased organization and stiffening of the protein network formed, originating from the intermolecular hydrophobic interactions of the curdlan with heating.

Conclusions

The characteristics of dispersion, gelation and the rheological properties of curdlan depended greatly on the recovery methods employed after its production, as the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) and the commercial pre-gelled curdlan dispersed better in water, acted as thickeners and formed

more homogeneous gels. The use of the pre-gelation method exerts a major influence on the size of the particles obtained and in the presence of NaCl, which contributes significantly to the dispersion and gelation characteristics described. However, the recovery method employed did not influence the structure of the polysaccharide. Even the pre-gelled commercial curdlans and those produced by the microorganism through the pre-gelation method had significantly different technological properties. The curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) showed a greater thickening and water and oil holding capacity than the pre-gelled commercial curdlan, while the latter demonstrated a considerably greater gelling capacity, that is related to degree of polymerization of the polysaccharide. As a consequence, although both types of curdlan increased firmness, viscosity and reduced the syneresis of yogurts, the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) caused a greater increase in the parameters, but also showed a less stable gel formation than the pre-gelled commercial curdlan. The commercial curdlan caused less significant effect in the firmness and syneresis of yogurts tested. Nevertheless, the curdlans recovered by the pre-gelation method provided rigid gels with a stable structure, allowing improvements in the texture of a range of products, and therefore, has great potential for application in the food industry.

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Author Contributions

Conceived and designed the experiments: CSM GM. Performed the experiments: CSM TTS VCF LNK SBSF. Analyzed the data: CSM MLB GM. Contributed reagents/materials/analysis tools: MLB GM. Wrote the paper: CSM GM.

References

1. Zhang R, Edgar K. Properties, chemistry and applications of the bioactive polysaccharide curdlan. Biomacromolecules. 2014; 15: 1079-1096.

2. Donot F, Fontana A, Baccou JC, Schorr-Galindo S. Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. Carbohydr Polym. 2012; 87: 951-962.

3. Martinez CO, Ruiz SP, Fenelon VC, Morais GR, Baesso ML, Matioli G. Characterization of curdlan produced by *Agrobacterium* sp. IFO 13140 cells immobilized in loofa sponge matrix, and application of this biopolymer in the development of functional yogurt. J Sci Food Agric. 2016; 96: 2410-2417.

4. Triveni R, Shamala TR, Rastogi NK. Optimised production and utilization of exopolysaccharide from *Agrobacterium radiobacter*. Process Biochem. 2001; 36: 787-795.

5. Lee I, Seo WT, Kim GJ, Kim MK, Park CS, Park YH. Production of curdlan using sucrose or sugar cane molasses by two-step fed-bactch cultivation of *Agrobacterium* species. J Ind Microbiol Biotechnol. 1997; 18: 255-259.

6. Wu C, Yuan C, Chen S, Liu D, Ye X, Hu Y. The effect of curdlan on the rheological properties of restructured ribbonfish (*Trichiurus* spp.) meat gel. Food Chem. 2015; 179: 222-231.

7. Li J, Zhu L, Lu G, Zhan X, Lin C, Zheng Z. Curdlan β-1,3-glucooligosaccharides induce the defense responses against *Phytophthora infestans* infection of potato (*Solanum tuberosum* L. cv. McCain G1) leaf cells. PLoS ONE. 2014; 9: e97197.

8. Wang M, Chen C, Sun G, Wang W, Fang H. Effects of curdlan on the color, syneresis, cooking qualities and textural properties of potato starch noodles. Starke. 2010; 62: 429-434.

9. Chen C, Wang R, Sun G, Fang H, Ma D, Yi S. Effects of high pressure level and holding time on properties of duck muscle gels containing 1% curdlan. Innov Food Sci Emerg Technol. 2010; 11: 538-542.

10. Funami T, Nishinari K. Gelling characteristics of curdlan aqueous dispersions in the presence of salts. Food Hydrocoll. 2007; 21: 59-65.

776 11. Chel-Guerrero L, Pérez-Flores V, Betancur-Ancona D, Dávila-Ortiz G. Functional
777 properties of flours and protein isolates from *Phaseolus lunatus* and *Canavalia*
778 *ensiformis* seeds. J Agric Food Chem. 2002; 50: 584-591.
779
780 12. Martinez CO, Ruiz SP, Nogueira MT, Bona E, Portilho M, Matioli G. Effective
781 immobilization of *Agrobacterium* sp. IFO 13140 cells in loofa sponge for curdlan
782 biosynthesis. Molecules. 2015; 20: 7957-7973.
783
784 13. Shin H, Liu L, Kim M, Park Y, Chen R. Metabolic engineering of *Agrobacterium*
785 sp. ATCC 31749 for curdlan production. J Ind Microbiol Biotechnol. 2016; 43: 1323-
786 1331.
787
788 14. Gagnon M, Lafleur M. From curdlan powder to the triple helix gel structure: an
789 attenuated total reflection-infrared study of the gelation process. Appl Spectrosc. 2007;
790 61: 374-378.
791
792 15. Fenelon VC, Aguiar MFA, Miyoshi JH, Martinez CO, Matioli G. Ultrafiltration
793 system for cyclodextrin production in repetitive batches by CGTase from *Bacillus*
794 *firmus* strain 37. Bioprocess Biosyst Eng. 2015; 38: 1291-1301.
795
796 16. Dubois M, Gilles KA, Hamilton JK, Reber PA, Smith F. Colorimetric method for
797 determination of sugars and related substances. Anal Chem. 1956; 28: 350-356.
798
799 17. AOAC International. Official Methods of Analysis of AOAC International (method
800 926.12). 1996.

18. AOAC International. Official Methods of Analysis of AOAC International (method 985.35). 1995.

19. Hirashima M, Takaya T, Nishinari K. DSC and rheological studies on aqueous dispersions of curdlan. Thermochim Acta. 1997; 306: 109-114.

20. Keogh MK, O'Kennedy BT. Rheology of stirred yogurt as affected by added milk fat, protein and hydrocolloids. J Food Sci. 1998; 63: 108-112.

21. Jin Y, Zhang H, Yin Y, Nishinari K. Comparison of curdlan and its carboxymethylated derivative by means of Rheology, DSC, and AFM. Carbohydr Res. 2006; 341: 90-99.

22. Mikkelsen MS, Jespersen BM, Moller BL, Laerke HN, Larsen FH, Engelsen SB. Comparative spectroscopic and rheological studies on crude and purified soluble barley and oat β-glucan preparations. Food Res Int. 2010; 43: 2417-2424.

23. Novák M, Synytsya A, Gedeon O, Slepička P, Procházka V, Synytsya A, et al. Yeast β(1-3),(1-6)-D-glucan films: preparation and characterization of some structural and physical properties. Carbohydr Polym. 2012; 87: 2496-2504.

24. Marchessault RH, Deslandes Y. Finestructure of (1→3)-β-D-glucans: curdlan and paramylon. Carbohydr Res. 1979; 75: 231-242.

825 25. Che Man YB, Irwandi J, Abdullah WJW. Effect of different types of maltodextrin
826 and drying methods on physico-chemical and sensory properties of encapsulated durian
827 flavour. J Sci Food Agric. 1999; 79: 1075-1080.
828
829 26. Favaro-Trindade CS, Santana AS, Monterrey-Quintero ES, Trindade MA, Netto
830 FM. The use of spray drying technology to reduce bitter taste of casein hydrolysate.
831 Food Hydrocoll. 2010; 24: 336-340.
832
833 27. Spicer EJF, Goldenthal EI, Ikeda T. A toxicological assessment of curdlan. Food
834 Chem Toxicol. 1999; 37: 455-479.
835
836 28. Funami T, Funami M, Yada H, Nakao Y. A rheological study on the effects of
837 heating rate and dispersing method on the gelling characteristics of curdlan aqueous
838 dispersions. Food Hydrocoll. 2000; 14: 509-518.
839
840 29. Zhang H, Nishinari K, Williams MAK, Foster TJ, Norton IT. A molecular
841 description of the gelation mechanism of curdlan. Int J Biol Macromol. 2002; 30: 7-16.
842
843 30. Wu J, Zhan X, Liu H, Zheng Z. Enhanced production of curdlan by *Alcaligenes*
844 *faecalis* by selective feeding with ammonia water during the cell growth phase of
845 fermentation. Chin J Biotechnol. 2008; 24: 1035-1039.
846
847 31. Saitô H, Yokoi M, Yoshioka Y. Effect of hydration on conformational change or
848 stabilization of (1→3)-β-D-glucans of various chain lengths in the solid state as studied

by high-resolution solid-state ^{13}C NMR spectroscopy. Macromolecules. 1989; 22: 3892-3898.

32. Nakanishi I, Kimura K, Kusui S, Yamazaki E. Complex formation of gel-forming bacterial (1→3)-β-D-glucans (curdlan-type polisaccharides) with dyes in aqueous solution. Carbohydr Res. 1974; 32: 47-52.

33. Seguchi M, Kusunose C. Lipophilization of curdlan granules by heat-treatment or chlorination. Food Hydrocoll. 2001; 15: 177-183.

34. Sciarini LS, Maldonado F, Ribotta PD, Pérez GT, León AE. Chemical composition and functional properties of *Gleditsia triacanthos* gum. Food Hydrocoll. 2009; 23: 306-313.

35. Chan TWD, Tang KY. Analysis of a bioactive β-(1→3) polyssacharide (curdlan) using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2003; 17: 887-896.

Supporting information captions

S1 Appendix. Curdlan interaction with aniline blue dye.

Solutions of the following concentrations of pre-gelled commercial curdlan and of curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gellation method) were prepared in NaOH 5 mmol L^{-1}: 0, 50, 100 and 150 µg mL^{-1}. These solutions were mixed with an equal volume of phosphate buffer (0.5 mol L^{-1}, pH 7) containing 160 mg L^{-1} of aniline blue and kept for 120 min at room temperature. Then, the absorbance of the mixtures was measured at 590 nm. The analysis was performed in triplicate.

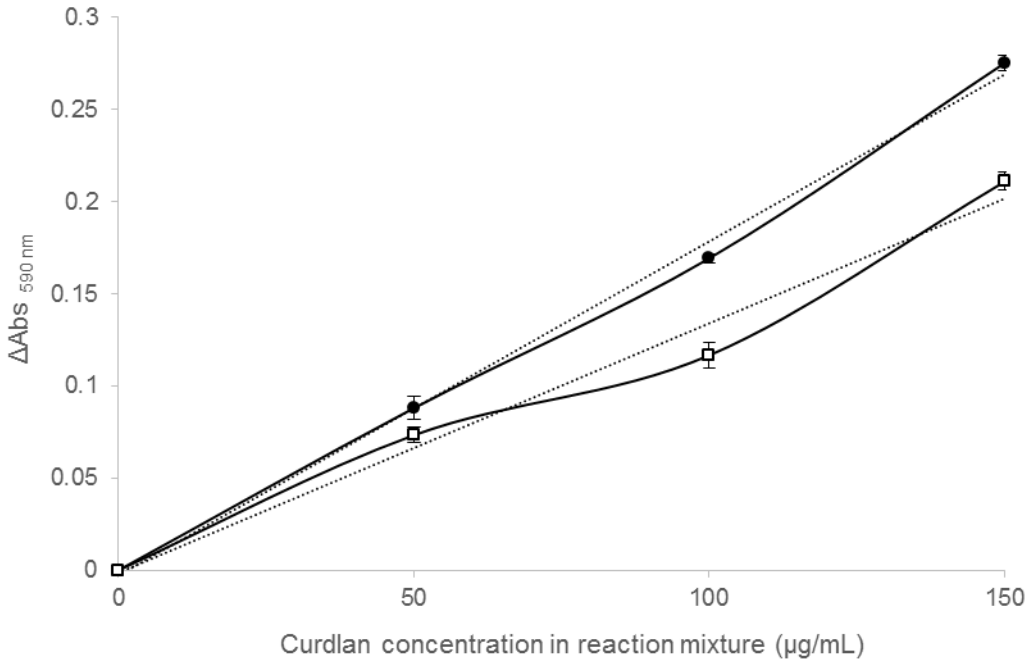

Fig S1. Relationship between absorbance difference and concentration of curdlans. Pre-gelled commercial curdlan (●) and curdlan produced by *Agrobacterium* sp. IFO 13140 recovered by the pre-gellation method (□).

ARTICLE 2

Use of FT-IR, FT-Raman and thermal analysis to evaluate the gel formation of curdlan produced by *Agrobacterium* sp. IFO 13140 and determination of its rheological properties with food applicability

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ABSTRACT

Curdlan is a linear polysaccharide composed of glucose units joined by β-(1,3) bonds that possesses unique gelation properties. This study aimed to characterize the structure and evaluate the gelling properties of curdlan produced by *Agrobacterium* sp.

IFO 13140 and its gels, as well as apply it in food. FT-Raman analysis highlighted the structural changes that occurred during the formation of gels, with variations related to the hydrogen bonds and hydrophobic interactions, which occur with the formation of the low-set and high-set gels, respectively. Rheological analysis showed that the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium sp*. IFO 13140 differed in terms of gelation properties, which depends of the degree of polymerization of the polysaccharide, but when applied to pasta products, both improved the texture parameters. The curdlan gels were found to have great potential as gelling agents to improve texture, water retention capacity and stability of food products.

Keywords: microbial polysaccharide, gelling agent, β-glucan, texture improver, thickener.

1 INTRODUCTION

Curdlan is a neutral linear polysaccharide composed of repeating glucose units linked by β-(1,3) bonds, and is classified as a β-glucan. It was discovered in 1966 when Harada and collaborators investigated the production of succinoglycan using a non-pathogenic bacillus found in soil. The microorganism, *Alcaligenes faecalis* variety *myxogenes* (today named *Agrobacterium* sp. by the ATCC), produced curdlan in an unexpected form by a mutant strain known as 10C3K (Amemura, Hisamatsu & Harada, 1977). This mutant strain became the precursor of others that preserved curdlan gel-forming ability. Amongst these strains are ATCC 21680 (or IFO 13140), ATCC 31749 and ATCC 31750 (McIntosh, Stone & Stanisich, 2005).

The molecule was named curdlan due to its capacity to curdle when heated. This property has allowed it to be used as a gelling agent for improving texture, water retention capacity and thermal stability of several food products (Shih, Yu, Hsieh & Wu, 2009). Curdlan is one of the few bacterial additives approved by the US Food and Drug Administration (FDA). Its gelling capacity has major potential for applications in food and pharmaceutical industries; curdlan gels can be used as fat replacers and they are colorless, flavorless and indigestible (Gagnon & Lafleur, 2007). Curdlan is therefore a useful additive for the preparation of pasta products, frozen foods and canned meats, being biodegradable, edible and non-toxic to humans and the environment (Shih et al., 2009).

A number of studies of the application of curdlan have recently been carried out in a range of industrial sectors. In the food industry, its use has been reported as a texture enhancer (Wang, Chen, Sun, Wang & Fang, 2010), fat replacer (Chen, Wang, Sun, Fan, Ma & Yi, 2010), encapsulant of active ingredients (Ferreira, Faria, Grosso & Mercadante, 2009), for the lowering oil uptake during deep-fat frying (Funami, Funami, Tawada & Nakao, 1999), and even as a potential prebiotic ingredient in rat feeding (Shimizu, Tsuchihashi, Kudoh, Wada, Takita & Innami, 2001). In the biomedical area, recent works have employed curdlan and its derivatives to prepare nanoparticles for application as release-controlled drug vehicles (Na, Park, Kim & Bae, 2000; Popescu, Pelin, Butnaru, Fundueanu & Suflet, 2013). Curdlan has also been recently used in the preparation of edible blend films (Wu et al., 2012) and as a support matrix for enzyme immobilization (Saudagar & Singhal, 2004).

In the food industry, curdlan is of great interest because of its solubility and gel formation properties. It is insoluble in water, but its aqueous dispersion forms two types of gels through heating, which are known as low-set or high-set. Low-set gel is obtained

by heating the dispersion at temperatures between 55 and 60 °C, with subsequent cooling to below 40 °C, and is thermo-reversible. High-set gel is obtained by heating the aqueous dispersion to above 80 °C and is irreversible (Funami & Nishinari, 2007).

Many factors such as the form of recovery of the polysaccharide from the medium, concentration, temperature, heating time and rate, as well as the dispersion method, can influence the conformational structure of curdlan and consequently affect its mechanical and gelling properties. The transition temperature of the gel from thermo-reversible to irreversible is dependent on its concentration and can also depend on the molecular weight of the molecule (Funami & Nishinari, 2007). Even curdlans produced by the same strain can differ in terms of mechanical properties depending on the conditions of culture of the microorganism. Thus, the structural study of curdlan and its gelling mechanisms and properties are essential to better define its use in the food industry.

The structure and gelling mechanisms of curdlan have been studied by many authors using a variety of methods. Techniques such as carbon nuclear magnetic resonance (^{13}C-NMR) (Saitô, Ohki & Sasaki, 1977), infrared spectroscopy (FT-IR) (Gagnon & Lafleur, 2007), X-ray diffraction (Harada, Okuyama, Konno, Koreeda & Harada, 1994), electronic microscopy (Marchessault & Deslandes, 1979), and differential scanning calorimetry (Hirashima, Takaya & Nishinari, 1997), have been used to identify both the chemical bonds involved in the gelling process and the structural conformation of the molecule. However, the use of Raman spectroscopy (FT-Raman) for the study of the molecule and its gels is both rare and original, and in accordance with Synytsya, Čopíková, Matějka and Machovič (2003), FT-IR and FT-Raman techniques are complementary for the structural analysis of carbohydrates.

Considering the interest in the gelling properties of curdlan for food application, the present study aimed to characterize the structure of curdlan produced by *Agrobacterium* sp. IFO 13140 and evaluate its gels properties. The mechanisms of formation of the curdlan gels were studied by FT-IR and FT-Raman spectroscopies, as well as their formation temperature by thermal analysis. In addition, the application of curdlan in homemade pasta was evaluated.

2 MATERIALS AND METHODS

2.1 Materials

The bacterial strain *Agrobacterium* sp. IFO 13140 was acquired from the Institute for Fermentation of Osaka (Japan) in lyophilized form. Commercial curdlan was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). According to the supplier, commercial curdlan is produced as a water-insoluble linear polyssacharide without branching by *Alcaligenes faecalis* var. *myxogenes*. The other reagents were of analytical grade.

2.2 Production of curdlan by *Agrobacterium* sp. IFO 13140

The reactivation of the microorganism was conducted using the culture medium proposed by the supplier (g/L): polypeptone (10), yeast extract (2), $MgSO_4.7H_2O$ (1) and pH 7. Thirty milligrams of the lyophilized bacteria were incubated in 100 mL of the medium at 30 °C and 120 rpm for 48 h. The free cells were recovered through centrifugation at 6000 ×g for 10 min, washed with 0.9% NaCl (w/v) and transferred to the production medium. The production of curdlan was performed in Erlenmeyer flasks containing 100 mL of the liquid medium described by Martinez, Ruiz, Nogueira, Bona,

Portilho and Matioli (2015) (g/L): glucose (50), KH_2PO_4 (2.7), NH_4Cl (1.6), $MgSO_4$ (0.5), pH 7 and trace elements (10 mL/L). The composition of the trace elements (g/L) in HCl 0.1 mol/L was: $FeCl_3.6H_2O$ (1), $MnCl_2.4H_2O$ (1), $CaCl_2$ (1), ZnCl (1) and $CaCO_3$ (0.03). The reactivated microorganisms were transferred to the production medium and maintained at 30 °C and 150 rpm for 5 days. Curdlan production was carried out in 5 batches, each containing 10 Erlenmeyer flasks with production medium.

To recover curdlan from the medium, NaOH 3 mol/L was added to the Erlenmeyer flasks containing the production medium at a ratio of 1.8:1 (NaOH:medium) to solubilize curdlan. Subsequently, the mixture was centrifuged at 18000 ×g for 15 min at 4 °C to separate the cell pellets. The curdlan present in the supernatant was precipitated by the addition of HCl 3 mol/L to pH 6-7 and recovered by centrifugation at 18000 ×g for 15 min at 4 °C. It was then washed three times with distilled water (20 °C) and subsequently lyophilized. The curdlan yield (the relation between the amount of curdlan produced and the initial amount of glucose) was also calculated. The commercial curdlan was subjected to the same treatment, and was therefore described as pre-gelled commercial curdlan to be differentiated from commercial curdlan. According to Gagnon and Lafleur (2007), commercial curdlan is purified by dissolution in a strong alkaline solution and dried in a spray-dryer, then washed with water until neutralization.

2.3 Preparation of curdlan dispersions and gels

The dispersions of the commercial pre-gelled curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 were prepared at a concentration of 2% (w/v) in water, in accordance with Hirashima et al. (1997), with modifications. The water-insoluble polysaccharide curdlan was dispersed in water using a mixer at room temperature for 5 min, sonicated for 10 min and then stirred in a magnetic stirrer for 12 h at 40 °C. The

dispersions were kept in a water bath at 61 °C/1 h to prepare the low-set gel and at 95 °C/1 h for preparation of the high-set gel. The temperatures used to gel formation were according to Gagnon and Lafleur (2007), with slight modifications due to the concentration of curdlan dispersions. After heating, samples were kept under refrigeration or lyophilized for further analysis.

2.4 Structural characterization of curdlan and its gels by FT-IR and FT-Raman

Samples of the commercial pre-gelled curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140, their lyophilized dispersions and gels, and also a sample of the commercial curdlan, were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR) and Fourier transform Raman scattering infrared spectroscopy (FT-Raman).

The FT-IR spectra of the samples were obtained using a Fourier Transform infrared spectrometer (Vertex 70v model, Bruker, Germany). The spectral region was 400-4000 cm^{-1} with 128 scans and a resolution of 2 cm^{-1}. The samples were mixed in KBr powder and pellets were made to perform the measurements. The Raman spectra of the samples were obtained using a Fourier Transform infrared spectrometer (Vertex 70v model with Ram module II, Bruker, Germany) equipped with a Germanium detector cooled with liquid nitrogen. A Nd:YAG laser was used for excitation at 1064 nm. The spectra were based an average of 200 scans with a resolution of 4 cm^{-1}. Both FT-IR and FT-Raman analysis were performed in duplicate.

2.5 Thermal analysis of curdlan dispersions

Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG) were performed on the dispersions (2%) of the commercial pre-gelled curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140, prepared as described in section 2.3. The

analyses were conducted in duplicate using a Simultaneous System of DSC/TGA Thermal Analysis (STA 409 PG Luxx model, Netzsch-Gerätebau GmbH, Selb/Bavaria, Germany). Samples were heated from 20 to 160 °C at a rate of 2° C/min under a nitrogen atmosphere (flow rate 30 mL/min).

2.6 Determination of number-average degree of polymerization (DP_n) of curdlans

The determination of the degree of polymerization of commercial curdlan, pre-gelled commercial curdlan and curdlan produced by *Agrobacterium* sp. IFO 13140 was performed according to the methodology established by Zhang and Lynd (2005) to determine the DP_n of insoluble cellulose. By this method, the DP_n is calculated as the ratio of glucosyl monomer concentration determined by the phenol-sulfuric acid method divided by the reducing-end concentration determined by the modified 2,2'-bicinchoninate (BCA) method. The assay was performed in quadruplicate.

2.7 Rheological characteristics of curdlan dispersions and gels

The dispersions of the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 at a concentration of 2% were prepared as described in section 2.3. The samples were prepared 24 h prior to analysis and were analyzed in a controlled stress rotational rheometer (HAAKE MARS II model, Thermo Fisher Scientific Inc., Newington, Germany), with steel cone/plate geometry (60 mm, separated by a fixed distance of 0.052 mm in the center). The samples were applied to the lower plate of the rheometer very carefully and allowed to equilibrate for 60 s prior to analysis. First, the linear viscoelastic region (the region in which stress is directly proportional to strain and the G' modulus remain constant) for each sample was determined at 20 °C. After this determination, the elastic (G') and viscous (G") modulus,

and the dynamic viscosity were measured depending on temperature (20-60 °C) at a frequency of 10 Hz. Subsequently, the same parameters, both for the dispersions and the gels of curdlans were evaluated at a temperature of 20 °C, with variations in frequency (0.1 to 10 Hz), and with application of a constant stress of 10 mPa. The curves of the temperature sweep test and of the dynamic oscillatory frequency test were then calculated using the RheoWin 4.10.000 software program (HAAKE software, Thermo Fisher Scientific Inc., Newington, Germany). In each case, the dynamic rheological properties of at least four replicate samples were determined (Hirashima et al., 1997).

2.8 Curdlan application in homemade pasta products

2.8.1 Preparation of homemade pasta products

To prepare the pasta products, wheat flour, curdlan (1%), water (30%), salt (1.4%) and eggs (40%) were used. The amounts of each ingredient are described in relation to wheat flour. With the exception of the curdlan, the ingredients were purchased in local market. Four pasta product formulations were prepared: A) without curdlan; B) with commercial curdlan; C) with pre-gelled commercial curdlan; D) with curdlan produced by *Agrobacterium* sp. IFO 13140. First, the dry ingredients (except curdlan) were mixed together. The curdlan was hydrated in water with a mixer and added to the dry ingredients, together with the eggs. The pasta products were kneaded and molded with a cylinder (3 mm thick) and cut and stored under refrigeration for further analysis.

2.8.2 Cooking characteristics of pasta products

The cooking of the pasta product formulations was conducted according to Wang et al. (2010), with variation only in cooking time due to differences in thickness and type of cereal product. The parameters evaluated were cooking loss (CL) and cooked weight (CW). Approximately 3 g of pasta were weighed and boiled in 200 mL of boiling deionized water for 30 min. The container remained closed during cooking to prevent loss by evaporation. The cooked pasta products were rinsed with 50 mL of distilled water to eliminate particles released during cooking that remained adhered in some way, and dried with a paper towel to remove excess water from its surface. The CL was measured by draining the cooking water and drying to constant weight at 105 °C, and expressed as the percentage of solids lost in cooking (Eq. 1). The CW was calculated and expressed as a percentage of the mass gained during cooking (Eq. 2).

$$CL\ (\%) = \frac{w_{after\ drying}}{w_{initial}} \times 100 \quad (1)$$

$$CW\ (\%) = \frac{w_{cooked}}{w_{initial}} \times 100 \quad (2)$$

Where, $w_{after\ drying}$ is the weight of solids obtained after draining the cooking water and drying to constant weight, $w_{initial}$ is the weight of the pasta before cooking and w_{cooked} is the weight of the pasta after cooking. The analysis was performed in triplicate.

2.8.3 Texture profile analysis (TPA) and tensile strength of the pasta products

For texture profile analysis of the pasta products, a TA-XT Plus texturometer (Stable Micro Systems, Godalming, UK) equipped with a version of the Texture Expert software (Stable Micro Systems, Godalming, UK) was used. The pasta products were prepared as described in section 2.8.1 and manually cut into 4 × 4 cm pieces (length × width). After, the pasta were cooked in boiling water for 30 min, cooled in cold water

(20 °C, 10 min) and subsequently dried with paper towels. A 35 mm cylindrical probe was used for the TPA test (ref. P/35, Stable Micro Systems). The probe compresses a single strip of pasta dough with a constant deformation rate of 1 mm/s to 70% of initial thickness. The probe was withdrawn and remained stationary for 10 s before a second compression was performed to 70% of the original thickness, thus obtaining the TPA curve, from which the hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness parameters were calculated. The measurements were performed in quadruplicate for each formulation. For the tensile strength test, the texturometer was equipped with traction claws (ref. A/TG, Stable Micro Systems) and the test conducted at a speed of 0.5 mm/s. The pasta products were prepared as described in section 2.8.1 and manually cut into 10 × 5 cm pieces (length × width). After, the pasta were cooked during 30 min (3 h prior to analysis, to remove moisture from the surface) and subsequently dried with paper towels. The assay was performed in triplicate for each formulation.

2.9 Statistical analysis

The data obtained were analyzed by analysis of variance (ANOVA). Means were compared with the Tukey Test ($p<0.05$) using the Statistica 8.0/2008 software package (Stat Soft, Inc., Tulsa, USA).

3 RESULTS AND DISCUSSION

Curdlan production by *Agrobacterium* sp. IFO 13140 achieved 20.0 ± 0.8 g/L, which corresponds to a curdlan yield (the relation between curdlan production and the initial amount of glucose) of 44 ± 2%. When discovering the production of curdlan by *Alcaligenes faecalis* var. *myxogenes* 10C3K, Harada, Fujimori, Hirose and Masada

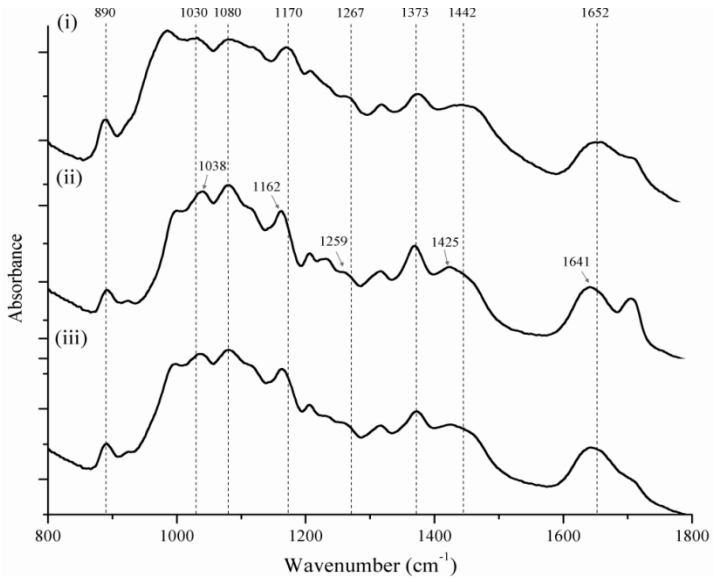
(1966) established a culture medium that resulted in a high polysaccharide yield, achieving 19.8 g/L in a medium containing 40 g/L of glucose, a result very similar to that obtained in the present study.

In their review of exopolysaccharides of industrial interest, Donot, Fontana, Baccou and Schorr-Galindo (2012) found that strains of *Agrobacterium* sp. can produce 5.02 to 76 g/L of curdlan depending on the strain and culture conditions. Authors who worked with smaller containers and without continuous control of process parameters, such as Portilho, Matioli, Zanin, Moraes and Scamparini (2006), obtained a production of approximately 20 g/L of curdlan by *Agrobacterium* sp. IFO 13140 and 22 g/L by *Agrobacterium* sp. ATCC 31749, values that are very close to those found in the present study, which shows that *Agrobacterium* sp. IFO 13140 is a high yield strain for the production of curdlan. Authors who worked with higher volumes in a fermenter with continuous control of process parameters obtained the highest values of curdlan production. Kim, Ryu, Choi, Rhee and Lee (2003), using a mutant strain of *Agrobacterium* sp. ATCC 31750 in a 300 L fermenter achieved a production of 76 g/L.

3.1 Structural characterization of curdlan and its gels by FT-IR and FT-Raman

Figure 1 shows the FT-IR spectra of the commercial curdlan, the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 (Fig. 1A), of the dispersion of the pre-gelled commercial curdlan and its gels (Fig. 1B) and of the dispersion of curdlan produced by *Agrobacterium* sp. IFO 13140 and its gels (Fig. 1C).

(A)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(ii)
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1000
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1400
1600
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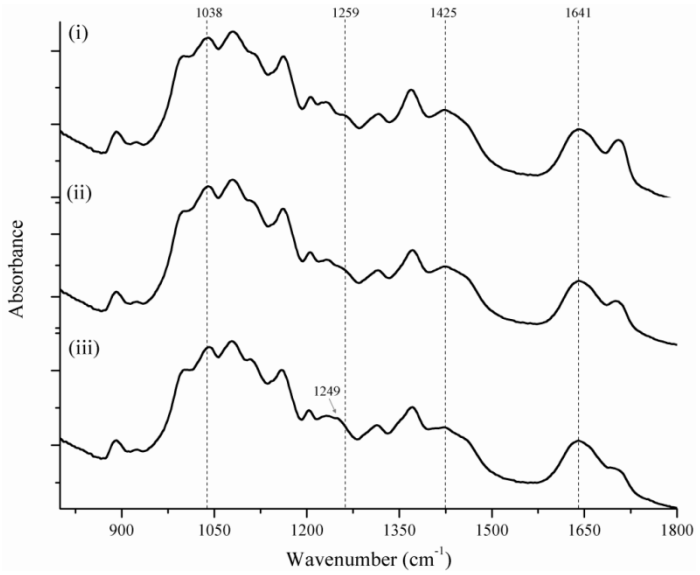
Wavenumber (cm-1)

(B)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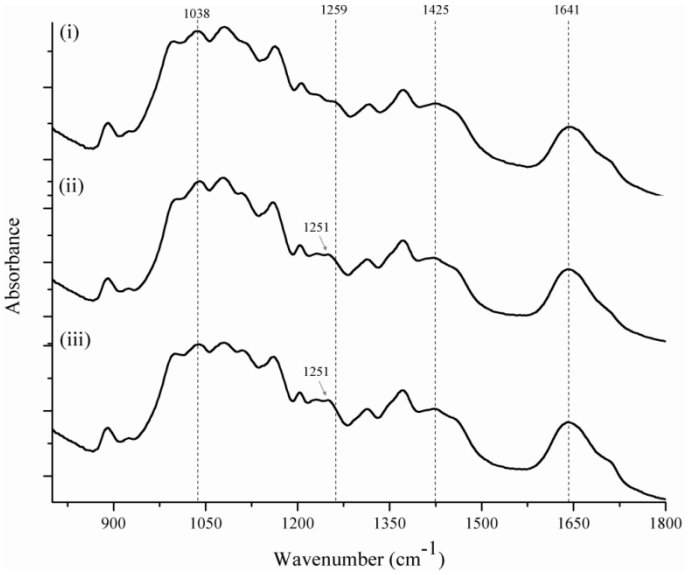
Wavenumber (cm-1)

(C)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1650
1800
Wavenumber (cm-1)

298

Figure 1: FT-IR spectra: (A) curdlans – (i) commercial, (ii) pre-gelled commercial, (iii) produced by *Agrobacterium* sp. IFO 13140; (B) dispersion of pre-gelled commercial curdlan (i) without heat treatment, (ii) heated to 61 °C/1 h, (iii) heated to 95 °C/1 h; (C) dispersion of curdlan produced by *Agrobacterium* sp. IFO 13140 (i) without heat treatment, (ii) heated to 61 °C/1 h, (iii) heated to 95 °C/1 h. The dotted lines show the characteristic peaks of the curdlans tested.

In the commercial curdlan spectrum (Fig. 1Ai) the most significant peaks for the characterization of the polysaccharide are demonstrated and their tentative assignments are described: the peak at 890 cm^{-1}, is attributed to the C_1–H deformation of the formation of the β-glycosidic bond, and the peak at approximately 1170 cm^{-1}, is probably attributed to the vibration C_1–O–C_3 of the same glycosidic bond (Gagnon & Lafleur, 2007). The peak at 1030 cm^{-1} is associated with the vibration of the C_1–O–C_5 bond of the ring of the glucose molecule (Wu, Cai & Sun, 2012). The peaks at 1080, 1267 and 1373 cm^{-1} are related to the vibrations of C–O, C–OH and C–H, respectively (Jin, Zhang, Yin & Nishinari, 2006). The last peaks, at 1442 and 1652 cm^{-1} are associated to the vibrations of the CH_2 groupings (carbon 6) and the association between the water and the curdlan molecule, resulting in vibrations of the H–O–H grouping, respectively (Popescu et al., 2013).

When pre-gelled, the commercial curdlan displayed the same variations in the FT-IR spectrum as the curdlan produced by *Agrobacterium* sp. IFO 13140 (Figures 1Aii and 1Aiii), revealing a structural similarity between the commercial curdlan and that produced by the microorganism. The differences between the spectra of commercial curdlan and the curdlans submitted to pre-gelling (pre-gelled commercial curdlan and the one produced by *Agrobacterium* sp. IFO 13140), which are some displacements of

the peaks at 1030, 1170, 1267 and 1652 cm^{-1} of the original molecule, can be related to the initial solubilization of curdlan in NaOH (that leads to water incorporation in the molecule) and subsequent neutralization with HCl (that causes the restoration of hydrogen bonds) due to the pre-gelation process. The bands at 1030, 1170 and 1267 cm^{-1} are associated to vibrations of C–O or C–OH of curdlan molecules, while the band around 1640 cm^{-1} is associated with water.

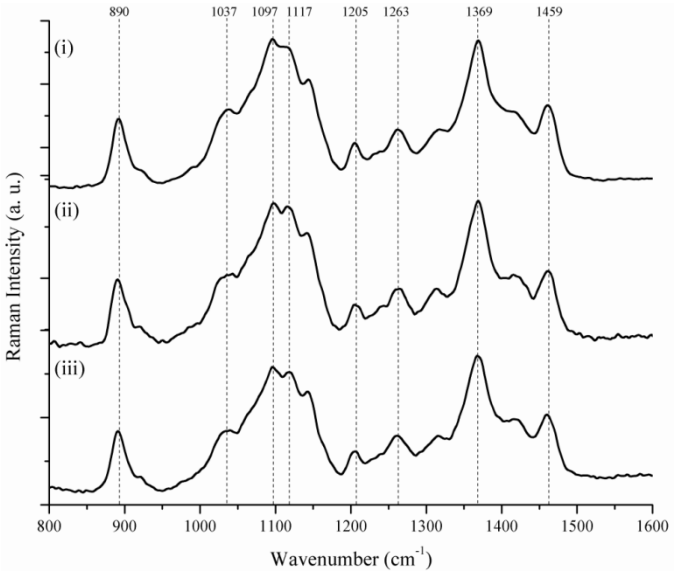
Figures 1B and 1C show the FT-IR spectra of curdlan dispersions and its gels. Modifications were envisaged, especially at the peaks at 1030, 1267 and 1442 cm^{-1} for the gels formed, which are related to the vibrations of the C_1–O–C_5, C–OH and –CH_2 bonds, involved in the gelling processes. However, modifications had already appeared in these peaks in both dispersions (Figs. 1Bi and 1Ci), and these were maintained in the gels formed. As such, it is difficult to identify structural modifications in curdlan related to its gelling using the FT-IR technique employed in this study. This is firstly due to the fact that the obtaining or pre-gelling of curdlan involves the reorganization of the hydrogen bonds of the molecule, a phenomenon that also occurs with the formation of the low-set gel, and secondly due to the spectra obtained presenting significant overlaying of the peaks, making the study of structure difficult.

Gagnon and Lafleur (2007) studied the processes of hydration and gelling of curdlan molecule using the FT-IR–ATR technique and obtained similar results to those found in the present study. When analyzing the region of 850 to 1200 cm^{-1}, they identified the appearance of characteristic peaks of curdlan molecule, especially at 890 and 1170 cm^{-1} and also perceived that the hydration of the molecule significantly modified the spectra of this region, as it is where the vibrations of the C–OH bonds of the carbohydrate appear. Also, the authors observed that the low-set gel presented an identical spectrum to the hydrated molecule, considering that the mechanism of

formation of the gel also occurs through the structuration of hydrogen bonds, and that the high-set gel possesses subtle differences, making it difficult to identify the structural modifications responsible for gelling.

According to Synytsya et al. (2003), Raman spectroscopy is widely used for structural investigations of polysaccharides, being evident that the FT-IR and FT-Raman spectroscopies are complementary for the structural analysis of carbohydrates. Some vibrational bands, which are either very weak or overlaid by other stronger bands and, therefore, cannot be detected using IR, can be identified or studied in FT-Raman spectroscopy and vice-versa. In the case of the FT-IR spectra obtained in the present study for curdlan and its gels, the region between 800 and 1600 cm^{-1} is replete with overlaid bands, while the FT-Raman spectra are clearer and the peaks better defined in this region. Figure 2 shows the FT-Raman spectra of commercial curdlan, pre-gelled commercial curdlan and curdlan produced by *Agrobacterium* sp. IFO 13140 (Fig. 2A), of the dispersion of the pre-gelled commercial curdlan and its gels (Fig. 2B) and of the dispersion of curdlan produced by *Agrobacterium* sp. IFO 13140 and its gels (Fig. 2C).

(A)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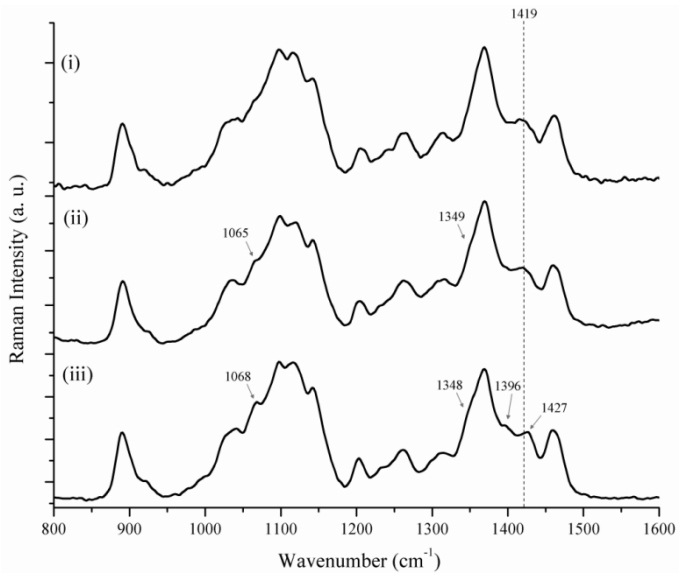
Wavenumber (cm-1)

(B)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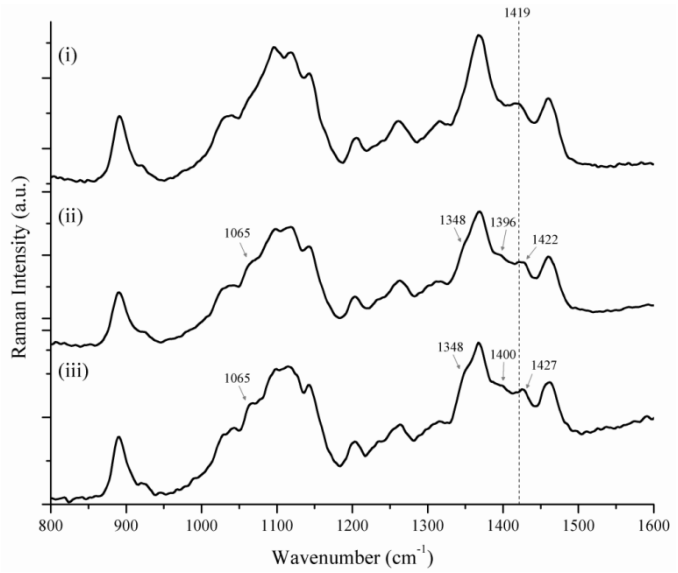
Wavenumber (cm-1)

(C)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(iii)
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1400
1427
Raman Intensity (a.u.)
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900
1000
1100
1200
1300
1400
1500
1600
Wavenumber (cm-1)

374

Figure 2: FT-Raman spectra: (A) curdlans – (i) commercial, (ii) pre-gelled commercial, (iii) produced by *Agrobacterium* sp. IFO 13140; (B) dispersion of pre-gelled commercial curdlan (i) without heat treatment, (ii) heated to 61 °C/1 h, (iii) heated to 95 °C/1 h; (C) dispersion of curdlan produced by *Agrobacterium* sp. IFO 13140 (i) without heat treatment, (ii) heated to 61 °C/1 h, (iii) heated to 95 °C/1 h. The dotted lines show the characteristic peaks of the curdlans tested.

Figure 2A shows the most significant FT-Raman peaks for the characterization of polysaccharide. Again, the peak at 890 cm^{-1} is attributed to the C_1–H deformation of the formation of the glucoside bond in the β form. The peak at 1037 cm^{-1} is attributed to the stretching of the C–C and C–OH bonds of the molecule (Synytsya et al., 2003), while the peaks at 1097 and 1117 cm^{-1} are related to the symmetric and asymmetric stretching of C–O–C of the glycosidic bonds. According to Kizil and Irudayaraj (2007), the peak at 1205 cm^{-1} is attributed to the vibrations of the C–C–H bonds and the peak at 1263 cm^{-1} is the result of the vibrations of the CH_2OH group of the side chain of the glucoses. Finally, the peak at 1369 cm^{-1} is attributed to the angular deformation of the CH and C_3–OH bonds, and the peak at 1459 cm^{-1} is found in the angular deformation region of the CH_2 grouping.

Remaining with Figure 2A, it can be seen that the three samples have the same structure. Then, Figures 2B and 2C can provide evidences of the structural variations due to the formation of gels in the commercial pre-gelled curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140. With the formation of low-set gel (Figs. 2Bii and 2Cii), there is the appearance of a shoulder at 1065 cm^{-1}, attributed to the stretching of the C–O and C–C bonds of the glucose ring, a shoulder at 1349 cm^{-1}, related to the angular deformation of the C–OH bond and the twisting of the CH_2

radical, and another shoulder at 1396 cm^{-1} (more evident in the curdlan gel produced by the microorganism), which can be attributed to the angular bending of the CH_2 radical and the deformation of the C–H and C–OH bonds (Kizil & Irudayaraj, 2007). These variations in these spectra are related to the formation mechanism of the low-set gel. According to Gagnon and Lafleur (2007), the low-set gel can be considered an assembly of swollen particles that interact via hydrogen bonding. The main conformation in these gels is the single helix structure. Additionaly, Tako and Hanashiro (1997) propose a more specific mechanism that retains this single helix structure: the hydrogen bonding between the oxygen of the glucose ring (C_1–O–C_5) and the hydroxyl of carbon 4, or between hydroxyls of the CH_2 groupings of carbon 6.

The formation of the high-set gel is evidenced by the accentuation of the formation of the shoulders described for the low-set gel, added to the displacement of the peak at 1419 cm^{-1} to 1427 cm^{-1}. This peak is related to the angular deformation of the CH_2 grouping of the glycoses of the molecule. As described by many authors (Gagnon & Lafleur, 2007; Saitô et al., 1977; Tako & Hanashiro, 1997) the high-set gel is formed by intermolecular hydrophobic interactions which are associated with the formation of a triple helix conformation. Tako and Hanashiro (1997) propose a more specific mechanism that preserves this triple helix conformation: the intermolecular hydrophobic interactions between CH_2 groupings of C-6. Additionaly, Saitô et al. (1977) observed by ^{13}C NMR some shift of C-4 signals, which is compatible with an intramolecular hydrogen bond between O-4'---O-5 and are ascribed to a region of single helical conformation. The same authors also observed very broad ^{13}C resonance peaks of C-1–C-5 and C-6 that are ascribed to the triple-helical junction zones for the gel structure and their vicinities. Also, Takeda, Yasuoka, Kasai and Harada (1978) stated by X-ray diffraction studies that by heat-treatment, the single helical molecules of curdlan

425 in water change their structure to triple-stranded helices, showing a behavior that
426 suggest the existence of a compact hydrophobic structure after heat-treatment. All these
427 results are in accordance with the mechanisms described above for formation of low-set
428 and high-set gels of curdlan and also give support to the ones proposed by Tako and
429 Hanashiro (1997). The gel formation mechanisms proposed by Tako and Hanashiro
430 (1997) which are in agreement with the FT-Raman variations of this work are shown in
431 Supplementary material 1.
432

433 **3.2 Thermal analysis (DSC and TG) of curdlan dispersions**

434 Figure 3 shows the DSC (Fig. 3A), TG (Fig. 3B) and DTG (Fig. 3C) data of the
435 dispersions of the pre-gelled commercial curdlan and curdlan produced by
436 *Agrobacterium* sp. IFO 13140, in a concentration of 2%. In Fig. 3A, both samples
437 displayed two well defined peaks, with the former being between 40 and 55 °C and the
438 latter between 70 and 80 °C. The two samples showed great variation in peak
439 temperature, which made it necessary to analyze the degree of polymerization (DP_n), as
440 according to Zhang, Huang, Nishinari, Watase and Konno, 2000, the magnitude of the
441 DSC peaks and the temperature of the peaks are susceptible to changes depending on
442 the concentration, molecular weight and experimental conditions. The DP_n values
443 obtained were 334 ± 8 for the pre-gelled commercial curdlan and 232 ± 10 for the
444 curdlan produced by microorganism, which corresponds to molecular masses of
445 approximately 54000 and 38000, respectively, and which explains why the peaks of the
446 two dispersions were not at the same temperature (the higher molecular weights lead to
447 higher transition temperatures). A dispersion of commercial curdlan was not evaluated
448 in thermal analysis because the commercial curdlan (DP_n = 332 ± 12) did not form
449 homogeneous dispersions and gels with the methodology employed in section 2.3.

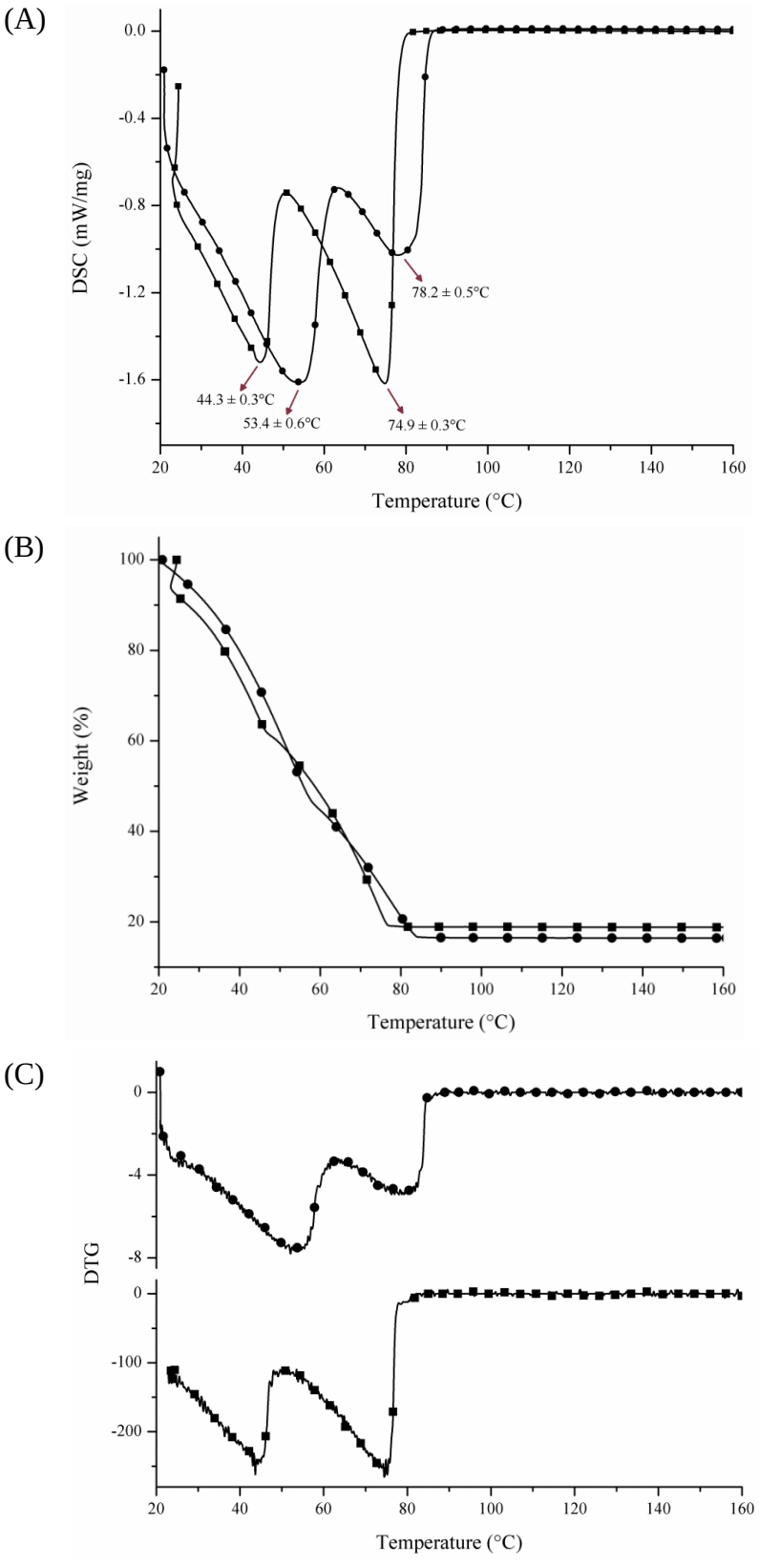


Figure 3: DSC (A), TG (B) and DTG (C) data of the dispersions of pre-gelled commercial curdlan (●) and curdlan produced by *Agrobacterium* sp. IFO 13140 (■) in a concentration of 2%.

In Fig. 3A, the first peak is due to the swelling of curdlan caused by the breaking of some hydrogen bonds, the first step of low-set gel formation. A number of studies (Harada et al., 1994; Hirashima et al. 1997; Jin et al., 2006; Konno & Harada, 1991; Zhang, Nishinari, Williams, Foster & Norton, 2002; Zhang et al., 2000) have performed DSC of curdlan gels in aqueous dispersions of 1, 2, or 4%, with a heating rate of 1 or 2 °C/min. All these works found that between 40 and 60 °C a clear endothermic peak appears due to swelling of curdlan molecules. Hirashima et al. (1997), for example, observed an endothermic peak at close to 58 °C, which shifted slightly with the increasing concentration of the curdlan dispersion (5 to 15%). The second peak shown in Fig. 3A for both dispersions (between 70 and 80 °C) is due to the hydrophobic interactions between curdlan molecules. Konno and Harada (1991) reported the appearance of a wide and shallow peak between 70 and 105 °C, which indicates the structural transition resulting from the formation of a firm gel stabilized by hydrophobic interactions. In the present study, the peak that represents the formation of this gel is thin and clearly defined.

According to Gagnon and Lafleur (2007), when an aqueous dispersion of curdlan is heated, two types of gel may be formed depending on the heating temperature. The first is a thermo-irreversible (~80 °C) high-set gel, maintained by intermolecular hydrophobic interactions, and the second is a thermo-reversible (~55 °C) low-set gel, maintained by intramolecular hydrogen bonds. The curdlan chains can adopt two helical conformations: single or triple helices. The gelling property of the polysaccharide is also attributed to transformations of its helical arrangement at different temperatures. At room temperature and in the low-set gel, the structure is predominantly formed by single helix curdlan chains, which are more strongly

associated in the low-set gel. In the high-set gel, the structure is predominantly formed by associated triple helices, forming an organized gel configuration.

The temperature for formation of the gels indicated in Fig. 3A can also be identified in Fig. 3B, which represents the loss of mass data of the curdlan dispersion samples during heating. For both samples, two distinct stages of loss of mass appeared at temperatures close to the endothermic peaks of DSC. In the first step, at up to 46.6 and 58.4 °C for the curdlan produced by *Agrobacterium* sp. IFO 13140 and the pre-gelled commercial curdlan, respectively, the weight loss is more pronounced, as the curdlan molecules are disorganized and swollen, which facilitates water loss. In the second stage, at temperatures up to 77 and 84.2 °C for the curdlan produced by *Agrobacterium* sp. IFO 13140 and the pre-gelled commercial curdlan, respectively, the loss of mass was a little less pronounced, as with the formation of a firm gel it is more difficult for water to become detached from the structure formed.

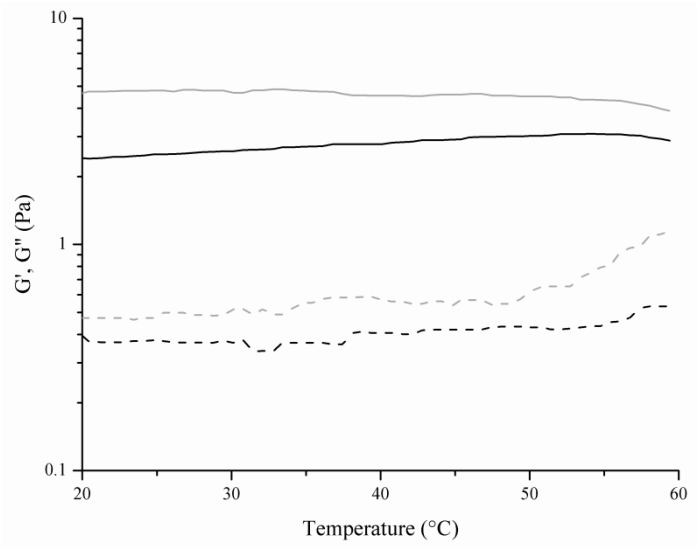
The two temperature stages seen in Fig. 3B, in which the mass loss was observed, can be better understood by Fig. 3C, which shows differential mass loss thermograms (DTG) of the curdlans, obtained from the weight loss curves (Fig. 3B). This mathematical procedure provides the rate of mass loss with increasing temperature. As a result, it can be seen that the DSC curves are recovered in the DTG thermograms of the samples, evidencing the approximate transition temperatures for the two gel formations for both the curdlan produced by *Agrobacterium* sp. IFO 13140 (~44 and 74 °C), and for the pre-gelled commercial curdlan (~54 and 78 °C).

3.3. Rheological characteristics of curdlan dispersions and gels

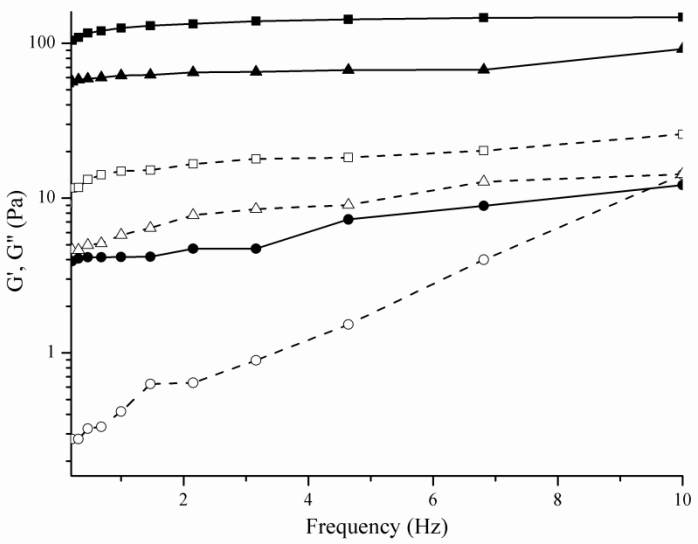
Determining some technological properties of polysaccharides is of great importance when predicting their possible applications. To better define the use of

curdlan and its gels as thickeners and gelling agents in the food industry, a rheological study that elucidate their mechanical properties is essential. Figure 4 shows the rheological data of the dispersion samples of the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 based on temperature (Figure 4A) and the oscillatory rheology of the curdlan dispersions and gels (Figures 4B and 4C). Once again, dispersions and gels of commercial curdlan were not evaluated in rheological analysis because the commercial curdlan did not form homogeneous dispersions and gels with the methodology employed in section 2.3.

(A)


10
1
0.1
G', G" (Pa)
20
30
40
50
60
Temperature (°C)

(B)


100
10
1
G', G" (Pa)
2
4
6
8
10
Frequency (Hz)

(C)

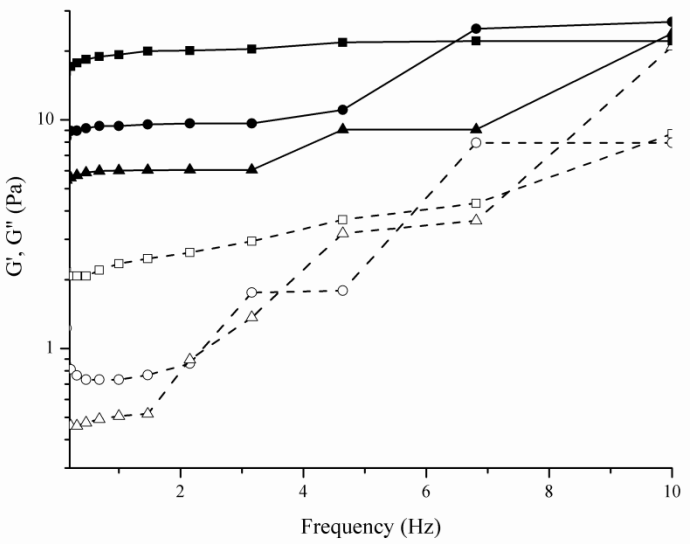

10
1
G', G" (Pa)
2
4
6
8
10
Frequency (Hz)

Figure 4: Temperature dependency of the G' (continuous line) and G" (dotted line) modules for the dispersions of pre-gelled commercial curdlan (black line) and of curdlan produced by *Agrobacterium* sp. IFO 13140 (grey line) (A); the frequency dependency of the modules G' (continuous line) and G" (dotted line) for the dispersions of pre-gelled commercial curdlan (B) and of curdlan produced by *Agrobacterium* sp. IFO 13140 (C) without heat treatment (●), heated to 61 °C/1 h (▲) and heated to 95 °C/1 h (■).

In Figure 4A it can be seen that the dispersions of the pre-gelled commercial curdlan and the curdlan produced by *Agrobacterium* sp. IFO 13140 display elastic behavior in their gels, as the elastic module (G') is greater than the viscous module (G"). Also, the curdlan produced by *Agrobacterium* sp. IFO 13140 presented higher values for both modules, as well as higher values for dynamic viscosity (data not shown), indicating that it has greater thickening potential than the pre-gelled commercial curdlan, which can be due to its lower DP_n, that facilitates the swelling or hydration of the molecule. The increase in temperature up to 60 °C did not have a widely significant effect on the rheological parameters of the dispersions (Fig. 4A). This is because, in the pre-gelling process, the neutralization of the alkaline solution of curdlan with acid results in a gelatinous precipitate, which has similar properties to those of the low-set gel (Kanzawa, Harada, Koreeda & Harada, 1987).

The G' values slightly decreased for the curdlan produced by the microorganism with increasing temperature. A similar result was found by Salah et al. (2011), evaluating the rheological properties of commercial curdlan depending on temperature (up to 65 °C), who observed that increasing the temperature reduced the viscosity of the curdlan. According to the authors, this is due to the fact that the temperature affects the conformational structure of the polysaccharide in solution so that it undergoes a

553 transition from a rigid and organized structure to a disordered structure. For the pre-
554 gelled commercial curdlan the G' values increased slightly. This temperature
555 dependence shows that the pre-gelled commercial curdlan mobility is lower than the
556 mobility of curdlan produced by the microorganism, and its chains are therefore more
557 rigid. As such, the commercial curdlan possesses a more rigid structure than that
558 produced by *Agrobacterium* sp. IFO 13140, demonstrating greater structural stability
559 when subjected to temperature variation. Similar results were obtained by Zhang et al.
560 (2002), who when evaluating the rheological behavior of 2% curdlan dispersions
561 depending on temperature found that this module experienced a slight increase as
562 temperature increased.

563 The rheological parameters of the curdlan dispersions and their gels were
564 evaluated according to frequency (Figs. 4B and 4C). Once again, the dispersion of
565 curdlan (without being submitted to heat treatment) produced by *Agrobacterium* sp. IFO
566 13140 presented higher values for both G' and G" modules, as well as higher values for
567 dynamic viscosity (data not shown), indicating that it has greater thickening potential
568 than the pre-gelled commercial curdlan. Despite this fact, the commercial curdlan had a
569 much greater gelling capacity, as the G' and G" values for its high-set gel reached 100
570 and 10 Pa, respectively, while for the high-set gel of the curdlan produced by the
571 microorganism these values remained only slightly superior to the dispersion without
572 heat treatment. The great difference between gelling capacities of the curdlans is related
573 to their distinct DP_n (or molecular weight). The extent of forming the triple-stranded
574 helices may simply depend upon the molecular weight of the polysaccharide. Thus,
575 higher degree of cross-linking may be achieved for samples with larger DP_n. In other
576 words, the gel forming ability of curdlan increases with increasing DP_n of the
577 polysaccharide (Nakanishi, Kimura, Kusui & Yamazaki, 1974). Also, according to

578 Ogawa and Tsurugi (1973), DP_n around 200 may be the lower limit to form gels in
579 aqueous media (DP_n of curdlan produced by the microorganism is 232 ± 10).

580 Still in Figs. 4B and 4C, it was verified that the parameters G' and G" increased
581 with the formation of gels, especially the parameter G', which is the measure of the
582 energy stored within the gel network. This shows that the interlacing between the
583 curdlan molecules, which forms a three-dimensional gel network, becomes stronger
584 with increased temperature. Additionally, the values of the G"/G' relationship based on
585 frequency were greater for samples that were not submitted to heat treatment, and
586 declined as the temperature used for gel formation increased. For example, for the
587 dispersion sample of the pre-gelled commercial curdlan at 2%, the G"/G' values were
588 0.15 ± 0.01 to 1.2 ± 0.1 between the frequencies of 0.1 to 10 Hz for the sample without
589 heat treatment and 0.045 ± 0.009 to 0.18 ± 0.03 for the sample treated at 95 °C/1 h.
590 Analogous results were found for the dispersion samples of the curdlan produced by
591 microorganism at 2%. As the relationship G"/G' is a measure of the dynamic
592 characteristics of the intermolecular bonds in the gel network, low values indicate that
593 the gel reacts to stress in a relatively more elastic than viscous manner. This result
594 suggests that the number of elastically active chains of curdlan molecules increased with
595 gel formation, making the gel an elastic structure. Similar results were found by
596 Hirashima et al. (1997), who assessed the rheological parameters of 2% curdlan
597 dispersions, and observed an increase, notably in G', as temperature increased, showing
598 the hardness of the gel formed, and also observed the reduction of the G"/G'
599 relationship.

600 The curdlan dispersions and their gels displayed an increase in rheological
601 parameters as the frequency applied was increased, but became more linear when higher
602 temperatures were employed for forming the gels (Figs. 4B and 4C), corroborating the

fact that the hardness obtained by the high-set gels makes this a stable structure for use in various products.

3.4 Application of curdlan in homemade pasta products

Curdlan is an ingredient of great interest to both the food and food supplement industries because, in addition to its ability to gel when heated, it is the most concentrated source of β-(1,3)-insoluble glucan available, and so is considered a dietary fiber. The addition of curdlan to foods therefore has many advantages. It can contribute to a variety of textures in foods, depending on the heat treatment applied, the concentration and the other ingredients present in the product. Among its main properties of interaction with other ingredients, it has a high water absorption capacity, and so can help obtain products with higher humidity and consequently a higher yield. It also has a high capacity of fat absorption, making it an important structural agent for food, especially in increasing palatability and the extension of shelf life (Jezequel, 1998). These properties influence various attributes of homemade pasta products.

According to Wang et al. (2010), the attributes of cooking loss and cooked weight are of great importance for cooked pasta products and consist of indicators of the loss of solids and the water absorbed during cooking, respectively. The addition of curdlan did not influence the cooking loss (CL) as the four pasta products evaluated (without curdlan, with commercial curdlan, with pre-gelled commercial curdlan and with curdlan produced by *Agrobacterium* sp. IFO 13140) presented values between 4.6 ± 0.9% and 5.9 ± 0.5% for this parameter, with no statistically significant difference ($p<0.05$). However, the use of curdlan increased statistically significantly ($p<0.05$) the water absorption or the cooked weight (CW) of the pasta products, with the pre-gelled commercial curdlan and the curdlan produced by the microorganism presenting similar

628 CW values of 175 ± 4% and 173 ± 3%, respectively. The commercial curdlan had no
629 effect on the cooked weight parameter, most likely because it is not easily homogenized
630 in the other ingredients. The CW value for the pasta products with commercial curdlan
631 was 158 ± 5%, a value statistically equal ($p<0.05$) to the pasta products without curdlan
632 (156 ± 5%).

633 There is a great difficulty in homogenizing or dispersing curdlan in food
634 products because it is insoluble in water. Therefore, it is necessary to use an efficient
635 homogenizer and, according to Zhang et al. (2002) and Jin et al. (2006), apply a further
636 stirring of the dispersion prior to the carrying out of applications requiring uniformity.
637 Both pre-gelled commercial curdlan and curdlan produced by *Agrobacterium* sp. IFO
638 13140 disperse easily in water when subjected to stirring and, when heated at 95 °C,
639 form a firm and homogeneous gel. However, the commercial curdlan do not easily
640 disperse in water and, even being gelled after heating at 95 °C, it occurs with a phase
641 separation in the gel formed. Other authors (Marchessault & Deslandes, 1979) have
642 previously identified the formation of a non-homogenous gel from curdlan in its native
643 form. Thus, the characteristics of dispersion of curdlan depend greatly on the recovery
644 method employed after its production. In the pre-gelling process, the neutralization of
645 the alkaline solution of curdlan with acid results in a gelatinous precipitate (Kanzawa et
646 al., 1987), which is much easier to rehydrate than commercial curdlan after dried
647 (commercial curdlan is neutralized with water and precipitates in the form of particles).
648 Thus, the use of the pre-gelling method in curdlans, which significantly increases the
649 ease application of the polysaccharide in food matrices and consequently results in
650 significant effects in the texture characteristics of pastas, is extremely important.

651 Wang et al. (2010) added different amounts of curdlan to macaroni pasta and
652 obtained similar results to the present study for the cooking loss parameter, since the

addition of curdlan in concentrations of up to 1% did not change the values found. However, the same author found that an increase in the amount of curdlan added to the pasta products resulted in a decrease in cooked weight, which varied from the result of this work. It is likely that the lack of prior treatment of the curdlan is the reason for this result, as pre-gelation allows a better dispersion of the polysaccharide in the pasta products. Comparing the cooked weight data for the samples without curdlan and with commercial curdlan in this study, no variation in the attribute was noted.

Commercial curdlan and the one produced by *Agrobacterium* sp. IFO 13140 are odorless, tasteless and have a very light color (light grayish yellow color). Supplementary material 2 exhibits a picture of both samples. Therefore, the addition of curdlan caused no significant effects on the flavor and appearance of homemade pasta products. Table 1 presents the texture parameters for these products prepared with and without curdlan. The addition of pre-gelled commercial curdlan and curdlan produced by *Agrobacterium* sp. IFO 13140 caused a significant increase in the hardness, adhesiveness and gumminess of the pasta, and a less significant increase in chewiness. This is because, with gelling occurring homogeneously in the pasta products, they become firmer and more adhesive in the mouth during chewing, and maintains a more persistent density, meaning that it spends more time in the mouth.

677 **Table 1:** Texture parameters of homemade pasta products with and without curdlan.
678 Values indicate mean ± standard-deviation.

679

Texture parameter	Sample			
	A	**B**	**C**	**D**
Hardness (N)	$135^{b} \pm 4$	$140^{b} \pm 6$	$183^{a} \pm 2$	$181^{a} \pm 9$
Cohesiveness	$0.86^{a} \pm 0.01$	$0.79^{b} \pm 0.01$	$0.73^{b} \pm 0.05$	$0.78^{b} \pm 0.01$
Adhesiveness (N × mm)	$6^{c} \pm 4$	$37^{b} \pm 8$	$60^{a} \pm 8$	$58^{a} \pm 7$
Springiness (mm)	$0.98^{a} \pm 0.01$	$0.94^{b} \pm 0.02$	$0.93^{b} \pm 0.02$	$0.95^{ab} \pm 0.01$
Gumminess (× 10^2 N)	$117^{b} \pm 3$	$110^{b} \pm 3$	$134^{a} \pm 9$	$142^{a} \pm 8$
Chewiness (× 10^2 N)	$115^{bc} \pm 2$	$105^{c} \pm 4$	$125^{ab} \pm 10$	$134^{a} \pm 7$
Tensile strength (N)	$28^{a} \pm 1$	$28.4^{a} \pm 0.4$	$29.4^{a} + 0.5$	$28.3^{a} \pm 0.3$
Extensibility (mm)	$11^{b} \pm 1$	$14^{b} \pm 1$	$13^{b} \pm 2$	$20^{a} \pm 2$

680 [a,b,c] Means within the same line with different letters are significantly different ($p < 0.05$).
681 Pasta product formulations: A) without curdlan; B) with commercial curdlan; C) with pre-gelled
682 commercial curdlan; D) with curdlan produced by *Agrobacterium* sp. IFO 13140.

683

684 The addition of commercial curdlan to the mass did not affect its hardness or
685 gumminess, but caused an increase (even if less significant than the other curdlans) in
686 adhesiveness. In terms of cohesiveness, the parameter related to degree of compression
687 of the food between the teeth before it breaks, all the curdlans caused the same reduction
688 in this parameter. The addition of curdlan had little influence on the parameters of
689 springiness, chewiness and tensile strength of the pastas. These changes are due to the
690 fact that the irreversible gel of curdlan is a firmer gel than it is elastic, and has greater
691 influence on parameters related to chewing force.

692 Wang et al. (2010) evaluated the firmness and tensile strength of the pasta
693 products with the addition of different concentrations of curdlan, and noted that adding
694 curdlan of over 0.3% increased the firmness of the pasta products and also the tensile
695 strength thereof. The authors also state that texture is a determining factor in pasta

quality: high firmness indicates high chewiness and high tensile strength indicates high elasticity, which are desirable characteristics for cooked pasta products. Oishi et al. (2009) added various concentrations of curdlan (up to 2%) to pasta products with hypoallergenic wheat flour (in which gluten is partially hydrolyzed by enzymes) and noticed that the addition of curdlan to this pasta increased the breaking strain and the hardness of the pasta in addition to inhibiting cooking loss, as the concentration of the curdlan added increased. The authors explain that this is due to the formation of an irreversible gel which occurs at cooking temperatures.

4 CONCLUSIONS

The data obtained from structural analysis revealed a similarity between the curdlan produced by *Agrobacterium* sp. IFO 13140 and the commercial curdlan. Among the analyses performed, the FT-Raman technique proved to be especially useful for the study of structural changes resulting from the formation of the curdlan gels, showing the hydrogen bonds and hydrophobic interactions that occur with the formation of the low-set and high-set gels, respectively, which could not be observed in FT-IR analysis. Despite the structural similarities, the gelling properties of the curdlans displayed significant differences, which are related to the difference between their degrees of polymerization. The curdlan produced by *Agrobacterium* sp. IFO 13140, which has the lowest DP_n, has a greater thickening capacity than the pre-gelled commercial curdlan, while the latter has a considerably greater gelling capacity. In spite of that, both types of curdlan increased the hardness and water absorption of pasta products, unlike commercial curdlan. The characteristics of dispersion and consequently gelation and rheological properties of curdlan depend greatly on the recovery methods employed

after its production, as the curdlan produced by *Agrobacterium* sp. IFO 13140 and the commercial pre-gelled curdlan disperse better in water than the commercial curdlan in its native form and are easily to disperse during their food application. In an innovative manner, the use of pre-gelling method has been proven to be very important for a more effective use of curdlan in foods. Nevertheless, the curdlan gels, especially the high-set gel, had a rigid, stable and elastic structure, displaying great potential for use as a gelling agent to improve the texture, water retention capacity and thermal stability of several food products.

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6 REFERENCES

Amemura, A., Hisamatsu, M., & Harada, T. (1977). Spontaneous mutation of polysaccharide production in *Alcaligenes faecalis* var. *myxogenes* 10C3. *Applied and environmental Microbiology*, 34, 617–620.

Chen, C., Wang, R., Sun, G., Hongmei, F., Ma, D., & Yi, S. (2010). Effects of high pressure level and holding time on properties of duck muscle gels containing 1% curdlan. *Innovative Food Science and Emerging Technologies*, 11, 538–542.

Donot, F., Fontana, A., Baccou, J. C., & Schorr-Galindo, S. (2012). Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers*, 87, 951–962.

Ferreira, D. S., Faria, A. F., Grosso, C. R. F., & Mercadante, A. Z. (2009). Encapsulation of blackberry anthocyanins by thermal gelation of curdlan. *Journal of the Brazilian Chemical Society*, 20, 1908–1915.

Funami, T., & Nishinari, K. (2007). Gelling characteristics of curdlan aqueous dispersions in the presence of salts. *Food Hydrocolloids*, 21, 59–65.

Funami, T., Funami, M., Tawada, T., & Nakao, Y. (1999). Decreasing oil uptake of doughnuts during deep-fat frying using curdlan. *Journal of Food Science*, 64, 883–888.

Gagnon, M., & Lafleur, M. (2007). From curdlan powder to the triple helix gel structure: an attenuated total reflection-infrared study of the gelation process. *Applied Spectroscopy*, 61, 374–378.

Harada, T., Fujimori, K., Hirose, S., & Masada, M. (1966). Growth and β-glucan 10C3K production by a mutant of *Alcaligenes faecalis* var. *myxogenes* in defined medium. *Agricultural and Biological Chemistry*, 30, 764–769.

Harada, T., Okuyama, K., Konno, A., Koreeda, A., & Harada, A. (1994). Effect of heating on formation of curdlan gels. *Carbohydrate Polymers*, 24, 101–106.

Hirashima, M., Takaya, T., & Nishinari, K. (1997). DSC and rheological studies on aqueous dispersions of curdlan. *Thermochimica Acta*, 306, 109–114.

Jezequel, V. (1998). Curdlan: a new functional β-glucan. *Cereal Foods World*, 43, 361–364.

Jin, Y., Zhang, H., Yin, Y., & Nishinari, K. (2006). Comparison of curdlan and its carboxymethylated derivative by means of Rheology, DSC, and AFM. *Carbohydrate Research*, 341, 90–99.

Kanzawa, Y., Harada, T., Koreeda, T. A., & Harada, A. (1987). Curdlan gel formed by neutralizing its alkaline solution. *Agricultural and Biological Chemistry*, 51, 1839–1843.

Kim, M., Ryu, K., Choi, W., Rhee, Y., & Lee, I. (2003). Enhanced production of (1→3)-β-D-glucan by a mutant strain of *Agrobacterium* species. *Biochemical Engineering Journal*, 16, 163–168.

Kizil, R, & Irudayaraj, J. (2007). Rapid evaluation and discrimination of γ-irradiated carbohydrates using FT-Raman spectroscopy and canonical discriminant analysis. *Journal of the Science of Food and Agriculture*, 87, 1244–1251.

Konno, A., & Harada, T. (1991). Thermal properties of curdlan in aqueous suspension and curdlan gel. *Food Hydrocolloids*, 5, 427–434.

Marchessault, R. H., & Deslandes, Y. (1979). Finestructure of (1→3)-β-D-glucans: curdlan and paramylon. *Carbohydrate Research*, 75, 231–242.

Martinez, C. O., Ruiz, S. P., Nogueira, M. T., Bona, E., Portilho, M., & Matioli, G. (2015). Effective immobilization of *Agrobacterium* sp. IFO 13140 cells in loofa sponge for curdlan biosynthesis. *Molecules*, 20, 7957–7973.

McIntosh, M., Stone, B. A., & Stanisich, V. A. (2005). Curdlan and other bacterial (1→3)-β-D-glucans. *Applied Microbiology and Biotechnology*, 68, 163–173.

Na, K., Park, K., Kim, S. W., & Bae, Y. H. (2000). Self-assembled hydrogel nanoparticles from curdlan derivatives: Characterization, anti-cancer drug release, and interaction with a hepatoma cell line (HepG2). *Journal of Controlled Release*, 69, 225−236.

Nakanishi, I., Kimura, K., Kusui, S., & Yamazaki, E. (1974). Complex formation of gel-forming bacterial (1→3)-β-D-glucans (curdlan-type polysaccharides) with dyes in aqueous solution. *Carbohydrate Research*, 32, 47–52.

Ogawa, K., & Tsurugi, J. (1973). The dependence of the conformation of a (1→3)-β-D-glucan on chain-length in alkaline solution. *Carbohydrate Research*, 29, 397–403.

Oishi, Y., Udagawa, H., Shinozaki, Y., Moriyama, M., Taniguchi, H., Kobayashi-Hattori, K., Arai, S., & Takita, T. (2009). Preparation of hypoallergenic wheat flour

819 noodles and evaluation of their physical properties. *Food Science and Technology*
820 *Research*, 15, 39–44.
821
822 Popescu, I., Pelin, I. M., Butnaru, M., Fundueanu, G., & Suflet, D. M. (2013).
823 Phosphorylated curdlan microgels. Preparation, characterization, and in vitro drug
824 release studies. *Carbohydrate Polymers*, 94, 889–898.
825
826 Portilho, M., Matioli, G., Zanin, G. M., Moraes, F. F., & Scamparini, A. R. P. (2006).
827 Production of insoluble exopolysaccharide of *Agrobacterium* sp. (ATTC 31749 and IFO
828 13140). *Applied Biochemistry and Biotechnology*, 129–132, 864–869.
829
830 Saitô, H., Ohki, T., & Sasaki, T. (1977). A ^{13}C nuclear magnetic resonance study of gel-
831 forming (1→3)-β-D-glucans. Evidence of the presence of single-helical conformation in
832 a resilient gel of a curdlan-type polysaccharide 13140 from *Alcaligenes faecalis* var.
833 *myxogenes* IFO 13140. *Biochemistry*, 16, 908–914.
834
835 Salah, R. B., Jaouadi, B., Bouaziz, A., Chaari, K., Blecker, C., Derrouane, C., Attia, H.,
836 & Besbes, S. (2011). Fermentation of date palm juice by curdlan gum production from
837 *Rhizobium radiobacter* ATCC 6466^{TM}: purification, rheological and physico-chemical
838 characterization. *LWT - Food Science and Technology*, 44, 1026–1034.
839
840 Saudagar, P. S., & Singhal, R. S. (2004). Curdlan as a support matrix for immobilization
841 of enzyme. *Carbohydrate Polymers*, 56, 483–488.
842

Shih, I., Yu, J., Hsieh, C., & Wu, J. (2009). Production and characterization of curdlan by *Agrobacterium* sp. *Biochemical Engineering Journal*, 43, 33–40.

Shimizu, J., Tsuchihashi, N., Kudoh, K., Wada, M., Takita, T., & Innami, S. (2001). Dietary curdlan increases proliferation of bifidobacteria in the cecum of rats. *Bioscience, Biotechnology, and Biochemistry*, 65, 466–469.

Synytsya, A., Čopíková, J., Matějka, P., & Machovič, V. (2003). Fourier transform Raman and infrared spectroscopy of pectins. *Carbohydrate Polymers*, 54, 97–106.

Takeda, H., Yasuoka, N., Kasai, N., & Harada, T. (1978). X-ray structural studies of (1→3)-β-D-glucan (curdlan). *Polymer Journal*, 10, 365–368.

Tako, M., & Hanashiro, I. (1997). Evidence for a conformational transition in curdlan. *Polymer Gels and Networks*, 5, 241–250.

Wang, M., Chen, C., Sun, G., Wang, W., & Fang, H. (2010). Effects of curdlan on the color, syneresis, cooking qualities and textural properties of potato starch noodles. *Starch*, 62, 429–434.

Wu, C., Peng, S., Wen, C., Wang, X., Fan, L., Deng, R., & Pang, J. (2012). Structural characterization and properties of konjac glucomannan/curdlan blend films. *Carbohydrate Polymers*, 89, 497– 503.

867 Wu, S., Cai, R., & Sun, Y. (2012). Degradation of curdlan using hydrogen peroxide.
868 *Food Chemistry*, 135, 2436–2438.
869
870 Zhang, H., Huang, L., Nishinari, K., Watase, M., & Konno. (2000). Thermal
871 measurements of curdlan in aqueous suspension during gelation. *Food Hydrocolloids*,
872 14, 121–124.
873
874 Zhang, H., Nishinari, K., Williams, M. A. K., Foster, T. J., & Norton, I. T. (2002). A
875 molecular description of the gelation mechanism of curdlan. *International Journal of*
876 *Biological Macromolecules*, 30, 7–16.
877
878 Zhang, Y. P., & Lynd, L. R. (2005). Determination of the number-average degree of
879 polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis.
880 *Biomacromolecules*, 6, 1510–1515.
881
882

883 **Supplementary material 1**

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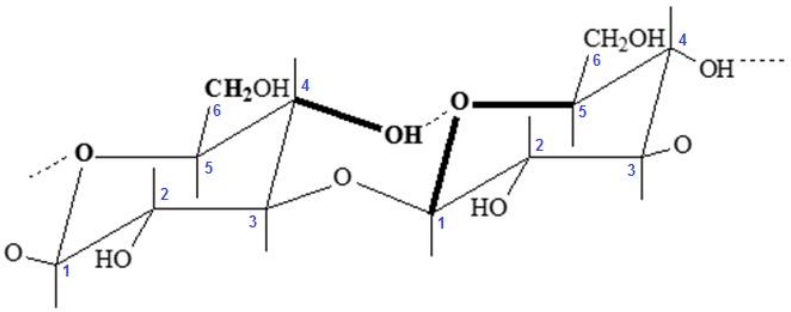
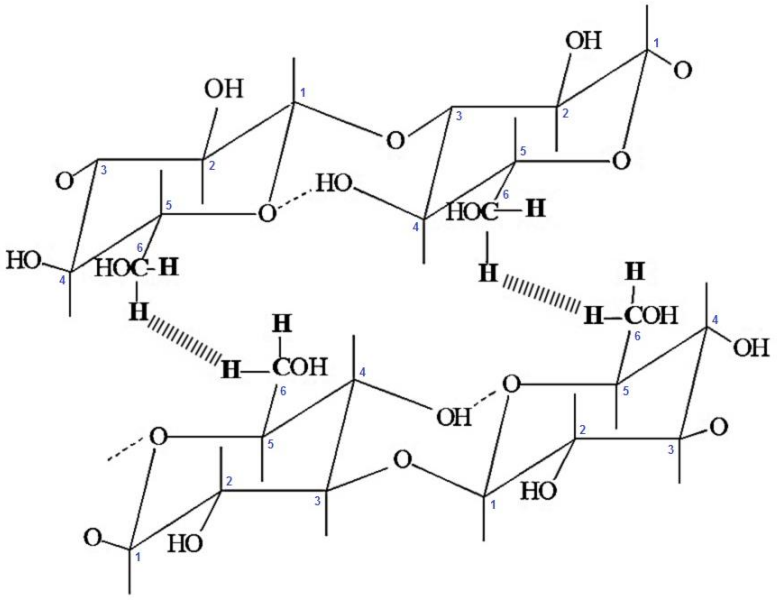
(A)



(B)



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886 **Figure S1:** Formation mechanisms of (A) low-set and (B) high-set gels described by

887 Tako and Hanashiro (1997) and suggested in the present study by FT-Raman.

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889

890

891 **Supplementary material 2**

892

(A)

(B)

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894 **Figure S2:** Pictures of (A) commercial curdlan and (B) of curdlan produced by

895 *Agrobacterium* sp. IFO 13140.

ANEXOS

ANEXO 1: Comprovante de aceite/publicação (Plos One)

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RESEARCH ARTICLE

Description of recovery method used for curdlan produced by *Agrobacterium* sp. IFO 13140 and its relation to the morphology and physicochemical and technological properties of the polysaccharide

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Abstract

Curdlan is a linear polysaccharide considered a dietary fiber and with gelation properties. This study evaluated the structure, morphology and the physicochemical and technological properties of curdlan produced by *Agrobacterium* sp. IFO 13140 recovered by pre-gelation and precipitation methods. Commercial curdlan submitted or otherwise to the pre-gelation process was also evaluated. The data obtained from structural analysis revealed a similarity between the curdlan produced by *Agrobacterium* sp. IFO 13140 (recovered by both methods) and the commercial curdlans. The results showed that the curdlans evaluated differed significantly in terms of dispersibility and gelation, and only the pre-gelled ones had significant potential for food application, because this method influence on the size of the particles and in the presence of NaCl. In terms of technological properties, the curdlan produced by *Agrobacterium* sp. IFO 13140 (pre-gelation method) had a greater water and oil holding capacity (64% and 98% greater, respectively) and a greater thickening capacity than the pre-gelled commercial curdlan. The pre-gelled commercial curdlan displayed a greater gelling capacity at 95˚C than the others. When applied to food, only the pre-gelled curdlans improved the texture parameters of yogurts and reduced syneresis. The curdlan gels, which are rigid and stable in structure, demonstrated potential for improving the texture of food products, with potential industrial use.

ANEXO 2: Normas (Plos One)

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Short title	100 characters	State the topic of the study	Cigarette Smoke Exposure and Innate Immunity SODIS and Childhood Diarrhoea

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ANEXO 3: Normas (Food Chemistry)

FOOD CHEMISTRY

AUTHOR INFORMATION PACK

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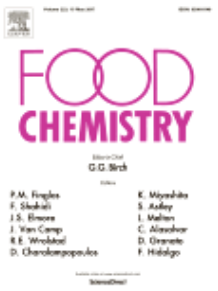

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Food Chemistry publishes original research papers dealing with the advancement of the **chemistry** and **biochemistry** of **foods** or the analytical methods/ approach used. All papers should focus on the novelty of the research carried out.

Topics include:

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– Methods concerned with the chemical forms in food, nutrient bioavailability and nutritional status;

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AUDIENCE

Food technologists, scientists and chemists

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