

Effects of Protein Isolate from Hyacinth Beans (*Lablab purpureus* (L.) Sweet)

Seeds on Cake Characteristics

Achmad SUBAGIO^{1*} and Naofumi MORITA²

¹ Laboratory of Chemistry and Biochemistry of Agricultural Products, Faculty of Agricultural Technology, University of Jember, Jl. Kalimantan I Jember 68121 Indonesia

² Laboratory of Food Chemistry, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Nakaku, Sakai, Osaka 593-8531, Japan

Received June 28, 2006; Accepted August 16, 2007

Effects of protein isolate prepared from hyacinth bean (*Lablab purpureus* (L.) Sweet) seeds on cake characteristics were studied. The addition of up to 1% of the protein isolate from hyacinth bean seeds could improve the baking properties of the cake. The volume development and specific volume of the cake with 1% protein isolate were 206.0%, and 2.63 ml/g, whereas those of the control were 160.3% and 2.17 ml/g. Isolate additions of up to 1% softened the cake, as indicated by decreases in texture values, i.e., 184, 112, 100 and 89 g of force for the control, 0.5, 0.75 and 1.0% protein isolate, respectively. However, when more than 1% protein isolate was added, the cake quality tended to decrease compared with that at 1%. Moreover, the more protein isolate was added, the more vivid the colour and the lower the staling rate. Therefore, the protein isolate from hyacinth beans merits further assessment as a practical food additive.

Keywords: cake improver, hyacinth bean, protein isolate

Introduction

Indonesia is rich in non-oilseed legumes, such as hyacinth bean (*Lablab purpureus* (L.) Sweet), which is commonly planted in marginal land with less of attention. According to Stephens (1994), hyacinth bean has many local names, such as *lablab*, *bonavist*, Chinese flowering, Egyptian, Pharaoh, shink, wild field, and Indian bean. In Indonesia, the local name of this bean is *koro komak*. It is generally considered to have originated in Southeast Asia. However, some authorities place its origin in Africa, where it has been known since the eighth century. Presently, it is widely cultivated throughout the tropics and subtropics. According to Indonesian farmers, productivity of hyacinth beans plant on dry land can reach to 1000–1200 kg of dry seeds/ha. Unfortunately, no data is available for the total production of the seeds in Indonesia. In some hyacinth bean-producing areas, the young pods are boiled separately as a vegetable, or together with corn as an soup. The dry seeds are cooked together with rice after soaking water for one night to supply protein. The seeds are also

used as raw materials of tempeh, an traditional Indonesian fermented food usually made from soybeans.

Previous study (Subagio, 2006) has reported that hyacinth bean seeds have a moderate protein content ($17.1 \pm 1.5\%$), and low concentration of HCN (1.1 ± 0.1 mg/100 g). However, before the seeds can be used as food, treatment is needed to reduce their anti-nutritional components, such as trypsin inhibitor (0.15 ± 0.02 TIU/mg) and phytate (18.9 ± 0.2 mg/g). Using an isoelectric method followed by washing with ethanol, protein isolate produced from the seeds had light colour, neutral odour, high protein content ($89.78 \pm 0.82\%$), and low ash ($2.97 \pm 0.36\%$). The protein isolate had also good functional properties such as solubility, foaming capacity, and emulsifying activity. However, the yield was low (7.38 ± 0.2 g per 100 g of the seeds) (Subagio, 2006).

Cake is a food system that requires good emulsion performance and foam development. A sponge-cake batter with emulsifier can be whipped in a first stage process, in which the incorporation and stability of air bubbles are improved, resulting in a finer dispersion and higher cake quality (Richardson *et al.*, 2002). Accordingly, the addition of protein isolate from hyacinth bean seeds, which has good capacities as

*To whom correspondence should be addressed.

E-mail: subagio@ftp.unej.ac.id

an emulsifier and foaming agent, may improve the quality of cake. The aim of this study was to investigate the influence of protein isolate from hyacinth bean seeds on baking properties, second Maillard reaction products, colour and sensory properties of cake. The staling rate of the cake was also studied by the measurement of texture during storage.

Materials and Methods

Materials Hyacinth beans used in this study were collected from a farm in Bondowoso City, East Java, Indonesia. After arrival in the laboratory, seeds were sorted to remove immature and defective beans. The sorted beans were then stored in a cold room (4–6°C) until use. Wheat flour, sugar, shortening, egg, and emulsifier (Ryoto Ester SP, Mitsubishi Chemical Ltd., Japan) were obtained from local market. The chemicals and solvents used were of guaranteed grade.

Preparation of the protein isolate Protein isolate was prepared using isoelectric method as reported previously (Subagio, 2006). The protein isolate had $89.78 \pm 0.82\%$ of protein on dry basis.

Cake preparation A standard cake formulation was used for the study (Table 1). The protein isolate was added in concentrations of 0, 0.5, 0.75, 1.0, 1.25 and 1.5% against wheat flour. The dry ingredients were combined and sifted well. Egg, crystal sugar and emulsifier were whipped for 6 min at high speed using a Philips HR 1500 (Indonesia) mixer. The fat and flour mixture was transferred to the whipped egg, and mixed manually until homogenized. Cake batter was then transferred to a cake pan and baked at 180°C for 60 min using an oven (Selecta Oven, Spain).

Measurement of cake characteristics Loaf volume of the cake was determined using the seed displacement method. Specific volume of cake was calculated by dividing volume to weight of the cake. Baking loss of cake was expressed using the percentage of decrement of cake weight after baking and the weight of cake batter. Firmness or texture of cake was determined using a Rheometer (RHEOTEX Type SD-700, Japan) with a standard plunger at 7 mm of penetra-

tion.

To evaluate the colour of the seeds and the protein isolate, Chromameter CR-100 (Minolta, Japan) was used with C illumination for daylight, and an averaging mode to produce 8 replications per sample. The instrument was calibrated externally with a standard white tile, and the tristimulus coordinates L^* , a^* , and b^* (CIE Lab colour scale) system was applied to express the colour (McGuire, 1992; Voss, 1992; Gonnet, 1999).

Secondary products of the Maillard reaction in the cake were measured. A sample of cake (0.5 g) was stirred in ethanol (10 ml) for 10 min and centrifuged ($2500 \times g$ for 10 min). The supernatant was collected, and the residue was re-extracted twice using the spectrophotometer method of Lericci *et al.* (1990, with some modifications) with ethanol. The collected supernatant was combined, and the volume was adjusted to 30 ml using ethanol. Absorbance of the supernatant was measured at a wavelength of 420 nm by spectrophotometer (PRIM, Secomam, France) to express the concentration of the Maillard reaction secondary product.

Staling rate Staling rate of the cake was determined by storing the cake in a box placed at room temperature for 5 days. Each day, cake texture was measured by Rheometer (RHEOTEX). Changes in the texture value were calculated by subtracting the texture value of the cake after storage with before storage. To prevent microbial spoilage, the box was exposed to UV light for 15 min after opening.

Sensory evaluation A trained panel of 25 judges carried out the sensory evaluation of cake samples by assigning scores for crumb cell uniformity (1 = uneven, 5 = even), colour (1 = in vivid, 5 = vivid), taste (1 = foreign, 5 = typical), aroma (1 = foreign, 5 = typical), texture (1 = very firm, 5 = very soft), mouth feel (1 = tough, 5 = very tender), and overall preference (1 = very bad, 5 = very good).

Statistical analysis Statistical analysis of data was performed using analysis of variance (ANOVA) on 6 experimental groups in triplicate. The experimental groups were then separated by using Duncan's new multiple range test, as

Table 1. Cake formulation.

Ingredients	g
Wheat flour	75
Sugar	75
Egg	75
Shortening	30
Emulsifier (Ryoto Ester SP)	1
Protein isolate*	0, 0.375, 0.5625, 0.75, 0.9375 and 1.125

*Calculated as percentage of wheat flour: 0, 0.5, 0.75, 1.0, 1.25 and 1.5%.

Table 2. Effect of the addition of protein isolate from hyacinth bean seeds on certain cake characteristics*.

Protein isolate	Volume development (%)	Specific volume (ml/g)	Baking loss (%)	Texture (g force)
0%	160.3 c	2.17 c	17.25 a	184 a
0.5%	185.0 ab	2.33 b	14.70 a	112 c
0.75%	189.0 ab	2.44 ab	14.55 a	100 cd
1.00%	206.0 a	2.63 a	15.50 a	89 d
1.25%	188.7 ab	2.50 ab	16.20 a	148 b
1.50%	180.7 bc	2.44 ab	16.50 a	165 ab

*Values in the same column followed by different letter showed significant differences ($P < 0.05$). Number of experiments: 3.

described by O'Mahony (1986).

Results and Discussion

Cake characteristics The effects of addition of protein isolate from hyacinth beans on the baking properties of cake are presented in Table 2. The addition of the protein isolate in low concentrations could increase the volume development of the cake. However, the volume development would decrease when the protein isolate was added at high concentrations. The addition of 0.5, 0.75, and 1.0 % could increase the volume development to 185.0, 189.0, and 206.0 %, respectively. These values are much higher than that of the control (160.3%). However, the volume development decreased to 188.7 and 180.7% by the addition of 1.25 and 1.5 % protein isolate, respectively. The increase in volume development may be related to the foaming capability of the protein isolate of hyacinth bean seeds, which can reach 232 ml/g (Subagio, 2006). The volume of cake is affected by the gas expansion that occurs during mixing (Kilara, 1994). Since the protein isolate could decrease the surface tension of gas/liquid, the addition of the isolate can increase the foam formation. However, the addition at high level will disrupt the gluten matrix, resulting in the decrease of volume after baking, since the structure of the cake was not sufficient to retain the original volume.

Accordingly, the addition of protein isolate from hyacinth bean seeds had a similar effect on the specific volume of the cake. As shown in Table 2, addition at a low level could significantly increase the specific volume of the cake. The addition of 0.5, 0.75, and 1.0 % increased the specific volume to 2.33, 2.44, and 2.63 ml/g, respectively. These values were 7.4, 12.4 and 21.2 % higher than that of the control (2.17 ml/g). However, the specific volume would significantly decrease to 2.50 and 2.44 ml/g when 1.25 and 1.5%, respectively, of protein isolate was added. Similar to volume development,

the increase in the specific volume may be due to the foaming capability of the protein isolate of hyacinth bean seeds, which can reach 232 ml/g (Subagio, 2006).

Moreover, the addition of protein isolates from hyacinth bean seeds did not affect the baking loss of the cake significantly (Table 2). Although protein isolate from hyacinth bean seeds have a high water holding capacity (WHC) of 321% (Subagio, 2006), the addition did not significantly affect the baking loss. This may be due to the high temperature (180 °C) for baking, which could annul the effect on WHC increment. Since the baking losses of the samples were not significantly different, the specific gravity of the cake was markedly affected by changes in the cake volume influenced by the addition of the protein isolate.

Table 2 also shows the effect of the addition of protein isolate from hyacinth bean seeds on cake texture. The addition of up to 1% isolate softened the cake as shown by the following texture values: 184, 112, 100 and 89 g force for 0 (control), 0.5, 0.75 and 1.0 % isolate, respectively. However, the value of the cake texture increased to 148 and 165 g force when the concentration of the isolate was 1.25 and 1.5%, respectively. Therefore, the addition of up to 1% isolate could soften the cake, but more than 1% could harden the cake. Cake texture is influenced by the three-dimensional structure, and the size and distribution of gas cells in the cake. The protein isolate from hyacinth bean seeds might decrease the surface tension of the colloidal system, resulting in a decrease in the globule size of the foam during cake mixing. This would improve the distribution of gas cells in the cake, resulting in shortening of the cake texture, as shown in cross section of the cake (Figure 1). However, since the protein isolate could disturb the gluten matrix, the addition of the isolate at high levels might harden the cake texture significantly.

The data on the effects of protein isolate from hyacinth bean seeds on the cake colour are presented in Table 3. As is

shown in this table, addition of the protein isolate resulted in cake colour becoming more brownish red, as expressed by H values. Cake without addition of the protein isolate had H value of $67.23 \pm 2.33^\circ$, whereas with addition of 0.5, 0.75, 1.0, 1.25 and 1.5% of the protein isolate, the H values of cake colour were 63.43 ± 3.51 , 60.50 ± 2.25 , 56.54 ± 1.37 , 56.56 ± 2.89 and $54.59 \pm 3.04^\circ$, respectively. Since H values are the angles of dots having coordinate of a^* and b^* , the addition of protein isolate would increase the value of a^* , the degree of redness, and would decrease the value of b^* , the degree of yellowness (Table 3).

These phenomena coincided with the results of analysis of secondary products of the Maillard reaction, which are pigments bearing a brownish red colour, as shown in Figure 2. Addition of 1% protein isolate increased the secondary products to nearly two times of that of the control. However, the addition of more than 1.25% did not further increase the values of the secondary products. The Maillard reaction involves the reaction of aldehydes with amines, and through numerous reactions, dark pigments (melanoidins) are formed (Bailey, 1998). Therefore, the addition of protein isolate would increase the concentration of the reactant, especially the amine compounds, resulting in more melanoidins, and more intense brownish red colour. However, when the addi-

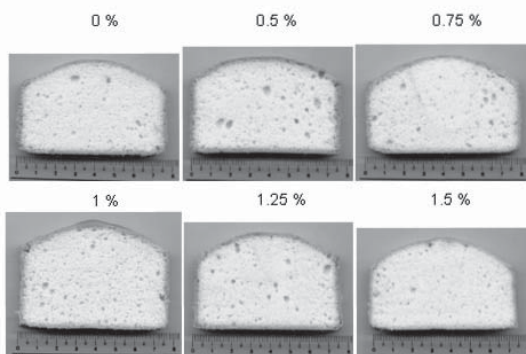


Fig. 1. Cross sections of cakes with various concentrations of protein isolate from hyacinth bean seeds.

tion of the protein isolates would provide amine compound in the imbalance amount with the aldehyde compounds, the Maillard reaction would not increase further, as shown in the 1.25 and 1.5% isolate results.

Staling rate Further observation of the cake textures during storage showed that the more protein isolate was added, the lower the rate of staling. The addition of the isolate decreased changes in the texture value of the cake during storage (Figure 3A). As a result, although the addition of the protein isolate up to 1% could soften cake texture, while more than 1% could harden the texture (Table 2 and Figure 3B-solid bar), the texture remained unchanged four days after baking (Figure 3B-open bar). Therefore, the more protein isolate was added to the cake, the lower the staling rate. These results agreed with the findings of Eliasson and Larsson (1993), which clarified that protein affects the staling rate in several ways: (a) protein-starch interactions decrease the retrogradation of the starch; (b) protein affects the rheological properties in the continuous phase; and (c) protein affects the distribution of water.

Sensory properties Sensory properties of the cake af-

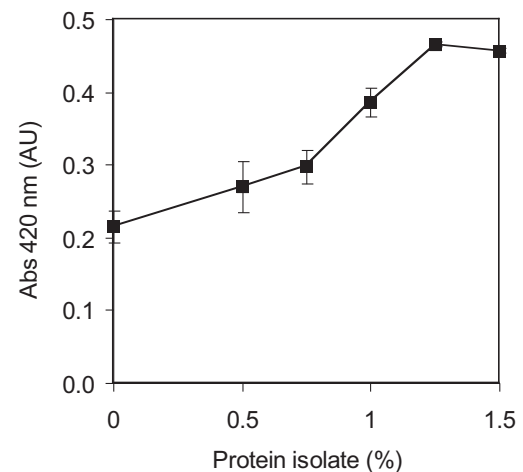


Fig. 2. Effect of the addition of protein isolate from hyacinth bean seeds on the Maillard reaction in cake. Number of experiments: 3.

Table 3. Effects of protein isolate from hyacinth bean seeds on cake colour.

Protein isolate	Colour parameters				
	L	a^*	b^*	c^*	H
0%	78.67 ± 0.18	6.61 ± 0.32	15.82 ± 1.14	17.16 ± 0.94	67.23 ± 2.33
0.5%	78.47 ± 1.13	7.05 ± 0.16	13.32 ± 2.52	15.99 ± 2.31	63.43 ± 3.51
0.75%	77.57 ± 0.99	7.34 ± 0.82	13.09 ± 2.40	15.02 ± 2.47	60.50 ± 2.25
1.00%	77.28 ± 0.21	7.75 ± 0.82	11.77 ± 1.60	14.09 ± 1.77	56.54 ± 1.37
1.25%	78.18 ± 0.44	7.71 ± 0.86	11.74 ± 1.76	14.06 ± 1.82	56.56 ± 2.89
1.50%	77.10 ± 0.04	8.35 ± 0.47	11.78 ± 1.08	14.45 ± 0.90	54.59 ± 3.04

Number of experiments: 3.

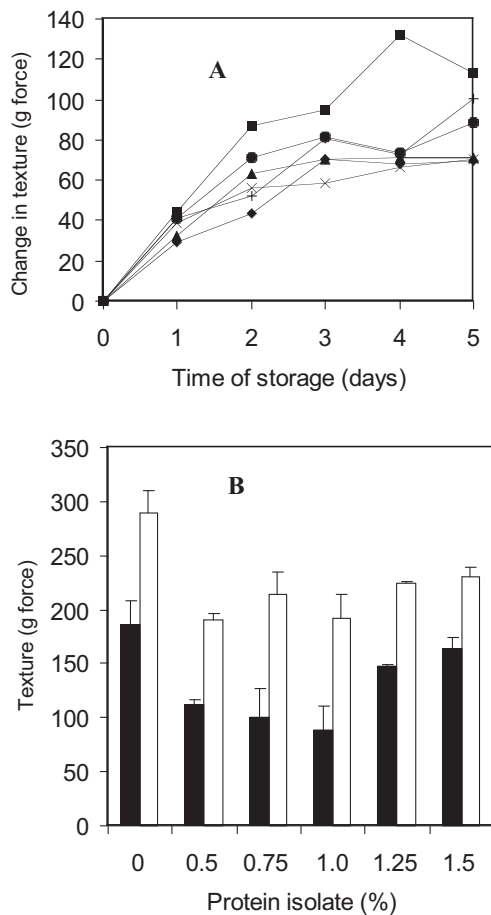


Fig. 3. Effects of the addition of protein isolate from hyacinth bean seed on staling rate of cake. (A) Changes of cake texture during storage. ■ : Control; ◆ : 0.5%; ▲ : 0.75%; × : 1.0%; ● : 1.25%; and + : 1.5% of the isolate. (B) Cake texture values after 0 (solid) to 4 days (open) of storage. Number of experiments: 3.

ter baking affected by the addition of the protein isolate are shown in Figure 4. The panellists clearly judged that addition of the protein isolate had significant effects on colour, texture, mouth feel, uniformity (Figure 4A), and overall preference (Figure 4B). However, the addition did not have a significant effect on the taste and aroma of the cake (Figure 4A). These results coincided with the physical and chemical analysis as described in previous sections.

Conclusion

In conclusion, the addition of the protein isolate from hyacinth bean seeds in concentrations up to 1% could improve the properties of the cake by increasing loaf volume, increasing specific volume, softening the texture and improving overall preference. When protein isolate of more than 1% was added, these properties tended to decrease compared with the cake with 1% isolate. However, the more protein isolate was added, the more vivid the colour and the lower

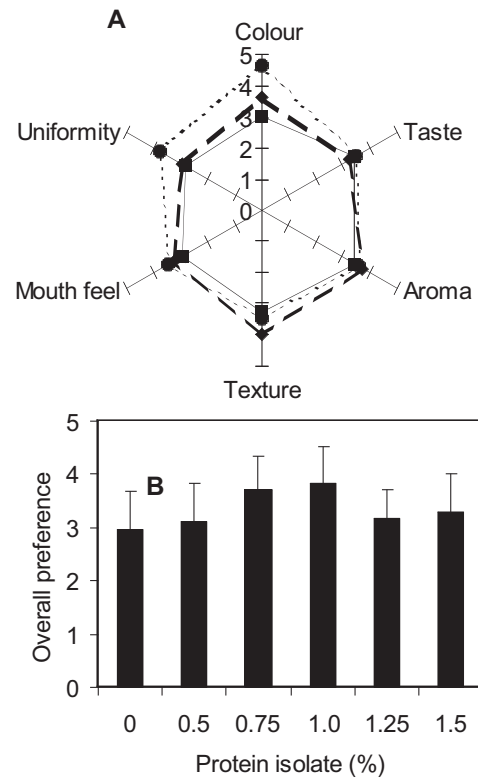


Fig. 4. Effects of the addition of protein isolate from hyacinth bean seed on sensory properties of cake. (A) Descriptive properties of three cakes (■ : Control; ◆ : 1.0%; and ● : 1.5%); (B): Overall preference. Number of experiments: 3.

the rate of staling.

At present, we could not elucidate the mechanism of the improving effects of protein isolate from hyacinth bean seeds for cake making. However, the results of the present study indicate that this isolate has merit for further assessment as a practical and functional food additive.

Acknowledgements Achmad Subagio was supported by the International Foundation for Science (Sweden), the Committee on Scientific and Technological Cooperation of the Organization of Islamic Conference (Pakistan), and the Prohibition of Chemical Weapons (The Netherlands).

References

- Bailey, M. E., (1998). Maillard reactions and meat flavour development. In: *Flavour of Meat, Meat Products and Seafoods* (edited by S. Shahidi). pp. 267-289. London, UK: Blackie Academic & Professional.
- Eliasson, A. C., & Larsson, K., (1993). *Cereals in Breadmaking*. New York, USA: Marcel Dekker, Inc.
- Gonnet, J. (1999). Colour effects of co-pigmentation of anthocyanins revisited 2. A colourimetric look at the solutions of cyanin copigmented by rutin using the CIELAB scale. *Food Chem*, **66**, 387-394.

- Kilara, A. (1994). Whey protein functionality. In: Protein Functionality in Food Systems (edited by N. S. Hettiarachchy, and G. R. Ziegler). pp. 325-355. New York, USA: Marcel Dekker, Inc.
- Lerici, C. R., Barbanti, D., Monzano, M., & Cherubini, S. (1990). Early indicators of chemical changes in food due to non-enzymatic and enzymatic browning reactions 1. *Lebensmittel Wissenschaftund Technology*, **23**, 289-294.
- McGuire, R.G. (1992). Reporting of objective colour measurements. *Horticulture Science*, **27**, 1254-1255.
- O'Mahony, M. (1986). Sensory Evaluation of Food. New York, USA: Marcel Dekker, Inc.
- Richardson, G., Langton, M., Faldt, P., & Hermanson, A. M. (2002). Microstructure of α -crystalline emulsifier and their influence on air incorporation on cake batter. *Cereal Chem*, **79**, 546-552.
- Stephens, J. M. (1994). Bean, Hyacinth -- *Dolichos lablab* L., or *Lablab purpureus* (L.) Sweet. Florida, USA: University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS).
- Subagio A. (2006). Characterization of hyacinth bean (*Lablab purpureus* (L.) Sweet) seeds from Indonesia and their protein isolate. *Food Chem*, **95**, 65-70.
- Voss, D. H. (1992). Relating colorimeter measurement of plant colour to the Royal Horticultural Society colour chart. *Horticulture Science*, **27**, 1256-1260.