Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

Physicochemical and functional properties of starch and germinated flours from *Dolichos lablab*

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Abstract

The objective of this research was to investigate the physicochemical and functional properties of native and germinated flours from lablab (*Dolichos lablab*) bean. The bean was germinated at 25ºC for 12, 24, 36 and 48 h. Non-germination bean (native flour) and native lablab starch were used as control. Results showed that protein content of flour increased with an increase of germination time which ranged from 25.7-28.5% db. Pasting properties of lablab starch were higher in peak viscosity (3941 cP), breakdown (919 cP), final viscosity (5670cP) and setback (2649 cP) than other flour samples. Germination showed significant effect ($p<0.05$) on the pasting properties of flours as reflected by lower in peak viscosity of flours. When compared to native flour, α-galactosides contents in 24h and 48h germinated lablab flours decreased by about 57.9% and 77.5%, respectively. Water, oil and butter absorption capacity of germinated flours did not significantly change ($p\geq0.05$) after germination. This study suggested that germination improved the nutritional value of lablab flour by lower α-galactosides contents with small effect on the functional properties.

Keywords: Lablab (*Dolichos lablab*) bean, flour, starch, germination,α-galactosides

1. Introduction

Bean is an excellent source of protein (20-25%), complex carbohydrate (50-60%) and good sources of mineral, vitamin and polysaturated free fatty acids (Chung et al., 2008). Lablab (*Dolichos lablab*) bean is one of legume that has been grown in several areas in the Northern part of Thailand. It can be used as a good source of protein and further processed to flour and starch for several food applications. However, general bean products contain alpha (α)-galactosides, which are oligosaccharides of the raffinose family (e.g. raffinose, stachyose and verbascose) or galactosyl-sucrose oligosaccharides (GSO). The α-galactosides are anti-nutritional factor of the bean because it is unable to break down by human digestive enzymes. This problem leads to the production of CO_2, CH_4 and H_2, resulting in flatulence and other digestive disorders after consumption of legume seeds.

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Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

There are several methods to reduce the α-galactosides in legumes. Jangchud and Bunnag (2001) found that soaking of red kidney beans for 12 h and boiling in pressure cooker for 15 min reduced the raffinose and stachyose by about 47% and 44%, respectively. Germination is another method that has been suggested as an effective treatment to remove anti-nutritional factors (Valverde et al., 1998). The mechanism of reduction of anti-nutritionalfactors is due to the enzyme α-D-galactosidase activity. During germination, this enzyme breaks down α-galactosides to sucrose and glucose as energy source for plant growing (Kadlec et al., 2006). They reported that α-galactosides contents in germinated pea seeds decreased from 3.6 to 0.7 g/100 g flour (80.5% reduction) after three days of germination. Ramakrishna et al. (2006) also found that germination was more effective method in reducing trypsin inhibitor activity than various cooking treatments of lablab bean.

Although many researchers have been studied in the extraction and some properties of lablab bean starch (Ramakrishna et al., 2006; Nwokwacha, 2010), there is limited information of germination effect on the physicochemical and functional properties of lablab bean flour. Therefore, the research aimed to investigate the effect of germination on the physicochemical, α-galactosides and functional properties of lablab bean flour as compared to lablab bean starch.

2. Materials and Methods

2.1 Materials

Lablab seed was purchased from Chaiprakarn, Chiang Mai province, Thailand. Soybean oil (Morakot brand, Morakot Industrial Public Co Ltd., Thailand) and unsalted butter (Allowrie brand, United Dairy Foods Co Ltd., Thailand) were used in this experiment.

2.1.1 Preparation of Native (NF) and Germinated Lablab Bean Flours (GF)

Native lablab flour was prepared from dehulled seeds and milled using a hammer mill. The flours were sieved through a 125-mesh sieve, vacuum packed in laminated aluminum bags and stored at -18ºC until further use.

The germinated flour was prepared following the method of Martin-Cabrejas et al. (2008) with slightly modification. For each batch, five hundred grams of lablab seeds were washed with 0.7% sodium hypochlorite solution and then soaked in 1,500 mL tab water at 30^{o}C for 6 h. After that, the excess water was removed. The drained lablab seeds were placed on moist cotton cloth and allowed to germinate at 25±2ºC for 12, 24, 36 and 48 h which called 12GF, 24GF, 36GF and 48GF, respectively. The germinated lablab samples were dried at 50ºC

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

for 24 h, milled and packed in laminated aluminum bag. Samples were stored at -18ºC before further analysis.

2.1.2 Preparation of Lablab Bean Starch (LS)

Starch was isolated following the modified method of Ovando-Martinez et al. (2011). Peeled lablab seeds were blended with water at the ratio of1:2 for 2 min using a blender (HP3A, Blendtec, USA). The slurry was filtered through a cotton cloth and sieved through a 170-mesh sieve. The filtrate was collected after standing undisturbed for 3 h. The starch was re-suspended in water, and allowed to settle; this process was repeated three times. The collected starch was oven-dried at 40ºC for 24 h, finally ground, sieved through a 120-mesh sieve, packed in laminated aluminum bag, and stored at -18ºC until further use.

2.2 Color and Water Activity Measurement

The color value (L*, a* and b*) of flour and starch samples were measured using a Hunter Color Meter (model SSE343, Color Quest II, USA). Water activity was measured using a water activity analyzer (AQUA LAB model series 3, Decagon Device Inc., Pullman, USA).The measurement was done in triplicates.

2.3 Determination of Chemical Composition

All flour and starch samples were estimated for their moisture, fat, protein (N×6.25), ash and fiber contents according to the methods of AOAC (2000). Carbohydrate content was calculated by difference. The measurement was done in triplicates.

2.4 Pasting Behavior Determination

Pasting characteristics of samples were determined using a rapid visco analyzer (RVA-4D, Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) following the method of Phimolsiripol et al. (2011).The suspension was heat from 50ºC to 95ºC then held at 95ºC and cooled to 50ºC for 13 min using the standard profile according to the method 76.21 of AACC. Pasting temperature, peak viscosity, breakdown, final viscosity and setback from peak were recorded. All measurements were performed in triplicates.

2.5 Water, Oil and Butter Absorption Capacity Measurement

Water, oil and butter absorption capacity of lablab flour and starch samples were measured in triplicate according to the modified method by Jitngarmkusol et al. (2008). One

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

gram (db) of each sample into a pre-weighed centrifuge mixed by vortex with 10 mL distilled water, oil or butter for 1 min. The solution allowed to stand for 30 min, centrifuged (2000*g*, 25min). The supernatant was poured and reweighed the sample. The water, oil and butter absorption capacities were expressed as the number of grams of water oil or butter bound by gram of flour (db).Soybean oil and butter were used to compare the different types of oil. Soybean oil represented higher polyunsaturated fatty acid and butter represented higher saturated fatty acid.

2.6 Swelling and Solubility Measurement

Swelling power was determined in triplicate using 0.1 g (db) of flour sample by the modified method of Schoch (1964). Flour sample was weighed into a centrifuge tube with cap which 15 mL distilled water was added. The tubes were heated and shake at 85ºC for 30 min. The swollen samples were centrifuged at 2,200 rpm for 15 min. The supernatant was poured into aluminum can, dried at 100ºC and then weighed (W_l). The sediment in tube was weighed (W_s). The water soluble index (WSI*)* and swelling power (SP) were calculated as follows:

$$WSI = \frac{(W_1)\times 100}{0.1} \qquad (1)$$

$$SP = \frac{(W_s)\times 100}{0.1\times(100-WSI)} \qquad (2)$$

2.7 Emulsion Activity and Stability Measurement

Emulsion activity and stability of flour and starch samples were determined in triplicate according to method of Jitngarmkusol et al. (2008). One gram (db) of flour sample was dispersed in 50 mL distilled water was homogenized at 3,400 rpm for 30 s. Flour suspension was homogenized with 25 mL soybean oil for 30 s. Then added another 25 mL of soybean and homogenized for 90 s. Emulsified sample was divided equally into two centrifuge tubes. The first tube was centrifuged at 1100*g* for 5 min and the volume of the remaining emulsion was measured.The measurement was done in triplicates. Emulsifying activity (EA) was calculated as follows:

$$EA(\%) = \frac{V_1}{V_2}\times 100 \qquad (3)$$

V_1 = volume of emulsified layer

V_2 = volume of emulsion before centrifuge

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

Second tubes was heated at 85ºC for 15 min, cooled to room temperature and centrifuged at 1100*g* for 5 min. Emulsion stability (ES) was calculated as follows:

$$ES(\%) = \frac{V_3}{V_2} \times 100 \qquad (4)$$

V_3 = volume of remaining emulsified layer

2.8 Alpha-galactosides Analysis

Galactosyl-sucrose oligosaccharides as α-galactosides including raffinose, sucrose and glucose were determined according to the method of McCleary et al. (2006) using a test kit of Magezyme. The measurement was done in triplicates.

2.9 Statistical analysis

One-way analysis of variance (ANOVA) and Tukey HSD all-pairwise comparisons test were used for comparing of the data at significance 95% confidence interval ($p<0.05$) using the SPSS statistical program (version 16.0, SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1 Color

The color of lablab bean flours was white with high L* values (92.48-94.39) as shown in Table 1. The L* value of LS (97.34) was significantly higher ($p<0.05$) than those of other flours. Longer period of germination (36 and 48 h) resulted in lower L* of flour. This is probably due to enzymatic browning occurs during germination. For a* value, LS showed significantly higher ($p<0.05$) than others, indicating the highest greenness in color of starch. The b* values of flour ranged from 8.4-9.9 (Table 1).

Table 1. CIE color values (L*, a* and b*) of NF, GF and LS.

Sample	L*	a*	b*
NF	94.39±0.17^{b}	-0.36±0.02^{ab}	9.79±0.34^{a}
12GF	93.97±0.51^{b}	-0.38±0.04^{b}	8.68±0.43^{bc}
24GF	93.95±0.35^{b}	-0.43±0.03^{b}	8.40±0.27^{c}
36GF	92.94±0.58^{c}	-0.38±0.07^{b}	9.23±0.50^{ab}
48GF	92.48±0.48^{c}	-0.37±0.04^{ab}	9.92±0.73^{a}
LS	97.34±0.27^{a}	-0.29±0.05^{a}	1.76±0.13^{d}

The different letters in the same column mean significantly different ($p<0.05$)

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

3.2 Chemical composition

Protein content of flour significantly increased ($p<0.05$) with germination effect which ranged from 25.66-28.48% db. The highest protein contents were found in the 36GF and 48GF as shown in Table 2. The results are similar with report of Chinma et al. (2009) who found that protein content of germinated brown tiger nut increased from 10.6-12.4% after 48 h germination. The increasing of protein contents is due to production of some amino acids from protein synthesis during germination. Fat content of all samples ranged from 0.14-1.49%. Ash content ranged from 0.02-10.17%. The LS showed the lowest values of fat (0.14%) and ash (0.02%) due to the extraction process. Crude fiber content also increased with an increase of germination period (1.0-2.1%). The result supported by the study carried out by Uwaegbute et al. (2000) and Chinma et al. (2009) that crude fiber apparently increased with germination time.

Germination had significant effect ($p<0.05$) on carbohydrate content. The 48GF had the lowest carbohydrate content (68.73%). The decrease of carbohydrate may be attributed to the increasing of α-amylase activity, leading to break down of complex carbohydrates to sugars for growing seed during the early stage of germination (Chinma et al., 2009). The LS showed the highest carbohydrate content (88.45%).

Water activity represents water content that microorganisms can use for growth. Lower than 0.6 of water activity indicates microbiological safety from bacteria, yeast or mold. Water activity of lablab bean flour and starch ranged from 0.243-0.455.

3.3 Pasting behavior

The pasting properties of all samples obtained from RVA are presented in Table 3. The germination process had significant effect ($p<0.05$) on the pasting characteristics of lablab bean flour. Germination had no significant effect ($p\geq0.05$) on the pasting temperature of flours which ranged from 86.48-87.58ºC. Peak viscosity of lablab bean flour varied from 619-1018 cP. The longer germination period would affect viscosity of flour. Breakdown of lablab bean flour ranged from 14-49 cP. The 48GF showed significantly higher ($p<0.05$) breakdown than that of NF. This indicates that longer germination provides higher stability of starch granule under high shear condition. For final viscosity, it indicates the ability of samples to form a paste (Kaur and Singh, 2005). Longer germination significant decreased ($p<0.05$) the final viscosity of germinate flours from 1580 cP for NF to 56-1378 cP for 12GF, 24GF, 36GF and 48GF. Setback from peak is useful indicator of starch retrogradation (Phimolsiripol et al., 2011) and this value represents the syneresis of flours when cooling of cooked flours (Kaur and Singh, 2005). Longer germination

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

time significant decreased ($p<0.05$) the setback values by about 23-176% when compared to NF.

Overall, the LS showed the highest values of all viscosities (peak, trough, breakdown, final viscosity, setback from peak). Similar results also found in the report of Jangchud et al. (2003). The results showed that LS had peak viscosity (3941 cP), trough (3021 cP), breakdown (919 cP), final viscosity (5670 cP) and breakdown (2649 cP) higher than NF and GF. In fact, the pasting behavior depends on granule, amylose and amylopectin contents in carbohydrate. Higher purification of starch content showed the greater viscosity.

Table 2. Chemical compositions of NF, GF and LS.

Sample	Fat (%db)	Protein (%db)	Ash (%db)	Crude fiber (%db)	Carbohydrate (%db)
NF	0.81±0.13^{c}	25.66±0.30^{c}	0.12±0.04^{a}	1.00±0.42^{b}	67.20±0.44^{bc}
12GF	1.02±0.07^{b}	27.44±0.49^{b}	0.17±0.14^{a}	1.81±0.42^{ab}	66.89±0.17^{bc}
24GF	0.69±0.15^{c}	27.23±0.44^{b}	0.14±0.07^{a}	1.26±0.10^{ab}	67.72±0.11^{b}
36GF	1.49±0.20^{a}	28.48±0.08^{a}	0.03±0.02^{a}	2.14±0.40^{a}	65.59±0.45^{c}
48GF	0.76±0.04^{c}	28.38±0.42^{a}	0.03±0.02^{a}	2.10±0.56^{a}	66.45±0.88^{c}
LS	0.14±0.03^{d}	N.D.	0.02±0.02^{a}	1.18±0.19^{ab}	88.45±0.86^{a}

The different letters in the same column mean significantly different ($p<0.05$). N.D. means not detected.

Table 3. Pasting properties of NF, GF and LS.

Properties	NF	12GF	24GF	36GF	48GF	LS
PT (ºC)	87.0±1.0^{a}	86.5±1.5^{a}	86.9±1.2^{a}	87.6±0.9^{a}	87.6±0.6^{a}	82.7±0.4^{b}
PV (cP)	1009.7±28.3^{b}	948.7±65.3^{b}	1018.8±93.9^{b}	755.2±86.2^{c}	619.2±55.3^{d}	3941.0±56.4^{a}
Trough (cP)	995.3±26.2^{b}	929.7±59.6^{b}	978.8±69.9^{b}	712.2±81.2^{c}	570.2±44.9^{d}	3021.2±42.8^{a}
BD (cP)	14.3±7.4^{c}	19.0±6.3^{bc}	40.0±24.3^{bc}	43.0±12.5^{bc}	49.0±14.6^{b}	919.8±32.0^{a}
FV (cP)	1580.2±51.5^{b}	1378.3±86.7^{c}	1360.5±74.7^{c}	938.5±85.4^{d}	56.5±53.5^{e}	5670.2±121.5^{a}
SB (cP)	584.8±34.8^{b}	448.7±31.7^{c}	381.7±9.7^{c}	226.3±15.0^{d}	186.3±9.8^{d}	2649.0±122.9^{a}

The different letters in the same row mean significantly different ($p<0.05$). PT, PV, BD, FV and SB indicate pasting temperature, peak viscosity, breakdown, final viscosity and setback from peak, respectively.

3.4 Water, oil and butter absorption capacity

The water (WAC), oil (OAC) and butter absorption capacity (BAC) values of lablab flours and lablab starch are presented in Table 4. The longer germination time had no significant effect ($p \geq 0.05$) on the WAC, OAC and BAC. In general, the WAC is due to the

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

functional group of protein. Hydrophobic interactions may cause the different level of WAC (Chau and Cheung, 1998). In addition, Phimolsiripol et al. (2011) also stated that protein and carbohydrate components can be promote the WAC since these constituents contain hydrophilic parts, such as polar or charged side chains. Jangchud et al. (2003) also found that flour could absorb more water than starch.

3.5 Swelling, solubility, emulsion activity and stability

WSI and SP of the NF were the highest values (27.12% and 11.54 g/g dry weight), respectively. Germination decreased the WSI and SP of flours as shown in Table 5. Nwokocha et al. (2005) found that the SP of lablab bean starch ranged from 30-35%, whereas our result from Table 5 showed that the SP of lablab flours and starch ranged from 8.71-13.16 g/g dry weight. Differences in SP of starchy materials can be attributed to starch content, the presence of impurities (e.g., proteins and lipids) and pre-treatment and processing history (Jangchud et al., 2003). For the EA, it represents the ability of flour can emulsify oil. The results showed that germination had no significant effect ($p \geq 0.05$) in both EA and ES. The LS was the highest of the EA (57.30%) and ES (64.15%), which may depend on carbohydrate content. This indicates that the LS showed the good emulsion activity and stability. This is probably due to some types of polysaccharides can support stabilization of the emulsion by increasing the viscosity of the system (AACC). Lowal (2005) also found that the EA and ES of native lablab flour were 54.3% and 53.9%, respectively, which were higher than our observation. This may cause by different variety of lablab bean. Chau et al. (1998) found that the EA and ES of lablab flour were lower than those of soybean flour

Table 4. Water, oil and butter absorption capacity (g water/g dry sample) of NF, GF and LS.

Flours	WAC	OAC	BAC
NF	6.40±0.14^{a}	6.29±0.22^{a}	6.64±0.38^{a}
12GF	6.12±0.21^{ab}	6.13±0.24^{ab}	6.24±0.26^{ab}
24GF	6.20±0.12^{ab}	6.15±0.12^{ab}	6.21±0.16^{b}
36GF	6.34±0.21^{a}	6.10±0.20^{ab}	6.23±0.16^{ab}
48GF	6.20±0.13ab	5.86±0.26b	6.25±0.19ab
LS	5.94±0.31b	5.91±0.21b	6.43±0.17ab

The different letters in the same column mean significantly different ($p<0.05$).

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

Table 5. Swelling, solubility, emulsion activity and stability of NF, GF and LS.

Sample	WSI (%)	SP (g/g dry weight)	EA (%)	ES (%)
NF	27.12 ± 1.83^{a}	11.54± 1.13^{ab}	48.33 ± 1.44^{b}	46.83 ± 3.88^{b}
12GF	14.03 ± 2.63^{b}	8.71± 0.59^{c}	48.83 ± 1.26^{ab}	48.00 ± 1.80^{b}
24GF	19.39± 3.58^{ab}	10.47± 0.45^{bc}	53.00 ± 6.08^{ab}	52.50 ± 6.61^{ab}
36GF	16.42± 2.46^{ab}	10.31± 0.55^{bc}	45.67 ± 1.15^{b}	48.00 ± 1.80^{b}
48GF	20.20± 3.93^{ab}	11.36± 0.73^{abc}	44.67 ± 0.58^{b}	45.00 ± 2.50^{b}
LS	21.26± 9.08^{ab}	13.16± 1.72^{a}	57.30 ± 3.99^{a}	64.15 ± 7.58^{a}

The different letters in the same column mean significantly different ($p<0.05$)

3.6 α-galactosides analysis

Germination resulted in significant decrease of α-galactosides content as shown in Fig. 1. The α-galactosides of the NF was the highest value (4.23g/100 g flour), whereas the reduction of α-galactosides was found in germinated flour. Longer germination period reduced the α-galactosides content. The germinated flour as 12, 24, 36 and 48 h contained lower than 23.87, 57.92, 75.41 and 77.54%, respectively of the α-galactosides when compared to the NF sample. Ramakrishna et al. (2006) also reported that raffinose and starchyose contents in lablab bean seeds after 32 h germination reduced by about 50% from native seed. Kadlec et al. (2006) stated that α-galactosides contents in pea seeds reduced by 79.8% from original value after three days of germination. Kadlec et al. (2008) proposed that the activation of enzyme α-D-galactosidase help to the increasing of sucrose levels during the germination process due to the fact that raffinose, starchyose and verbascose are converted into sucrose, raffinose and stachyose, respectively this can be seen that the sucrose content increased when germination was applied.

4. Conclusion

Germination could improve the nutritional value of lablab bean by reduction of anti-nutrition factor and increase of protein content. The germinated flour for 36 h had the highest protein content (28.35%) and could reduce α-galactosides 75.41% from the native lablab bean flour. It is suggested that germinated flour can be used as a good source of protein with lower anti-nutritional factor and small effect on the functional properties. However, the applications of lablab bean flour and starch is required to further investigation.

Food and Applied Bioscience Journal, 2013, vol.1(2): 69-80

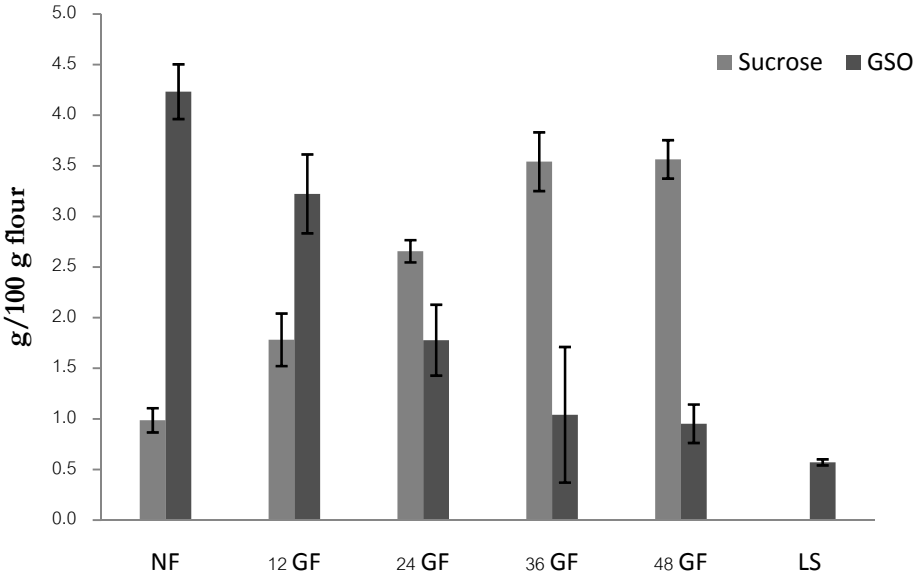

Figure 1. Galactosyl-sucrose oligosaccharides (GSO) and sucrose contents of NF, GF and LS.

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