CHAPTER V
RESULTS

5.1 Preliminary trial for extraction condition

As shown in Table 5.1, percentage yield of okra gum powder significantly increased from 16.40±4.35 to 38.98±0.57 with higher temperature and longer period of extraction ($p \leq 0.05$). The extraction condition at 90 ºC for 60 min particularly gave the highest yield (38.98±0.57%). On the other hand, the poorest condition was 70 ºC for 10 min. In addition, the longer period of extraction gave the significantly higher %yield of okra gum powder. Moreover, there is also the interaction between temperature and period of extraction that affected the obtained %yield, as show in Table 5.2.

After drying at 60 ºC for 18 h, the water activity value of okra gum powder samples ranged from 0.28±0.03 to 0.39±0.07. The longer extraction time as well as higher extracting temperature significantly decreased the A_w of obtained okra gum powder. Besides, the water activity value of okra gum powder from 90 ºC extraction was the lowest whereas other temperature extractions were slightly higher than that from extraction at 90 ºC relating to significant difference of %yield of okra gum powder ($p \leq 0.05$) was found in different levels of the temperature. Interaction between time and temperature was found not affecting the A_w of okra pectin in this study (Table 5.2).

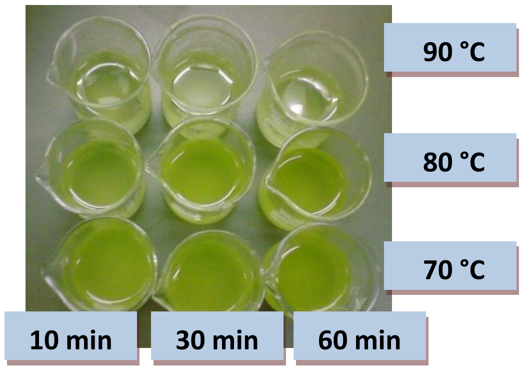

Figure 5.1 Okra gum solutions from different extraction conditions

The samples showed a pH value in the range of 6.08±0.20-6.33±0.16. No significant differences of okra gum solution pH ($p>0.05$) were found in temperature and period of extraction. Regarding the color of okra gum, the L* value of okra gum powder sample extracted at higher temperature was higher when compared with other conditions. L* value of okra gum solution was significantly different in temperature and extraction time ($p<0.05$). While the a* values were in the range of 2.63±0.21 to 4.07±0.82 and the b* values were in the range of 22.79±0.25 to 30.29±1.17. No significant differences of a* and b* were found in the temperature and period of extraction ($p>0.05$). Conversely, the color of sample tended to be yellow-green brown as shown in Figure 5.1.

The viscosity of solution of the okra gum extracted at 90 °C was higher than that from extraction condition at 80 °C. The lowest of viscosity value was obtained from the gum extracted at 70 °C. As compared to the 10-min extracted gum, 60-min extracted gum gave a higher viscosity while 30-min extracted gum gave a lower viscosity. The higher temperature and longer extraction time significantly increased the viscosity (Table 5.2). However, there was no interaction between those 2 extraction factors (Table 5.2).

Table 5.1 %yield[1], A_w[1], pH[1], color value[1] and viscosity[1] of okra gum at different conditions by variable of temperature and time of extraction

Characteristics		Extraction Condition								
		70 °C			80 °C			90 °C		
		10 min	30 min	60 min	10 min	30 min	60 min	10 min	30 min	60 min
%Yield		16.40 ±4.35	20.52 ±0.49	26.46 ±5.14	24.46±0.12	25.84 ±0.41	29.20±0.85	20.54±4.08	27.91±1.76	38.98±0.57
A_w		0.388 ±.066	0.345 ±.006	0.349 ±.092	0.377±.021	0.298 ±.019	0.298±.050	0.342±.039	0.280±.020	0.279±.033
pH		6.26±.29	6.28±.41	6.56±.07	6.26±.53	6.32±.31	6.32±.28	6.33±.16	6.28±.23	6.08±.20
Color value	L*	12.80 ±.78	13.32 ±1.67	14.70 ±0.61	25.09±1.28	27.52 ±7.45	23.51±4.90	30.24±2.02	26.90±1.33	30.80±4.48
	a*	3.48±.26	2.76±.76	3.46±1.20	4.07±.82	3.25±1.22	2.86±.76	2.63±.21	3.59±.31	2.65±.16
	b*	25.83 ±1.07	22.79±.25	27.71 ±1.37	30.29±1.17	27.85 ±1.05	26.12±.29	27.18±.10	28.60±2.61	23.88±.16
Viscosity (cPs)		100.767 ±35.90	177.50 ±12.38	157.167 ±7.64	103.017 ±18.95	164.833 ±29.37	209.833 ±18.74	210.167 ±52.68	259.83 ±49.50	300.833 ±14.85

[1]Mean±SD of triplicate analyses

Overall, extraction condition at 90 °C for 60 min provided the highest yield and highest viscosity in solution therefore this condition was selected for preparing okra gum. In a subsequent experiment, the resulting gum was precipitated with ethanol to obtain okra pectin.

Table 5.2 Analysis of Variance for the effects of extraction temperature and time on the yield, Aw, pH, color values and viscosity of okra gum

Factors		Temperature		Time		Temperature*Time	
		F value	Sig.	F value	Sig.	F value	Sig.
%Yield		20.164	0.000	37.262	0.000	4.932	0.007
Aw		3.870	0.040	5.232	0.016	0.165	0.953
pH		.429	0.657	0.037	0.964	0.759	0.565
Color value	L*	7.395	0.005	0.005	0.995	0.173	0.949
	a*	0.180	0.837	0.152	0.860	0.448	0.772
	b*	0.849	0.444	0.448	0.646	1.097	0.388
Viscosity (cPs)		7.913	0.003	4.076	0.035	0.247	0.908

5.2 Physical properties of okra gum and pectin

5.2.1 % yield of okra gum and pectin

The percentage yield of the okra gum extracted at 90 °C for 60 min and the precipitated pectin powder varied somewhat among the three batches (Table 5.3). The percentage yield of okra gum powder was between 39.4 and 49.5% while that of okra pectin powder was 10.5 to 14.9 %. The percentage yield of okra gum powder was 3.5 times of okra pectin powder.

Table 5.3 The percentage yield of okra gum and pectin powder (dry weight basis)

Replication	%Yield	
	Okra gum powder	Okra pectin powder
1	47.7	12.2
2	49.5	14.9
3	39.4	10.5
Mean (SD)	45.6±5.4	12.7±2.2

5.2.2 Pectin content of okra gum (dry weight basis)

From Table 5.4 it could be calculated that 100 g of okra gum powder contained 29.7 g okra pectin. In other words, about one third of okra gum existed as pectin.

Table 5.4 The percentage of okra pectin in okra gum powder

Okra gum solution[1] (g)	Okra pectin powder[1] (g)	Okra gum powder [1] (g)	% pectin in gum[1]
440.0	4.0	13.5	29.7

[1]Mean of duplicate analyses

5.2.3 Viscosity of okra gum and pectin

From Table 5.5 the viscosity of okra gum and pectin solutions at 1% concentration was 43.1±4.7 cPs and 715±49 cPs, respectively at shear rate 12-14 s^{-1}. Compared to okra gum solution, the viscosity of okra pectin solution was about sixteen times higher. Okra gum solution at a concentration of 1%, 2% and 3% (w/v) and okra pectin solution at a concentration of 0.25%, 0.50%, 1% and 1.5% (w/v) exhibited a steady increase in the consistency index with increasing concentration. Okra gum and pectin solutions showed the highest consistency index at 3% and 1.5% (w/v) concentration, respectively. At 1% concentration, the consistency index of okra pectin solution was about thirty seven times higher than that of okra gum solution. Flow behavior index of okra gum and pectin solutions decreased with increasing concentration with the flow behavior index of okra pectin solution being lower than that of okra gum solution.

Table 5.5 Viscosity[1], consistency index[2] (k) and flow behavior index[2] (n) of okra gum and pectin solution

	Concentration (%w/v)	Viscosity (cPs) at shear rate12-14 s^{-1}	Consistency index (Pa.s^n)	Flow behavior index
Okra gum	1.00	43.1±4.7	126.21	0.570
	2.00	N/A	595.66	0.440
	3.00	N/A	1,524.76	0.366
Okra pectin	0.25	N/A	231.85	0.509
	0.50	N/A	977.24	0.381
	1.00	715.0±49.0	4,688.13	0.296
	1.50	N/A	11,089.19	0.235

[1]Mean±SD of triplicate analyses
[2]Mean of duplicate analyses
N/A = Not analyzed

5.3 Chemical properties of okra gum and pectin

5.3.1 Galacturonic acid content

Table 5.6 shows that okra pectin contained more than 50% of GalA content which is the main component of okra pectin. On the other hand, okra gum contains a lower GalA than okra pectin at 13%.

5.3.2 Degree of methylation

DM of samples was determined according to the methods described in Section 4.5.2.

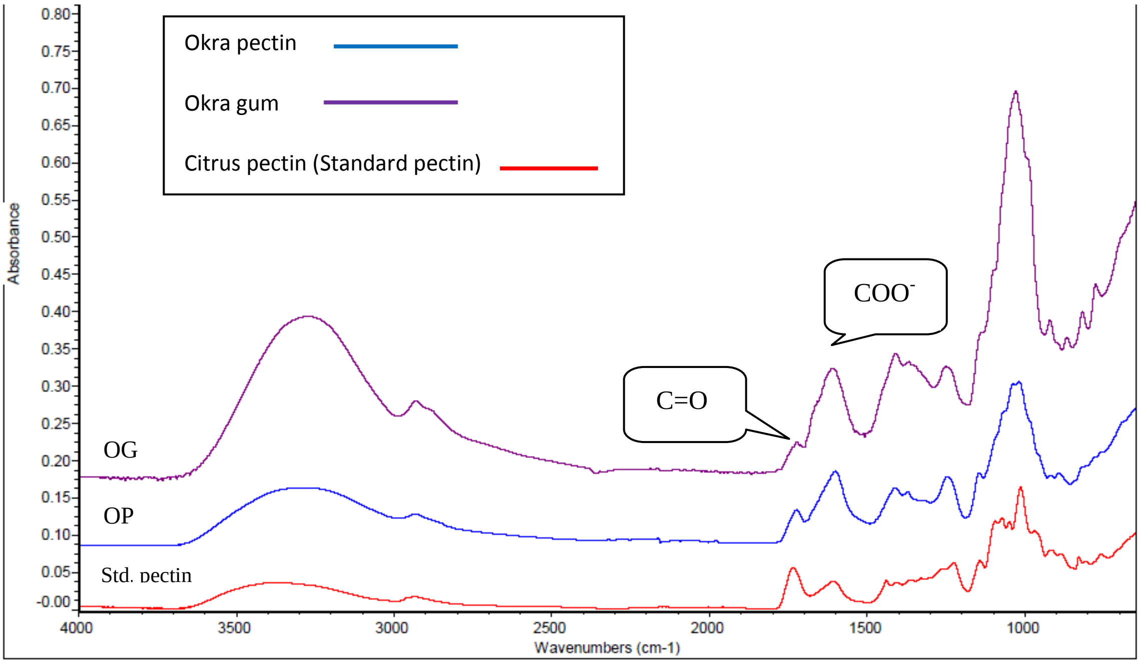

Figure5.2 The spectra of okra gum, pectin and citrus pectin obtain from FTIR

Fourier Transform Infrared spectroscopy (FTIR) was used to determine DM of okra gum and pectin. Figure5.2 shows the FTIR spectrum of okra gum and pectin, compared to that of standard citrus pectin with 62-66% DE. The absorption pattern of okra gum and pectin spectra was similar to standard citrus pectin spectra. The region which identified the major chemical groups of pectin polysaccharides was fingerprinted in the range of 950 and 1200 cm^{-1}.

The DM of okra gum and pectin was determined using a spectral region between 1500 and 1750 cm^{-1}. The range of this spectral area clearly presented two peaks. The first peak at 1730 cm^{-1} assigned to ester carbonyl groups and the second peak at 1600 cm^{-1} related to free carboxyl groups. Besides, Figure 5.2 shows that the intensity absorbance of okra gum and pectin at 1730 cm^{-1} was lower than intensity absorbance at 1600 cm^{-1}. Moreover, the okra gum absorbance at 1730 cm^{-1} was lower than that of okra pectin absorbance. Compared to standard citrus pectin, both okra gum and pectin had a lower intensity absorbance. DM of okra gum and pectin was calculated using peak area between 1600 and 1760 cm^{-1}. FTIR analysis showed the DM of okra gum and pectin at 32.35±0.78% and 41.51±1.15%, respectively. Okra pectin had a higher DM than okra gum in line with their intensity absorbance.

In addition, the measurement of ^1H NMR spectrometry, which shows five anomeric proton signals at δ_H 5, δ_H 4, δ_H 3, δ_H 2 and δ_H 1 was also performed. The $\delta_H 1+_H 5$ signal shows methyl ester group (-COOMe), δ_H 5 identifies carboxyl group (-COO) and $\delta_H 2$ presents methoxy group ($-OCH_3$). The DM is calculated by the ratio of area of esterified carboxylic groups and carboxylic groups. DM of okra gum and pectin by NMR was 45.47±4.87% and 32.11±2.52 %, respectively. The DM of okra gum was greater than okra pectin. However, comparison of FTIR analysis and NMR spectroscopy data for okra pectin sample demonstrated that FTIR analysis gave higher results than NMR spectroscopy.

5.3.3 Degree of acetylation

The DAc was determined by methods described in Section 4.5.2. The DAc by NMR was calculated by the ratio of area of signal at 1.5 – 2.0 ppm which is acetyl group and total signal area (δ_H 5, δ_H 4, δ_H 3, δ_H 2 and δ_H 1). %DAc of okra gum and pectin was 5.69±3.13% and 12.33±0.77%, respectively. Both okra gum and pectin had a high DAc with okra pectin showing a higher DAc than okra gum. Colorimetric determination revealed that the DAc of okra gum and pectin was 6.05±0.08% and 17.60±0.44%, respectively. The DAc of okra pectin by this method was greater than that of okra gum, similar to the NMR results. Nevertheless, the colorimetric method gave a higher value than NMR analysis.

5.3.4 Intrinsic viscosity

Intrinsic viscosity, a measure of the hydrodynamic volume occupied by a molecule, is a measure of the capacity of a polymer molecule to enhance the viscosity (91). Figures 5.3 and 5.4 show a plot of η_i (reduced viscosity) or ln (η_r)/C versus C for okra gum and pectin solution obtained after extrapolation to zero concentration with Huggins equation **(♦)** and Kraemer equation **(■)**. The intrinsic viscosity was determined by intersection point of the two equations.

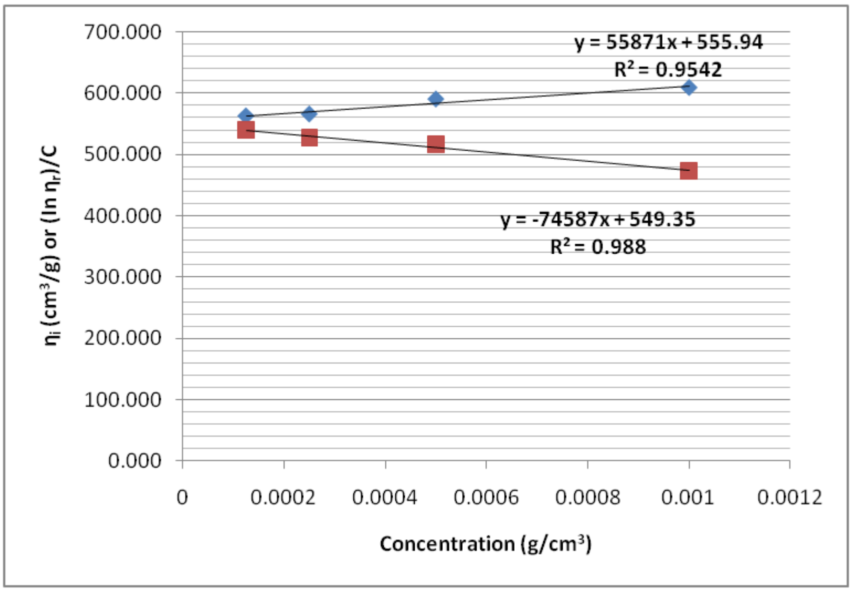

Figure 5.3 Relationship between η_i (cm^3/g) or (In η_r)/C and concentration (g/cm^3) of okra gum in 0.1 M NaCl solution. (♦) represents Huggins plot and (■) represents Kraemer plot.

The intrinsic viscosity of okra gum and pectin in 0.1 M NaCl solution was determined to be 586.57±95.44 and 1,852.90±86.59 cm^3/g, respectively. Okra pectin exhibited approximately three times higher intrinsic viscosity than okra gum.

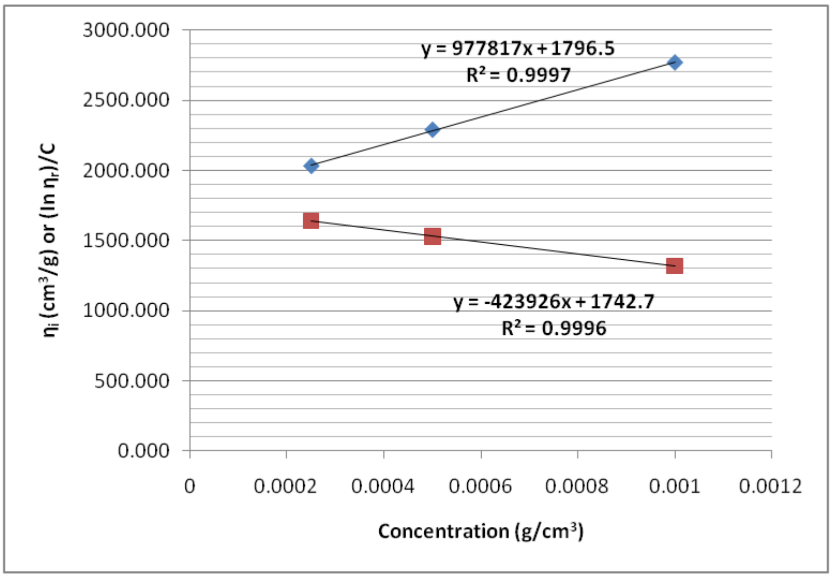

Figure 5.4 Relationship between η_i (cm^3/g) or (In η_r)/C and concentration (g/cm^3) of okra pectin in 0.1 M NaCl solution. (♦) represents Huggins plot and (■) represents Kraemer plot.

5.3.5 Average molecular weight

Molecular weight of the sample in 0.1 M NaCl solution was determined indirectly through the value of intrinsic viscosity using Mark-Houwink-Sakurada equation; $[\eta] = K(M_w)^{\alpha}$ where K and α are 0.0436 and 0.78, respectively (88). The average molecular weight of okra gum and pectin was 189.26±51.20and 848.85±35.01 kDa, respectively. The average molecular weight of okra pectin was greater than that of okra gum.

Table 5.6 Galacturonic content[1], % degree of methylation[1] (%DM), % degree of acetylation[1] (%DAc), intrinsic viscosity[1] and average molecular weight[1] of okra gum and pectin.

		Okra gum	Okra pectin
%Galacturonic acid content (g)		13.39±1.40	53.11±4.06
%DM	NMR	45.47±4.87	32.11±2.52
	FTIR	32.35±0.78	41.51±1.15
%DAc	NMR	5.69±3.13	12.33±0.77
	Colorimetric method	6.05±0.08	17.60±0.44
Intrinsic viscosity (cm^3/g)		586.57±95.44	1,852.90±86.59
Averages molecular weight (kDa)		189.26±51.20	848.85±35.01

[1]Mean (SD) of triplicate analyses

5.3.6 Relative free sugar content

From sugar content analysis using phenol-sulfuric test, okra gum and pectin contain sugar at the concentration of 82.16% and 69.84%, respectively, which implied the higher free sugar content in okra gum. It should be noted that the percentage of sugar presents in okra gum and pectin were higher than that of citrus pectin (57.00%).

5.3.7 Soluble and insoluble dietary fiber

Fresh okra contains both soluble and insoluble dietary fiber at 4.79% and 4.96%, respectively. After extraction soluble dietary fiber content of okra gum changed from 4.79 % in the fresh raw material to 64.67% in the gum powder and to 84.03% in the pectin. Insoluble dietary fiber content also changed accordingly. Okra gum powder contained smaller amount of soluble dietary fiber than okra pectin powder but its insoluble dietary fiber content was greater than that of okra pectin (Table 5.7).

Table 5.7 Soluble[1] and insoluble[1] dietary fiber content in fresh okra, okra pectin and gum powder

Content (per 100g)	Fresh okra	Okra gum powder	Okra pectin powder
Soluble dietary fiber	4.79±0.11	64.67±0.74	84.03±1.22
Insoluble dietary fiber	4.96±0.18	31.99±0.23	15.22±0.88

[1]Mean±SD of duplicate analyses

5.4 Application of okra gum and pectin

5.4.1 Non fat pasteurized chocolate milk

a. Sensory evaluation

All formulas of non-fat pasteurized chocolate milk with and without addition of okra gum and pectin were screened for sensory acceptability by 15 untrained panelists aged between 20 and 50 years old following the methods described in Section 4.6.3.

Table 5.8 shows the sensory acceptability results of non-fat pasteurized chocolate milk added with okra gum and pectin and the control formula in terms of color, odor, mouth feel and overall acceptability. There were no significant differences ($p \leq 0.05$) in color, odor and overall acceptability among the control formula and non-fat pasteurized chocolate milk containing okra gum and pectin. For mouth feel, there were significant differences ($p \leq 0.05$) between the control formula and non-fat pasteurized chocolate milk with okra pectin while there were no significant differences between non-fat pasteurized chocolate milk with okra pectin and okra gum as well as that with okra gum and control. Both okra gum and pectin increased the viscosity of product, while okra pectin seemed to provide greater viscosity to the milk than okra gum as expected from the higher viscosity of okra pectin solution reported in Table 5.5.

Table 5.8 Sensory scores of non fat pasteurized chocolate milk using okra gum and pectin as thickening agent at a concentration of 0.15% and 0.075%, respectively[1,2]

Formula	Sensory properties				
	Color[3]	Odor[3]	Mouth feel[3]	Overall acceptability[3]	Consistency[4]
Control	7.40±1.18[a]	7.07±1.22[a]	7.13±1.13[a]	7.33±0.98[a]	2.67±0.62[a]
Okra gum	7.40±1.12[a]	6.20±1.78[a]	6.20±1.42[ab]	6.47±1.41[a]	3.00±0.54[ab]
Okra pectin	7.60±1.18[a]	6.40±1.60[a]	6.07±1.34[b]	6.33±1.54[a]	3.47±0.74[b]

[1] Mean ± SD (n=15)

[2] Mean in the same column followed by a different letter (a-b) were significantly different (p<0.05)

[3] Nine point hedonic scale (9=like extremely, 5= neither like nor dislike, 1= dislike extremely)

[4] Five point just-about-right scale (5=much too thick, 3=just-about-right, 1=much too thin)

The just-about-right sensory results of non-fat pasteurized chocolate milk added with okra gum and the control formula as well as the formula with okra gum and with pectin were not significantly different ($p \leq 0.05$) in terms of consistency. Significant differences of the just-about-right score ($p \leq 0.05$) were found in the consistency of non-fat pasteurized chocolate milk containing okra pectin and the control formula.

Overall, the overall acceptability scores were higher than 6.00 showing that all formulated non-fat pasteurized chocolate milk was acceptable to panelists. The non-fat pasteurized chocolate milk added with okra gum and pectin was accepted by the panelists in terms of color, odor, mouth feel and overall acceptability.

Moreover, okra gum and pectin helped to improve consistency and mouth feel of the milk compared with control.

b. Storage test

Non-fat pasteurized chocolate milk added with okra gum and pectin and the control formula were stored at 4 °C in a refrigerator for 3 days.

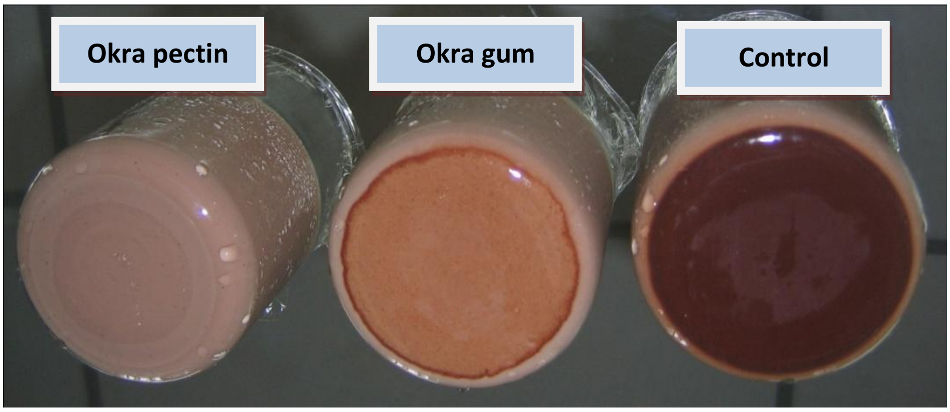

Figure 5.5 Precipitation of chocolate powder in non-fat pasteurized chocolate milk added with okra gum and pectin and the control formula after storage at 4 °C in a refrigerator for 3 days (Bottom view)

Figure 5.5 illustrates precipitation of chocolate powder in non-fat pasteurized chocolate milk after storage at 4 °C in a refrigerator for 3 days. In the control formula of non-fat pasteurized chocolate milk, a large portion of chocolate powder was found at the bottom of the container at the end of the storage period. In the case of addition of okra gum, the participation of chocolate powder was much less. Interestingly, non-fat pasteurized chocolate milk with okra pectin did not have any precipitation of chocolate powder. The extent of precipitation (weight basis) is given in Figure 5.6.

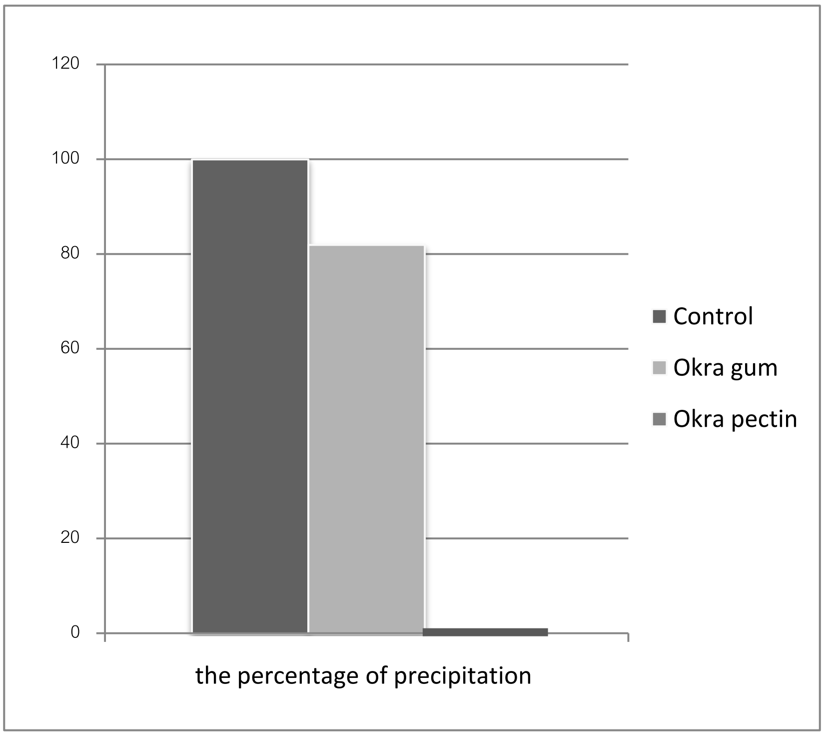

Figure 5.6 Percentage of precipitation of chocolate powder from non-fat pasteurized chocolate milk added with okra gum and pectin and the control formula after storage at 4 °C in a refrigerator for 3 days

Phase separation of non-fat pasteurized chocolate milk occurred after storage at 4 °C in refrigerator for 3 days. This was observed in non-fat pasteurized chocolate milk containing okra gum phase while the control formula and the formula with okra pectin showed no phase separation (Table 5.9).

Table 5.9 Phase separation of non-fat pasteurized chocolate milk added with okra gum and pectin and the control formula after storage at 4 °C in a refrigerator for 3 days

Non-fat pasteurized Chocolate milk	Phase separation of chocolate milk		
	days		
	1	2	3
Control	x	x	x
okra gum	x	x	√
okra pectin	x	x	x

X = Not separated and √ = Separated into 2 layers

5.4.2 Orange-flavored beverage

a. Sensory evaluation

All formulas of orange-flavored beverage were screened for sensory acceptability by 15 untrained panelists aged between 20 and 50 years old following the methods described in Section 4.6.3.

The sensory acceptability results of orange-flavored beverage added with okra gum and pectin and the control formula in terms of color, odor, mouth feel and overall acceptability appear in Table 5.10. There were no significant differences ($p \leq 0.05$) in color, odor and overall acceptability among the control formula and orange-flavored beverage containing okra gum and pectin. For mouth feel significant differences ($p \leq 0.05$) were found among the control formula and orange-flavored beverage with okra pectin and okra gum. Both okra gum and pectin provided viscosity to orange-flavored beverage.

Table 5.10 Sensory scores of orange-flavored beverage using okra gum and pectin as thickening agent at a concentration of 0.15% and 0.075%, respectively[1,2]

Formula	Sensory properties				
	Color[3]	Odor[3]	Mouth feel[3]	Overall acceptability[3]	Consistency[4]
Control	6.53±1.846[a]	5.80±1.424[a]	6.40±1.724[a]	6.13±1.846[a]	2.53±0.640[a]
Okra gum	6.33±2.093[a]	5.53±1.302[a]	4.80±1.424[b]	5.33±1.496[a]	3.53±0.640[b]
Okra pectin	6.40±1.639[a]	5.53±1.356[a]	5.60±1.404[c]	5.47±1.407[a]	3.27±0.594[b]

[1] Mean ± SD (n=15)

[2] Mean in the same column followed by a different letter (a-c) were significantly different ($p<0.05$)

[3] Nine point hedonic scale (9=like extremely, 5= neither like nor dislike, 1= dislike extremely)

[4] Five point just-about-right scale (5=much too thick, 3=just-about-right, 1=much too thin)

The just-about-right sensory results of orange-flavored beverage added with okra gum and the control formula were significantly different ($p\leq0.05$) in terms of consistency. No significant differences ($p\leq0.05$) were found in the just-about-right consistency scores between the orange-flavored beverage with okra pectin and okra gum.