Production of Food Grade Thickener From *Benincasa hispida*

Noraziah A.Y. and Theng C.C.

***Abstract*—*Benincasa hispida* is a fruit which has huge potential as the source of polysaccharides, which lead to its capability to become a good main material of thickener. Commercial thickener nowadays will bring lots of side effect to our health. However, the properties of *B. hispida* thickener differ from other common thickeners; they will contribute benefits to men's health. The objectives of this study are to determine the polysaccharides content in skin, pulp and seeds of *B. hispida*, to study the effects of pH towards the viscosity of the thickener and also to compare the properties of *B. hispida* produced thickener with commercial thickeners. In this study, the composition of polysaccharides in skin, pulp and seeds of *B. hispida* will be analyzed. The properties of serum separation will be tested using the comparative method of stability of holding water in the chilli sauce as a food model system versus the time between *B. hispida* with commercialized thickener. The one that able to homogenized the food system for a longer period will be have more excel properties in curbing serum separation from happening. From the result indicates that the *B. hispida*'s seeds consists highest content of polysaccharides and pH 7 have the highest viscosity with low serum separation. This will portrays the suitability of *B. hispida* to be used as source of thickener.**

***Index Terms*—*Benincasa hispida*, Starch, Thickener, Viscosity**

I. Introduction

Food thickeners or thickening agents are used in food to absorb liquid of the food excluding changing its physical or chemical properties. Food thickener is added by the food industries to provide high quality foods with consistent properties and shelf stability and appealing that would attract consumers' interest in the product. Food thickener plays the main role in controlling the shelf life of products, mouthfeel and texture such as coffee creamers, ice cream, mayonnaise and others [1], [2].

Hydrocolloids are extensively used in thicken food systems [3]. The primary reason behind the ample use of hydrocolloids in foods is their ability to modify the rheology of food systems. This includes two basic properties of food systems that is, flow behavior or viscosity and mechanical solid property or texture. The modification of texture and viscosity of food systems helps modify its sensory properties. For that reason, hydrocolloids are used as significant food additives to perform specific purposes. Hydrocolloids are diverse group of long chain polymers, which are polysaccharides and proteins, characterized by their property of forming viscous dispersions when dispersed in water [4].

Gums and stabilizers have non-Newtonian rheology and they impart non-Newtonian character to the emulsions even when the amount of the dispersed phase is low [5]. For consumers' acceptability, the long-term stability of sauces is also very important. Hydrocolloids is a substances that easily dissolve in cold and warm water as well as able apply in modeling structure in order for thickening the food [6]. The principles of hydrocolloids action is increase the solution's viscosity and enhance gel creation such as stabilizing, thickening, and emulsifying [6], [7]. Thickeners which posses below C^*, is Newtonian behavior, their viscosity is independent of the rate of shear and if it is above C^*, it will show non- Newtonian behavior where will create low shear Newtonian plateau, shear thinning region and high shear Newtonian plateau [8]. The thickening effects that produced from the hydrocolloids are depending on the types of hydrocolloid used and the pH of the food system. In dilute dispersion, individual molecules of hydrocolloid will at first able to move freely and do not shows thickening while in concentrated system. These molecules will later begin to come into contact with one another, thus the movement of molecules becomes restricted.

In nature, all natural gums have unique properties but also have inherent limitations and insufficiency causes the overall utilization in gums [4]. The combination of the thickeners will produce the improved characteristic of the products in term of viscosity, shear rate, flexibility, salvation and ease of deformation of particles. The nature of the biopolymer mixture is cause by two different hydrocolloid molecules which are associates with each other or to non association mixed.

The current food market demands functional food and healthy products, using natural additives that provide the final product with a healthy added value. In addition, the food thickener that used in the industry is being carefully regulated by the Food and Drug Administration (FDA) to ensure their proper usage and labeling [9].The dossiers of each food additive added must be showed so that will not mislead the consumer and bring harms to them [10]. Moreover, as the health awareness is increasing among the consumers, the usage of natural food thickener without limiting the dosage is favored. Food hydrocolloids do not have their own regulation category, food hydrocolloid are simply regulated as food additives. Food additive is any substances which is not normally consumed as a foodstuff but is added to a foodstuff

A.Y. Noraziah is with Faculty of Chemical and Natural Resources Engineering Universiti Malaysia Pahang, 26300, Kuantan, Pahang Darul Makmur, Malaysia (e-mail: noraziahay@gmail.com).
C.C. Theng is with Faculty of Chemical and Natural Resources Engineering Universiti Malaysia Pahang, 26300, Kuantan, Pahang Darul Makmur, Malaysia (e-mail: cheechengtheng@yahoo.com)

in term of colors, thickener, emulsifier, preservatives, antioxidants, acidity regulators and flavor enhancers.

Each type of foodstuff that have certain amount of permitted of thickener must follow the good manufacturing practice (GMP) to prevent the hazardous to the consumer when exceed the certain amount of thickener [11]. For example, carrageenan as a thickener in many products such as ice cream and yogurt. If the carrageenan is over amount of GMP, it will causes stomach upset, deterioration of mammary gland tissues and may cause carcinogenic [12]. Therefore, regulatory approval of food ingredient is critical in Food safety such as thickener in food should be approved by FDA before the products are on the market.

Currently, *B. hispida* have gained much popularity and interest among researchers due to their potential uses as functional food ingredients. *B.hispida* consists of valuable nutrients and health benefits over others thickeners. *B. hispida* is belongs to the family of Cucurbitacae and originally cultivated in South East Asia (south of China, east of India, west of New Guinea and north of Australia). The *B. hispida* fruits on ground and has hairy leaves when the fruits are young, the skin is hairy. The fruit loses its hair and get waxy coat when matures. Various health benefits have been found in *B.hispida* fruits especially it is rich in minerals and vitamins. Minerals and vitamins are very important nutrients that are essentials to our body for daily functions. Minerals and vitamins can be obtained via our daily intake food. *B. hispida* fruit in matured fruits of *B. hispida* contain minerals such as sodium (Na), calcium (Ca) and phosphorus (P) and other composition. Sodium (Na) is important to human body because it required in order maintaining the equilibrium of fluids [13], calcium (Ca) is a micronutrient which essential to human health [14]. It acts as a diverse biological function in the human body and serves as a second messenger for nearly every biological process such as stabilizes proteins. If our bodies have deficient amounts of calcium (Ca), it will trigger a large number of diseases and disorders. Phosphorus (P) is vital nutrient in energy metabolism as it get involves in production, storage and transfer of energy [15]. Thus, *B. hispida* is known as one of the most valuable plants in Cucurbit family. Each part of *B. hispida* has their uses. For example the young shoots, leaves and lowers can be eaten, seeds can act as snacks and the wax of the fruits is scraped off to make candles [16].

Polysaccharide is the major component in thickener that able to make the substances become colloidal form [17], [18], [19], [20]. Besides, parameter that need to consider is the viscosity of thickener, temperature, concentration of thickener that added, pH, ionic strength and also the type of agitation that consider speed and size of spindle [21]. This research will study the potential of *Benincasa hispida* to be a food grade thickener by measuring the polysaccharides content and effect of pH towards viscosity of thickener produced by *B. hispida*.

II. MATERIAL AND METHODS

A. Preparation of Benincasa hispida

Each part of *B. hispida* (seeds, skin and pulp) was washed and dried in oven at 50 °C for one day to remove moisture content to be less than 20% of moisture content.

B. Floor preparation for seeds, skin and pulp

Dried seeds were soaked in solution of sodium hydroxide (5g/100ml) and citric acid (5g/100ml) respectively, each for 2 minutes and washed with distilled water. Then the seeds were dried in oven at 50 °C overnight and until moisture content is less than 13g/100g. Seeds are blended. Next, the dry sample passed through a sieve with 0.16 mm mesh size. The flour packed in plastic bag and kept at room temperature about 25 °C until further use.

Pulp dried immersed in 1.2 L of 96% ethanol at 80°C about 20 minutes. Mixture was homogenized using a blender until it was homogenous with the solvent. The homogenous solution was filtered through the nylon mesh. The solid residue was suspended and stirred in 1.5 L of ethanol at 80°C. The solution was filtered again and the process of filtration was repeated until 4 times. Finally, the *B. hispida* alcohol insoluble residue (BAIR) was produced then dried the BAIR in oven at 60°C overnight. The BAIR was stored at -20°C for further analysis. The same steps were repeated with other part of *B.hispida*.

C. Starch Isolation

Flour immersed in 0.05M sodium hydroxide solution and constantly stirred for 6 hour for the first extraction. Slurries are centrifuge at 3000xg for 20 minutes at 4°C. The supernatant was drained and the upper browned sediment was scrapped. Second extraction was carried out by the upper browned sediment with a 0.05M sodium hydroxide solution. After that extracted samples were mixed with distilled water and filtered by a sieve with 0.071 mm mesh size to eliminate the fibers. The filtrate was neutralized with 0.1 mol/L hydrochloric acid to pH 7 and centrifuge at 3000xg for 20 minutes at 4°C. The supernatant was drained and scraped the upper brown sediment was scrapped. The upper brown sediment was washed with distilled water 3 times and centrifuged at 3000xg for 20 minutes at 4°C. The starch cake collected after centrifuge was dried at 50°C for 12 hour in oven. Then, the starch was grounded with a mortar and pestle. The grounded starch had to pass through a sieve with 0.16 mm mesh size. The starches were packed in a plastic bag and kept at room temperature for further analysis.

D. Chili sauce preparation

Wash chilies with tap water. The chilies and pickling garlics were steamed for 30 minutes. This step was to remove the seeds from the chilies. Then, blend the seedless chilies and onion with vinegar (acetic acid) until fine. Sugar, salt were added with 1g/100ml of starch at 50°C. Then, pasteurized at 100°C for 10 minutes and immediately filled the hot filling into bottles with screw caps and cooled at room temperature. This procedure was to prepare chili alone without starch to act as a controller. The procedure was repeated again to mix with chili combine with corn starch, chili with potato starch or chili with *B. hispida* starch at 37°C. Observe the food model system for 4 weeks.

E. Screening of polysaccharides content

The polysaccharides content was analyzed using ultraviolet viscometer spectrometer (UV-Vis, U-1800 Spectrophotometer). All measurement was done in duplicate.

F. pH test

Arabic gum is categorized as low viscosity gum and the most favorable pH is 4.5 before lose its viscosity [22]. Tragacanth gum is classified as medium viscosity gum, it is most stable at pH 4 up to pH 8 [23]. Guar Gum is classified as high viscosity gum, the pH 7 to pH 9 is its best pH to produce viscosity stability [24]. The sample of *B. hispida* was analyzed within the range of pH 4 to pH 10. Added acidic and alkaline solution in *B. hispida* starch sampling to determine whether the thickener would be malfunction at certain range of viscosity.

G. Viscosity test

Powder that produced from *B. hispida* was added with distilled water. Add 10g of *B. hispida* with 100ml of distilled water. Viscosity of powder determined by using Brookfield DV-III Ultra Programmable Rheometer. In order to observe the duration of time taken affects the powder loose viscous by a graph plotted using variances between viscosities versus time. Operating condition of rotational viscometer would be set at the speed of 180 rpm at 25°C.

III. RESULT AND DISCUSSION

A. Raw material of thickener B. hispida: skin, seed, and pulp

Seed and pulp have low alkaline while the skin has low acidic pH. Previously, there is no concrete record frame work done for analysis of each parts of *B. hispida* in term of pH value. The only reported pH value is from pulp that is 5.5 [24], which is very similar with research's result which are 5.53 (1% w/v) and 5.88 (10% w/v). The samples prepared from seed, pulp and skin were kept in powder form with moisture content less than 20 % in order to preserve them from any microorganism growth on its surface.

Starch content for *B. hispida* is quite low. From these 3 samples, seed shows the highest content of starch follow by skin and lastly is pulp as shown in Fig. 1. High content of starch in *B. hispida* seed can be supported by other vegetable or fruit such as its properties is similar to its family such as pumpkin seed, rice bean seed (56.58%), pea (40%), black gram (45%), red bean (46%), araucaria (64%) [25], [26], [27]. Besides, Fig. 2 proved the seeds also show the highest viscosity compare to skin and pulp samples. Viscosity of the *B. hispida* seeds proved that the seed is potential source of thickener as it is proven contain highest polysaccharide compare with others. This experiment carry out on *B. hispida* with starch at 1% (w/v %) concentration and 10% (w/v %) concentration.

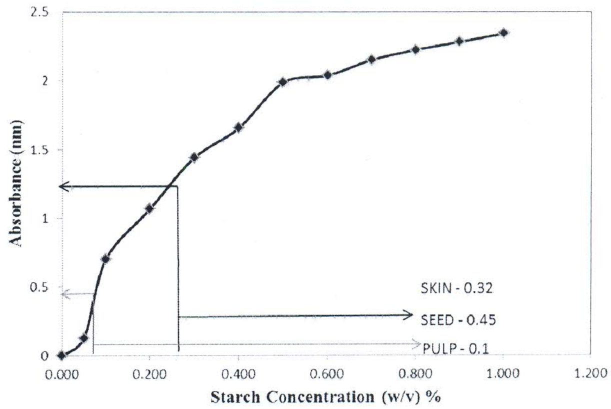


Fig. 1. Absorbance (nm) versus pure starch concentration (%) at 1.0 w/v % where skin (at 25°C, pH 6.7), seeds (at 25°C, pH 8.67) and pulp (at 25°C, pH 5.53).

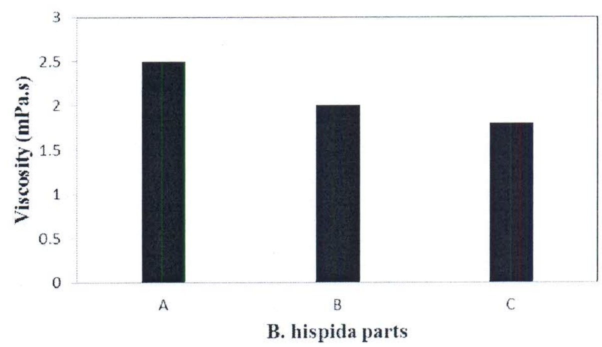


Fig. 2. Viscosity (mPa.s) of each part *B.hispida* fruits a) A-Seeds (pH 8.67) b) B-Skin (pH 6.7) c) C- Pulp (pH 5.53) at 25°C, 1.0 w/v % at 180 rpm stirring speed

C. Effect of pH towards viscosity

Most thickener functions within an optimal pH range. The *B.hispida* seed starch viscosity is affected when the variation pH occur. Fig. 3 illustrates that at neutral pH 7 gives highest viscosity at 180 rpm stirring speed at 100°C. Based on the result obtained, it indicates that the starch viscosity of *B.hispida* seed is not much affected when varied in pH. In view of that pH variation in lower concentration, the seed's starch viscosity will not give much different compare to 10 times higher of the concentration. pH is more significant towards the viscosity of the solution which jotted down 2 mPa.s at 1.0 w/v % , as shown on Fig. 3 and 141.467 mPa.s 1.0 w/v % for Fig. 4. Fig. 4 proved that at neutral pH 7 gives highest starch viscosity which also be proven from the result in Fig. 3. By increasing the concentration of *B. hispida* starch it affected the significant pH values. Different thickeners have different application, due to differences in taste, clarity, and their responses to chemical and physical conditions. The thickeners vary widely in the pH requirements such as methylcellulose and hydroxypropyl methylcellulose are stable over a pH range of 3.0 to 11.0 [28]. The high methoxyl pectins only gel within narrow range of 2.5 to 3.8 pH [28]. Acid conditions may cause some hydrocolloids to form a precipitate.

Thickener such as carrageenan solutions become unstable and loss of viscosity due to polymer chain cleavage at pH below 6. Besides that, gelatin loss its viscosity when the pH condition is greater than 7.5 [18]. For acidic foods, arrowroot is a better choice compare to the cornstarch, which cornstarch loses thickening potency in acidic mixtures [28]. At acidic pH levels below 4.5, guar gum has sharply reduced its aqueous solubility, thus also reducing its thickening capability [18]. On the other hand, when in frozen condition (<0°C), tapioca or arrowroot are preferable over cornstarch, which they will not turns spongy when frozen like cornstarch [14]. This proves that the pH value is affected according to the sample of starch. By using the cross linking of starch, we can produce thickener that able to provide the better tolerance in acidic condition.

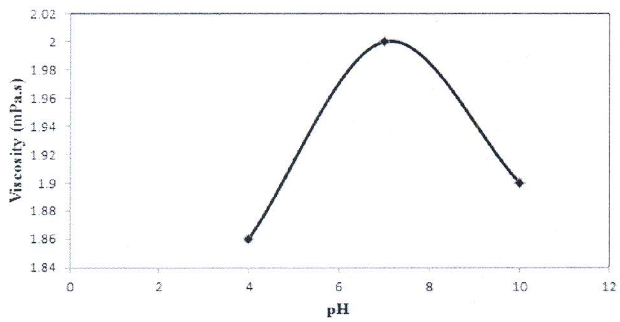


Fig. 3. Viscosity versus pH at 1.0 w/v % seeds, 180rpm stirring speed and 100°C

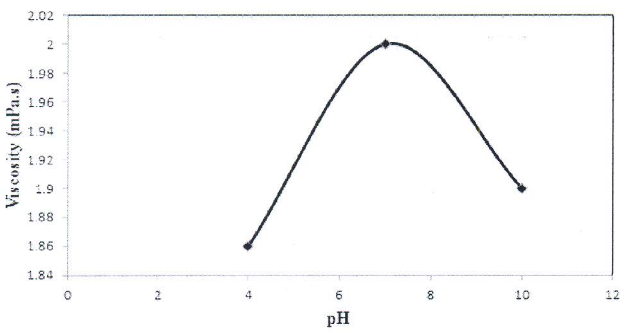


Fig. 4. Viscosity versus pH at 10 w/v % seeds, 180rpm stirring speed and 100°C

D. Serum separation

Serum separation occurs when the solids begin to settle out of solution, leaving the clear, straw-colored serum as layer on top of the product. The water separation acts as important sensory attribute. The water separation decreased with the addition level of hydrocolloid and will increase with increasing storage duration [29]. A more homogenized and stable solution reduces serum separation. In this study, the speed of the chilli sauce mixing acts as a parallel parameter.

Serum separation in chili sauce increased with the extension of storage duration of up to 4 weeks at 37°C is shown in Fig. 5. At the first week, chili sauce with potato starch and *B.hispida's* starch exhibited no serum separation while control and chili sauce with corn starch portrayed the evidence of serum separation.

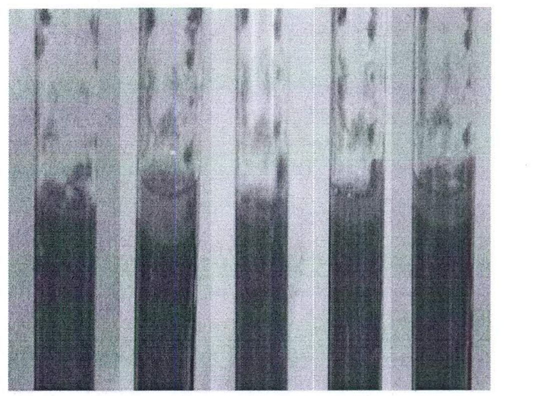
Serum separation can be a significant problem in liquid products. Preventing serum separation requires that the insoluble particles remain in a stable suspension throughout the serum. Generally at higher viscosity, less serum separation occurs. The potato starch which consist the highest viscosity at about 3000 mPa.s in 5% starch concentration which is categorized in high viscosity thickener while compare to corn starch which only exhibit 100 mPa.s [27]. From this statement proved that the consistency of potato starch is better compare with corn starch.

The results obtained indicated that chili sauce with potato starch had lower levels of serum separation than others starch. The stability sequence of sample is decreasing from potato starch follow by *B.hispida* seed starch, corn starch and lastly is control. The stability of the starch sample contributes to the uniformity or consistency of a product under a variety of conditions encountered during processing, storage or use. From the analysis proved that each sample are able to prevent separation by slowing or preventing the movement of particles either droplets of immiscible liquids, air or insoluble solids. The starch poses as a thickener when causing increase in viscosity, which leads to slow down of the separation process. The control in this research shows the serum content is no longer held in place, the control exhibit syneresis. The reaction time in 4 weeks can be summarized as follows:

Decreasing stability →

Potato starch>*B. hipida* seed starch> Corn starch> Control

As matter of fact that *B.hispida* seed display as a potential thickener which fall in medium viscosity gum.



Initial a) b) c) d)

Fig. 5. Serum separation of chili sauce in test tube after storage at 37°C for 4 weeks. a) Control was chili sauce without starch, b) Potato starch chili sauce, c) *B.hispida's* seed starch chili sauce, d) Corn starch chili sauce

IV. Conclusion

As a conclusion *B.hispida* seed starch is classified as medium viscosity gums. The level of serum separation during storage for 4 weeks was decreasing stability as follow Potato Starch>*B. hipida's* seed Starch> Corn starch> Control. *B. hispida* as a potential source of high value components for thickener food industry.

For this research, the sample must be well stirred because it is significant to the viscosity measurement. This is because the unhomogenized sample will affect the measurement. For future study it is recommended treating with different volume fractions (e.g., 30-70%, 40-60% and etc), study the effect of size distribution of dispersed phase on thickener stability, and optimize the condition in large sample quantity to obtained higher viscosity of the thickener and study the class of *B. hispida* seed starch.

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A.Y. Noraziah. This author became a Member (M) of BEM, IEM, ICheme. Borned in Malacca, Malaysia on 18 August 1983. Next, the author's educational background is listed. Acquired the first degree at International Islamic University of Malaysia (B.Eng Biochemical and Biotechnology, Hons.) and pursued master level at Universiti sains Malaysia (MSc. Chemical Engineering, Hons.). The specialization and research interest in biochemical processing, fermentation and bioprocess, food technology, enzyme, and tissue culture.