Effect of Inulin Fortification on Qualities of Sacred Lotus Seed-soybean Milk

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Abstract
In recent years, plant-based milk from various sources is a trending beverage among health-conscious consumers as well as those with lactose intolerance or cows' milk allergy. Lotus seeds are rich in vitamins, nutrients, and bioactive compounds. Soy milk is the most popular plant-based milk since it is inexpensive and contains a lot of nutrients and essential vitamins, minerals, and phenolic compounds. Inulin can be used as a low-calorie sweetener, fat replacer, and texture improver. Moreover, inulin is also a prebiotic compound. Therefore, the objectives of this research were to study the effect of inulin concentration on physical qualities, chemical composition, and microbiological qualities of lotus seed-soybean milk. Lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin was produced. The microstructure, physical stability, viscosity, proximate composition, and microbiological quality were evaluated. The micrographs showed that lotus seed-soybean milk containing 0, 0.5, and 1 wt% inulin had small particles dispersed throughout the continuous phase. On the contrary, lotus seed-soybean milk containing 1.5 wt% inulin had the flocculation of particles with a significantly higher viscosity than other samples. Moreover, lotus seed-soybean milk containing 0.5 and 1 wt% inulin had a sediment phase height significantly lower than other samples. Therefore, one percent inulin was the highest concentration producing the most stable lotus seed-soybean milk in this study. The microbiological quality of lotus seed-soybean milk containing 1 wt% inulin was accepted following the standard as specified by the Notification of the Ministry of Public Health (No. 356) on hermetically sealed beverages.

Keywords: *plant-based milk, sacred lotus seed, soybean, inulin*

1. Introduction
In recent years, plant-based milk from various sources such as soybean, almond, rice, etc. is a trending beverage among health-conscious consumers as well as those who are unable to fully digest lactose in cows' milk (lactose intolerance) or allergic to cows' milk.

Nelumbo nucifera, commonly known as lotus, sacred lotus, or water lily, is an aquatic perennial flower ubiquitously found in Thailand, and its flowers are a significant part of Buddhist traditions, rituals, and ceremonies of Thai people. Literally, all parts of the lotus plant can be used for many purposes as foods, functional foods, and medicines especially the lotus seeds (Bangar, Dunno, Kumar, Mostafa & Maqsood, 2022; Lin, Zhang, Cao, Damaris & Yang, 2019). Lotus seeds are rich in vitamins (B1, B2, B6, C, and E), nutrients, and bioactive compounds. The seed is useful due to its good physiological functions, antioxidant activity, and immune-boosting properties with 16-21% of total protein which can be used as a substitute for meat protein in plant-based products or vegan protein shakes (Lin et al., 2019; Mukherjee, Mukherjee, Maji, Rai & Heinrich, 2009). Previously, the lotus seed milk was produced in a separate preliminary study. Lotus seed milk was very unstable to phase separation. Therefore, soy milk was mixed with lotus seed milk in this research to improve the physical qualities of the product. Soy milk was used in this research since it is the most popular plant-based milk. In addition, it is inexpensive and contains a lot of nutrients. Soy milk is a good source of protein, with a protein profile almost similar to cows' milk, essential vitamins, and minerals. It also contains phenolic compounds with antioxidant properties, isoflavones, trypsin, lectins, lecithin, and phytic acids that may help improve human health by raising the high-density lipoprotein (HDL) level and reducing the risk of many diseases (Arora, Dogra, Dogra & Goyal, 2018; Bhuiyan, Saleh, Hossain, Jahan & Ali, 2016; Jiang, Cai & Xu, 2013; Saini & Morya, 2021).

Inulin is a fructose-containing polysaccharide that belongs to the fructo-oligosaccharide group of compounds. It is used as an ingredient in various foods. Moreover, inulin is odorless, water-soluble, and resistant to heat and acidity. Due to its significant functional properties,it can be used as a low-calorie sweetener, fat replacer, and texture improver. Inulin is also a prebiotic compound since it is not digested in the human small intestine (Gibson, 2004; Tungland & Meyer, 2002).

Previously the lotus seed-soybean milk had been successfully developed in a separate preliminary study to be a new alternative for health-conscious consumers including those who are vegetarians and to help increase the market value of lotus seeds. Therefore, this research focused on the investigation of the effect of inulin fortification on the qualities of this milk.

2. Objectives

1) To study the effect of inulin concentration on physical qualities of lotus seed-soybean milk
2) To evaluate the proximate composition of lotus seed-soybean milk containing inulin
3) To evaluate the microbiological qualities of lotus seed-soybean milk containing inulin

3. Materials and Methods

3.1 Preparation of milk

Dried lotus seeds (*Nelumbo nucifera*) (Tawan Produce Co. Ltd., Samut Prakan, Thailand) were purchased from a local grocery. The seeds were soaked in tap water for 6 h. After drained, they were washed in running tap water. The cleaned seeds were blended with water at a ratio of 1:7 (by weight) for 10 min. in a blender (SHARP EMC-15, Thai City Electric Co. Ltd., Bangkok, Thailand) with a 2 L blending container. The obtained mixture was then filtered through double-layer straining cloth to obtain the homogeneous liquid milk.

Dried soybeans (Thanya Farm, Co. Ltd., Nonthaburi, Thailand) were also bought from a local grocery. The soybean milk was made by the processing method similarly used in processing lotus seed milk. The difference was only the ratio of the beans to water which was 1:3 (by weight).

The lotus seed milk and the soybean milk were mixed at a ratio of 7:3 (by weight) and 0.05 wt% guar gum (Chemipan Co. Ltd., Bangkok, Thailand) as a stabilizer was added.

3.2 Inulin fortification

Inulin (Fuji FF, Fuji Nihon Thai Inulin Co. Ltd., Ban Pong, Ratchaburi) was incorporated into the milk at levels of 0 (control sample), 0.5, 1, and 1.5 % (by weight). The milk samples were subjected to homogenization treatment using a stand-mounted laboratory homogenizer equipped with a fine stator slot dispersing generator (Ystral X 10/25, Ystral GmbH Maschinenbau + Processtechnik, Ballrechten-Dottingen, Germany) operated at a speed of 11,000 rpm for 10 min. Caramel-flavored syrup (Annapolis Co. Ltd., Pathum Thani, Thailand) was added (5% by weight) for flavor improvement. The milk samples were then pasteurized, heated at 72 °C for 10 min. and instantly filled into a glass bottle. Then, a metal cap was screwed on firmly to create a hermetically sealed pack. The bottle was cooled down in an iced water bath and refrigerated at 4°C for further analysis.

3.3 Microstructure

The milk samples were observed through a light microscope (Motic BA200, Motic Microscopes, Texas, USA) to evaluate their microstructure. One drop of each sample was placed by a plastic transfer pipette on a 1 mm thick glass microscope slide (25x75 mm in width and length) and covered by a cover slip. Twenty watts quartz halogen lamp installed in the microscope at the maximum intensity was used as an illumination source. A combination of eyepiece and objective lens at 10x and 40x magnification were employed. The micrographs of the sample were obtained by a smartphone equipped with a digital camera attached to the eyepiece of the microscope.

3.4 Physical stability

The physical stability of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin was determined by observing phase separation in a sample during storage. Eighty mL of each milk sample was filled in a 100 mL glass graduated cylinder, sealed by polyvinyl chloride (PVC) cling film, and stored in a refrigerator at a temperature of 4°C for 10 days. The height of sediment phase at the bottom layer of the tube was measured using a digital vernier caliper expressed in centimeters (cm) after 10 days of storage.

3.5 Viscosity

The viscosity of the milk samples was measured using a rotational dial reading viscometer (Brookfield LVT, AMETEK Brookfield Co. Ltd., Massachusetts, USA). Five hundred mL of each milk sample at a temperature of 4°C was filled in a 600 mL beaker, rested for 10 minutes, then measured with a suitable combination of spindle number and speed (RPM), and then torque in percentage was recorded. Viscosity expressed in centipoise (cP) was calculated from torque using a related spindle factor.

3.6 Proximate composition

An analysis of proximate compositions was performed for the selected milk sample according to the AOAC standard (AOAC, 2012). These included moisture, protein, lipid, crude fiber, ash, and carbohydrate (by calculation).

3.7 Microbiological quality

An analysis of the microbiological qualities of the selected milk sample was conducted following the standard as specified by the Notification of the Ministry of Public Health (No. 356) on hermetically sealed beverages (Thai Ministry of Public Health, 2013). These included total bacterial count (CFU/mL), yeast and mold count (CFU/mL), and Coliform count (MPN/100 mL).

3.8 Statistical analysis

All experiments were carried out at least twice and the data were subjected to the analysis of variance (ANOVA) using SPSS statistical software. Duncan's Multiple Range Test comparisons at P value ≤ 0.05 were performed to determine significant differences.

4. Results and Discussion

4.1 Microstructure

The effect of inulin concentration on physical qualities including optical microstructure, phase separation, and viscosity of lotus seed-soybean milk was investigated. The microstructures of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin was observed using optical microscope. The optical micrographs showed that microstructures of lotus seed-soybean milk containing 0, 0.5, and 1 wt% inulin had small particles that dispersed throughout the continuous phase (Figure 1). All the samples were mainly constituted by water, lotus seed and soybean fragmented materials, and guar gum as stabilizer, which were composed by the continuous and dispersed phases. For the continuous phase, the main constituents included water and water-soluble substances, which could not be seen through the optical microscopy. The particles present in the dispersed phase were insoluble substances from lotus seeds and soybeans. In addition, the guar gum and inulin were partially dissolved in water; therefore, the insoluble particles remained as the dispersed phase (Guimaraes et al., 2018; Leite, Augusto & Cristianini, 2015; Ronkart et al., 2010).

However, the optical microstructure of lotus seed-soybean milk containing 1.5 wt% inulin had a flocculation of particles (Figure 1). The changes in lotus seed-soybean milk microstructure, when 1.5 wt% inulin was added, could be attributed to the interactions between polysaccharides, proteins, and water were high enough resulting in the flocculation phenomena (Corredig, Sharafbafi & Kristo, 2011; Guimaraes et al., 2018). This complex behavior could be a consequence of the attraction forces between insoluble polysaccharides and proteins in the dispersed phase, and the depletion of these molecules in continuous phase resulting in the depletion flocculation (Guimaraes et al., 2018; Radford & Dickinson, 2004; Sittikijyothin, Sampaio & Goncalves, 2010).

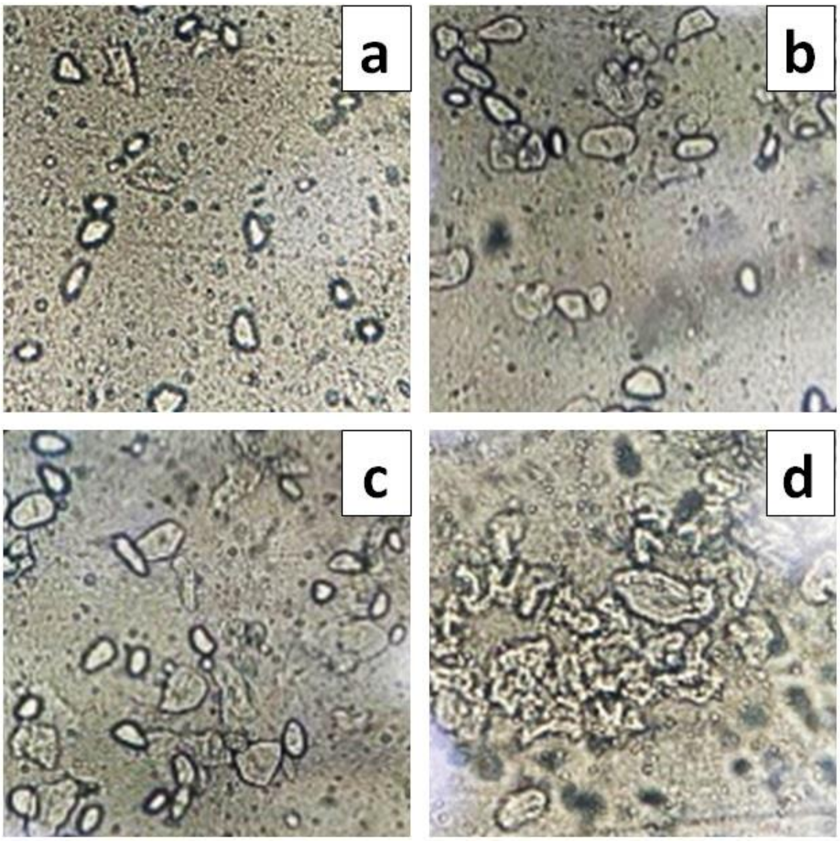

Figure 1 Optical microstructures of lotus seed-soybean milk containing 0 (a), 0.5 (b), 1 (c), and 1.5 (d) wt% inulin

4.2 Physical stability

The kinetic stability of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin was evaluated through the phase separation. The heights of the sediment phases at the bottom layers of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin was measured during the 10-day storage. The results showed that lotus seed-soybean milk containing 0.5 and 1 wt% inulin had sediment phase heights significantly lower ($P \leq 0.05$) than the lotus seed-soybean milk without inulin (Figure 2). This might be due to the sedimentation of particles declined by inulin acting as a stabilizer (Rad, Delshadian, Arefhosseini, Alipour & Jafarabadi, 2012)

However, lotus seed-soybean milk containing 1.5 wt% inulin had a sediment phase height significantly higher ($P \leq 0.05$) than lotus seed-soybean milk containing 0, 0.5, and 1 wt% inulin (Figure 2). This could be attributed to lotus seed-soybean milk containing 1.5 wt% inulin with depletion flocculation particles (Figure 1) that were likely separated into two phases including a concentrated particle phase and a depletant-enriched serum phase (Radford & Dickinson, 2004; Repin, Scanlon & Fulcher, 2012).

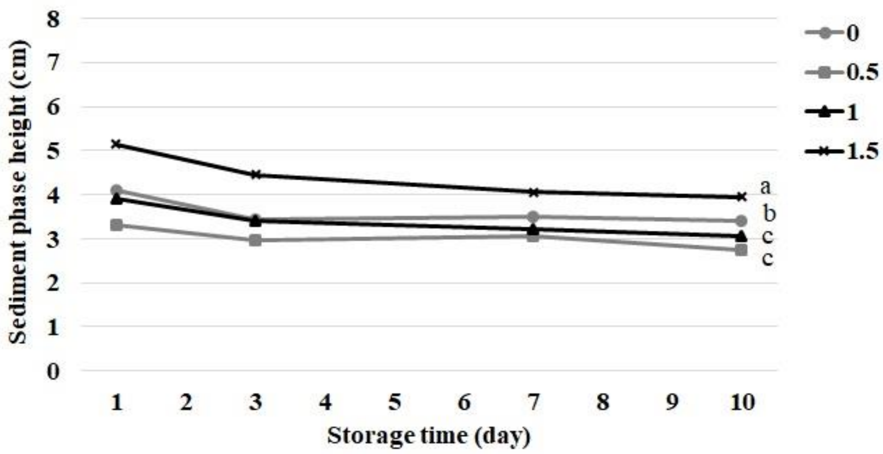

Figure 2 Sediment phase heights of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin ([a,b] indicates the significant difference among treatments with different inulin concentrations, $P \leq 0.05$)

4.3 Viscosity

The viscosity of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin was evaluated using Brookfield viscometer. The results showed that an addition of 0.5 and 1 wt% inulin did not significantly affect ($P > 0.05$) the viscosity of lotus seed-soybean milk compared to the control without inulin. However, lotus seed-soybean milk containing 1.5 wt% inulin had a viscosity significantly higher ($P \leq 0.05$) than lotus seed-soybean milk containing 0, 0.5, and 1 wt% inulin (Table 1). Similar studies evaluated the effect of inulin on the viscosity of milk beverage. Guimaraes et al. (2018) and Villegas and Costell (2007) found that an increase in the concentration of inulin led to the increasing viscosity of milk beverage. This could be attributed to the hygroscopic nature of inulin that it forms a gel-like network with the liquid phase of milk beverage (Rad et al., 2012).

Table 1 Viscosities of lotus seed-soybean milk containing 0, 0.5, 1, and 1.5 wt% inulin

Inulin concentration (wt%)	Viscosity (cP)
0	$6.50^{b} \pm 0.17$
0.5	$7.25^{b} \pm 0.30$
1	$7.75^{b} \pm 0.35$
1.5	$19.50^{a} \pm 0.71$

[a,b] Indicates the significant difference between treatments with different inulin concentrations, $P \leq 0.05$

The lotus seed-soybean milk containing 1 wt% inulin was selected for the next part of the experiment because 1 wt% inulin was the highest concentration to produce the most stable lotus seed-soybean milk in this study.

4.4 Proximate composition

The proximate compositions of lotus seed-soybean milk containing 1 wt% inulin was evaluated according to the AOAC standard. Table 2 shows the proximate compositions of lotus seed-soybean milk containing 1 wt% inulin including moisture content, ash content, crude protein, crude fat, crude fiber, and total carbohydrate. From the literature review, the proximate compositions of lotus seed are 61.3-70.1 wt% carbohydrates, 16.2-28.19 wt% proteins, 0.22-3.68 wt% lipids, 8.17-14 wt% water, and 3.07-4.1 wt% ash (Bangar et al., 2022; Zhang et al., 2015).

Table 2 Proximate compositions of lotus seed-soybean milk containing 1 wt% inulin

Proximate composition	Value (wt%)
Moisture content	89.48±0.18
Ash content	0.38±0.05
Crude protein	1.81±0.08
Crude fat	1.63±0.40
Crude fiber	0.18±0.01
Total carbohydrate	6.7±0.55

4.5 Microbiological quality

The microbiological quality of the lotus seed-soybean milk containing 1 wt**%** inulin was conducted following the standard as specified by the Notification of the Ministry of Public Health **(**No**.** 356**)** on hermetically sealed beverages**.** Table 3 shows the microbiological quality of lotus seed-soybean milk containing 1 wt% inulin including total bacterial count **(**CFU/mL**)**, yeast and mold count **(**CFU/mL**)**, and Coliform count **(**MPN/100 mL**).** The results showed that microbiological quality of lotus seed-soybean milk containing 1 wt% inulin was accepted following the standard as specified b7y the Notification of the Ministry of Public Health **(**No**.** 356**)** on hermetically sealed beverages **(**Thai Ministry of Public Health, 2013**).**

Table 3 Microbiological quality of lotus seed-soybean milk containing 1 wt% inulin

Microorganism tests	Value
Total bacterial count (CFU/ml)	Not detected
Yeast and mold (CFU/ml)	Not detected
Coliform count (MPN/100ml)	< 3

5. Conclusion

Since no previous research investigated the effect of inulin on qualities of lotus seed-soybean milk, an effort was made in this research in order to determine microstructure, physical stability, viscosity, proximate composition, and microbiological quality of the lotus seed-soybean milk containing inulin. The results indicated that the addition of inulin at low concentrations (0.5 and 1 wt%) could improve the physical stability of lotus seed-soybean milk providing significantly ($P \leq 0.05$) lowest sediment phase heights. In addition, the optical microstructures and viscosities of lotus seed-soybean milk containing 0.5 and 1 wt% inulin were not significantly different ($P > 0.05$) compared to the control without inulin. On the contrary, the addition of inulin at a high concentration (1.5 wt%) could adversely affect the physical stability of lotus seed-soybean milk providing significantly ($P \leq 0.05$) highest sediment phase heights. Moreover, lotus seed-soybean milk containing 1.5 wt% inulin had unstable depletion flocculation particles and significantly ($P \leq 0.05$) highest viscosities. One percent was the highest concentration of inulin to produce the most stable lotus seed-soybean milk in this study. The findings from this research could be used for further studies on sensory quality to provide some insights into the impact of inulin on the sensory quality of lotus seed-soybean milk.

6. Acknowledgements

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7. References

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