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Research Article
Effects of Xanthan Gum and Protein Isolates on the Functional Properties of Lablab Bean (*Dolichos lablab*) Seeds Starch

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Abstract

Background and Objective: Lablab bean seeds (*Dolichos lablab*) contain high protein and resistant starch. The resistant starch possesses an important function for health. However, it has poor functional properties, so its use in food products is limited. The purpose of this study was to improve the functional properties of starch from lablab bean seeds by interacting it with proteins and gum xanthan. **Materials and Methods:** Starch was isolated from the flour that has been made free from fat and protein. The interaction between lablab bean seed starch with protein fraction and xanthan gum was conducted using the following steps: Five gram protein fraction was mixed with xanthan gum in the amounts of 0.1, 0.2 and 0.3 g and without xanthan gum as a control. The mixture was diluted in 100 mL water using a 250 mL beaker glass and was brought to a pH of 7 using 0.1 M NaOH . The solution was stirred for 10 min until homogeneous. A total of100 g (dry weight) of lablab bean seed starch was added to each treatment and stirred. In all treatments, the mixture was heated to 120°C for 2 h and cooled in a desiccator for 1 h. The flours were crushed and sieved with a (100 sized) mash and were observed. A randomized block design was used for this study. **Results:** The functional properties of starch after interacting with protein (5%) and gum xanthan (0.1-0.3%) were better than those without interaction, especially in functionality related to viscosity, swelling power, water holding capacity and emulsion capacity; however, the gelatinization temperature rose above 80°C while starch only requires 76°C. **Conclusion:** This study found that functional properties of starch interacted with 5% protein and 0.2% gum xanthan yielded the best flour. It is recommended to be used as an ingredient for food products such as noodles, bread, biscuits, snacks and others.

Key words: Starch, xanthan gum, lablab bean seeds, functional properties, amylose content, food products

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Starch from bean seeds is a type of starch that is resistant to digestion (resistant starch) because it is slowly digested and absorbed[1]. The seeds of lablab bean contain 68% carbohydrate, which is dominated by starch[2]. The starch of lablab bean seeds is a type of carbohydrate that is resistant to digestion because of its high amylose content. The resistant starch has an essential physiological role in human health because it has physiological effects that are similar to fibrous foods[3]. Starchy foods in the diet, such as rice, bread, cakes and noodles, consist mostly of easily digestible starch. The problem is that starch that is easily digested contributes to chronic diseases in humans and animals. Therefore, starch resistant to digestive enzymes is widely studied[4]. Natural starch from seeds of beans, including lablab bean seeds, has some constraints if it is used as food ingredients because it has poor functional properties. Some researchers[5] have investigated the interaction of starch with proteins and its contribution to food texture. Zhang and Hamaker[6] studied the three-way interaction between starch, protein and lipids that affect viscosity profiles using isolated sorghum starch, whey protein isolate and free fatty acids. It has been reported that bean starch, especially mung bean (*Vigna radiata*) starch, possesses a high-quality raw material for the production of noodles and other food industries[7].

The aim of this study was to improve the functional properties of starch from lablab bean seeds that are interacted with proteins and gum xanthan as a binding agent so that lablab bean seeds can be used as food ingredients. It is expected that the ingredients can be implemented for the development of food products such as noodles, bread, biscuits, snacks and others.

MATERIALS AND METHODS

The primary ingredients include lablab bean seeds obtained from the experimental garden of the Faculty of Agriculture from Widyagama University of Malang. Gum xanthan (food grade) was obtained from Chemical company in Makmur Sejati Malang Indonesia. All materials for chemical analyses were pure, class analysis materials.

Starch preparation: Starch isolation from flour that is free from fat and protein was conducted as follows : The flour was neutralized with 0.1 M HCl until the pH was 7, flour was washed with distilled water three times to clean up the remaining NaOH and NaCl in the flour. The separation of starch was carried out by depositing the flour in water for 12 h. The precipitate was separated, dried and crushed. The flour was then sifted with a size of 100 mash.

Preparation of protein isolate: Protein isolation was performed according to a previous method described by Sukamto *et al.*[8]

The interaction between lablab bean seed starch, protein fraction and xanthan gum was carried out as follows: Five grams of protein fraction was mixed with xanthan gum in 0.1, 0.2 and 0.3 g and without xanthan gum as a control. The mixtures were diluted with distilled water to 100 mL and were brought to a pH of 7 by adding 0.1 M NaOH. The solution was stirred using a magnetic stir barr for 10 min until it was homogeneous. The starch of lablab bean seeds (100 g dry weight) was added to each treatment and stirred. The samples were heated in an oven at 120°C for 2 h to evaporate the water and make cause interaction. Samples were cooled in a desiccator for 1 h and were subsequently crushed and sieved with a 100 (size) mash and observed. A randomized block design was used for the study.

Yield of starch: The yield of starch (%) from the flour was calculated using the following formula:

$$\text{Starch yield (\%)} = \frac{\text{The starch extract}}{\text{The amount of flour}} \times 100$$

A proximate analysis was conducted using the AOAC methods[9]: The moisture content was obtained by heating the sample until it reached 105°C in the oven until the samples reached a constant weight. The protein was determined by total nitrogen. Then, total nitrogen was calculated by multiplying the protein by 6.25. The lipids was determined by soxhlet methods and ash content was obtained by heating sample in an oven at 550°C until the sample transformed into the ash. All proximate analysis was determined based on the starch and flour from lablab bean seeds.

The structure of the granules: The microstructure was observed using a TM 3000 Hitachi Scanning Electron Microscope (SEM) manufactured by Hitachi High-Technologies Corporation Tokyo Japan. Micrographs were used to determine the morphology of the granules[10].

Swelling power: The expanding ability was observed using Daramola and Osanyinlusi[11] methods, which were modified as follows: 0.1 g of sample was weighed and put in a test tube and 10 mL distilled water was added. The mixture was heated in a water bath at 50, 60, 70, 80, 90 and 100°C for 30 min at each temperature. The tube and its contents were centrifuged at a rate of 1500 rpm for 20 min. The supernatant was separated by decantation and the starch paste was weighed. Swelling power was calculated using the following formula:

$$\text{Swelling power} = \frac{\text{Weight of the starch paste}}{\text{Weight of dry starch}}$$

Temperature of gelatinization: The temperature of gelatinization was measured as follows: Briefly, 1 g of the sample was put into a 20 mL beaker glass and 10 mL water was added. The dispersed sample was heated on a hot plate. The temperature of gelatinization was determined using a thermometer placed on the suspension as the gel occurred[12].

Water Holding Capacity (WHC): The water absorption capacity was measured as follows: five gram of the sample was added to the water until it reached 80% w/v in the centrifugation tube. The tube was weighed before the sample was added. The tube containing the sample and water was shaken using a vortex mixer for 2 min. It was then centrifuged at 400 rpm for 20 min. The supernatant was decanted, then, the weight of the tube and the sample was weighed. The weight is calculated and expressed as the weight of the water-bound by a dry sample of 100 g[13].

Emulsion capacity: Emulsion Capacity was observed using Omojola *et al*.[12] methods as follows: Briefly, 1 g of the sample was added with 5 mL H_2O and it was dispersed with a vortex mixer for 30 sec. It was then added to 5 mL vegetable oil and shaken for 30 min. The suspension was centrifuged at 1600 rpm for 5 min. Separate oil volumes were read from within a scaled centrifuge tube. Emulsion capacity is the amount of oil emulsified per gram of the sample.

Viscosity: The sample (4%) was stirred and heated to various temperatures (50-100°C). The viscosity change was determined using an Elcometer Viscometer.

RESULTS AND DISCUSSION

Yield starch and proximate composition: The yield of starch from lablab bean seeds was 50.8±2% and the results of the proximate analysis of flour and starch are presented in Table 1. Starch yield from lablab bean seeds is higher than the amount of starch extracted from winged beans, peanut seeds and Pinto beans. Sung and Stone[14] reported the starch extracted from winged beans is 25.3% and is 13.8 and 9.6% from groundnut seeds and Pinto beans, respectively. The starch content of lablab bean seeds is still higher than the starch content of red beans, which was isolated and measured by Shimelis *et al*.[15]. The amount of starch extracted from lablab bean seeds is similar to the starch content isolated from mung bean. The yield of starch extracted from mung bean seeds ranges from 54.73-57.99%[16].

Table 1: Proximate compositions of starch and flour from lablab bean seeds

	Amount (g/100 g)	
Components	Flour	Starch
Water	11.40±0.50	11.20±0.05
Fat	1.49±0.05	0.20±0.02
Crude Fiber	10.79±0.50	1.50±0.04
Protein	20.86±1.00	1.00±0.05
Ash	3.78±0.55	0.18±0.02

Lablab bean seeds starch has a chemical component that is very different from the flour (Table 1). The proximate composition of lablab bean seeds starch is similar to the starch isolated from mung bean seeds, which is reported by Sung and Stone[14], having a moisture content of 9.7%, protein 0.8%, fat 1 and 0.1% ash, respectively.

Microstructures of granular: The microstructure of granules resulted from the interaction between starch with protein and gum xanthan, which was observed using a Scanning Electron Microscopy (SEM) and is presented in Fig. 1. The interaction between starch with protein and gum xanthan (at a pH of 7) indicates that the use of gum xanthan affects the structure of the granule. The greater the concentration of xanthan gum that is used, the larger the starch granules that are bound by protein and gum xanthan (Fig. 1). Similar research has been conducted by Nayebzadeh *et al*.[17], who investigated interactions between whey globulin, xanthan gums and polysaccharides (at a pH of 7) and revealed that polysaccharides are bound in globulin tissue.

Swelling power (SP): The effect of gum xanthan on the swelling behavior of the starch interaction with protein indicates that there is a change in the amount of water absorbed into the starch granules during heating. The behavior of water absorption into the interaction product increased from 80-90°C (Fig. 2). This condition is related to the starch gelatinization properties of the lablab bean seeds, which is relatively high at 89.16°C (Fig. 3a). Omojola[13] explained that the behavior of water absorption into a material increases with temperature. The results

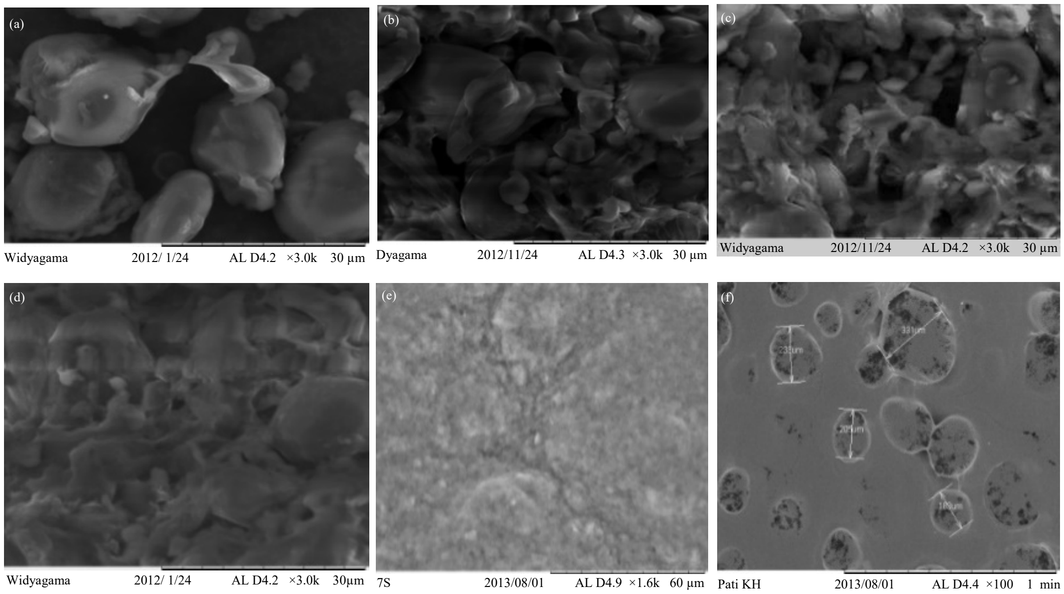

Fig. 1(a-f): The scanning electron microscopy of granular flour (1200× magnification) and xanthan gum (XG) 100× magnification (a) Microstructures of starch of lablab bean seeds at 1200× magnification, (b) Microstructures of interaction starch with protein 5% and XG 0.1%, (c) Microstructures of interaction starch with protein 5% and XG 0.2%, (d) Microstructures of interaction starch with protein 5% and XG 0.3%, (e) Microstructures of protein isolate of lablab bean seeds and (f) Microstructures of XG

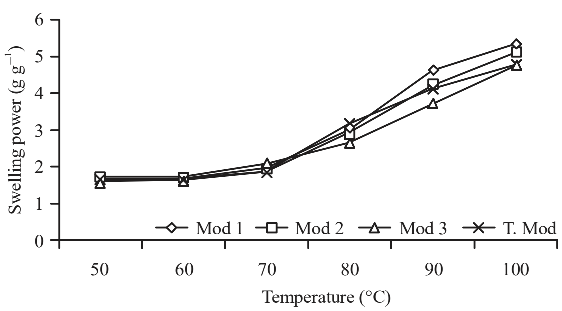

Fig. 2: Effect of protein and xanthan gum on the swelling power (SP) of lablab bean seeds starch

Mod 1: Protein 5% and XG 0.1%, Mod 2: Protein 5% and XG 0.2%, Mod 3: Protein 5% and XG 0.3%, T. Mod: Starch of lablab bean seeds as a control

revealed the effect of gum xanthan on interaction products between starch and protein tends to increase after the temperature exceeds from 70-100°C. We assumed that gum xanthan can serve to maintain the stability of the interaction products. The existence of the branches of mannose acetate, mannose and glucuronic acid in the molecular structure of gum xanthan causes the formation of strong colloidal tissue so that the viscosity is relatively stable against temperature and pH changes[18].

Gelatinization temperature, water holding capacity and emulsion capacity: The results of the gum xanthan effect on gelatinization temperature, water holding capacity (WHC) and emulsion capacity (EC) of the starch interaction product from lablab bean seeds with protein are presented in Fig. 3a-c. The temperature of gelatinization in the interaction product is higher than the temperature of gelatinized starch without the interaction. This is due to the stability of starch after it is interacted with proteins and gum xanthan.

Water holding capacity: The ability to absorb and retain water from the interaction of starch with protein and gum xanthan tends to be higher than before this interaction (Fig. 3b). The concentration of gum xanthan's ability to absorb water is increased. Water absorption occurs when there is an interaction between the solvent (water) and solid (interaction product). Water absorption capacity increased in the presence of protein and gum xanthan. Both materials also have hydrophilic functional groups. Chavan *et al.*[19] explained that differences in the water-binding capacity of protein isolates also depend on the characteristics of protein conformation and the balance of hydrophilic and hydrophobic amino acids. In addition, the presence of gum xanthan possesses a good

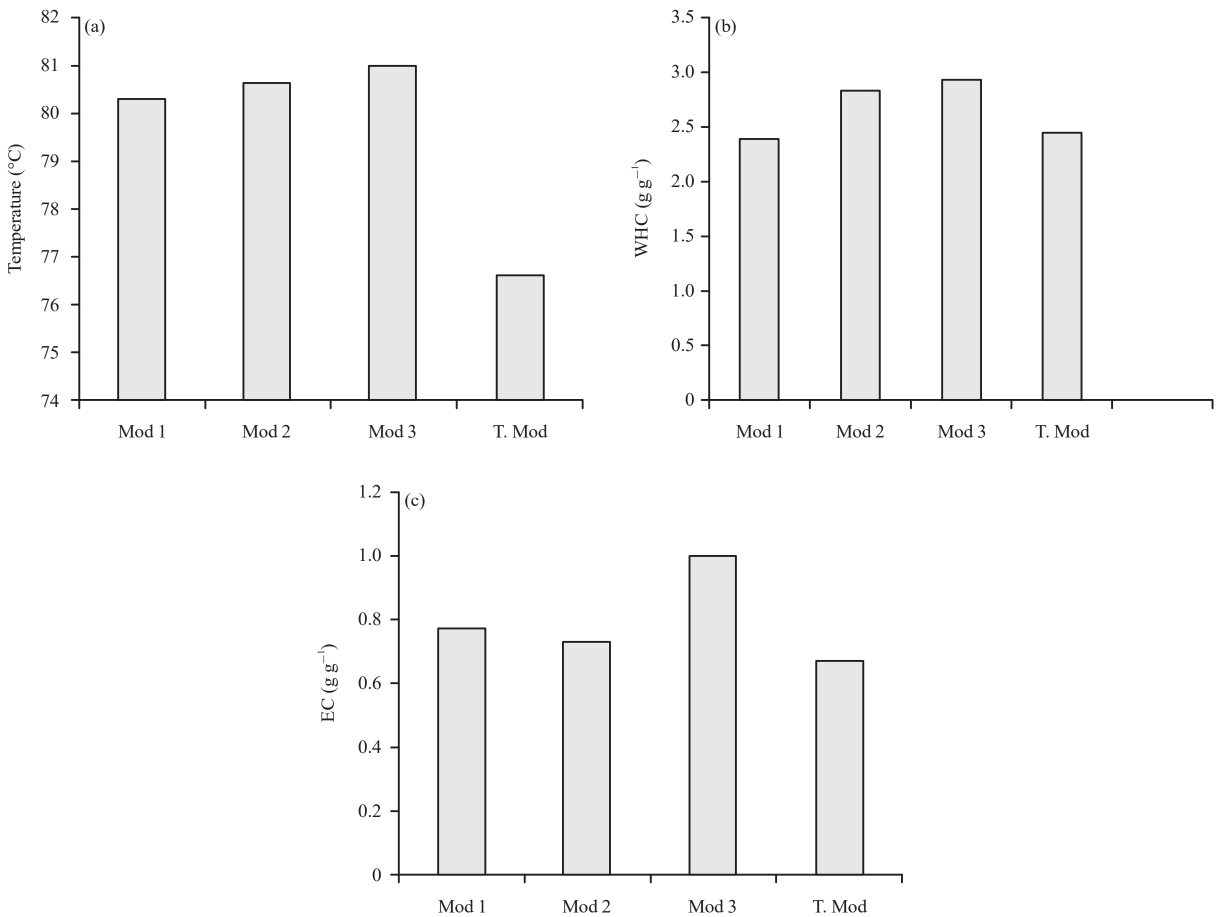

Fig. 3(a-c): Effect of xanthan gum and protein on (a) Gelatinization temperature, (b) Water holding capacity and (c) Emulsion capacity of starch

degree of solubility, thus it easily interacts with water. This condition will also improve the ability of the material to bind water.

Emulsion capacity (EC): Emulsion capacity (EC) reflects the product's ability to interact with water and oil during the formation of emulsions. The EC of starch interacting with protein and gum xanthan exhibits a tendency to increase the emulsion capacity (Fig. 3c). Increased emulsion capacity is caused by polypeptide folds of globular protein molecules being more open. This enables hydrophobic and hydrophilic groups, which are initially protected, to more easily move to the surface. The macromolecular deformation causes the surface response to become larger and interact with non-polar and polar solvents such as oil and water.

Viscosity: The average viscosity of lablab bean seed starch after interacting with protein and xanthan gum is greater than the viscosity before the interaction (Fig. 4). The increased

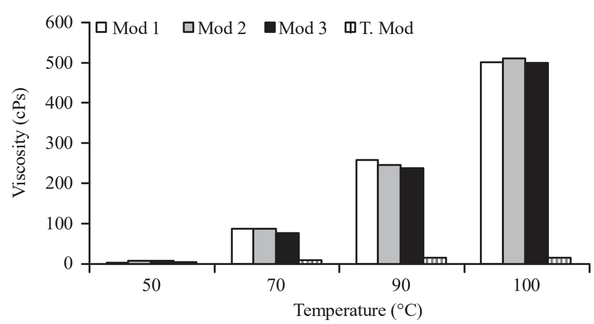

Fig. 4: Effect of xanthan gum and protein on viscosity of starch at temperature 50-100°C

viscosity is due to the influence of the molecule and intermolecular charges of proteins that promote the opening of folds between protein molecules. The repulsive force between protein molecules will encourage the opening of molecular protein chains and cause a reduction of interactions between protein molecules. This condition stimulates the interaction between protein molecules, which weakens and forms a non-cooperative complex that is reversible between the protein and xanthan gum, making it more soluble in water. Such conditions increase viscosity.

CONCLUSION

Lablab bean seeds have potential as a source of amylose starch, of which more than 50.8% have been successfully isolated from the lablab bean flour. The interaction product between starch, protein (5%) and gum xanthan (0.1-0.3%) is able to improve its functional properties, especially viscosity, swelling power (SP), water holding capacity (WHC) and emulsion capacity (EC), although gelatinization temperature rises to above 80°C, while starch gelatinization temperature is only 76°C. Based on the observations of granular microstructure and its functional properties, starch combined with 5% protein and 0.2% gum xanthan is the best interaction and is most appropriate for food ingredients.

We suggest that further research be conducted on the implementation of this ingredient for the development of food products such as noodles, bread, biscuits, snacks and others.

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REFERENCES

1. Roman, L. and M.M. Martinez, 2019. Structural basis of resistant starch (RS) in bread: Natural and commercial alternatives. Foods, Vol. 8, No. 7, 10.3390/foods8070267.
2. Sukamto, Aulanni'am and Sudiyono, 2009. [Functional properties of the interaction product between globulin of 7S fraction of lablab bean (*Dolichos lablab*) with Xantan gum]. J. Teknologi Industri Pangan, 20: 117-123, (In Indonesian).
3. Rahim, A., Haryadi, M.N. Cahyanto and Y. Pranoto, 2012. Characteristics of butyrylated arenga starch prepared at different reaction time and butyric anhydride concentration. Int. Food Res. J., 19: 1655-1660.
4. Birt, D.F., T. Boylston, S. Hendrich, J.L. Jane and J. Hollis *et al*., 2013. Resistant starch: Promise for improving human health. Adv. Nutr.: Int. Rev. J., 4: 587-601.
5. Bravo-Núñez, Á., R. Garzón, C.M. Rosell and M. Gómez, 2019. Evaluation of starch–protein interactions as a function of pH. Foods, Vol. 8, No. 5. 10.3390/foods8050155.
6. Zhang, G. and B.R. Hamaker, 2003. A three component interaction among starch, protein and free fatty acids revealed by pasting profiles. J. Agric. Food Chem., 51: 2797-2800.
7. Zhu, J.H., N.U. Haase and W. Kempf, 1990. Investigations on the laboratory scale separation of mung bean starch. Starch, 42: 1-4.
8. Sukamto, M. Sui, Sudiyono, P.D. Galih and F. Karim, 2019. Effects of pepsin on the separation of the globulin 7S and 11S fractions from lablab bean (*Komak*) seeds (*Dolichos lablab*) and its functional properties. Pak. J. Nutr., 18: 783-790.
9. Horwitz, W. and G.W. Latimer, Jr., 2005. Official Methods of Analysis of AOAC International. 18th Edn., AOAC International, Gaithersburg, Maryland, USA Pages: 94.
10. Nand, A.V., R.P. Charan, D. Rohindra and J.R. Khurma, 2008. Isolation and properties of starch from some local cultivars of cassava and taro in Fiji. South Pac. J. Nat. Sci., 26: 45-48.
11. Daramola, B. and S.A. Osanyinlusi, 2006. Investigation on modification of cassava starch using active components of ginger roots (*Zingiber officinale* Roscoe). Afr. J. Biotechnol., 5: 917-920.
12. Moses, O.O, T.O. Abayomi, O.A. Michael and B.A. Adedayo, 2012. Preparation and physicochemical characterization of icacina starch citrate – a potential pharmaceutical / industrial starch. Agric. Biol. J. North Am., 3: 11-16.
13. Omojola, M.O., N. Manu and S.A. Thomas, 2012. Effect of cross linking on the physicochemical properties of cola starch. Afr. J. Food Sci., 6: 91-95.
14. Sung, W.C. and M. Stone, 2004. Characterization of legume starches and their noodle quality. J. Mar. Sci. Technol., 12: 25-32.
15. Shimelis, E.A., M. Meaza and S.K. Rakshit, 2006. Physico-chemical properties, pasting behavior and functional characteristics of flours and starches from improved bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. Agric. Eng. Int.: CIGR J., Vol. 8.
16. Li, W., C. Shu, P. Zhang and Q. Shen, 2011. Properties of starch separated from ten mung bean varieties and seeds processing characteristics. Food Bioprocess Technol., 4: 814-821.
17. Nayebzadeh, K., J. Chen and S.M.M. Mousavi, 2007. Interactions of WPI and xanthan in microstructure and rheological properties of gels and emulsions. Int. J. Food Eng., Vol. 3, No. 4. 10.2202/1556-3758.1104.
18. Fariaa, S., C.L.d.O. Petkowicz, S.A.L.d. Morais, M.G.H. Terrones, M.M.d. Resende, F.P.d. França and V.L. Cardoso, 2011. Characterization of xanthan gum produced from sugar cane broth. Carbohydr. Polym., 86: 469-476.
19. Chavan, U.D., D.B. Mckenzie and F. Shahidi, 2001. Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). Food Chem., 74: 177-187.