

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chemical composition of Bambara groundnut

The chemical compositions of Bambara groundnut flour and starch are presented in Table 4.1. The Bambara groundnut flour contained 64.49% carbohydrate, 16.36% protein, 5.71% fat, 4.17% ash and 2.78% crude fiber content. The major components of Bambara groundnut flour is carbohydrate and protein. This result is comparable to the research of Piyarat (2008) in that this variety of Bambara groundnut contained higher protein content and lower fat content than that of Bambara groundnut flour from Pattalung province, Thailand. However, some Bambara groundnuts that produced in other areas contain slightly higher protein than this variety. For instance, the proteins of Bambara groundnut in Nigeria was observed to be 18.83% (Alhassan, Dangambo, & Mikail, 2015). Lipid content of Bambara groundnut in Nigeria were slightly higher (approx. 6.1%) (Anhwange & Atoo, 2015). Many studies reported that Bambara groundnut is an excellent source of high essential amino acids, fiber and carbohydrate but low in lipid content. (Minka & Bruneteau, 2000) (Oyeyinka & Oyeyinka, 2018). The chemical composition of Bambara groundnut from the same area but difference in genotypes was slightly difference. (Oyeyinka, Singh, Adebola, Gerrano, & Amonsou, 2015). This indicated that the environment and genotypes may influence the composition of BG.

For the chemical compositions of the isolated starch from Bambara groundnut, had low protein, fat, ash, and fiber content (Table 4.1). This indicated that this isolation method (alkaline method) gives the relatively pure starch.

Table 4.1 Chemical compositions of starch and flour from Bambara groundnut

Composition (%)	Flour	Starch
Moisture	6.49 ± 2.67	14.78 ± 2.12
Fat	5.71 ± 0.98	1.32 ± 0.43
Protein	16.36 ± 1.73	0.11 ± 0.86
Ash	4.17 ± 2.10	0.60 ± 2.34
Crude fiber	2.78 ± 2.89	0.49 ± 2.77
Carbohydrate	64.49 ± 0.75	82.7 ± 0.91
Amylose	ND	19.50

ND = not determined

4.2 Fatty acid profile of Bambara groundnut flour

The free fatty acid profile of Bambara groundnut oil was analyzed by gas chromatography (GC). It shows that fifty fatty acids were found in Bambara groundnut oil (Table 4.2). Both of saturated and unsaturated fatty acid were founded. The highest fatty acid found in Bambara groundnut was linoleic acid (39.54%), followed by oleic acid (19.56%), palmitic acid (18.85%) and stearic acid (6.57%). This result is in agreement with other reports. The linoleic acid is the highest concentration in Bambara groundnut oils. However, fatty acid profile of BG was different depending on variety, origin, climate and environmental condition (Aremu, Mamman, & Adebisi, 2013; Yao et al., 2015). Most of legume seed oils contain lower level of saturated fatty acid than unsaturated fatty acid. The important saturated fatty acid found in this BG was palmitic acid and stearic acid. Legume seed oils is the good source of essential unsaturated fatty acids especially linoleic and α -linolenic. (Khrisanapant, Kebede, Leong, & Oey, 2019)

Table 4.2 Fatty acid composition of Bambara groundnut oils (% w/w)

Common name	Numeric formula	Percentage
Myristic acid	C14:0	0.09
Pentadecanoic acid	C15:0	0.04

Table 4.2 Fatty acid composition of Bambara groundnut oils (% w/w) (Cont.)

Common name	Numeric formula	Percentage
Palmitic acid	C16:0	18.85
Palmitoleic acid	C16:1	0.07
Stearic acid	C18:0	6.57
Oleic acid	C18:1n9c	19.56
Linoleic acid	C18:2n6c	39.54
Arachidic acid	C20:0	2.02
Linolenic acid	C18:3n3	4.87
Heneicosanoic acid	C21:0	0.05
cis-11,14-Eicosadienoic acid	C20:2	0.05
Behenic acid	C22:0	5.40
cis-11, 14, 17 -Eicosatrienoic acid	C20:3n3	0.17
Triosanoic acid	C23:0	0.17
Lignoceric acid	C24:0	2.56

The fatty acids found in the Bambara groundnut oil sample were differenced in the number of carbon, double bond, and structure. So, these factors affected the ability of amylose-lipid complex formation. The important fatty acids found in Bambara groundnut oils that could have the ability to form complex with amylose in Bambara groundnut starch are linoleic, oleic, palmitic, and stearic.

Oyeyinka (2016) studied the effect of high-pressure homogenization on physicochemical properties of Bambara groundnut starch complexed with different fatty acids. High-pressure homogenization was used to prepare the complexed sample. The complex index (CI) value showed that the CI of Bambara groundnut starch-palmitic acid and stearic acid was higher than that of oleic and linoleic at the same condition. This result indicated that complex formation was dependent on the type of fatty acids.

4.3 Hydrolysis of Bambara Groundnut (BG) oil

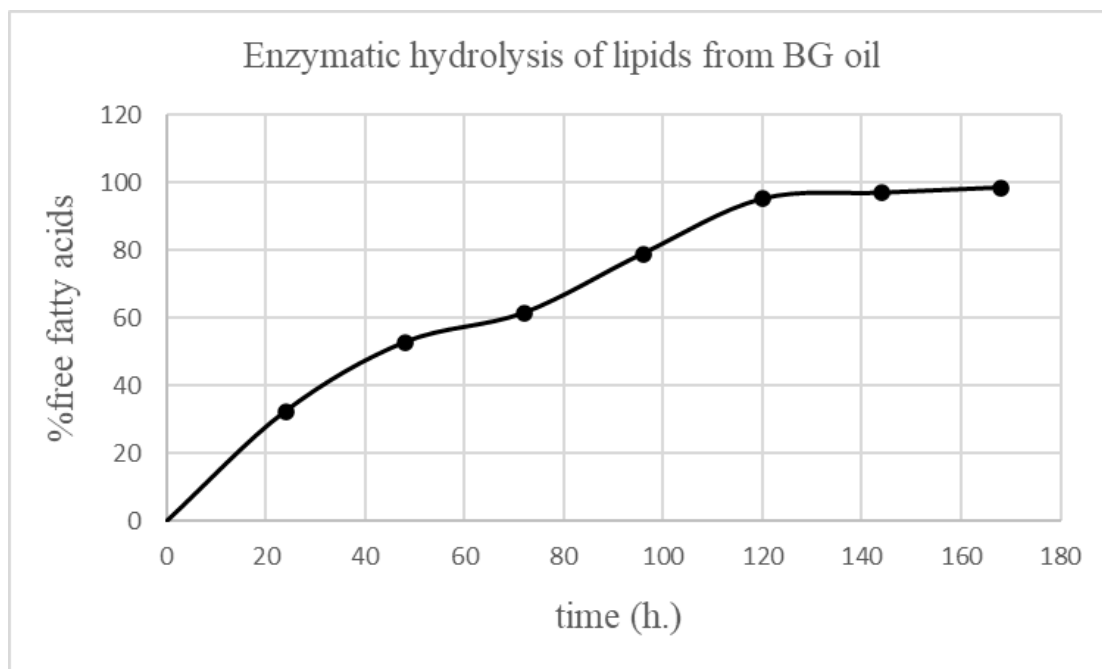


Figure 4.1 Enzymatic hydrolysis of BG oil

Lipase enzyme is the enzyme that was used to breakdown triglycerides into fatty acids and glycerol. In this study, lipase enzyme was used in the hydrolytic reaction to convert triglyceride in Bambara groundnut oils into fatty acids. Figure 4.1 showed the percentage of free fatty acid release after hydrolysis with lipase at 37°C. The results show that when incubation time was longer, free fatty acids were increased. At 120 to 168 h., the percentage of free fatty acid was close to 100% in which it indicated the maximum point. So, Bambara groundnut hydrolyzed oils after incubated at 37°C for 120 h was used to prepare the starch-complexed formation.

4.4 Preliminary study on starch-lipids complex formation using ethanol

4.4.1 Complex index value

The complex index value of the modified flour prepared in 25% and 50% ethanol is present in Figure 4.2. The sample without ethanol and hydrolyzed oils was

used to be a control sample. The sample without hydrolyzed oils in 25% ethanol exhibited the CI value of 74.38%, indicating that ethanol may form complex with free amylose in defatted flour. The sample prepared in 50% ethanol also showed a similar result. The sample without hydrolyzed oils in 50% ethanol exhibited the CI value of 86.34%. As concentration of hydrolyzed oil was increased, the CI value of both 25% and 50% ethanol was also increased to 98%; then it did not change after the concentration of 4% oil.

4.4.2 Crystalline structure

The X-ray diffractogram of native Bambara groundnut flour showed a doublet peak at 2theta of 17°, 18° and a singlet peak at 15°, 20°, and 23° (Figure 4.3), suggesting an A-type crystalline pattern. This results is similar to the study of Kaptso (2015) and Sirivongpaisal (2008).

For the complexed sample, all of the sample showed the similar diffraction patterns (figure 4.4) in which is the major peak was formed at 2theta of 12.8, 19.7 and the minor peak at 2theta of 7.5. These three peaks indicated the V-amylose diffraction peaks. (Wokadala, Ray, & Emmambux, 2012). This result suggested that the complexation was formed in BG flour. However, the WAXS pattern still showed some peaks of A-type (doublet peak at 17°, 18° and singlet peak at 15°, 20°, and 23°), that the complexed sample still contained an A-type crystalline structure.

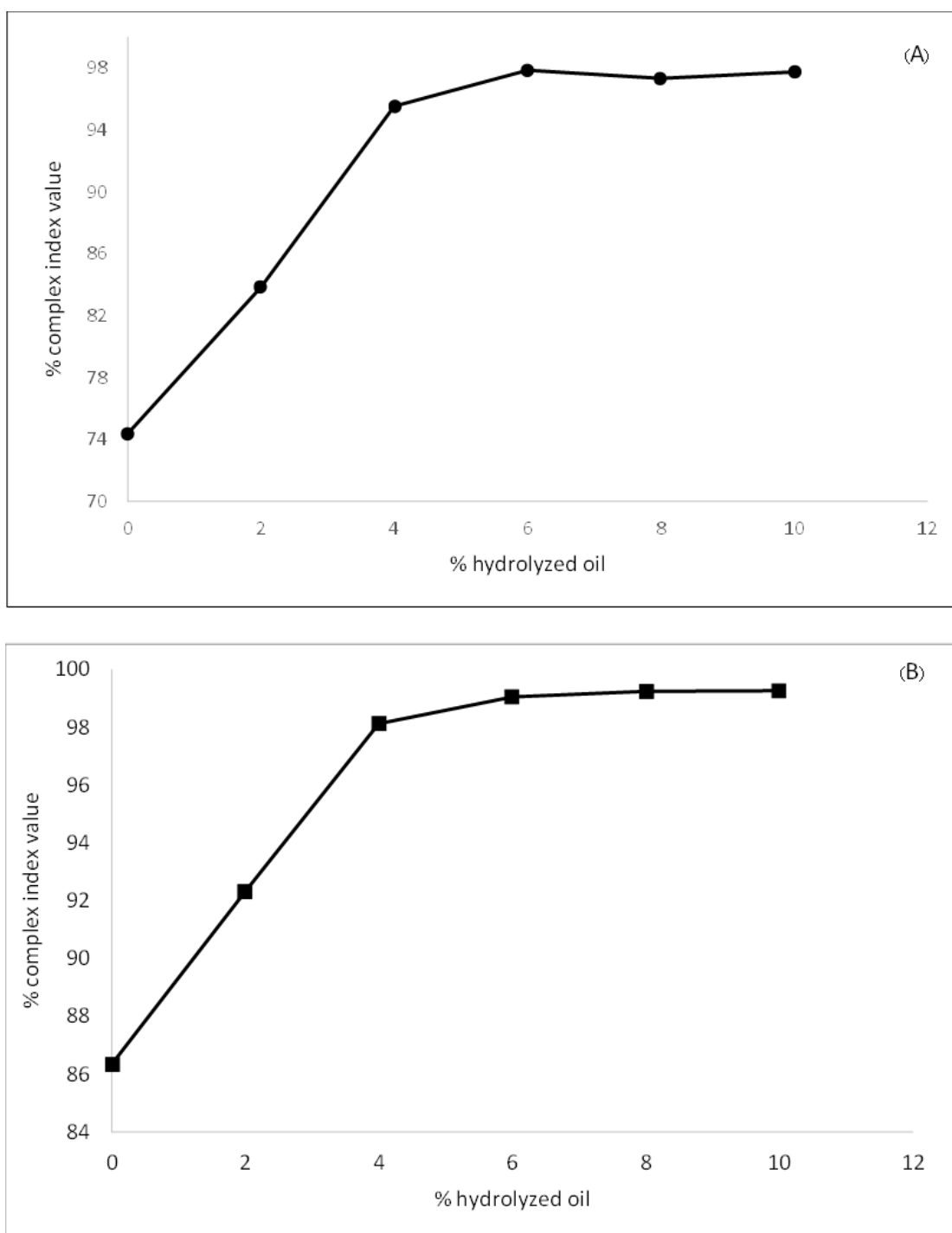


Figure 4.2 The complex index value of Bambara groundnut flour (A);Modified Bambara Groundnut flour was prepared in 25% ethanol (B);Modified Bambara Groundnut flour was prepared in 50% ethanol

Sarifudin et al. (2020) reported that starch granules that was incubated in ethanol solution showed a V-type peak, They reported that when the temperature was increased, the crystallinity of native starch decreased and the V-type crystallinity was increased, the critical temperature to initiate the V-type crystalline structure formation of the amylose-ethanol complex was at 86 °C (Sarifudin, Keeratiburana, Soontaranon, Tangsathitkulchai, & Tongta, 2020).

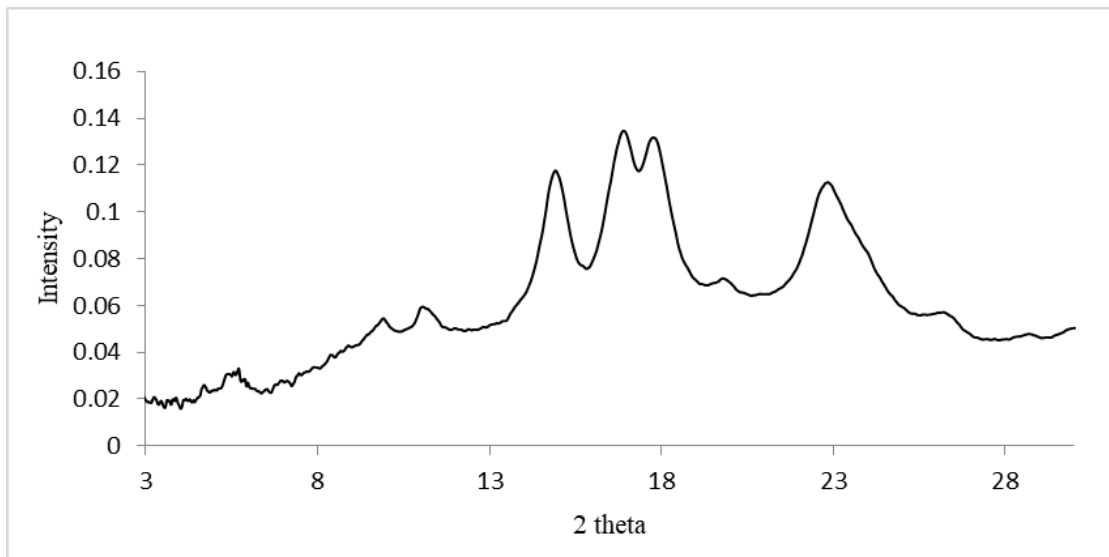
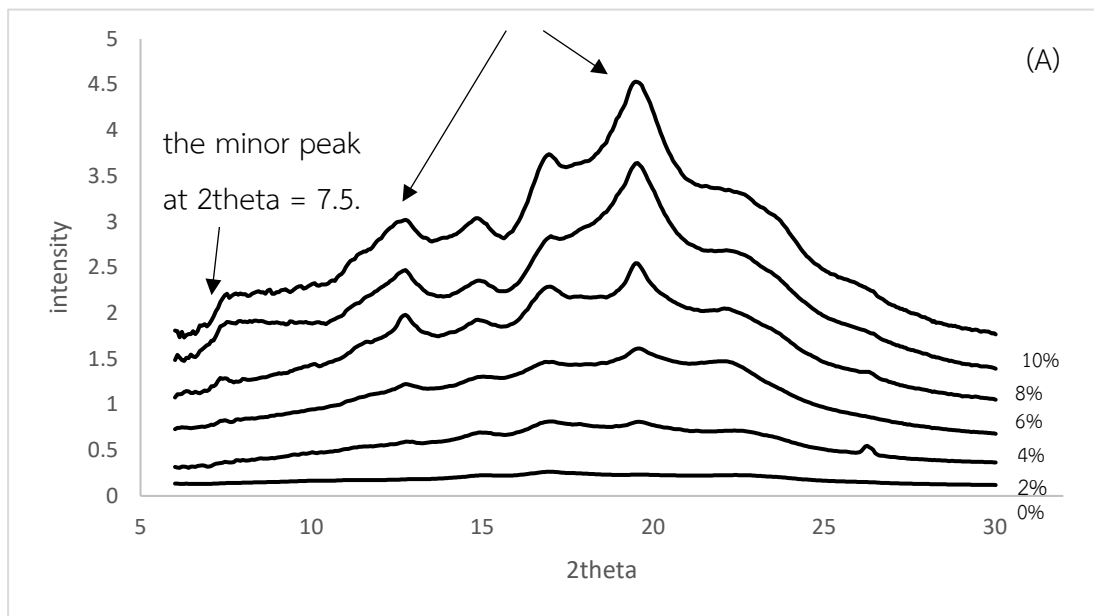


Figure 4.3 The WAXS pattern of native Bambara groundnut flour

the major peak
at $2\theta = 12.8, 19.7$



the major peak
at $2\theta = 12.8, 19.7$

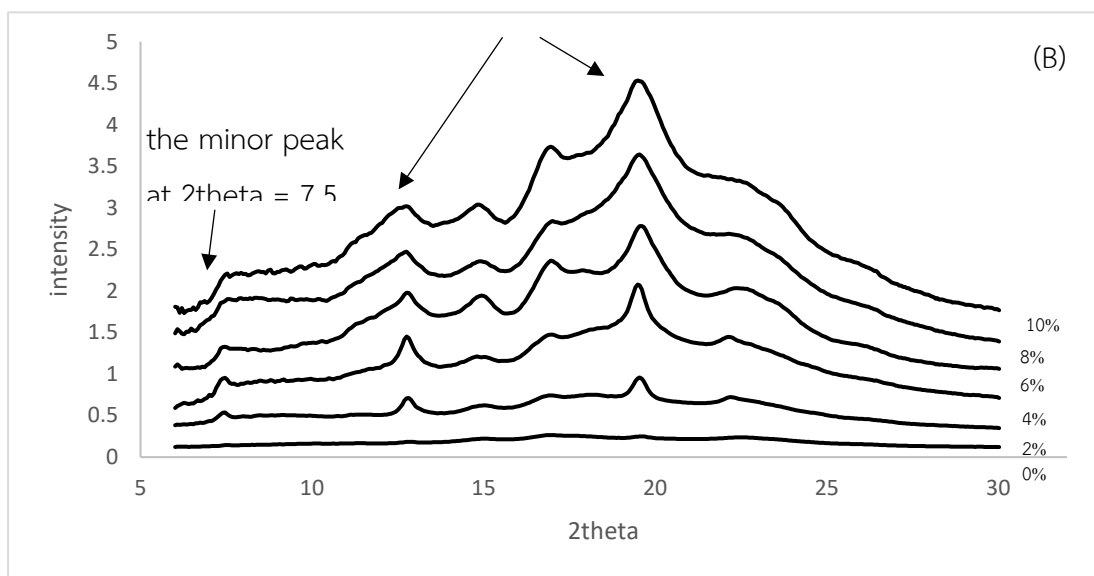


Figure 4.4 The WAXS pattern of complexed Bambara groundnut (A)starch and (B)flour with different hydrolyzed oils concentration (0, 2.5, 5, 7.5, 10, 12.5, 15 and 20%).

4.4.3 Thermal properties

The thermal properties of modified Bambara groundnut flour was determined using differential scanning calorimetry (DSC). The DSC curve of all samples showed the same results in that there was no peak in their thermograms. This result confirms that ethanol competed with fatty acids to form complex with amylose in which it is known that ethanol-amylose complex is water soluble.

4.5 Modified Bambara groundnut flour

4.5.1 Complex index value

The amylose-lipid complex flour/starch that was prepared in ethanol-water suspension show a negative results of amylose-lipid complex formation, these sample preparation was conducted in a water suspension system. The result of CI value was shown in figure 4.5 A that the flour/starch samples which were added with the hydrolyzed oils at the concentration 2.5-12.5% showed an increase in complex index value when the concentration of Bambara groundnut hydrolyzed oils were increased. This results can be explained that at the hydrolyzed oil concentration of 2.5-12.5%, free amylose was still available to be able to complex with fatty acids. But, the CI value showed a plateau at the hydrolyzed oil concentration of 12.5-20%. This result suggested that all of the free amylose in the sample was used to form complexation with amylose.

Stearic acid was used to compare with BG hydrolyzed oils in order to study their ability of complexation between BG flour and starch. The CI values of amylose-stearic complex of BG starch (figure 4.5 B) was significantly higher than that of BG flour at the same concentration of hydrolyzed oils. This could be attributed to that the BG hydrolyzed oil contain several types of fatty acids (table 4.2) and the majority of fatty acids is linoleic acid (39.54%), oleic acid (19.56%), palmitic acid (18.85%), and stearic acid (6.57%), respectively. The degree of complexation of BG flour and starch with hydrolyzed oils was affected by fatty acids types.

Most of fatty acids in BG oils are the unsaturated fatty acid. The number of double bond in the aliphatic lipid chains affects the ability of complex formation. The saturated fatty acids are the linear chains that can go into the cavity of helix easier than the unsaturated fatty acids which could form a partial inclusion in the

helix cavity because of the kink structure of their lipid chains.(Joke A. Putseys, Derde, Lamberts, Goesaert, & Delcour, 2009; Yamada, Kato, Tamaki, Teranishi, & Hisamatsu, 1998)

Not only the lipid structure, but the component in the sample also affected the ability of complexation. The complex index value of starch complexation with BG hydrolyzed oils is higher than that of the flour (figure 4.5 A). This results may be explained by that the other components such as protein and fiber in BG flour could interrupt the complex formation between fatty acids and amylose. However, when stearic acid was used for complexation with starch or flour, they showed a similar result. This may be because stearic acid could form complexation easily as explained earlier. This indicates that the ability of complex formation between steric acid and amylose in starch and flour is greater than that of hydrolyzed BG oils.

Table 4.3 Hydrolyzed BG oils content on complex index value of Bambara groundnut flour and starch

Hydrolyzed oils (%)	Complex index value (%)					
	BG starch			BG defatted flour		
2.5	38.56 ^b	±	2.84	30.39 ^a	±	1.34
5	47.47 ^c	±	1.15	49.77 ^c	±	1.72
7.5	71.09 ^e	±	1.76	60.70 ^d	±	1.08
10	80.10 ^f	±	0.93	71.93 ^e	±	0.54
12.5	86.63 ^g	±	1.78	80.05 ^f	±	0.42
15	86.40 ^g	±	0.53	80.61 ^f	±	0.71
20	86.96 ^g	±	2.86	81.52 ^f	±	0.25

Table 4.4 Stearic acid content on complex index value of Bambara groundnut flour and starch

Stearic acid (%)	Complex index value (%)					
	BG starch			BG defatted flour		
0.625	62.15 ^b	±	1.33	58.40 ^a	±	0.72
1.25	72.72 ^c	±	0.86	71.39 ^c	±	0.38
2.5	78.40 ^d	±	0.73	78.55 ^d	±	1.88
3.75	89.67 ^f	±	0.63	86.62 ^e	±	1.17
5	94.24 ^g	±	0.36	93.43 ^g	±	0.60
10	94.12 ^g	±	0.13	93.54 ^g	±	0.81
15	95.02 ^g	±	1.36	93.67 ^g	±	0.30

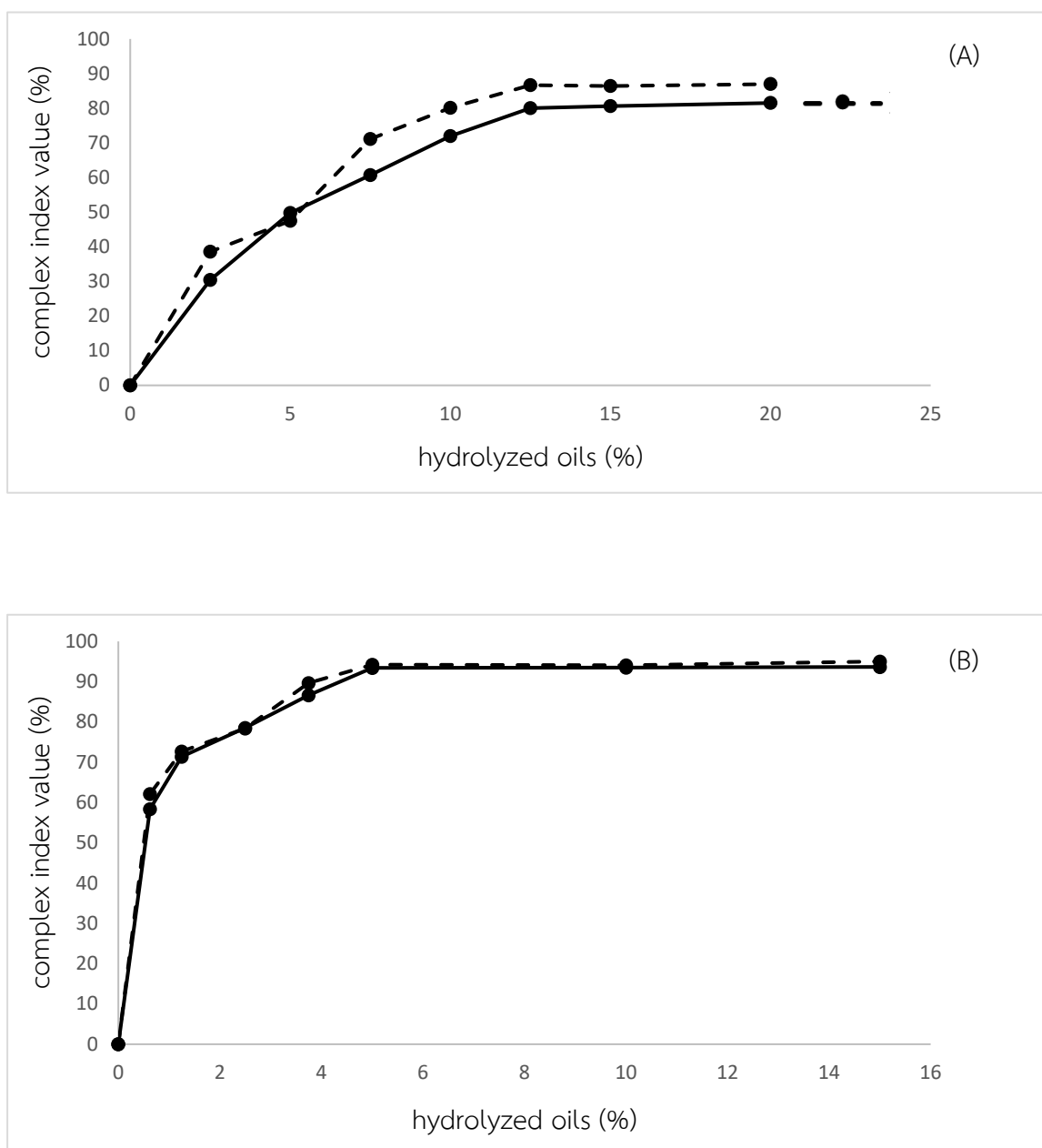


Figure 4.5 Complex index value of amylose-lipid complex of Bambara groundnut flour and starch; (A) BG hydrolyzed oil; (B)stearic acid (—●— BG starch; —●— BG flour)

4.5.2 Crystalline structure

The crystalline structure of starch is classified into three categories: A, B and C type. The X-ray diffractogram of native Bambara groundnut flour and starch showed a doublet peak at 2theta of 17°, 18° and a singlet peak at 15°, 20°, and 23° (Figure 4.6), suggesting an A-type crystalline pattern, This result is similar to the study

of Kaptso et al., (2015) and Sirivongpaisal, (2008). However, other studies have reported that on a C-type pattern was formed for Bambara groundnut starch (Oyeyinka, Singh, & Amonsou, 2017).

For the complexed sample, they show the similar diffraction pattern (figure 4.7) which is the major peaks at 2theta of 12.8, 19.7 and the minor peak at 2theta of 7.5. These three peaks indicated the V-amylose diffraction peaks (Wokadala et al., 2012). The percentage of crystallinity of the complexed samples showed in figure 4.8. The crystallinity of starch-BG fatty acid complex is higher than that of flour-BG fatty acid complex at the same content of hydrolyzed oil. This result is correlated with the complex index value. It suggest that Bambara groundnut starch had more ability to form complexes with fatty acids than Bambara groundnut flour.

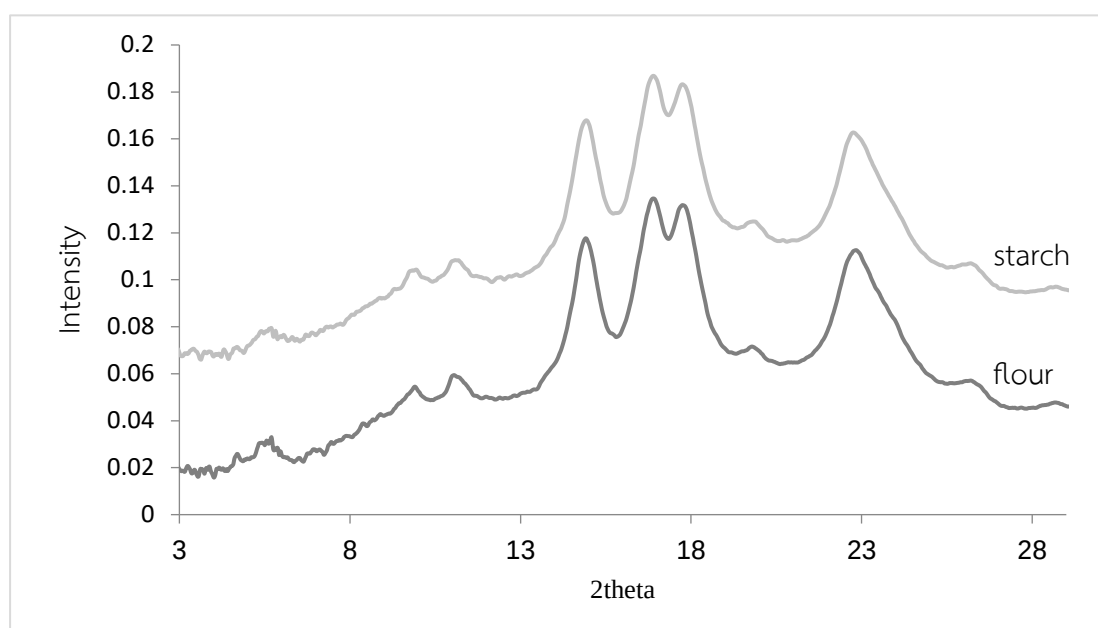


Figure 4.6. The WAXS pattern of native Bambara groundnut flour and starch

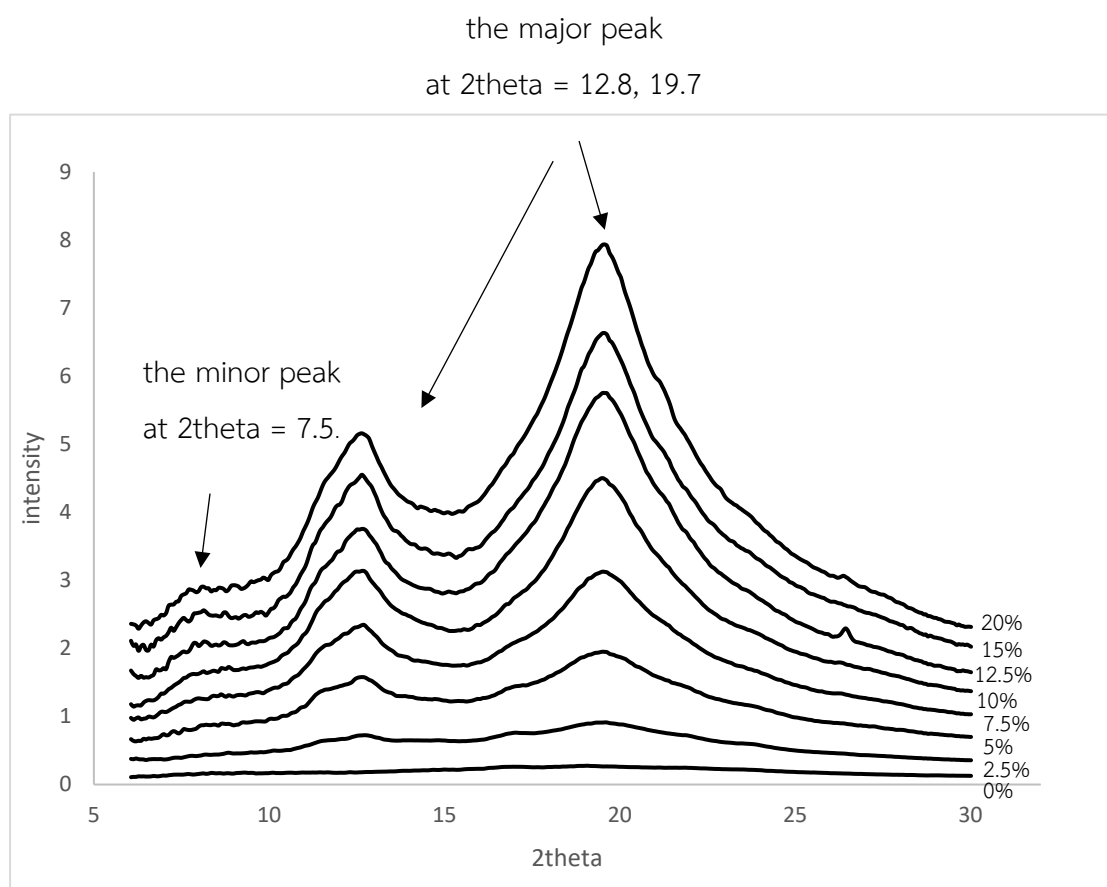


Figure 4.7. The WAXS pattern of complexed Bambara groundnut (A);starch, (B);flour with different hydrolyzed oil concentration (0, 2.5, 5, 7.5, 10, 12.5, 15 and 20%)

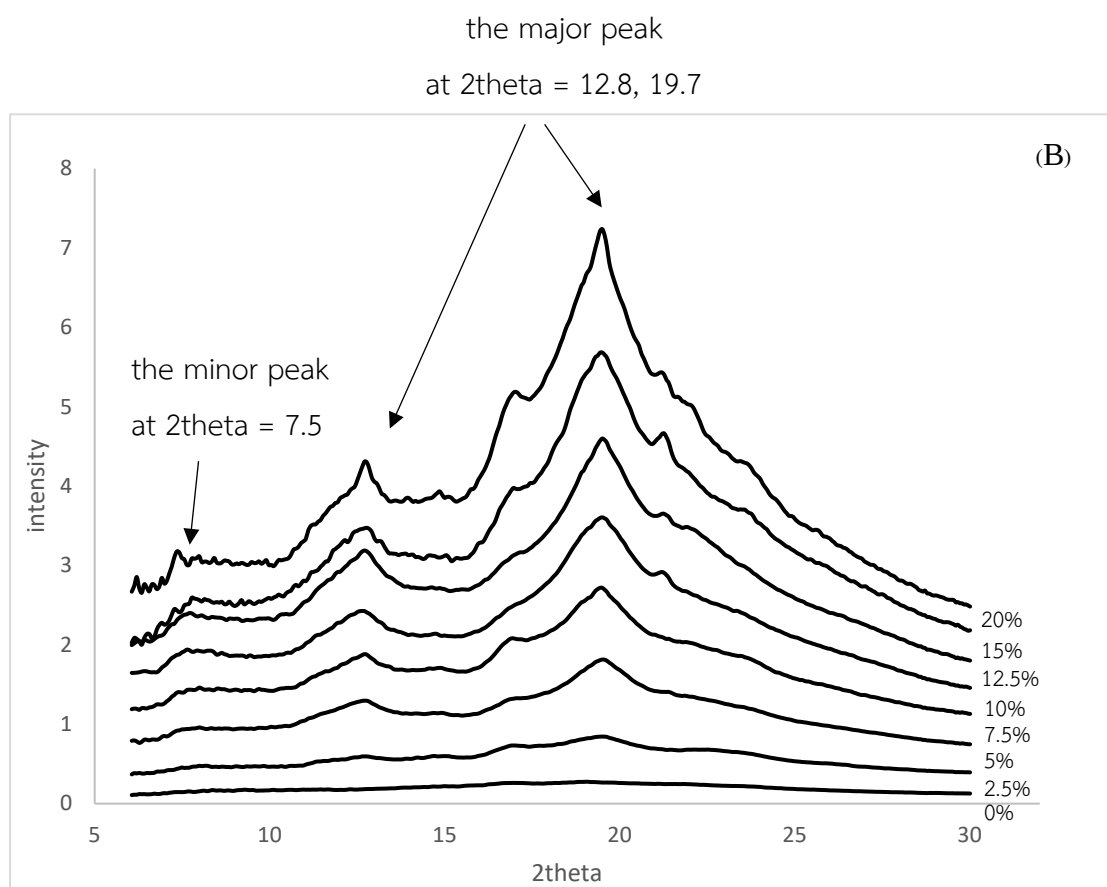


Figure 4.7. The WAXS pattern of complexed Bambara groundnut (A);starch, (B);flour with different hydrolyzed oil concentration (0, 2.5, 5, 7.5, 10, 12.5, 15 and 20%) (Cont.)

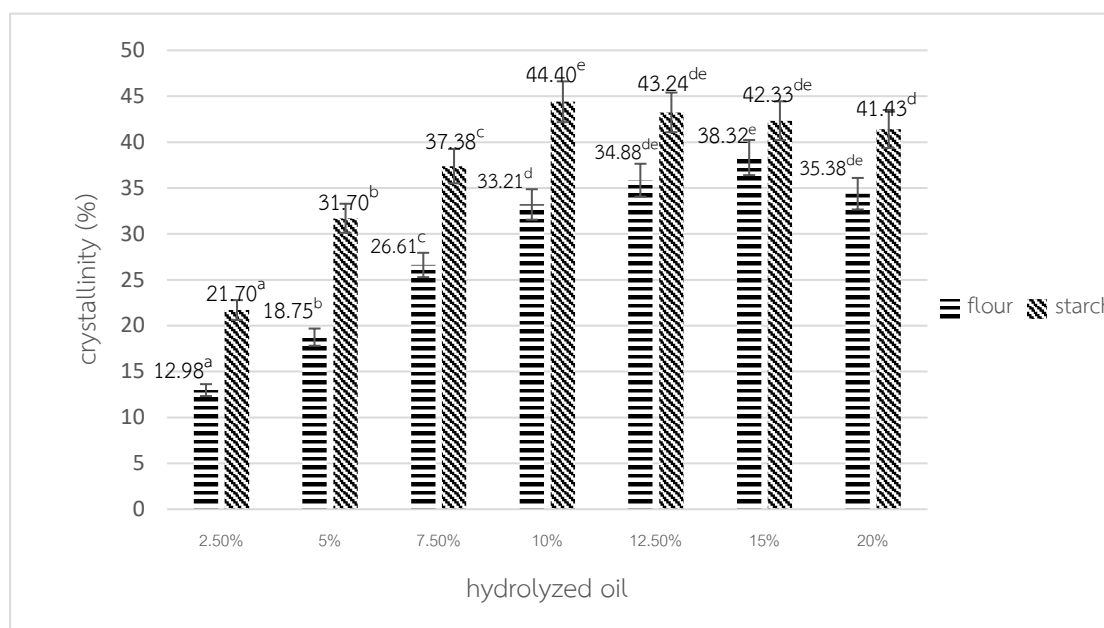


Figure 4.8. The percentage of crystallinity for the complexed sample

4.5.3 Thermal properties

The thermal properties of modified BG flour complexing with hydrolyzed oil and stearic acid are shown in table 4.5 and table 4.6, respectively. The peak temperature of all samples were not different. The flour-stearic complex has a peak temperature at 103°C, while the flour-BG hydrolyzed oil complex has a peak temperature at 99°C. Both of them showed the properties of type I complex (95°C-105°C)(J. A. Putseys, Lamberts, & Delcour, 2010). According to Biliaderis and Galloway (1989), a sufficient amount of energy was required to form the complexation of amylose. It needed to unwind the amylose helix and the guest molecules could be inserted. However, this complex may exist in an unordered state, which is known as a type I complex. However, the enthalpy was significantly increased with increasing the concentration of BG hydrolyzed oil or stearic acid. The melting temperature was related with the physical stability. The longer helical length of complex induced more hydrophobic interaction and a higher temperature to break the interaction was required. (Kawai, Takato, Sasaki, & Kajiwar, 2012) For the enthalpy, it refers to the amount of the complex (Kawai et al., 2012). This result was confirmed with the complex index value and percentage of crystallinity in that the higher concentration

of hydrolyzed BG hydrolyzed oil or stearic acid, the more amount of complex was increased.

Table.4.5 Thermal properties of complexed Bambara groundnut flour with different hydrolyzed oil concentrations

Sample	T _o		T _p		T _c		enthalpy	
2.5%	87.36	±0.76 ^{ab}	98.63	±1.16 ^a	108.23	±2.61 ^a	1.29	±0.50 ^a
5%	88.60	±1.30 ^{ab}	99.52	±1.55 ^a	109.00	±1.89 ^a	2.12	±0.57 ^b
7.5%	89.95	±0.69 ^{ab}	99.13	±0.33 ^a	108.12	±2.24 ^a	2.15	±0.26 ^b
10%	89.17	±2.52 ^{ab}	99.94	±0.76 ^a	106.63	±0.53 ^a	1.90	±0.04 ^{ab}
12.5%	85.86	±0.80 ^a	99.52	±0.25 ^a	106.29	±0.43 ^a	2.18	±0.25 ^b
15%	85.47	±0.30 ^a	99.10	±0.40 ^a	106.43	±0.52 ^a	2.20	±0.17 ^b
20%	86.11	±0.70 ^a	99.45	±0.32 ^a	107.72	±0.61 ^a	2.21	±0.44 ^b

Table.4.6 Thermal properties of complexed Bambara groundnut flour with different stearic acid concentrations

Sample	onset		Peak		endset		enthalpy	
2.5%	92.97	±5.64a	103.12	±0.88a	110.71	±0.82b	1.43	±0.63a
5%	87.41	±2.09a	104.18	±0.29a	109.82	±0.64b	2.91	±0.65b
10%	86.58	±1.28a	103.23	±0.41a	110.07	±0.30b	2.54	±0.43b
15%	92.63	±4.13a	102.98	±0.88a	108.00	±0.23a	2.58	±0.50b

4.6 Properties of modified Bambara groundnut flour

4.6.1 Starch fractions

Based on its *in vitro* enzymatic hydrolysis and the rate of glucose release, starch is divided into 3 types, which are rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The starch fraction of the modified flour is shown in table 4.7. RDS was decreased when compared with the control sample (0% FFA) and decreased with increasing of the concentration of fatty acid. SDS and RS were increase with the concentration of fatty acid increase and higher than the control sample. These results were linked with the complex index value

and enthalpy of complexed that was at high complexing ability and amount of complex, the SDS and RS were high.

This results agreed with a previous study. Oyeyinka (2017) reported the formation of lipid (stearic acid, linoleic acid and lysophosphatidylcholine) complexed in Bambara groundnut starch reduced the digestibility. They suggested that the digestibility depended of lipid chain length and degree of unsaturated, the long chain of lipids was form high crystalline and strongly. In addition, lipid prevented the hydration and swelling of starch. Holm (1983) reported that the steric hindrance and low in solubility of lipids was affected to decreasing of the susceptibility of digestion enzyme. In addition, double bond of unsaturated fatty acids induced a structural disturbance of complex formation and then increasing the susceptibility of enzyme digestion.(Holm et al., 1983; Oyeyinka et al., 2017).

The study of Kawai (2012) was difference. They reported that the amylose-saturated fatty acid complex was higher digestibility than the amylose-unsaturated fatty acid complex. They explained that the melting temperature of saturated fatty acid was higher than hydrolyzed temperature (37°C) so after enzyme hydrolysis, the complex between amylose and fatty acid was break. The free fatty acid was form self-crystalline. While, unsaturated fatty acid has lower melting point than 37°C, after enzyme hydrolysis, free fatty acid was formed complex with partial hydrolyzed amylose and short-chain of amylopectin. These affected to the susceptibility of digestion enzyme.

Table 4.7 Starch fraction of modified Bambara groundnut flour

Sample	RDS		SDS		RS	
0%	21.30	±0.67 ^b	75.87	±0.55 ^a	2.82	±0.89 ^a
2.5%	10.99	±0.42 ^a	79.63	±0.59 ^{bc}	9.38	±0.16 ^b
5%	11.63	±0.38 ^a	78.48	±1.13 ^b	9.88	±0.57 ^b
7.5%	11.97	±0.70 ^a	77.24	±0.19 ^b	10.79	±0.48 ^b
10%	11.57	±0.53 ^a	77.93	±0.54 ^b	10.50	±0.17 ^b
12.5%	11.59	±0.04 ^a	77.09	±0.29 ^b	11.32	±0.33 ^{bc}

Table 4.7 Starch fraction of modified Bambara groundnut flour (Cont.)

Sample	RDS		SDS		RS	
15%	11.56	$\pm 0.52^a$	77.39	$\pm 0.27^b$	11.05	$\pm 0.47^{bc}$
20%	11.48	$\pm 1.76^a$	77.41	$\pm 0.38^b$	11.11	$\pm 0.36^{bc}$

Values in the same column with the difference letters significantly different ($p < 0.05$)

4.6.2 Dynamic Rheology

A strain sweep was used to determine the linear viscoelastic region (LVR) and critical strain in oscillatory measurements. It is important that the material is stable and does not change during the sweep. The sample should be stable at the measurement temperature. A strain sweep test from a strain range of 0.1-100% at a constant frequency of 1 rad/s and 37°C was conducted to set the upper limit of the LVR. The results shows in Figure 4.9. The loss moduli and storage moduli have a relative constant value and do not change with the applied strain within the linear region. As the strain increased outside the LVR (>8%), the structure of the material changed because the high strain oscillation and a drop in the storage modulus was observed. From this experiment, 1% strain which is within LVR for all of the sample, was used in the frequency sweep test.

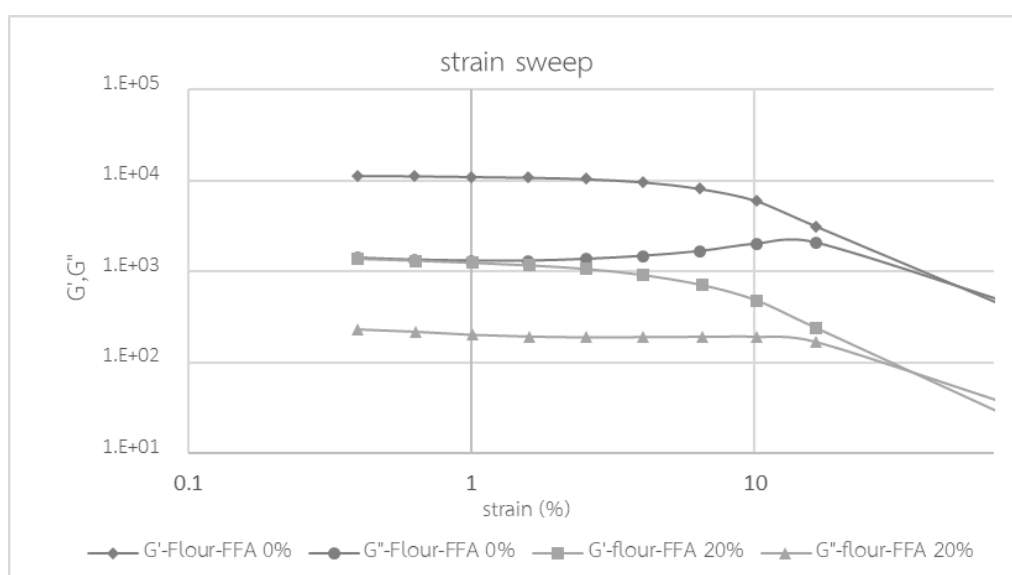


Figure 4.9 The strain sweep of BG flour complexed at 0% and 20% concentration of BG hydrolyzed oils

The viscoelastic behaviour of Bambara groundnut flour gels was affected by the complexation with fatty acids (Fig. 4.10 and 4.11). The storage moduli (G') value of BG flour gels were generally greater than their loss moduli (G'') value. The G' and G'' of all samples were significantly lower than the control, Bambara groundnut flour. Moreover, both G' and G'' were increased with the concentration of hydrolyzed oil was increased. This result is related with the loss tangent ($\tan \delta$) value. The $\tan \delta$ value is the ratio between viscosity and elastic modulus. From the results, the increase of $\tan \delta$ appeared when the concentration of hydrolyzed oils was increased (fig.4.12.). This might indicate that the non-gelling behavior of starch paste was observed with added hydrolyzed oils. This result can be explained that as the sample with a higher hydrolyzed oils content, the V-amylose structure was formed in a greater extent. This is confirmed by the results of CI, WAXS, and thermal properties. Thus, it is possible that the free amylose that could form double helices, junction zones and gel network during storage was reduced. The complex viscosity value of V-amylose complex sample was lower than that of native BG flour (fig 4.13). This result tend to reduce with higher BG hydrolyzed oils concentration. This result was related with G' and G'' value. The increase of fatty acid concentration affected the gel network formation ability of free amylose. The double helix formation of free amylose led to high viscosity of the starch paste.

A similar result was reported by Oyeyinka. (2016). They study the effect of high-pressure homogenization on rheological properties of Bambara groundnut starch complexed with different fatty acids. BG starch-fatty acid complexes produced gels with lower G' and G'' compared to their native starch gel counterparts. The reduction of G' and G'' in the presence of lipids has been associated with the formation of V-amylose complex. In addition, some research explained that amylose-fatty acid complexes have been suggested to either prevent the formation of free amylose or increase the spacing between junction zones. The higher level of V-amylose complex may also slow down the re-association of amylose molecules during short term storage.

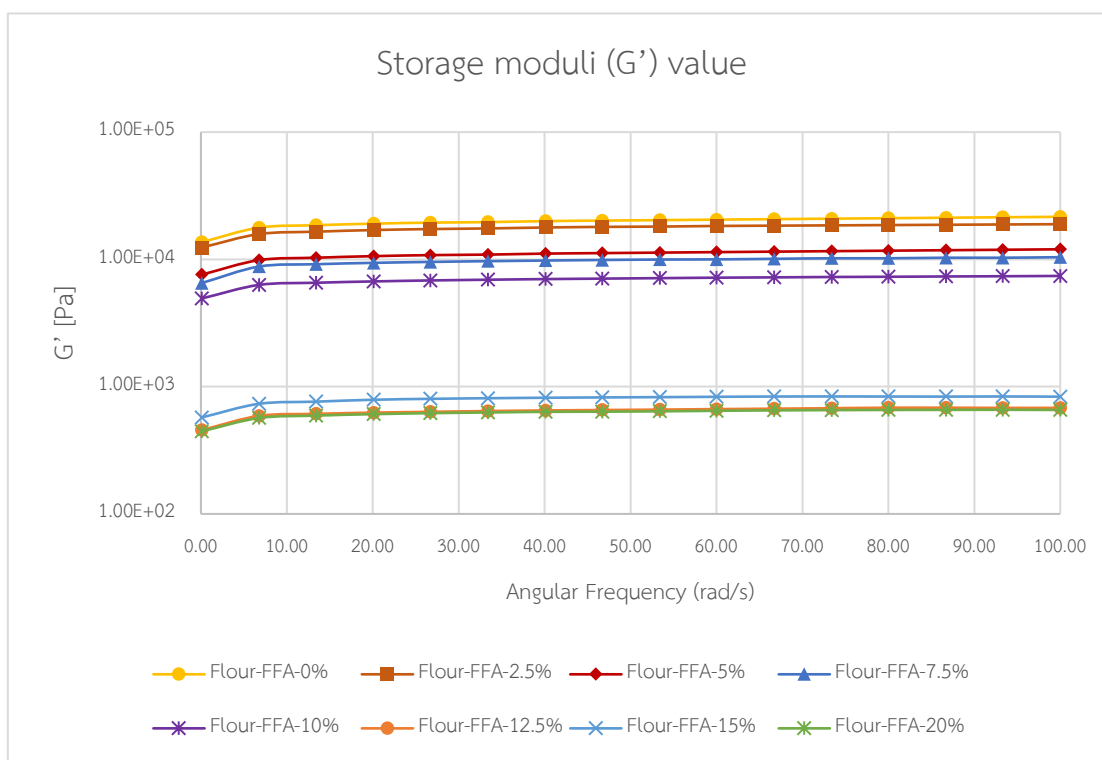


Figure 4.10 Effect of BG hydrolyzed oil on storage moduli (G') value of BG flour

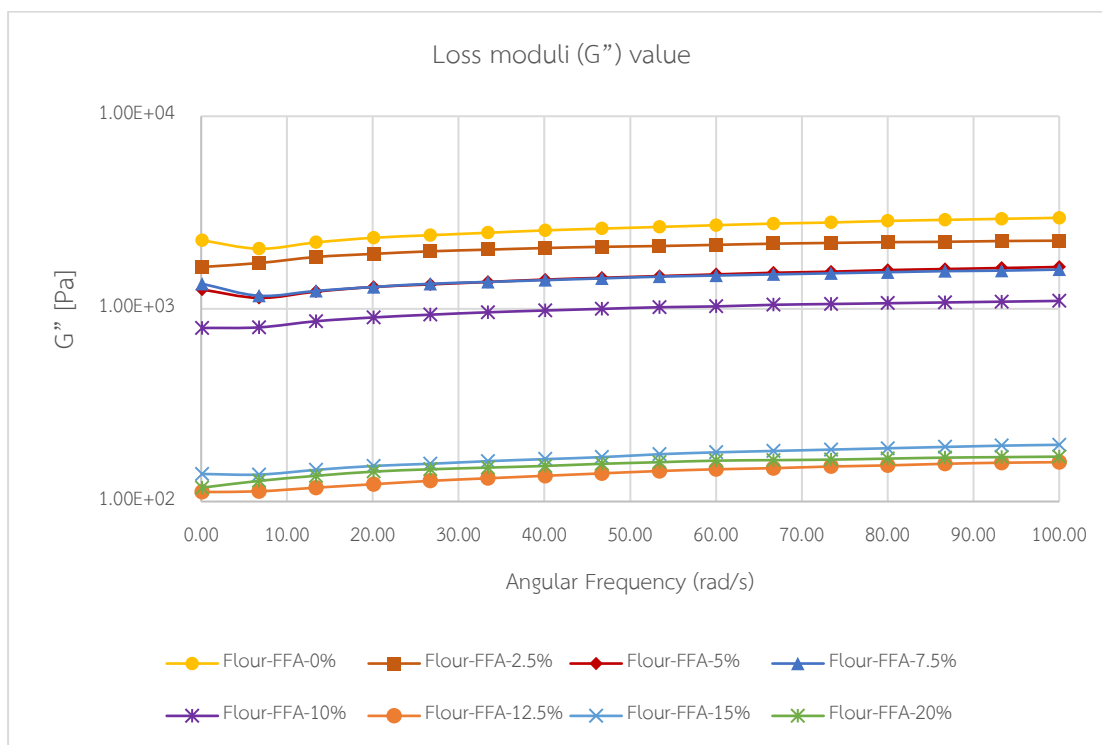


Figure 4.11 Effect of BG hydrolyzed oil on loss moduli (G'') value of BG flour

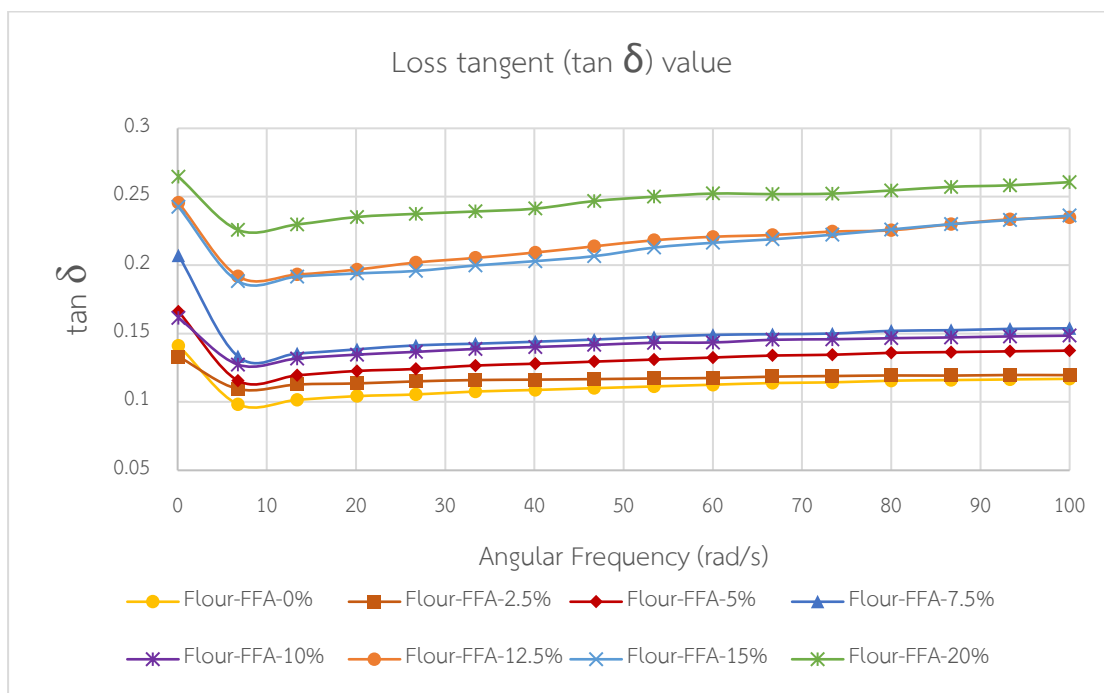


Figure 4.12 Effect of BG hydrolyzed oil on loss tangent ($\tan \delta$) value of BG flour

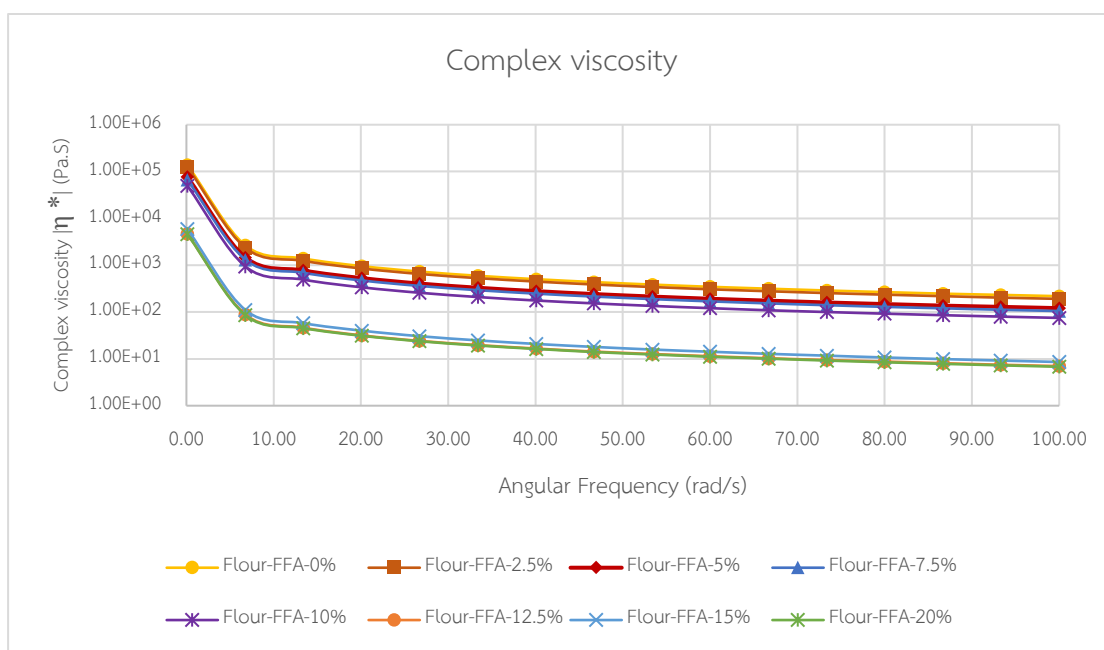


Figure 4.13 Effect of BG hydrolyzed oil on complex viscosity value of BG flour

4.7 References

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