International Journal of Agricultural Technology 2023 Vol. 19(3):1223-1234
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Inulin Gel Formation and Its Application in Frozen Plant-based Squid

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Polpued, R. and Khuenpet, K. (2023). Inulin Gel Formation and Its Application in Frozen Plant-based Squid. International Journal of Agricultural Technology 19(3):1223-1234.

Abstract An increase of inulin concentration from 20 to 40% (w/w) significantly increased both elastic modulus (G') and viscous modulus (G''). All inulin-water suspension samples formed the homogenous texture and the appearance of gel concentration specimens were changed from clear yellow solution to opaque white gel. The optimum preparation level of inulin gel was suggested to be 30% (w/w). In order to improve texture of plant-based squid, completely inulin gel was added to recipe. The finished products were kept in frozen condition (-18^{o}C). Texture profile analysis of cooked samples after freezing and thawing were measured. The result showed that the freezing point of both plant-based squids is -2.8 ^{o}C. The adding of inulin gel improved the texture profile, exhibited the lower hardness and cohesiveness after thawing, and able to comparable with commercial vegan squid.

Keywords: Freeze-thaw, Inulin gel, Plant-based, Squid

Introduction

Inulin has been potentially applied to the development of semi-solid food products because it is dietary fiber and able to improve the physicochemical properties of food. It has a unique range of molecular weight with the degree of polymerization (DP) varying from 2-60 (Berizi *et al*., 2017). Especially, long-chain inulin (DP > 23) is commonly used as a fat replacer because it can combine with water for obtaining a creamy white texture similar to the original fat (Beccard *et al*., 2019). There are several factors influence on the formation of inulin gel including inulin concentration, temperature preparation, shear rate, ionic strength, and some plant protein (Beccard *et al*., 2019; Bengoechea *et al*., 2019; Florowska *et al*., 2020). Normally, the critical concentration of inulin gel was suggested larger than 10-15% (w/w) due to the molecular density which promotes the crowding effect. Moreover, the higher inulin concentration creates the gel formation immediately because of the aggregation of biopolymer and provides stronger gel (Bengoechea *et al*., 2019).

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Currently, plant-based seafood consumption has become interesting because it can prevent the contamination of heavy metals, toxic substances, and some allergens as well as reduce unhealthy fats like cholesterol (Kazir and Livney, 2021). The improvement of alternative seafood is not only focused on the nutritional benefit but also should be taken into account the appearance, taste, and texture properties. Konjac glucomannan has an excellent structure and creates a thermal irreversible elastic gel which widely utilized in plant-based products (Jiang *et al*., 2019). Some studies indicated that the addition of konjac contributes caused an increase of hardness. However, using konjac alone results in a weak junction zone leading to a loose gel network with a low gel strength (He *et al*., 2012; Ran *et al*., 2022). Therefore, the combination of konjac with other ingredients such as hydrocolloids, starch and protein can improve the gel properties. Safaei *et al*. (2019) reported that prebiotic sausage produced from konjac, inulin, and starch had better cooking characteristics and overall acceptability. Moreover, the addition of inulin reduces the freezable water in rice starch gel during the freeze-thaw cycle. This was because inulin had a great water holding capacity and water bound to inulin was less freezable, so the ice crystal was reduced to form the gel through freezing condition, and less water release upon thawing. Inulin also interacts with amylose and amylopectin producing the complex structure, therefore the syneresis was inhibited. (Ye *et al*., 2018).

In accordance with Cui *et al*. (2019) revealed that frozen dough from gluten protein containing 2.5% konjac showed the highest water holding capacity and delay water migration as well as less holes caused by ice crystals (Cui *et al*., 2019). Besides, a long-chain could improve the stability of gel storage at low temperatures (Kou *et al*., 2018). Consequently, the objectives were to study the formation of inulin gel as a fat replacer and rheological properties for improving the frozen plant-based squid from konjac glucomannan.

Materials and methods

The experiment was conducted from June to September, 2022 in the laboratory at Thammasat University, Pathum Thani, Thailand. Konjac and inulin powder were supplied by Chemipan Corporation Co., Ltd., and DPO (Thailand) Ltd., Bangkok, Thailand. Methylcellulose was supported by Questex Co., Ltd., Samut Prakan, Thailand. Seasoning powder (Ajinomoto (Thailand) co. ltd, Samut Prakan, Thailand) and salt (Saha Pathanapibul Public Company Limited, Phra Nakhon Si Ayutthaya, Thailand) were purchased from supermarkets in Pathum Thani. Commercial vegan squid was obtained from

Mercy vegan house, Pathum Thani, Thailand which was made from plant protein and soy protein.

Inulin gel preparation

Inulin gel was prepared following Beccard *et al*. (2019) with some modifications. Long-chain inulin powder (Orafti®HP) was dissolved in the water at 60 ^{o}C with different concentrations of 20, 30, and 40% (w/w), respectively. Then, inulin suspension was independently homogenized by a hand blender (5KHB1231EWH, Kitchen Aid, Chaina) for 15 min until the clear yellow solution was obtained. After that, the mixture was stored at 4$\pm$1 ^{o}C for 24 h. The solution transformed to gel which the appearance was opaque white and it was called completely inulin gel. All products were photographed. Each inulin gel concentration was measured rheological properties including storage modulus (G'), loss modulus (G'') and complex viscosity over the temperature transition.

Frozen plant-based squid production

The production of plant-based squid was divided into three main steps. 1) Konjac solution preparation step; konjac were independly combined with water at the ratio of 1:2, then mixed all together to get the hydrated component. 2) Methylcellulose mixture preparation step; 8% methylcellulose, 11% rice bran oil, and 81% cold water were mixed by using a food processor (DoubleForce, Tefal, Thailand) at a high-speed level for 2 min until a firm and fluffy texture was obtained. 3) Last step is a formulation and freezing; the amount of prepared konjac solution, methylcellulose mixture, and inulin gel as shown in Table 1 were mixed together for 3 min. After that, seasoning powder and salt were added and blended for flavoring and providing a homogenous texture. Each mixture samples were packed into a squid mold around 30 g and stored at -18 ℃ for 24 h before analysis. All products were photographed. Then, frozen plant-based squid was cooked and analyzed the texture properties comparable with commercial vegan squid.

Quality determination

Rheological properties

Rheological properties of completely inulin gel samples after 24 h storage were determined according to the method of Bengoechea *et al*. (2019) with slight modification. A rotational rheometer (Kinexus Ultra plus, England) equipped with a rough stainless-steel parallel plate (diameter of 40 mm and a gap size of 1 mm) was applied. Small amplitude oscillatory shear measurements of temperature sweep were conducted at a frequency of 1 Hz and the strain of 0.1% to remain within the linear viscoelastic region (LVR). Inulin gel samples were loaded at 25 ^{o}C into an aluminum plate, covered the sample with paraffin oil and cooled down from 40 to -5 ℃ at the rate of 3 ℃/min, and then heated up again to 40 °C at the same rate. Storage modulus (G'), loss modulus (G'') and complex viscosity over the temperature transition was obtained.

Table 1. Formulations of frozen plant-based squid

Ingredients	Percentage of ingredient (% w/w)	
	F1 (control)	F2
Konjac solution	55	50
Methylcellulose mixture	40	40
Inulin gel	-	5
Seasoning powder	4	4
Salt	1	1
Total	100	100

Freezing curve

The plant-based squid was frozen in the freezer (SF-PC1497, Panasonic, Thailand) at -18 ^{o}C for 12 h. The core temperature of all samples was recorded every 30 s in real-time during the freezing process using a thermocouple (7 cm length) connected with a data logger (CMC-RP, Ellab, Denmark). The variation of the temperature with time as it is allowed to cool was plotted as a freezing curve.

Texture profile analysis

Texture properties of plant-based squid after frozen were analyzed as described by Wang *et al*. (2020) using texture analyzer TA-XTPlus (Stable Micro System, England) with a 50-mm aluminum cylindrical probe. The plant-based squid was steamed at 100^{o}C for 10 min before analysis. Cube samples (1x1x1 cm) were compressed in two cycles of 50% of their original height. The conditions were as follows: pre-test speed 1.0 mm/s, test speed 5.0 mm/s, post-test speed 5.0 mm/s. The texture characteristics including hardness (N), springiness, cohesiveness, and gumminess were determined. The texture analysis was performed in ten replicates.

Statistic analysis

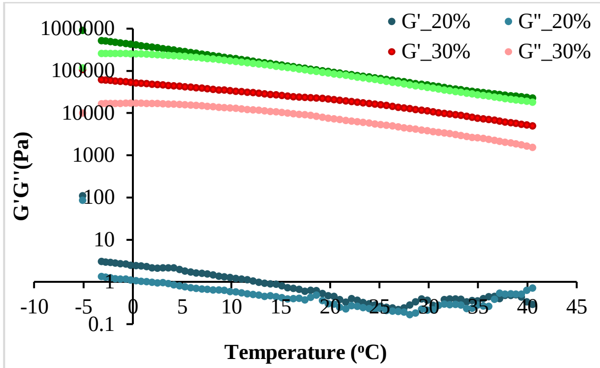
The experimental design was carried out using completely randomized design (CRD). The quality determinations were conducted in triplicate and the results were expressed as means with standard deviations. The data were analyzed using SPSS software version 22.0. One-way analysis of variance (ANOVA) was used to determine significant differences between formulations ($p < 0.05$). Multiple comparisons of means were performed by using Duncan's multiple range test.

Result

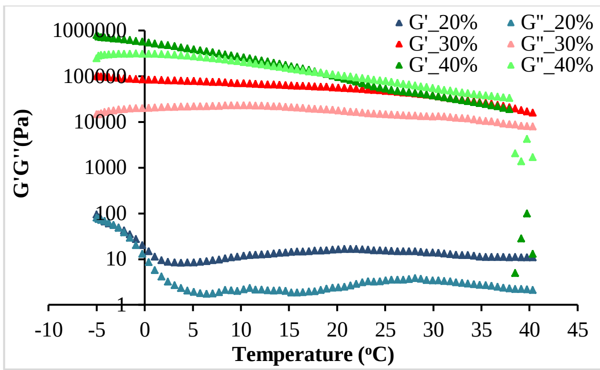
Rheological properties of inulin gel

The rheological properties of inulin gel specimens with different concentrations at 20, 30, and 40% (w/w) during the cooling (40-(-5) ^{o}C) and heating (-5) -40 ^{o}C) conditions are shown in Figure 1. The change in elastic modulus (G') and viscous modulus (G") of all samples were plotted as a function of temperature. The G' values of 20% and 30% inulin gel were higher than those of G" over the temperature transition (Figure 1a, 1b) which indicated that the samples exhibited the predominantly solid-like character or elastic properties and better gel structure. On the other hand, the G' and G" values of inulin gel containing 40% (w/w) are more stable. This sample had the highest G' value whereas the G'' was immediately reduced when the temperature was closed to 40 ^{o}C.

The shear viscosity for all levels of inulin concentration during the cooling-heating process is illustrated in Figure 2. The result revealed that viscosity had the same tendency with G' and G'' values. The 40% inulin gel showed the highest viscosity. In this study, the change of temperature extremely affected on viscosity of both 20 and 30% (w/w) inulin gel, except 40% (w/w). Moreover, the viscosity of 40% inulin gel was crossover during the temperature change indicating that the inulin gel structure remains unaffected and the gel network was stable.



(a) The value of G’ and G’’ when temperature decreases from 40-(-5) ^{o}C



(b) The value of G’ and G’’ when temperature increases from (-5)-40 ^{o}C

Figure 1. Rheological properties of inulin gel with different concentration at 20, 30 and 40% during the cooling (a) and heating (b) condition

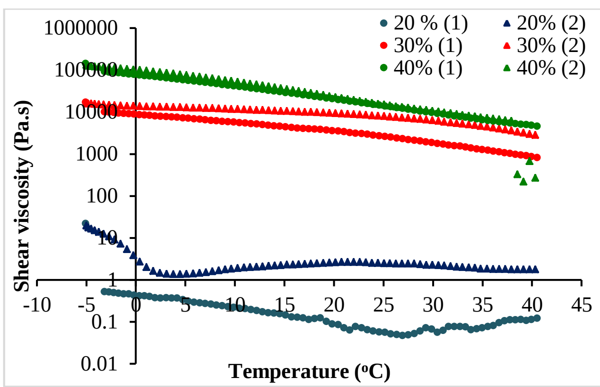


Figure 2. Shear viscosity of inulin gel with different concentration at 20, 30 and 40% during the cooling-heating condition, (1) Cooling condition by temperature decreases from 40-(-5) ^{o}C, (2) Heating condition by temperature increases from (-5)-40 ^{o}C

The appearance of completed set inulin gel with different concentrations at 20, 30, and 40% (w/w) are shown in Figure 3. Upon the inulin suspension was storage at a low temperature overnight, the solution was changed from clear yellow solution to opaque white gel. All samples showed smooth texture and homogeneous structure without some granular residues. Inulin gel containing 40% (w/w) seemed to be more rigid and compact than other samples whereas 20% inulin concentration was noticed in softness, juiciness, and become flow. From the previous study of the rheological properties of inulin gel, it was found that the appropriate concentration can be used as a fat substitute in plant-based squid products is inulin gel at a concentration of 30% (w/w) due to its high G' value, indicating the solid-like character and is stable during temperature changes. Therefore, it is suitable for use in frozen food products.

(a) (b) (c)

Figure 3. Inulin gel 20% (a), 30% (b) and 40% (c) (w/w)

Frozen Plant-based squid production

The appearance of plant-based squid made from konjac and konjac mixed with inulin gel before freezing and after thawing treatment comparable with commercial vegan squid is presented in Figure 4. The plant-based squid has white color whereas the commercial one contains yellow-brown color. The structure of all samples was slightly changed but it is still maintained the original shape as before freezing. Moreover, the cut-in strip on the surface is not clear and become fade after thawing.

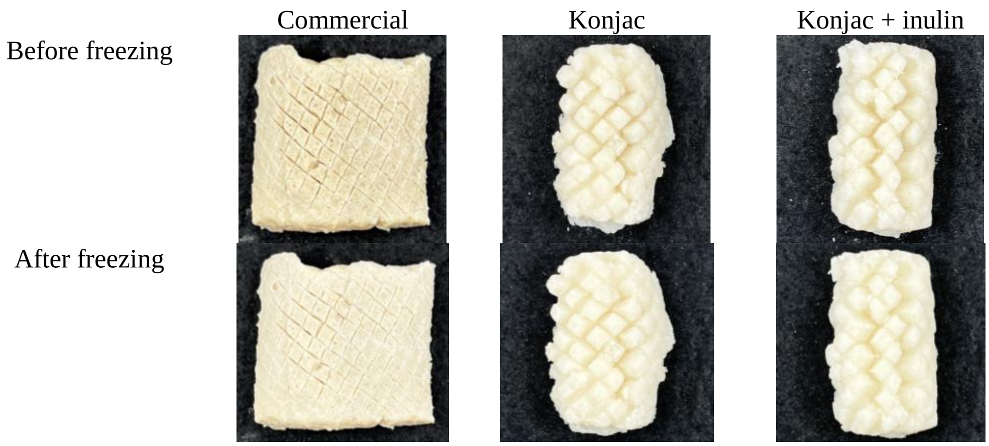


Figure 4. Commercial vegan squid and plant-based squid made from konjac and konjac mixed with inulin gel before freezing and after thawing samples

The plant-based squid was developed using 30% inulin gel combined with konjac glucomannan. The sample was subjected to a freeze treatment at -18 ^{o}C for 12 h. Temperature changes were recorded every 30 s until the product was completely frozen. The freezing curve of frozen plant-based squid from inulin gel is represented in Figure 5. At the initial state of around 10^{o}C, the sample was rapidly precooled for removing the sensible heat in 20-30 min. Then, the temperatures of plant-based squid were ploted both konjac (F1) and konjac with inulin gel (F2). All graphs were sharply lower to supercooling at -3.7 and -3.5 ^{o}C, respectively. After that, the nuclei formation (nucleation) occurred to form the ice crystal together with the temperature immediately increase to the freezing point around -2.8 ^{o}C, so the latent heat was removed (Tan *et al*., 2021). The result shows that temperature of frozen plant-based squid added only konjac was dropped faster than konjac mixed with inulin gel.

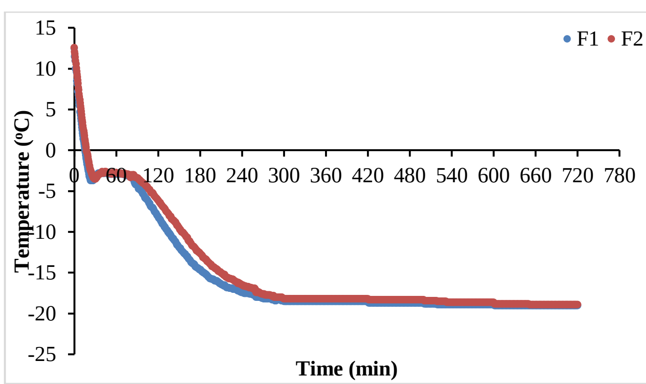


Figure 5. Freezing curves of plant-based squid using konjac (F1) and konjac plus inulin gel (F2)

The texture properties and mouthfeel play an important role in the development of plant-based food products. The texture profile of plant-based squid comparable with commercial vegan squid after thawing is represented in Table 2. The cohesiveness values of commercial product had the highest which accounted for 0.68 g followed by konjac mixed with inulin gel and only konjac squid, respectively. However, springiness and gumminess values were no significantly different of all samples.

Table 2. The texture profile of cooked plant-based squid compared with commercial product

Formula	Hardness (g)	Springiness (g.sec) [ns]	Cohesiveness	Gumminess [ns]
Commercial	780.15 + 63.75[b]	0.79 + 0.04	0.68 + 0.02[a]	527.69 + 51.70
Konjac	936.01 ± 145.46[a]	0.83 + 0.03	0.56 + 0.03[c]	525.20 + 86.67
Konjac + inulin gel	848.03 + 65.23[ab]	0.84 + 0.04	0.62 + 0.03[b]	524.34 + 45.87

[a,b,c] Different letters in the same column indicate that values are significantly different ($p<0.05$), [ns]: not significant different

Discussion

The reduction of temperature from 40 °C to -5 °C resulted in an increase of elastic modulus (G') and viscous modulus (G'') values. This is because the interaction between biopolymers in the gel system especially hydrogen bonds were created to form the three-dimensional network structure of the gel (Wang *et al*., 2020). When the inulin gel was heated again, the rheological properties gradually decreased because the higher temperature is the main factor that can change the systematic gel network to a disordered state (Wang *et al*., 2020). However, the G' value is well above the G'' indicating the behavior of gel. The high temperature also influenced the particles suspended in the solution through random movement, so-called Brownian motion, which easily interacts with other molecules as well as the loss of moisture content in the system, therefore the sample behaved as an elastic material (Kou *et al*., 2018).

The formation of the inulin gel depended on several factors such as inulin concentration, temperature, shear rate, and a divalent cation. (Bengoechea *et al*., 2019; Beccard *et al*., 2019). Normally, the inulin concentrations lower than 5 % (w/w) did not form the gel network because of the molecular density of inulin chains below the critical concentration to promote the mentioned crowding effect. The critical concentration was suggested at 10-15% (w/w) caused by thermal and shear application (Bengoechea *et al*., 2019). Subsequently, the inulin gel with 40% (w/w) provides a stronger gel. Kim *et al*.

(2001) reported that the suitable range for inulin gel formation is 20-30% (w/w). Moreover, the viscosity may relate to the hardness of the sample. The highest shear viscosity value was observed in 40% inulin concentration because the higher amount of long-chain inulin can form more easily junction zone producing a harder gel structure (Chiavaro *et al*., 2007).

The appearance of plant-based squid and commercial product was different because of the ingredient addition. Commercial vegan squid made from soy protein and plant protein providing the light-yellow color whereas plant-based squid produce form konjac and inulin gel which containing opaque white color. The freezing rate of plant-based squid made from konjac was faster than the addition of inulin gel. Konjac is a neutral polysaccharide that contains high molecular weight. It produces a weak junction zone which sensitive to heat or temperature change. Because the high viscosity of konjac may inhibit the aggregation of the molecule and slow down the formation of the junction zone. However, this phenomenon was better improved by other hydrocolloids addition (Safaei *et al*., 2019; He *et al*., 2012).

Konjac glucomannan was applied to various types of food products for increasing the strength of the gel. Safaei *et al*. (2019) revealed that the incorporation of konjac with inulin or starch also reduced undesired texture properties like exceeding in hardness and maintaining the adhesiveness and cohesiveness to the appropriate level more than using the konjac alone. In this study, the hardness of plant-based squid from konjac showed the trend of going down with the addition of inulin gel. Because inulin can decrease the syneresis effect of the gel during the freezing process by enhancing the water holding capacity and also reducing freezable water (Ye *et al*., 2018). Therefore, the water leakage was not observed in konjac mixed with inulin gel after freeze-thaw cycles.

According to the report of Wang *et al*. (2019), the hardness of gelatin gel was slightly softer when added inulin while the springiness remained. It was proved that inulin did not establish the direct chemical interaction with gelatin but it just embedded or fulfilled in the gel matrix, thereby providing excellent gel properties. Some studies revealed that inulin potentially promotes the mouthfeel and sensory properties of the gel food product. In comparison with commercial vegan squid, it had the highest cohesiveness value which means the internal structure has strong interaction. Because soy protein and other plant proteins in vegan squid were linked by several chemical interactions such as hydrogen bonds, disulfide bonds, and hydrophobic interactions promoting the powerful network structure (Chiang *et al*., 2019).

Acknowledgements

This research was supported by Thammasat University Research Fund, Contract No. TUFT 70/2564.

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(Received: 25 October 2022, accepted: 30 April 2023)