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Development of grass jelly processing using modified starches and higher efficient extraction method

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Abstract The grass jelly stalks was extracted using pressure cooker at 117°C for 30 minutes and followed by boiling under atmospheric pressure for 60 minutes to get the total solid content (%) in grass jelly solution resulted non-significantly different from the traditional boiling for 3 hours. The pea starch and 3 types of modified starches (KSA1502, ET50, and ESH15) at 5 % by solution weight were improved the grass jelly texture. The grass jelly from KSA1502 had hardness, springiness and gumminess that did not significantly different from the control (tapioca starch). Finally, the grass jelly solution was extracted by reducing grass jelly stalks for 30% from the control formula, and using 2% KSA1502 with gelatin 2.5 % together with varying tapioca starch to 4 levels as 0, 1, 2 and 3% by solution weight. It was found that the formula using 2% KSA1502 along with 1% tapioca starch and 2.5% gelatin by weight to receive the best texture properties and sensory scores in all attributes.

Keywords: Grass jelly, Modified starch, Gelatin, Tapioca starch, Pressure

Introduction

Grass jelly (also known as "Chao Kuay" in Thai) is made from the herb with scientific name of *Mesona chinensis* in the mint family (Lamiaceae) (Lim, 2012). Grass jelly is believed to be an herbal food to relieved heartburn, stomach pains, nausea, and indigestion. Its thirst-refreshing taste is also beneficial for health because of its water-soluble fiber (Handayani *et al*., 2017) can bind sugar and fat in the digestive system, so it helps preventing diseases such as diabetes, heart disease, muscle pain and stroke. This herb exhibits various biological activities, such as antioxidant, antihypertensive, inhibition of DNA damage, protection against heat stroke, and hepatoprotective effects (Liu *et al*., 2018).

Grass jelly is a famous refreshing dessert usually served with sugar syrup and water and crushed ice or ice cubes. Variations of the water mixture may

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also include milk, flavorings or other fruit toppings such as jackfruit, pineapple, banana, and other fruit. It is also used to make “black bean jelly” in China (Xiao *et al*., 2020).

Grass jelly is made by boiling the aged and slightly oxidized stalks and leaves of *Mesona chinensis*. The small twigs and leaves of the grass are boiled in water with sodium carbonate for three hours to extract pectin, lignin and other polysaccharides from the herbs and to evaporate water so that the final solution had the optimum concentration of total solid content for gel setting with the association of suitable starch type and concentration (Bush, 2006). After that the concentrate with herbs is pressed hard and filtered in tiny mesh to remove the mixed particles and squeeze out the juice. The starch solution made from appropriate amount of cassava, arrowroot starch and/or gelatin was added to the solution for binding with grass jelly polysaccharides extracted during boiling (Clarissa, 2016; Kreungngern and Chaikham, 2016). The juice is then cooled down into a gelatinous consistency then sliced to desired cubes and then immersed in syrup overnight to develop firmer texture of grass jelly.

Since the manufacturing of grass jelly comprised of the expensive herbs and the high cost of extraction process as it needs at least 3 hours for extraction time, this research aimed to study the efficient extraction method with less extraction time and energy, investigate the effect of native pea and modified starches on the grass jelly properties as well as study the optimum ratio of modified starch on grass jelly properties with 30% reduction of grass jelly stalks.

Materials and Methods

Grass jelly stalks (Wah Tai from Talad Thai, Pathumtani Province), gelatin (Bloom no. 250 from Chemipun Co., LTD), tapioca starch (Pla Thai 5 Dao), Pea starch, modified potato starch (KSA1502, Starch Acetate E1420), modified pea starch (ESH15, Hydroxy propyl starch E1440) and modified food starch (ET50, Hydroxy propyl distarch phosphate) from Emsland Asia Food Innovation Corp. Co., Ltd. were used in the experiments.

Traditional grass jelly (control formula) was prepared by following the grass jelly formulation of Wittayapanyanon (2012). 100 g of dried grass jelly stalk was washed and boiled in 5 liter of boiling water containing 3.43% (by grass jelly stalk weight) of sodium carbonate for 3 hours at 95^{o}C. The solution was squeezed through a filter cloth to get most of mucilage in the filtrate. The tapioca starch (5% of solution weight) was added to the filtrate immediately at 85 ^{o}C and stirred well for 3 minutes. The mixtures were poured into stainless steel tray and cooled down to 25 ^{o}C before kept in a refrigerator prior to analysis.

High pressure cooking preparation of grass jelly: The grass jelly prepared using high pressure cooker was following the same formula as the traditional grass jelly production but using the different amount of water and procedure. 100 g of dried grass jelly stalk was washed and boiled in 1,700 g of water in pressure cooker at 117^{o}C (Kuhn Rikon, Switzerland) for 30 minutes. The mixture was taken out from the pressure cooker to stainless steel pot and subsequently added 1,100 g of water containing 3.43% (by grass jelly stalk weight) of sodium carbonate and continue boiling as same as the traditional boiling at 95^{o}C for 1 hour. The solution was squeezed through a filter cloth to get most of mucilage in the filtrate. The gelling agent was added to the filtrate immediately at 85 ^{o}C and stirred well for 3 minutes. Pour the mixtures into stainless steel tray and cooled down to 25 ^{o}C before kept in a refrigerator prior to analysis.

The extraction time for traditional grass jelly was varied for 30-180 minutes whereas the extraction time for high pressure cooking was initially fixed for 30 minutes in high pressure cooker and then varied the boiling time for 0-60 minutes under atmospheric pressure. The effect of pea starch and 3 different modified starches (modified potato starch (KSA1502), modified pea starch (ESH15) and modified food starch (ET50)) on grass jelly properties was subsequently studied by totally substituting tapioca starch in the control formula (5% by solution weight) to improve grass jelly texture. In order to investigate an efficiency of gelling properties of KSA1502, the grass jelly was reproduced by decreasing grass jelly stalks for 30% from the control formula and determine the optimum percentage of tapioca starch by varying 0, 1, 2, and 3 % by solution weight with 2.5% gelatin and 2% KSA1502. The experimental design was a completely randomized design (CRD) with two replications for each. The results were reported as the mean value with standard deviation. Statistic was analyzed using SPSS for Windows and Duncan’s multiple range test (DMRT) was used for comparing the differences among mean values at the 95% confidence level ($p \leq 0.05$).

Determination of total solid content of grass jelly extract solution

The total solid content of extract solution from traditional and high pressure cooking procedure at various extraction time was analyzed according to AOAC (1997). The percentage of total solid was measured as the remaining weight of sample after drying in hot air oven at 105^{o}C overnight and was expressed as percentage of the wet sample. The criterion used to consider the optimum extraction time for pressure cooker is the minimum extraction time that gave the solid content of mixture closed to the extract from the traditional boiling at 3 hours.

Texture profile analysis (TPA) of grass jelly

Texture profile analysis was performed to evaluate the texture of the grass jelly using a Texture Analyzer TA-XT2i (Stable Microsystem, UK). Grass jelly samples were cut into 20 × 20 × 20 mm cubes for TPA measurement. A standard double-cycle program was used to compress the samples at a speed of 1 mm/sec with 50% deformation using a 50 mm diameter probe with 30 second waiting time before starting the second compression. Hardness (N), cohesiveness (no unit), springiness (mm) chewiness (Nm) and gumminess (N) were calculated by the software program. At least 5 samples were measured to obtain an average value of all texture parameters for each formulation (Blandino *et al*., 2013).

Lightness measurement of grass jelly

Lightness (L*) of the grass jelly was measured using colorimeter (Hunter Lab model Colorflex45/0, USA).

Sensory evaluation

Sensory evaluation was performed to select the best formulation using a 9-point Hedonic scale test of 50 people. Each panelist was asked to rate the liking of quality attributes according to color , glossy , flavor, texture , general liking of each sample using a 9-point hedonic scale (1 =dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither dislike nor like, 6=like slightly, 7= like moderately, 8 = like very much and 9 = like extremely). 2 pieces of grass jelly (2x2x2 cm) in syrup were served at room temperature.

Results

The total solid content (%) of grass jelly extract from two different methods at each extraction time was increased with the extraction time (Table 1). The high pressure cooking method showed higher total solid content (%) than traditional boiling at the same extraction time. The criterion ofthe optimum extraction time was the minimal time to give the solid content of the grass jelly extract which closedly to 1.0% (the total solid content of grass jelly extract from traditional method at 3 hours). It can be concluded that "boiling at atmospheric pressure for 3 hours" and "boiling under high pressure for 30 minute, followed by boiling under atmospheric pressure for 60 minute" gave the optimum solid content of the grass jelly extract.

Table 1. Total solid content of grass jelly extract from traditional and high pressure cooking method at various extraction time

Time(min)	Total solid content(%)	
	Traditional cooking	High pressure cooking
0	-	0.57[def] ± 0.04
15	-	0.68[cde] ± 0.13
30	0.39[f] ± 0.06	0.76[bcd] ± 0.03
45	-	0.91[ab] ± 0.08
60	0.49[ef] ± 0.06	1.00[a] ± 0.02
90	0.61[de] ± 0.09	-
120	0.71[bcde] ± 0.07	-
150	0.88[abc] ± 0.08	-
180	1.00[a] ± 0.23	-

[a,b,c,..] Mean values in a column with different letters are significantly different ($p \leq .05$).

The texture profile analysis (TPA) of grass jelly from both methods was evaluated at the optimum extraction time as shown in Table 2. The grass jelly texture from both methods showed no significant differences in hardness, springiness, cohesiveness, gumminess, and chewiness (P>0.05). Therefore, the high pressure cooking method was chosen for further studying in the effect of various kinds of starches on grass jelly properties.

Table 2. Texture profile analysis of grass jelly from traditional and high pressure methods at the optimum extraction time

Texture profiles	Traditional method	High pressure method
Hardness[ns] (N)	518.8 ± 50.9	512.7 ± 65.2
Springiness[ns]	0.88 ± 0.04	0.88 ± 0.04
Cohesiveness[ns]	0.59 ± 0.08	0.56 ± 0.12
Gumminess[ns] (N)	319.7 ± 93.27	300.61 ± 63.74
Chewiness[ns] (N)	252.70 ± 52.92	258.85 ± 89.47

[ns] Not significantly different (p>0.05).

The effect of pea starch and modified starches on grass jelly properties

The grass jelly that was substituted with pea starch that provided the highest hardness, while the one substituted with ESH15 gave the lowest hardness(Table 3). The hardness of grass jelly substituted with KSA1502 and

ET50 was not significantly (P>0.05) different from the control formula. Nevertheless, grass jelly substituted with KSA1502 had cohesiveness and gumminess greater than ET50.

The lightness (L*) of grass jelly substituted with pea starch and modified starches resulted that the grass jelly with pea starch presented the highest lightness, followed by the grass jelly with KSA1502 and ET50. The lightness of grass jelly with ESH15 was not significantly different to the control formula (p>0.05) but the textural properties of grass jelly with ESH15 was more soft than the control. KSA1502 was therefore chosen for further studying in the next experiment because cohesiveness and gumminess were the unique characteristic of grass jelly that KSA1502 had textural properties greater than ET50.

Table 3. Effect of pea starch and modified starches on texture properties of grass jelly

Starch	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
Control	518.80^{bc} ±50.90	0.88^{ab} ±0.04	0.59^{a} ±0.08	319.72^{a} ±93.27	252.70^{b} ±52.92
Pea	1494.18^{a} ±36.29	0.90^{a} ±0.01	0.27^{bc} ±0.06	415.78^{a} ±59.90	378.31^{a} ±49.05
KSA1502	446.63^{c} ±44.32	0.71^{c} ±0.01	0.34^{b} ±0.01	145.40^{b} ±27.21	104.20^{c} ±20.85
ESH15	161.40^{d} ±10.75	0.89^{ab} ±0.00	0.69^{a} ±0.01	110.79^{b} ±5.77	98.85^{c} ±5.16
ET50	558.90^{b} ±16.13	0.83^{b} ±0.03	0.22^{c} ±0.01	122.89^{b} ±9.10	102.42^{c} ±4.11

[a,b,c,..] Mean values in a column with different letters are significantly different ($p \leqslant .05$).

Table 4. Effect of pea starch and modified starches on the lightness (L*) of grass jelly

Starch	Lightness (L*)
Control	3.54^{a} ± 0.10
PEA	11.25^{c} ± 0.28
KSA1502	6.65^{b} ± 1.12
ESH15	4.84^{ab} ± 2.16
ET50	6.76^{b} ± 0.41

[a,b,c,..] Mean values in a column with different letters are significantly different ($p \leqslant .05$).

The optimum ratio of KSA1502 on grass jelly properties with 30% reduction of grass jelly stalks

The 30% value of grass jelly stalks was selected for this experiment because the control formula (5% tapioca starch) could not form gel structure due to low source of polysaccharides in grass jelly extract solution. The results of decreasing grass jelly stalks by varying 2 levels of KSA1502 as 2% and 4% by solution weight were shown in Table 5. Adding more KSA1502 concentration led to an increase in hardness, while the other textural properties were not significantly (P>0.05) different. Moreover, increasing the concentration of KSA1502 resulted in higher lightness that made grass jelly turned grey which was not a good appearance of grass jelly especially when using KSA1502 greater than 4% (Table 6).

Table 5. Effect of KSA1502 concentration on textural properties of grass jelly

KSA1502 (%)	Hardness (N)	Springiness[ns]	Cohesiveness[ns]	Gumminess[ns] (N)	Chewiness[ns] (N)
Control	518.80[a] ±50.9	0.88 ±0.41	0.59 ±0.08	319.72 ±93.27	252.70 ±52.92
2	219.63[b] ±22.19	0.83 ±0.02	0.58 ±003	142.99 ±29.08	123.50 ±33.01
4	308.94[b] ±53.12	0.87 ±0.01	0.63 ±0.04	196.65 ±46.83	171.75 ±42.61

[a,b,c,..] Mean values in a column with different letters are significantly different ($p \leq .05$);
[ns] Not significantly different (p>0.05).

Table 6. Effect of KSA1502 on lightness (L*) of grass jelly

KSA1502 (%)	Lightness (L*)
Control	3.54[c] ± 0.99
2	7.96[b] ± 0.45
4	10.55[a] ± 0.09

[a,b,c,..] Mean values in a column with different letters are significantly different (p ≤ .05).

The grass jelly properties from using KSA1502 with gelatin and tapioca starch

The grass jelly preparation with using 1% tapioca starch with 2.5% gelatin and 2% KSA1502 was not significantly different from 2% and 3% tapioca starch (p>0.05) (Table 7). However, grass jelly with 1% tapioca starch had the gumminess, chewiness, and hardness greater than grass jelly without tapioca. Therefore, grass jelly with 0% and 1% tapioca starch were chosen to perform sensory evaluation comparing with the control formula.

The sensory score of grass jelly with 0% and 1% of tapioca starch, 2% KSA1502, and 2.5% gelatin comparing with the control formula were shown in Table 8. The sensory evaluation resulted that consumer like the color and glossy of grass jelly without tapioca which did not significantly different from grass jelly with 1% tapioca starch and the control formula (5% tapioca starch). However, grass jelly with 1% tapioca starch got the significant highest score in flavour, texture and general liking among the others.

Table 7. Effect of tapioca starch concentration with using 2.5% gelatin and 2% KSA1502 on textural properties of grass jelly

Gelling agent (%)	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
Control	518.8^{bc} ±50.90	0.88^{b} ± 0.04	0.59^{b} ± 0.08	319.72^{b} ± 93.27	252.70^{c} ±59.92
Gelatin	96.71^{d} ±7.85	1.10^{a} ± 0.02	0.82^{a} ± 0.09	71.13^{c} ± 3.67	71.23^{d} ±0.59
0	389.67^{c} ± 4.67	0.95^{ab} ± 0.02	0.79^{a} ± 0.03	308.63^{b} ± 15.00	303.48^{c} ±6.72
1	648.49^{ab} ± 9.3	0.92^{ab} ± 0.01	0.68^{ab} ± 0.03	441.63^{ab} ± 0.14	405.93^{b} ±2.31
2	699.83^{a} ±79.38	0.94^{ab} ± 0.01	0.72^{ab} ± 0.02	499.55^{a} ± 43.73	467.04^{ab} ±34.1
3	788.52^{a} ± 7.92	0.93^{ab} ± 0.02	0.72^{ab} ± 0.04	570.20^{a} ± 86.45	530.55^{a} ±70.1

[a,b,c,..] Mean values in a column with different letters are significantly different ($p \leq .05$).

Table 8. Sensory evaluation of grass jelly with 0 and 1% tapioca starch, 2.5% gelatin and 2% KSA1502

Tapioca starch (%)	Characteristic				
	Color[ns]	Glossy[ns]	Flavor	Texture	General linking
Control	7.26 ± 1.44	7.24 ± 1.6	5.62^{b} ± 1.90	4.28^{c} ±1.69	4.74^{c} ± 1.64
0	7.10 ± 1.53	7.22 ± 1.00	5.90^{ab} ± 1.63	5.26^{b} ±1.90	5.72^{b} ± 1.40
1	6.96 ± 1.37	7.12 ± 1.19	6.36^{a} ± 1.48	6.20^{a} ±1.49	6.35^{a} ± 1.44

[a,b,c,..] Mean values in a column with different letters are significantly different ($p \leq .05$);
[ns] Not significantly different (p>0.05).

Discussion

The total solid of extract solution from both methods increased with the extraction time but the total solid from cooking grass jelly stalks using pressure cooker at 117^{o}C was higher than the solution from traditional cooking when comparing at the same extraction time. The result concured with the work of Korir *et al.* (2018), where they observed the increase in percentage of mucilage from root bark of *Malva verticillata* as an increase in extraction time. The comparison between using hot water and high pressure cooker for pectin extraction from stringy pulp of Nanga jackfruit (Juampa Krop) was studied by Wititsiri (2014). The result revealed that 5.69% yields was obtained by hot water for 60 minutes at 80°C whereas 24.63% yields was gained by using high vapor pressure for 30 minutes at 121°C which is in accordance with grass jelly extraction. Zaid *et al*. (2016) also reported that pectin yield from dragon fruit (*Hylocereus polyrhizus*) peels extraction increased with an increase in extraction time (30-120 minutes) and temperature (30-70^{o}C). It was due to the accumulation of thermal energy with the extraction time and temperature causes the boiling water or steam penetrate mucilaginous cell, enhance dissolution and transfer mucilage out of the cell. The extraction time to reach the total solid of 1.0 % for pressure cooker method (90 miunutes) was two times less than the traditional method (180 minutes). Using a pressure cooker during the first 30 minutes prior to 60 minutes of boiling at atmospheric pressure gave higher efficient extraction because the steam pressure inside pressure cooker weakened the cell wall of grass jelly stalk so that water vapour can easily diffused through the cell walls and washing out the mucilage in the cell content to the extract solution. The texture profile analysis (TPA) of grass jelly from traditional and high pressure methods evaluated at the optimum extraction time indicated that all texture properties of grass jelly were not significantly different ($p \geq 0.05$) because of the same total solid content obtained from both methods at the optimum extraction time which it could form the similar gel structure with 5% tapioca starch.

The substitution of tapioca starch with 4 different kinds of native and modified starches including pea starch, modified potato starch (KSA1502, Starch Acetate E1420), modified pea starch (ESH15, Hydroxy propyl starch E1440) and modified food starch (ET50, Hydroxy propyl distarch phosphate) in grass jelly formulation had a significant effect on the texture properties and lightness (L*) of the product.The grass jelly with pea starch provided the highest hardness since the native pea starch is a rich source of amylose (30-40%) that gave rise to the high extent of retrogradation (Ratnayakea *et al*., 2002). The pasting properties of modified pea starch (ESH15) were low heat viscosity after cooling down provided the lowest hardness of grass jelly texture as seen in Table 3. The low final viscosity and set back of this derivatized pea starch was from the introduction of hydroxypropyl ester groups in starch chains which help prevent retrogradation (BeMiller and Whistler, 2009). The modified potato starch (KSA1502) is starch acetate or thin boiling starch which has the functional properties of providing viscous solutions which forms gels after cooling and remain rather stable afterwards. The hardness of grass jelly prepared from KSA1502 was slightly less than that from hydroxyl propyl distarch phosphate (ET50) but both of them was not significantly ($p>0.05$) different from the control formula (tapioca starch). Since the ET50 is hydroxyl propyl distarch phosphate, a starch that was modified by a combination of substitution and cross-linking, hardness of grass jelly from ET50 was therefore higher than KSA1502. However, cohesiveness and gumminess of grass jelly from KSA1502 is close to the control better than from ET50. The lightness (L*) of grass jelly from Table 3 demonstrated that the grass jelly from high extent retrogradation starch (pea starch) showed the highest lightness which attributed to the reassociate process to form more ordered structures of disaggregated amylose and amylopectin chains (Wang *et al.*, 2015). In addition, both properties are the characteristics of this product, KSA1502 was therefore chosen to study the optimum ratio of KSA1502 on grass jelly properties with 30% reduction of grass jelly stalks.

The objective of reduction in grass jelly stalks for 30% from the control formula was to investigate the efficiency of KSA1502 in gelling properties without the help of mucilage from grass jelly stalk. The 2% and 4% KSA1502 were chosen to study the optimum percentage because 5% KSA1502 gave unacceptable color of grass jelly. The result in Table 5 indicated that adding more KSA1502 led to an increase in hardness and lightness of grass jelly which was in accordance with the findings of Kreungngern and Chaikhan (2016) in that hardness, cohesiveness, springiness, gumminess and lightness of grass jelly significantly increased with increasing concentration of potato flour from 3% to 6%. Although the 4% of KSA1502 gave the good hardness but the lightness of grass jelly was opaque and grey in color, 2%KSA1502 was therefore selected to mix with 2.5% gelatin and varied tapioca starch for 0-3% to find the best formulation of grass jelly. The texture properties of grass jelly in Table 7 indicated that combination of 2.5% gelatin and 1-3% tapioca starch gave the same texture properties in all attributes. Use of 2.5% getatin only (without tapioca starch) in grass jelly resulted in lowest hardness, gumminess and chewiness; in contrast, the springiness and cohesiveness showed the highest value. This is because of the unique properties of gelatin gel in which it is clear, elastic gel, syneresis free and thermoreversible (Imeson, 2010). The increase in tapioca starch from 0 to 1% with 2.5% gelatin and 2% KSA1502 caused an increase in hardness and chewiness. This findings were in accordance with Kreungngern and Chaikhan (2016) in that the G' and G" significantly increased with the increasing levels of gelling agents. This was probably due to a stronger gel system with more cross-link densities (Apicharsrangkoon and Ledward, 2002).

The sensory evaluation of grass jelly with 0 and 1% of tapioca starch with 2% KSA1502 and 2.5% gelatin revealed that 1% tapioca starch got the significant highest score in flavour, texture and general liking among the others. The products were accepted and got the hedonic score in terms of color, flavor, texture and general liking as like slightly to like moderately and in the attributes of glossy were scored as like slightly to like very much.

In summary, the higher efficient extraction method for grass jelly was boiling grass jelly stalks for 30 minutes in pressure cooker at 117^{o}C followed by boiling under atmospheric pressure for 60 minutes. The combination of gelling agent for grass jelly production with 30% reduction of grass jelly stalks was 2% KSA1502, 2.5% gelatin and 1.0% tapioca starch.

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