Characteristics and Rheological Properties of Freeze-dried Black Grass Jelly Prepared with Different Gelling Agents

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Abstract

Characterization of freeze-dried Black Grass jelly (Mesona procurebens Hemsl.) jelly in terms of physical and rheological properties was the main objective of this study. Black Grass jelly prepared with different gelling agents including (3.5%) tapioca starch (commercial formula or control sample), (2.5%) tapioca starch + (1%) guar gum, (1.0%) tapioca starch + (2.5%) gelatin and (0.5%) tapioca starch + (3%) maltodextrin. The Black Grass Jelly samples were then freeze-dried and analyzed for their water activity, color, rheological behaviors, and textural profile attributes. The results show that gelling agents had a significant effect on the qualities of freeze-dried Black Grass jelly, which has a low a_w (<0.2) classified as dry food that can be stored for a long time. Color parameters indicated that jelly combined with tapioca starch was apparently darker than the other samples. The gel had a medium density of branched-structure, suggested by the viscoelastic behaviors. From rheological, an increasing gelatin concentration increased the presence of a solid-like gel with the predominance of an elastic component. This increase is attributed to a higher polymer concentration contributing to form a stronger structure and a higher cross-linker concentration making the density of the spherical particles higher. The addition of a gelling agent affected the stability of the gel. The results confirmed that 1% tapioca + 2.5% gelatin addition could be appropriate to ensure the good gel qualities for freeze-dried Black Grass jelly production.

Keywords: *Black Grass Jelly, Gelling agent, Freeze-drying, Rheological behaviors, Texture property*

1. Introduction

Black Grass Jelly or Hsian-Tsao (*Mesona procurebens* Hemsl.), a herbal plant belonging to Labiatae family, is consumed as an herbal drink, a jelly-type dessert by Thai and Chinese for centuries (Kreungngern & Chaikham, 2016). However, it is used to treat heart attack, hypertension, diabetes, and muscle pains. (Yang *et al.*, 2008) and used as a remedy for treating heat-stroke in Chinese folk medicine. Black Grass jelly was previously demonstrated to have hepatoprotective and anti-hypertensive functions with oleanolic acid, ursolic acid identified as active components. (Huang *et al.*, 2016) Many health benefits of this herb, including lowering blood pressure and diuretic effect, were previously reported by Chen *et al.* (1996) and Widyaningsih *et al.* (2014). Besides, demonstrated that Hsian-Tsao leaf gum is a potent polar antioxidant that interacts with a wide range of species directly responsible for oxidative damage.

Black Grass Jelly is traditionally prepared through the addition of starch to the polysaccharide extract of the plant leaves. However, the gelling properties of polysaccharide gum from Black Grass Jelly leaves were found to be strongly influenced by various factors, including extraction conditions, starch types, and concentrations, as well as parts of the plant (Lai *et al.*, 2000). Generally, sodium bicarbonate or sodium carbonate was used to extract the polysaccharide gum in Hsian-Tsao his gum can be extracted by using sodium bicarbonate or sodium carbonate and is reported to interact with starch synergistically, resulting in a noticeable increase in viscosity and the formation of a thermoreversible gel (Kreungngern & Chaikham., 2016; Lai *et al.*, 2001). It is well-known that the addition of hydrocolloids may increase the viscosity of starch dispersions, influence the retrogradation rate, and prevent the syneresis of starch (Lai *et al.*, 2000; Liu and Eskin, 1998). Hsian-tsao gum was also reported to interact with starch synergistically, which resulted in a marked increase in viscosity and the formation of a thermo-reversible gel it is generally accepted that the added hydrocolloids result in a modification on the rheological properties of the starch system by influencing amylose gelation.

Freeze-dried products get more and more attention and are used directly as snack products or in a broad range of foods, such as dried soups, chocolate or breakfast cereals and cereal bars. The freeze-drying process better maintains the natural shape of the raw material and the nutritional and sensory properties compared to hot air drying since the product is stabilized by freezing and removing water-based on sublimation (Fellows, 2009). Freeze-drying is performed under vacuum and consists of three main steps: freezing, primary drying (sublimation) and secondary drying (desorption). In particular, freezing temperature and ice morphology can significantly affect the freeze-drying process by modulating the mass transfer and thus the rate of primary and secondary drying (Fauster *et al.,* 2019; Searles *et al*., 2001).

Texture is the most important characteristic of Black Grass jelly and the addition of several gelling agents including potato flour, agar and other hydrocolloids has been displayed to provide desirable texture, relating to the consumer acceptance. The most popular method for characterizing this property is oscillatory stress sweep measurement (Chaikham *et al.,* 2012).

In this research, the development of value-added products from Black Grass Jelly was studied to extend the shelf life longer (usual seven days). Besides, in trade with foreign countries, the weight of the product is very difficult to transport, and the product must be chilled all the time. Therefore, this paper aims to develop Black Grass jelly products prepared with different gelling agents including 3.5% tapioca starch (TS) (control sample), 2.5% TS + 1% guar gum, 1% TS + 2.5% gelatin and 0.5% TS + 3% maltodextrin used freeze-drying process. In order to test the quality, water activity, color measurement, rheological behaviors, textural profiles were examined. By applying freeze-drying technology to make the product of higher quality leads to an increase in the commercial value of Grass jelly products that consumers can choose to eat. Therefore, in order to extend the shelf life can store Black Grass jelly without chilling for the benefit of increasing distribution channels of Thai products to foreign countries.

2. Objectives

1. To develop Black Grass jelly products prepared with different gelling agents used freeze-drying process.
2. To test the quality of the examine water activity, color measurement, rheological behaviors, textural. Profiles.

3. Materials and Methods

3.1 Preparation

Dried Black Grass jelly leaves were purchased from a local market in Kamphaeng Phet province, Thailand. In brief, 100 g of dried leaves were washed and continuously extracted in 3 Litres of boiling water (95±5°C) containing 0.25% (W/V) sodium carbonate for six hours before filtering through a filter cloth. Afterward, the heated filtrate was mixed with various gelling agents including 3.5% tapioca starch (TS) (commercial formula or control group), 2.5% tapioca starch + 1% guar gum, 1% tapioca starch + 2.5% gelatin and 0.5% tapioca starch + 3% maltodextrin, and immediately stirred for 15 minutes. All mixtures were poured in the stainless steel plates before cooling down to the temperature of 25°C, then cut into pieces that have to 1x1x1 cm^3 subsequently kept in a refrigerator (~4°C) before analysis.

3.2 Freeze-drying process

80 kilograms of Black Grass Jelly samples were placed on stainless steel plates in a single layer, not in contact with each other, and frozen in an Ultra-Low temperature freezer (Low-temperature freezer, Freeze dry station, Thailand) at temperatures of −40±2°C for a minimum duration of 6 h. The frozen samples were placed onto shelves in a freeze drier (FD-150, Freeze dry station, Thailand). The condenser was pre-cooled to −25±2°C before the samples were placed in the freeze drier. The freeze-drying process was performed for 25 h, and the chamber pressure was maintained at 0.21±0.05 mbar throughout the drying process. After the freeze-drying process, the samples were stored in aluminum foil bags, which contains moisture absorbing sachets.

3.3 Water activity

The water activity (a_w) of freeze-dried Black Grass Jelly was measured by a Lab Swift-aw (NOVASINA, Switzerland) according to the method described by Cheng *et al.* (2017). The freeze-dried sample was ground and weighed about 1.5 g of water activity evaluation. All measurements were in duplicate and results presented with a standard deviation

3.4 Color measurement

A colorimeter Konica Minolta CR400 (Chroma meter, Japan) was used with a white plate standard to measure the color parameter of dried Black Grass Jelly samples. The terms of L^* (lightness), a^* (redness) and b^* (yellowness) values.

3.5 Dynamic viscoelastic properties of freeze-dried Black Grass jelly after rehydration

Freeze-dried Black Grass jelly sample was weighed about 1 g and submerged in 100 mL of hot water (90±5°C) for 5 min. Dynamic viscoelastic properties of rehydrated samples were determined according to Kreungngern & Chaikham (2016), using a controlled stress MCR 302 Modular Compact Rheometer (Anton Paar, USA) equipped with a 25 mm parallel steel plate measuring system and 1 mm gap width. An oscillatory frequency sweep assessment was performed using a stress range of 0.01-10 pa. Consequently, a linear viscoelastic region (LVR) of the sample was determined at the stress of 5 Pa. The frequency sweep testing was subsequently performed to measure storage modulus (G'), loss modulus (G'') and loss tangent ($\tan \delta = G''/G'$) over a frequency range of 0.1-20 Hz.

3.6 Texture profile analysis

Texture profile analysis (TPA) of freeze-dried Black Grass jelly after rehydration in hot water (95±5°C) was carried out using a texture analyzer (TA General, Brookfield engineering Labs Inc., USA) with a 4 kg-load cell. Experiments were evaluated by compression tests, which generated a plot of force (g) versus time (s). A 38.1 mm diameter TA 4/1000 probe was used to measure the textural probe and testing effects of gelling agents on hardness, cohesiveness, springiness, gumminess, and chewiness of jelly samples.

3.7 Statistical Analysis

The experimental design used was a complete randomized design (CRD). The data were presented as the mean values ± standard deviation. Statistical analyses were carried out using the IBM SPSS statistics 11 software using one-way ANOVA and Duncan's multiple range tests. The results were considered statistically significant at a 95% confidence interval ($p<0.05$).

4. Results and Discussion

4.1 Water activity (a_w)

During storage, freeze-dried Black Grass Jelly samples must be protected from heat, oxygen, light, and moisture. The moisture content of a product can be defined as the percentage weight of water in relation to the dry weight of the product. Concerning the viability of probiotics as well as spoilage organisms in foods, the term water activity is more important than the moisture content (Vesterlund *et al.*, 2012). a_w of the freeze-dried Black Grass Jelly samples added with various gelling agents Table 1 The amount of a_w It was found that dried Black Grass Jelly at the highest water activity tested a_w 0.18 the product was very stable. The variable a_w defines the free water in the product, which is available for microbe metabolic purposes.

Table 1 Water activity evaluation of freeze-dried Black Grass Jelly combined with different gelling agents

Gelling agents	**a_w (NS)**
3.5% tapioca starch	0.18±0.09
1% tapioca starch + 2.5% gelatin	0.10±0.01
2.5% tapioca starch + 1% guar gum	0.13±0.01
0.5% tapioca starch + 3% maltodextrin	0.15±0.02

The means with different small letters in the same column were significantly different ($p<0.05$)

In general, foods have a_w level in the range of <0.2 for very dry foods to 0.99 for moist and fresh foods. Microorganisms can keep their viability regardless of a_w, but for growth, bacteria require $a_w>0.8$ and yeasts and molds $a_w>0.6$. Dry foods, for example, milk powder and instant coffee, have a_w of 0.2, and dried fruits, biscuits, and dried pet foods have a_w of 0.4. The lower a_w, the better the stability of the Black Grass Jelly and dry food product, but in very low $a_w<0.1$, the oxidation of membrane lipids can also reduce viability (Viernstein *et al.,* 2005).

4.2 Color measurement

Lightness (L^*), redness (a^*) and yellowness (b^*) were determined for dried Black Grass Jelly added with various gelling agents including, 3.5% tapioca starch, 2.5% tapioca starch + 1% guar gum, 1.0% tapioca starch + 2.5% gelatin and 0.5% tapioca starch + 3% maltodextrin (Table 2) It was noticed that types and concentrations of gelling agents had a significant effect ($P\leq0.05$) on color parameters of products. In general, freeze-dried Black Grass Jelly combined with tapioca starch (control group) showed significantly higher ($P\leq0.05$) L^* (37.35) and a^* (1.76) values than the other batches (L^* = 36.07-36.96, a^*= 1.35-1.61) whereas the highest values of b^* (4.31) parameter in sample added 0.5% tapioca starch + 3% maltodextrin were observed. Overall, these results displayed that dried Black Grass Jelly including with 2.5% tapioca starch + 1% guar gum was apparently darker ($P\leq0.05$) than the rest, but this is a good characteristic (dark brown to black) for Black Grass Jelly (Lai *et al.,* 2000).

Table 2 Color attributes of freeze-dried Black Grass Jelly combined with different gelling agents

Gelling agents	***L****	***a****	***b****
3.5% tapioca starch	37.35±0.03^a	1.76±0.08^a	4.05±0.01^b
1% tapioca starch + 2.5% gelatin	36.63±0.03^c	1.49±0.03^c	3.58±0.02^c
2.5% tapioca starch + 1% guar gum	36.07±0.01^d	1.35±0.06^d	3.35±0.05^d
0.5% tapioca starch + 3% maltodextrin	36.96±0.02^b	1.61±0.05^b	4.31±0.07^a

The means with different small letters in the same column were significantly different ($p<0.05$)

4.3 Dynamic viscoelastic properties of freeze-dried Black Grass jelly after rehydration

Oscillatory viscoelastic measurement of storage modulus (G'), loss modulus (G''), and loss tangent ($\tan\delta$) are among parameters that characterize the gel system of freeze-dried Black Grass jelly have been rehydration with hot water (95±5°C). Suggested that G', G'' moduli are the measurement of the energy stored and dissipated in the material under test, white $\tan\delta$ is the ratio of G''/G' and represents of viscoelastic (Kreungngern & Chaikham., 2016).

Table 3 Dynamic viscoelastic properties of freeze-dried Black Grass jelly after rehydration at a frequency of 1 Hz of Black Grass jelly combined with different gelling agents

Gelling agents	*G´*	*G´´*	tan δ
3.5% tapioca starch	1766.40±77.69^{b}	214.65±37.51^{b}	0.12±0.01
1% tapioca starch + 2.5% gelatin	2084.90±78.41^{a}	276.98±44.11^{a}	0.13±0.02
2.5% tapioca starch + 1% guar gum	1360.76±39.34^{c}	164.28±43.13^{c}	0.12±0.03
0.5% tapioca starch + 3% maltodextrin	1265.78±26.08^{d}	168.89±26.85^{c}	0.13±0.02

The means with different small letters in the same column were significantly different ($p<0.05$)

Figure 1 and Table 3 depict dynamic viscoelastic behaviors in which amplitude sweep to determine the linear viscoelastic region of freeze-dried Black Grass Jelly after rehydration added with different gelling agents. In all products G´ was found much higher than G´´ and the difference between G´ and G´´ of each plot was relatively 1 log cycle, indicating the value tanδ <1 presence of a solid-like gel with the predominance of an elastic component (Almdal *et al.,* 1993). It was noticed that both moduli significantly increased ($P \leq 0.05$) with the increasing levels of gelling agents, particularly samples combined with 2.5% gelatin. It was probably due to a stronger gel system with more crosslink densities (Kreungngern & Chaikham, 2016). The rising of both G´ and G´´ in the jelly samples as affected by the increase of gelling agents levels was an indication of a true gel behavior structure with more solid-like structure (Kreungngern & Chaikham, 2016; Ross-Murphy, 1984)

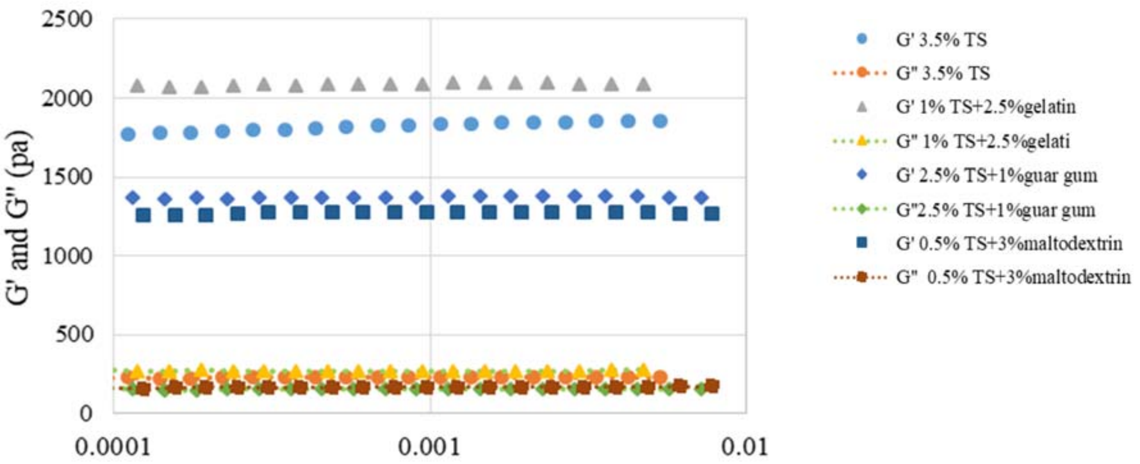

Figure 1 Strain amplitude sweep at frequency 1 Hz for freeze Black Grass jelly after rehydration combined with different gelling agents

The rising of both *G´* and *G´´* in the Black Grass Jelly samples as affected by the increase of gelling agents was an indication of a true gel behavior structure with a more solid-like structure. It is also confirmed by their loss tangent (tan δ) values that were lower than 1 (Table 3). It was noticed that both moduli significantly increased ($P \leq 0.05$) with the increasing levels of gelling agents, particularly sample combined with 1% tapioca starch + 2.5% gelatin. The results were similar to the findings of Satrapai and Suphantharika (2007), which reported that the magnitudes of G´ and G´´ increased with the increase of hydrocolloid concentration in prepared gels. Also, Chaisawang & Suphantharika (2005) found that both G´ and G´´ values for tapioca starch pastes increased with increasing the gum levels (such as guar and xanthan gums). That most gels or pastes containing gelling agents (or hydrocolloids) not only usually exhibit higher viscosities, but they also have increased dynamic moduli compared to gels prepared without gelling agents. The increases in dynamic moduli are often not as dramatic as are the increases in viscosity, but they are more indicative of the processes occurring within the gels (Kreungngern & Chaikham, 2016; BeMiller, 2011).

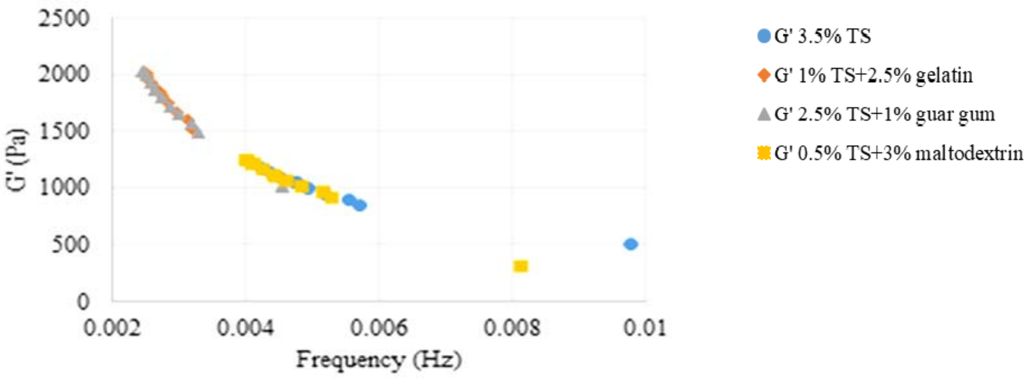

Figure 2 The frequency sweeps testing of freeze-dried Black Grass Jelly after rehydration combined with different gelling agents

Besides, the use of high-frequency waves causes the molecular structure of the gel to be shorter and smaller. Figure 2 shortens the ability to combine into a helix structure of the molecular chain (Jiamjariyatam, 2018). However, the tendency of gel strength will decrease as the frequency increases. Due to the loss of strength within the amylose structure from tapioca starch. Hydrogels herein shrank and decreased their volume after freeze-drying treatment and in turn reduced the mesh size due to the loss of ordered structures of starch gels. Regarding the size of amylose reassembled aggregates, ordered regions survived from freeze-drying due to the densely packed structures, while the size of amorphous regions decreased probably due to gel shrink and vapor destruction after freeze-drying treatment (Chi *et al.,* 2020).

Black Grass Jelly was traditionally prepared through an addition of starch to the polysaccharide extract of the plant leaf The results concerned with the cross-linking of Black Grass Jelly polysaccharide gum with starch have received particular attention. Lai *et al.* (2000), that the gelling properties and viscoelastic behaviors of this gum were found to be strongly influenced by various factors, including extraction conditions, starch types and concentrations as well as parts of the plant.

4.4 Texture profile analysis

Texture has been considered to be one of the most important attributes influencing consumers' final satisfaction with Black Grass Jelly (Aguirre *et al.,* 2018). Mechanical properties of Black Grass Jelly were obtained from a texture profile analysis (TPA) test using a texture analyzer. The added effects of gelling agents on hardness, cohesiveness, springiness, gumminess, and chewiness of Jelly samples have been rehydration with hot water (95±5°C) were evaluated. Textural parameters obtained from the force-time curve were described by Garrido *et al.* (2015). Hardness is defined as the force necessary to attain a given deformation. In the sensory analysis, it is the force required to compress food between molars in the first bite. Cohesiveness represents the strength of the internal bonds making up the body of the product. It is expected to be inversely proportional to the rate at which the material fractures under mechanical action. In other words, the lower the cohesiveness of a material, the more brittle it will be. Springiness is related to the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite. It represents the rate at which a deformed material goes back to its unreformed condition after the deforming force is removed. Gumminess represents the energy required to disintegrate a semi-solid food product to a state ready for swallowing. Chewiness the mouthfeel sensation of labored chewing due to sustained, elastic resistance from food (Kreungngern & Chaikham, 2016).

Table 4 Texture profile analysis (TPA) of freeze-dried Black Grass Jelly after rehydration combined with different gelling agents

Gelling agents	**Hardness** (N)	**Cohesiveness**	**Springiness** (mm) **(NS)**	**Gumminess** (N) **(NS)**	**Chewiness** (mJ) **(NS)**
3.5% tapioca starch	54.75±13.67^{ab}	0.86±0.05^{a}	3.43±0.24	47.25±10.78	1.57±0.32
1% tapioca starch + 2.5% gelatin	65.00±10.67^{a}	0.64±0.13^{b}	2.82±0.51	43.00±7.07	1.35±0.41
2.5% tapioca starch + 1% guar gum	50.00±4.58^{ab}	0.75±0.09^{ab}	3.26±0.65	37.33±1.52	1.20±0.28
0.5% tapioca starch + 3% maltodextrin	45.25±9.46^{b}	0.76±0.11^{ab}	3.08±0.10	34.25±7.27	1.03±0.20

The means with different small letters in the same column were significantly different ($p<0.05$)

From this study, Table 4 showed that hardness and cohesiveness values in the products significantly increased ($P\leq0.05$), except the springiness, gumminess, and chewiness values of Jelly combined with ager (Table 4). At the high concentration of gelling agents, freeze Black Grass Jelly with tapioca starch (control) addition had significantly higher ($P\leq0.05$) hardness and cohesiveness than the other products. However, the addition of gelatin to Black Grass Jelly will increase the hardness because the increased gelatin could make the structure of the gel junction hold the gelatinization of the gelatin. There is a cross-link between the polypeptide wires with two types of bonds the connection between the peptide groups with hydrogen bonds and a cross-link with disulfide bonds between the amino groups. Therefore, the higher the gelatin concentration, the more bonding between the bonds results in the gel being stronger (Periche *et al.,* 2014). Also, it was found springiness, gumminess, chewiness no significant differences ($P\leq0.05$) in texture attributes were found between gelling agents methods.

5. Conclusion

Freeze-dried Black Grass Jelly, which has a low water activity (<0.2) classified as dry food, can be stored for a long time. Color parameters indicated that jelly combined with 2.5% tapioca starch + 1% guar gum was apparently darker than the other samples. The gel had a medium density of branched-structure, suggested by the viscoelastic behaviors. From rheological behaviors and texture properties, the results confirmed that 1% Tapioca starch + 2.5% gelatin addition could be appropriate to ensure the good gel qualities for freeze-dried Black Grass Jelly production.

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