Purified Konjac Glucomannan as Thickener for Substituting Gelatin in Making Panna Cotta

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ABSTRACT

A thickener such as gelatin is essential in increasing viscosity and forming a gel system. Nowadays, gelatin is one of the most popular thickeners in food, pharmacy, and cosmetic products. Animal skins which lead to halal issues are still common sources of gelatin. Crude konjac flour (CKF) which is sourced from porang tubers and has a high degree of purity of glucomannan (GM) is a potential substitute for animal skins. Moreover, the use of KGM also supports national programs of processing agricultural products into finished products ready to export. This study applies five different CKF variants as panna cotta (PC) thickener: CKF from the fresh bulb of porang (*Amorphophallus oncophyllus* sp.) (CH-UP), CKF from chips of Porang (CH-CP), a native CKF, as well as laboratory-purified products of CKF (>90%-w of GM content) (DM-CKF-M3X-50, and DM-CKF-M3X-96), and commercially products of porang flour (K-TPO and K-TPM) were applied to make PC; substituting gelatin. Only CKF, DM-CKF-MX-50, and DM-CKF-M3X-96 successfully thickened PC in terms of appearances. Further, organoleptic analysis was applied to CKF, DM-CKF-MX-50, and DM-CKF-M3X-96. According to the organoleptic analysis, PC with CKF, DM-CKF-MX-50, and DM-CKF-M3X-96 was more preferred by the respondents than commercial PC. In overall, CKF application on PC exhibited improved sensory properties and tastes of PC.

Keywords: *Halal, Konjac glucomannan, Substitute, Thickener.*

1 Introduction

Glucomannan (GM) is a polysaccharide consisting of d-glucose and d-mannose as a monomer with β-1,4 glycosidic bonds [1]. It can be purified from Porang tubers using mechanical,

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enzymatic, or chemical processes such as extraction. Usually, it is used as a thickening and gelling agent due to its capability to form and stabilize the gel structure [1]. Its flour is used in Japan to produce shirataki noodles and konyaku jelly [1]. The physical and chemical properties of konjac glucomannan (KGM) are linearly correlated with the purity level of GM [2]. Therefore, the GM content is among the other parameters that need to be analyzed to classify the quality of Porang flour. Regarding the GM content, Porang flour can be named Crude Konjac Flour (CKF) or KGM. The CKF is when the GM content is between 60-85% (NY/T 494-2020). Meanwhile, higher GM content, ca. ≥85%, is called KGM. Unfortunately, the quality of CKF and KGM is still not regulated in SNI. In contrast, the Porang chip is regulated in SNI 7939:2020. NY/T 494-2020, a standard from the Republic of China for GM flour, is used as the standardized requirement for the KGM. The best quality of KGM requires 90% of GM at minimum.

Glucomannan can bind water, dissolve in water, swell, and have a neutral smell even when added with other ingredients. Therefore, they are a wide-use application of GM, i.e., additive in the noodles or pasta industry, drug delivery, tablet filler, bioadhesive, cellular therapy, materials for cell immobilization, encapsulation materials, films and membranes, coating materials, cosmetics, emulsifiers, and surfactants. The solubility of GM is usually represented by the degree of acetylation. The presence of the acetyl group in the GM can prevent intramolecular hydrogen bonding, thus enhancing the solubility of GM in water [3]. Therefore, the degree of acetylation can also be used to express the gelatinization [2]. Moreover, GM can be used as a woven reinforcement in the textile industry [4].

In the case of food industries, a thickener plays an essential role in increasing the viscosity and forming a gel system. It also functions as a binder to stabilize and thicken food mixed with water. Generally, the thickener agents are used as gelatin, carboxymethylcellulose (CMC), alginate, carrageenan, and gelatin [5]. At the same time, gelatine is the favorite. Gelatin is a commonly used stabilizer in the food and beverage industry. Nowadays, gelatin is made from pork and cow skins because the collagen from the Vertebrata family is not dissolved in water [6].

The demand for gelatin is increasing worldwide [7]. Annually, 326,000 tons of gelatin are produced, with 46% made from pig skin, 29.4% from cow skin, 23.1% from bone, and the rest from other sources [8]. Gelatin Global is estimated to reach USD 4.42 billion by 2026 [9]. However, high attention to health and religious issues in gelatin encourages researchers to discover alternative gelatin sources such as fish and insects [10].

Therefore, this research studies the application of lab-scale purified KGM for making panna cotta, a favorite Italian dessert. It substitutes the use of gelatin and evaluates the produced panna cotta. Glucomannan has more or less physical properties similar to gelatin. Subtiting the use of gelatin with a natural thickener such as glucomannan. Hence, halal processes are ensured, especially in the food, beverage, cosmetics, and drug industries, supporting the government program ‘processing agricultural products into finished products ready for export.’ Moreover, a sensory analysis covering the assessment of visual appearance, an expectation of the product, scent, flavor, melting degree, and texture is also conducted.

2 Materials and methods

2.1 Materials

Fresh bulbs of Porang and Crude Konjac Fluor (CKF) were donated from PT. ABS (Bogor, Indonesia), while the commercial product of Porang flour is bought commercially from an e-commerce platform. Chemicals such as ethanol (EtOH) food grade, phenyl hydrazine pure grade, $KMnO_4$, NaOH, NaCl, sodium bisulfite ($NaHSO_3$), concentrated of HCl, H_2SO_4, acetone, NH_4OH, $CaCl_2$ is bought commercially from Merck (Darmstadt, Germany). Glasses apparatus, a set of reflux glass apparatus, a hot plate stirrer, a set of titration apparatus, analytical balance, whipped cream, milk, gelatin, and cane sugar are also used in the experiment. The ingredients for making panna cotta, such as whipped cream, milk, gelatin, and cane sugar, were bought commercially at Sukolilo, Surabaya, Indonesia.

Table 1. This research used different kinds of konjac flour to make Panna cotta.

Group	Sample Description	Samples code
Crude Konjac Flour (CKF) from the fresh tuber of Porang (*Amorphophallus muelleri*)	CKF from fresh tuber - soaking in NaCl 15% (single-stage process extraction with EtOH 50% - 3 h)	CH-UP-15-S
	CKF from fresh tuber – soaking in NaCl 15% (multiple stage process extraction with EtOH 50% - 3 h)	CH-UP-15-M
CKF from Porang Chips donated from PT. ABS	CKF from chips – soaking in NaCl 15% (single stage process extraction with EtOH 50% - 3 h)	CH-CP-15-S
	CKF from fresh chips – soaking in NaCl 15% (multiple stage process extraction with EtOH 50% - 3 h)	CH-CP-15-M
CKF donated from PT. ABS	Native CKF	CKF
purified konjac glucomannan (KGM) within 3-stage of extraction steps using ethanol	KGM – 3 stages process extraction with EtOH 50%	DM-CKF-M3X-50
	KGM – 3 stages process extraction with EtOH 96%	DM-CKF-M3X-96
commercial product of Porang flour	Commercial product – Organic Flour of Porang	K-TPO
	Commercial product – Organic Flour of Porang for Shirataki noodles	K-TPM

Nine kinds of konjac flour made Panna cotta (Table 1). They are classified into five major groups: (1) Crude Konjac Flour (CKF) from fresh tuber of Porang (*Amorphophallus muelleri*) donated from PT. Anugerah Bumi Samudra (PT. ABS), Bogor – labeled as CH-UP; (2) CKF from chips of Porang donated from PT. ABS is labeled CH-CP; (3) native CKF was donated from PT. ABS – labeled as CKF; (4) purified konjac glucomannan (KGM) within 3-stage extraction steps using ethanol – labeled as DM-CKF; and (5) commercial product of Porang flour – labeled as K-TPX. In addition, the 3-stage extraction steps using ethanol is not within the scope of this paper and not further explained.

The first konjac flour, CH-UP, is made at our laboratory following our developed protocols from the Porang tubers donated by PT. ABS. Meanwhile, the second konjac flour, CH-CP, is made from Porang chips donated by PT. ABS. Those are both only different from the raw materials used. The CH-UP, CH-CP, and DM-CKF are developed at our laboratory (not yet published), while CKF is kindly donated from PT ABS and used as it is.

2.2 Methods

Panna cotta Recipe. A 200 g of whipping cream, 45 g of milk, 15 g of sugar, and 2/3 of a teaspoon of vanilla bean are mixed and stirred in the saucepan at medium heat. The stirring continues till all the sugar is well dissolved. Add 4.5 g of gelatin and continuously stir till homogenously dissolved. Subsequently, the mixture was heated to room temperature. Slowly add the remaining 50 g of whipping cream, gently stirring. Put the panna cotta dough in clear, clean, closed containers, and chill it in the refrigerator (at least 4 hours). Hence, panna cotta is finally ready to serve. Repeat all the steps using konjac flour as a raw material. In the case of using either CKF or KGM, only 1.5 g of gelation is used instead of 4.5 g. The remaining amount, i.e., 3 g, is substituted with konjac flour (Table 1).

Glucomannan Content Analysis. Analysis of glucomannan content was conducted based on the National Standard of Indonesia (Standar Nasional Indonesia/SNI) 7939:2020 [10]. Accurately weigh 1 g of flour sample (noted as Wo). The sample was placed in a 100 mL Erlenmeyer. Add 30 mL HCl (bd 1.025 or 2.05 M), boil stones, and conduct the reflux process for 3.5 hours. During the reflux process, keep the solution at 70-80 °C. Do the filtration process directly with filter paper and wash the supernatant with boiling water. Mix all the filtrates, add three drops of phenolphthalein indicator, and basify the filtrates by adding 10% NaOH till the solution color turns pink. Acidify the filtrate using concentrated acetic acid, and check the pH using litmus paper. Evaporate the solution into approximately 30 mL. Add 1.5 mL of phenylhydrazine, 1.5 mL of concentrated acetic acid, and 10 mL of distilled water to the concentrated solution. Allow the solution to reach room temperature, then put it in the refrigerator for a night. Filter the precipitate formed using a vacuum filter, then wash the supernatant several times with 15 mL of distilled water each. Finally, wash with 10 mL acetone. The final supernatant obtained was dried in the oven till its constant weight (noted as W1). Calculate the glucomannan content with the Eq. 1.

$$\boldsymbol{KM = \frac{\frac{2}{3}W_1}{W_0} x\ 100\%} \tag{1}$$

KM = percentage of glucomannan content; 2/3 = conversion factor of mannose of phenylhydrazine to mannose; Wo = initial weight of sample (g), and W1 = final weight of obtained supernatant (g).

Calcium Oxalate Analysis. Analysis of calcium oxalate content consists of 3 steps: digestion, precipitation of oxalate, and titration of permanganate. This protocol is based on SNI 7939:2020 [12].

Digestion steps. Accurately weigh a 2 g flour sample and put it in a 250 mL Erlenmeyer. Add 190 mL of distilled water and 10 mL of 6 M HCl. Heat the solution at 100 °C for an hour. Cool the solution and directly add water till it reaches 250 mL. Filtrate the final solution. Divide the filtrate equally, i.e., 125 mL each.

Precipitation of Oxalate steps. Add four drops of methyl red indicator to each filtrate portion (from the previous step). Gradually add a concentrated NH_4OH solution (drop by drop) until the solution color turns pink to a stable pale yellow. Subsequently, heat each filtrate at 90 °C, cool it, and filter it to remove the precipitation of iron ions. Reheat the iron-free filtrate at 90 °C and add 10 mL of 5% $CaCl_2$ solution. A continuous stirring is still conducted during the addition of $CaCl_2$. Cool the final solution and leave it overnight at five °C. A centrifugation step is conducted at 2500 rev/min for 5 min. Pipette the supernatant and redissolve it in 10 mL of H_2SO_4 solution 20% (v/v).

Permanganate titration steps. Combine the two filtrates to make 300 mL of total volume. Take only 125 mL of final filtrate for titration purposes and heat it until it is almost boiled. Titrate the solution with a standardized 0.05 M $KMnO_4$ solution till a pale pink color appears and is stable for 30 seconds. Calculate the calcium oxalate level based on Eq. 2.

$$\text{calcium oxalate}\ \left({}^{mg}/_{100\ g}\right) = \frac{KMnO_4(mL)\,x\ 0.00225\ x\ 2.4}{\text{weight of sample}\ (g)\,x\ 5}\,x\ 10^5 \quad (2)$$

The volume of titrant $KMnO_4$ is in milliliters (mL); 0.00225 = 1 cm^3 of 0.05 M $KMnO_4$ solution in proportion to 0.00225 g of anhydrous oxalic acid; 2.4 = a dilution factor (125 mL taken from 300 mL); 5 = $KMnO_4$ redox reaction

Table 2. Questionnaire sheet for organoleptic test of panna cotta

Have you ever eaten panna cotta?			**YES**		**NO**	
No	**QUESTIONS**	**1**	**2**	**3**	**4**	**5**
1	Does the panna cotta (sample at this survey) appearance look interesting?					
1 = Strongly dislike; 2 = Dislike; 3 = between like or dislike – Neutral; 4 = Like; 5 = Very much like						
2	Does the panna cotta (sample at this survey) have smell?					
1 = odourless; 2 = slightly smell; 3 = smellable; 4 = moderately smell; 5 = strong smell						
3	How does the panna cotta (sample at this survey) taste?					
1 = Strongly dislike; 2 = Dislike; 3 = between like or dislike – Neutral; 4 = Like; 5 = Very much like						
4	Does the panna cotta (sample at this survey) easily melt in the mouth?					
1 = hard; 2 = Not melt; 3 = Neutral; 4 = Slightly melt; 5 = well melt						
5	Does the texture of panna cotta (sample at this survey) appropriate as it is?					
1 = Very inappropriate; 2 = unsuitable; 3 = Either suitable or not suitable - Neutral; 4 = Suitable; 5 = Very suitable						
6	Does the texture of panna cotta (sample at this survey) appropriate as you expected? **for whom whose never eat panna cotta previously*					
1 = Very inappropriate; 2 = unsuitable; 3 = Either suitable or not suitable - Neutral; 4 = Suitable; 5 = Very suitable						

Organoleptic Test of Panna cotta. An organoleptic test is conducted on the prepared panna cotta using the konjac flour variance (Table 1). In comparison, for substituting the gelatin with different variances of konjac flour (Table 1), a variance of 1.5 or 3 g of gelatin is used, depending on the studied variable. An original panna cotta recipe used 4.5 gelatin. The panna cotta is prepared using the previously mentioned recipe (subsection 2.2, methods). Appearance, aroma, taste, melting, texture, and expectation of the panna cotta are the parameters observed in the organoleptic test. The organoleptic test was conducted with the completed questions, as shown in Table 2. The organoleptic test was conducted with visual appearance, the expectation of the product, scent, flavor, melting degree, and texture as the parameters assessed (Table 2). The value score starts from 1 = very unsuitable, while the maximum value is 5 = very suitable. Those higher score values indicate that the product is more likely to be preferred by the respondents. The test was conducted with thirty-five respondents with the age range of 20-22 years old. It was conducted at the Chemical Engineering Department from June to July 2022.

Microscopic Analysis. The presence of the oxalate in the konjac flour was observed using a binocular light microscope (Nikon, eclipse Ei). A slight amount of konjac flour was spilled with demineralized water, placed on the object glasses, and covered with cover glasses. The observed crystals were documented by digital camera.

3 Results and discussion

Panna cotta is prepared using all the konjac flour as described in Table 3. All the gelatin is replaced with variances of konjac flour in Table 3, meaning that 4.5 g of konjac flour is used. Unfortunately, only CKF, DM-CKF-MX-50, and DM-CKF-M3X-96 successfully made the panna cotta, especially regarding their appearance, while the rest did not. The konjac flour (CH-UP-15-S, CH-UP-15-M, CH-CP-15-S, CH-CP-15-M, K-TPO, and K-TPM) cannot be used as a substitute for gelatin, indicated by not well mixed between konjac flour with other ingredients. Hence, the panna cotta cannot be produced. The non-mixing condition is due to the low glucomannan (GM) content in those code samples of konjac flour, i.e., <90%. Therefore, the gelling capability is lower.

Table 3. Glucomannan and calcium oxalate content of the konjac flours which is applied in this research.

Raw Material for panna cotta making	Samples code	Glucomannan content (%-w)	calcium oxalate content (mg/100 g)	Panna cotta making
CKF donated from PT. ABS	CKF	64.12	27.81	√*
CKF from fresh tuber – soaking in NaCl 15% (single-stage process extraction with EtOH)	CH-UP-15-S	67.947	23.149	X*
CKF from fresh tuber – soaking in NaCl 15% (multiple-stage process extraction with EtOH)	CH-UP-15-M	86.826	15.381	X
CKF from chips – soaking in NaCl 15% (single-stage process extraction with EtOH)	CH-CP-15-S	77.427	34.950	X
CKF from fresh chips – soaking in NaCl 15% (multiple-stage process extraction with EtOH)	CH-CP-15-M	84.153	37.916	X
KGM – 3 stages process extraction with EtOH 50%	DM-CKF-M3X-50	99.420	12.42	√
KGM – 3 stages process extraction with EtOH 96%	DM-CKF-M3X-96	95.200	16.20	√
Commercial product – Organic Flour of Porang	K-TPO	7.588	38.34	X
Commercial product – Organic Flour of Porang for Shirataki noodles	K-TPM	37.01	6.75	X

*check sign (√) indicates that panna cotta was successfully made using the raw material as a substitution for gelatin. In contrast, the cross sign (X) indicates that panna cotta can not be made.

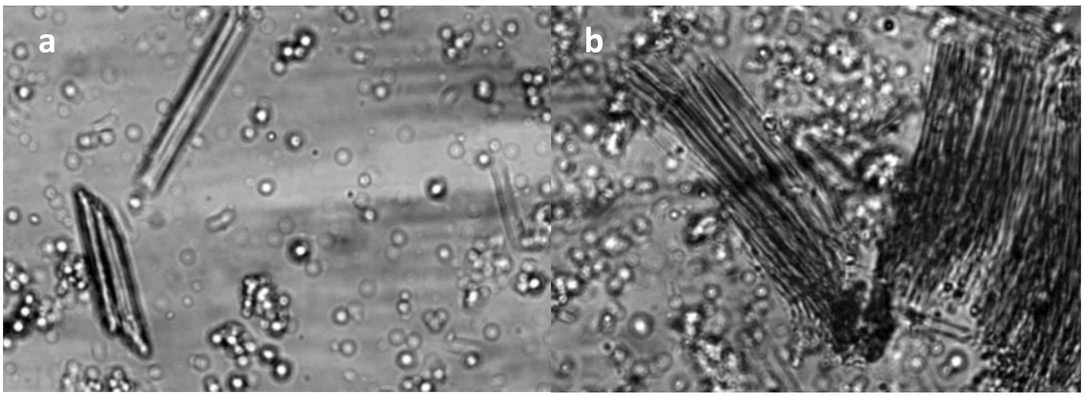
Therefore, in the further experiment, only CKF, DM-CKF-MX-50, and DM-CKF-M3X-96 were observed for panna cotta production and organoleptic test. When konjac flour is used to substitute gelatin for making panna cotta production, only 1.5 g of gelatin is used instead of 3.0 g for konjac flour. The ratio of gelatin to konjac flour is 0.5. This ratio gives the best result, visually observed, of panna cotta. This value is taken to keep the appearance, taste, and texture of the resulting panna cotta as the original one. The original panna cotta recipe used 4.5 g of gelatin. Moreover, 1.5 g of gelatin and 3.0 g of konjac flour resulted in an overly challenging panna cotta.

Code samples of CKF, DM-CKF-M3X-50, and DM-CKF-M3X-96 have 64.12, 99.42, and 95.20, respectively, of glucomannan content (Table 3). Though CKF has lower glucomannan content than CH-UP and CH-CP of konjac flour, its particle size is finer and uniform. Those help in the mixing process. Moreover, it can be a result of the high content of oxalate. A higher oxalate content may cause inflammation and irritation, giving an unpleasant taste in the mouth and throat, such as itchy and burnt. Hence, Porang flour with a high oxalate content is inedible to consume.

Microscopic observations clearly show the presence of oxalate crystals in the konjac flour (Fig. 1). They were CH-UP-15-S, native chips Porang, and CKF, respectively, for Fig. 1a, 1b, and 1c. Meanwhile, for CH-CP-15-M, the crystals are less observed.

Raphide crystals of oxalate are mainly observed (Fig.1a, 1b, and 1d). Raphide-shaped crystals look like a single needle as well as a bundle. Black long bundle straight edge raphide crystals are observed in Fig. 1a, fragile raphide crystals are observed in Fig. 1b, while in Fig. 1d, a long single raphide crystal is observed.

Fig. 1c shows that such a druse crystal is observed as a solid druse crystal. Accumulation of oxalate crystal at Porang (*Amorphophallus oncophyllus* sp.) is usually found as druse, raphide, prism, and styloid [12].



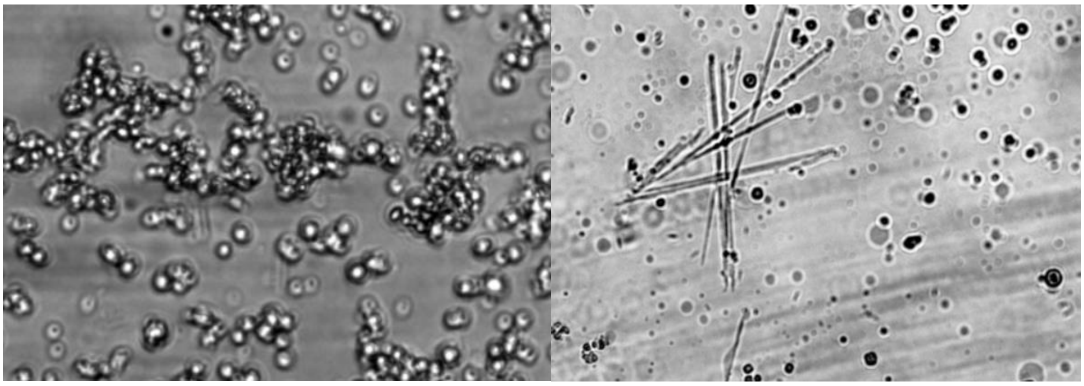


Fig. 1 Variations of observed oxalate crystals at (a) CKF from fresh tuber – soaking in NaCl 15% (single stage process extraction with EtOH) - CH-UP-15-S; (b) native chips Porang; (c) Crude Konjac Flour (CKF); and (d) CKF from fresh chips – soaking in NaCl 15% (multiple stage process extraction with EtOH) - CH-CP-15-M.

Furthermore, only three types of konjac flour (CKF, DM-CKF-M3X-50, and DM-CKF-M3X-96) are further applied to make panna cotta and pursue an organoleptic test. The ratio between the konjac flour and gelatin is observed; obtaining 1.5 g of gelatin and 3.0 g of konjac flour is the best ratio (Table 4), and the organoleptic test results are in Table 5.

Table 4. A combination of konjac flours and gelation is applied to make panna cotta.

Raw Material for panna cotta making	Ratio*	Sample code	Panna cotta making**
4.5 g gelatin (original recipe)	-	blank	√
1.5 g gelatin + 3.0 g CKF	0.5	A-1	√
3.0 g gelatin + 1.5 g DM-CKF-M3X-50	2	B-1	√
1.5 g gelatin + 3.0 g DM-CKF-M3X-50	0.5	A-2	√
1.5 g gelatin + 3.0 g DM-CKF-M3X-96	0.5	A-3	√

*Ratio between gelatin and konjac flour. **check sign (√) indicates that panna cotta was successfully made using the raw material as a substitution for gelatin. In contrast, the cross sign (X) indicates that panna cotta can not be made.

On average, a blank sample of panna cotta, i.e., used 4.5 g of gelatin without any substitution, gave the best organoleptic test. That was also observed in other parameters. Panna cotta with 3.0 g substitution of DM-CKF-M3X-50, sample A-2, has a similar visual appearance as the blank sample. Glucomannan can bind water and dissolve in water. Therefore, its resulting visual appearance looks like panna cotta. The gelling capacity of konjac flour is due to the hydrocolloid properties of glucomannan [1, 2].

Table 5. Organoleptic test result for produced panna cotta.

Sample code	Parameters observed						Average
	Appearance	Aroma	Taste	Melting	Texture	Expectation	
blank	3.70	3.72	4.39	4.37	4.05	4.08	4.05
A-1	3.55	2.74	3.45	3.23	3.00	3.00	3.16
B-1	3.69	3.11	3.61	3.53	3.56	3.55	3.51
A-2	3.78	3.44	3.53	3.56	3.78	3.55	3.61
A-3	3.68	3.00	3.48	3.81	3.61	3.35	3.49

Higher glucomannan content affects the texture. Hence, it is well related to the observed organoleptic test of panna cotta texture. A DM-CKF-M3X-50, sample A-2, with 99.42% glucomannan, gives the most challenging texture compared to CKF (sample A-1) and DM-CKF-M3X-96 (sample A-3). It contains 64.12 and 95.20% of glucomannan for CKF (sample A-1) and DM-CKF-M3X-96 (sample A-3), respectively. CKF (sample A-1) has the lowest glucomannan content. Therefore, the resulting mushy texture. Glucomannan and oxalate content in the konjac flour affects the success of the konjac flour used as a gelling agent and the particle size uniformity.

4 Conclusion

Not all variances of konjac flour, CKF, and KGM were successfully applied for substituting gelatin in making Panna Cotta (PC). Konjac Glucomannan (KGM) could not completely replace gelatin when making a PC. Glucomannan and oxalate content and the uniform particle size of konjac flour affect their application as a gelling agent. A ratio of 2:1 KGM: gelatin is the best. A KGM with 98.28%-w of GM, 3.58% of water content, 1.52% of ash, 12.42 mg of calcium oxalate/100 g, and 55.264 mPa.s of viscosity was applied for the PC. The produced PC has a score of 3.8 = visual appearance, 3,5 = customer expectation of the product visualization, 3.4 = scent, 3.5 = flavor, 3.6 = melting degree, and 3.8 = texture. The commercial PC has 3.7 = visual appearance, 4.2 = customer expectation of the product visualization, 3.5 = scent, 4.6 = flavor, 4.0 = melting degree, and 4.2 = texture. Improvement in KGM properties needs to be explored more for completely substituting gelatin in PC ingredients.

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