CHAPTER VI
DISCUSSION

6.1 Preliminary extraction condition

The percentage yield of okra gum powder tended to increase with an increase in temperature. Similar results were reported on extraction of mucilage from young fronds of *Asplenium australasicum* (J. Sm.) Hook showing its yield increased with increasing temperature of extraction (92). Moreover, not only high temperature but also long extraction time led to the higher percentage of yield due to the softening effects on tissues of okra which may help releasing gum from their tissues. Yujaroen P. *et al*. (93) showed that increasing time and temperature during extraction of sugar palm meat pectin gave a better yield. However, too long extraction time could make pectin decompose. Water activity of all powder samples was low and was well lower than the range required for microbiological activity. Nevertheless, one specific property of okra gum is that it is hygroscopic, so the powder tended to become moist easily. Suitable packaging and storage condition would help to extend the shelf life of okra gum. Chatchawal *et al*. (94) also observed that mucilage extracted from *Basella alba* Linn had a hygroscopic property and mucilage powder from hairy basil seeds was shown to be highly hygroscopic (95). Deionized water was used as a solvent in extraction, so the pH of okra gum solution was similar to the pH of deionized water. The results agreed well with a previous study that at 1% concentration hibiscus and okra mucilage solution obtained using water extraction gave a pH value of 6.5 and 6.1, respectively (96). Viscosity of okra gum increased when temperature and time of extraction was increased. On the other hand, Thanatcha *et al*. (97) showed that the viscosity of jujube cultivar Samros mucilage was lowered when a higher extraction temperature was applied. Moreover, a study on the viscosity of *Opuntia ficus* indica mucilage dispersions showed that their viscosity decreased with increasing temperature of extraction (98). Chen and Chen (99) also revealed that green laver mucilage had lower viscosity at higher temperature of extraction. Therefore, both period of extraction and temperature are important factors that affect pectin extraction

6.2 Physical properties of okra gum and pectin

Water extraction of okra gum gave a yield of 45.6±5.4% on a dry basis. The result of water extraction of ripe jujube mucilage at 60 °C gave the yield about 15.40% (% dry weight) (97). Therefore, okra appeared to be a good source of gum with a high extraction yield. Okra pectin was prepared by precipitation of okra gum with ethanol. After ethanol precipitation the yield of okra pectin was 12.7±2.2%. According to the result of Sengkhamparn *et al*. (10), okra polysaccharide obtained by hot buffer and dilute alkaline extraction gave a yield of 11.2% and 13.2%, respectively. Water extraction in this study seemed to give a similar yield to chemical extraction applied by others. This could then be a good alternative for the preparation of a food ingredient by using less chemical. Compared to the percentage yield of okra gum powder, the percentage yield of okra pectin powder was about 3.5 times lower than that of okra gum (Table 5.2). The result was in line with a subsequent experiment which showed that the pectin content of okra gum was about 30% (Table 5.3). Okra pectin was precipitated from gum solution with ethanol to remove other matters. Pigments, ash, mineral acid (100), monosaccharides and disaccharides (101) were eliminated by ethanol precipitation. Therefore, the yield of okra pectin was lower than that of okra gum.

The viscosity of okra gum solution was 43.1±4.7 cPs at 1% (W/V) concentration while the value reported for *Basella* mucilage was 132.33 cPs (94). In addition, the viscosity of 1% okra pectin solution was 715±49 cPs while *Abelmoschus esculentus* extracted with cold water containing 1% (w/v) sodium metabisulphate gave a viscosity of 225 cPs (102). The reason for the difference in viscosity could be from the difference in plant variety used as a starting material, season and location for cultivation as well as extraction process and condition. Compared to okra gum solution, okra pectin solution is more viscous (Table 5.4). The factors influencing the viscosity include concentration of solutes, molecular weight, intermolecular interactions, temperature, pH and the concentration of electrolytes. In this case, average molecular weight of okra pectin was 894.92 kDa, approximately 4 times greater than that of okra gum and the intrinsic viscosity of okra pectin was about 3 times higher than okra gum (Table 5.5). In general, a compound with high molecular weight is related to larger polymer molecules which make a solution viscous. As a result, okra pectin solution was more viscous than okra gum solution at the same condition. The higher concentration of okra gum and pectin solution also gave rise to the consistency index. Consistency index of okra gum solution was lower than okra pectin solution in relation to their respective viscosity. In the study of jujube mucilage extraction, the consistency index of jujube mucilage solution at concentrations 5% - 9% was about 783 to 6873 ($Pa.s^n$) which was in the same range of okra gum (Table 5.4) (96). Flow behavior index of okra pectin at concentration 0.25% and 0.5% was 0.509 and 0.381, respectively. This result was compatible with okra pectin extracted with hot buffer in a previous study with the flow behavior index at concentration 0.2% and 0.5% being 0.40 and 0.30, respectively (10). Moreover, when shear force increased, the viscosity and the flow behavior index of okra gum and pectin decreased. Hence, okra gum and pectin solution exhibited a non-Newtonian fluid property which is pseudoplastic type. Lengsfeld *et al*. (8) showed that okra mucilage obtained by water extraction displayed a pseudoplastic rheology property.

6.3 Chemical properties of okra gum and pectin

The GalA content of okra gum was 13.39±1.40% compare to the result of Wu *et al*. (103) which showed that okra mucilage contained about 24% GalA. In another study okra mucilage extracted with water contained 28.5% total uronic acid (8). Okra gum from the current study contained less than 50% GalA. Therefore, it could be a non-pectin polysaccharide with main components being other neutral sugars. Sugar beet pulp polysaccharides extracted with water at 40 °C for 30 min presented about 16.6% GalA (102). The GalA in okra pectin was observed about 53.11±4.06 % in accordance with okra pectin extracted using chelating agent which had approximately 63% GalA (10). Okra pectin contained more than 50% GalA which could be considered as the main component. Thus, okra pectin is possibly a pectin-like polysaccharide. Comparing between okra gum and pectin, okra pectin was precipitated with ethanol therefore its purity should be greater than okra gum, which reflected in the higher sugar content obtained from phenol-sulfuric test, as shown in section 5.3.6. Furthermore, the main component of okra mucilage could also include neutral sugars such as 27% rhamnose, 40 % galactose (103).

DM determined by NMR was 45.47% for okra gum and 32.11% for okra pectin. Such value is higher than that reported by Sengkhamparn *et al*. (10) whose okra pectin extracted with hot buffer and chelating agent contained 24% and 48% DM, respectively. It has been reported that water extraction gave higher esterified pectin with higher neutral sugar content than chelating agent extraction (28), which could be the reason for the greater DM of okra pectin in this study. Determination of DM by FTIR revealed %DM of okra gum and pectin at 32.35±0.78 and 41.51±1.15, respectively (Table 5.5). The % DM of okra gum by FTIR was lower than the %DM by NMR analysis because okra gum was not purified and its main composition was neutral sugars. The area of carboxylic group by FTIR increased, so comparing in the ratio of esterified group and carboxylic group gave a lower % DM than the value obtained by NMR analysis. In addition, the %DM of okra pectin by FTIR was higher than NMR determination due to a relatively high value of DAc (12.33±0.77%) (Table5.5). FTIR analysis of acetylated sample assumes the overlapping peak of acetyl C=O stretching of ester at 1740–1745 cm^{-1} in the same peak of ester carbonyl groups at 1730 cm^{-1}, so the peak area of ester carbonyl groups of okra pectin could be higher than the actual value. Duarte *et al*. (104) studied the DAc of chitin and chitosan by FTIR spectroscopy and found that highly acetylated samples might result in double peak in the NH bending region at 1663 and1626 cm^{-1} assigned to C=O (amide I) and NH bending (amide II). The DAc of okra gum and pectin by NMR was 5.69±3.13 and 12.33±0.77, respectively while %DAc of okra gum and pectin by colorimetric method was 6.05±0.08 and 17.60±0.44, respectively. A previous study reported that okra pectin extracted with chelating agent contained about 18 DAc by mole (10). Besides, okra polysaccharides contained acetyl group about 5.5% w/w but no evidence was mentioned about the precise position of acetyl group in polysaccharide (72).

Intrinsic viscosity of okra gum and pectin was found at 586.57±95.44 and 1,852.90±86.59 cm^3/g, respectively. Lih-Shiuh *et al*. (106) determined intrinsic viscosity of mucilage extracted from the young fronds of *Asplenium australasicum* using different extraction conditions. Water extraction gave the highest intrinsic viscosity at 290.7±28.3 cm^3/g and malva nut gum extracted by HCL showed the intrinsic viscosity at 1000 cm^3/g (107). Compared to okra pectin, intrinsic viscosity of okra gum was about 3 times lower than okra pectin (Table 5.6) because okra gum composed of neutral sugars and one third of it was pectin. Urias-Orona *et al*. (108) found that chickpea husk pectin extracted using acid had intrinsic viscosity about 374 cm^3/g. Due to size, macromolecules have a very considerable impact on the viscosity of solution, so average molecular weight is related to intrinsic viscosity. In this study, an average molecule weight of okra pectin was 848.85±35.01 kDa, about 1.5 times greater than okra gum (Table 5.5). According to other studied, fresh okra pods extracted with water had an average molecular weight of 1380 kDa (8) and pectin extracted from citrus peel had an average molecular weight in the range of 50 to 2000 kDa (109). From the results of average molecular weight, the structure of okra pectin appeared to be larger than okra gum. Similar findings were suggested in okra extracted with hot buffer. It composed of highly branched rhamnogalacturonan (RG I) containing high levels of acetyl groups and short galactose side chains (10). An average molecular weight was obtained from Mark-Houwink-Sakurada equation using K and α of pectin. Since okra gum contained about one third of its weight as pectin, the average molecular weight of okra gum was estimated from this equation. Generally, soluble dietary fiber composes of mucilage, gums and pectin. After extraction both the amount of soluble and insoluble dietary fiber increased from the fresh fruits due to a concentration effect.

Amount of soluble fiber increased about 14 – 17 times of fresh fruit, while insoluble fiber became 3-7 times of fresh fruit. Okra pectin contained more soluble dietary fiber than okra gum (Table 5.6) due to the ethanol precipitation process. The results agreed with a study which reported that soluble fiber content of ripe jujube was 2.85±0.05% and after preparation into mucilage powder form, soluble fiber increased to 89.76 ±0.03% (96).

6.4 Application of okra gum and pectin

Since okra gum and pectin contained many acetyl groups and branched side chains, they are unable to form gel. Nevertheless, they can provide viscosity to the solution. In this study, okra gum and pectin were added in food products as thickening agent and stabilizer. In non fat pasteurized chocolate milk, okra gum and pectin were used as to provide desired texture and to prevent precipitation of chocolate powder. The amount of okra gum added into chocolate milk was higher than okra pectin because okra gum provided lower viscosity than okra pectin. Sensory acceptability scores of non fat pasteurized chocolate milk with okra gum and pectin ranged from like slightly to like moderately. The color, odor and overall acceptability scores of chocolate milk with okra gum and pectin did not differ significantly from the control product. However, mouth feel score of chocolate milk with okra pectin was significantly lower than the control ($p \leq 0.05$). In just-about-right test, chocolate milk with okra gum provided a suitable body or consistency while the control product was too thin (low consistency). The chocolate milk with okra pectin gave a slightly higher consistency but did not differ significantly from the chocolate milk with okra gum. Moreover, okra pectin was not only efficient as a thickening agent but also delayed precipitation of chocolate powder after storage in a refrigerator for 3 days. This property, however, was not observed in chocolate milk added with okra gum. Therefore, only okra pectin exhibited a potential for application in this type of food product.

In orange-flavored beverage with okra gum and pectin, the sensory acceptability ranged from dislike slightly to like slightly. The color, odor and overall acceptability scores of orange-flavored beverage with okra gum and pectin was not significantly different from the control product. On the other hand, mouth feel score of orange-flavored beverage with okra gum and pectin was significantly lower than the control ($p \leq 0.05$) due to the system of orange-flavored beverage composed of sugar, water and citric acid causing a gel-like or slimy body which was not desirable. Nevertheless, in just-about-right test, orange-flavored beverage with okra gum and pectin provided better body or consistency than the control although the panelist judged the products as slightly too thick. Therefore, it could be possible to use okra gum and pectin to add body to this type of beverage, particularly at a lower concentration which may be better in terms of mouth feel.