



Development of Pumpkin Pudding-like Food Using Soybeans [*Glycine max* (L.) Merr.]

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to develop an enhanced value-added pudding-like food with soybeans to improve quality of everyday life for peoples suffering from egg and milk allergies.

Study Design: Preparation of pudding-like foods, improvement of its quality, setting up sensory evaluation and determination of proximate composition.

Place and Duration of Study: Yamagata University, Yamagata, Japan, from November 2017 to October 2018.

Methodology: Optimal condition was investigated to prepare pudding-like food as follows: 1. adding water rate to raw soybeans, 2. bitter content, 3. Heating time and temperature of soymilk, 4. addition of sugars to improve the taste, 5. addition of pumpkin as a secondary ingredient to improve nutritive value. The physicochemical properties of pudding-like food were compared to those of commercially available puddings.

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Results: Best processing condition was as follows: 1. Soymilk was prepared by addition of four volumes of water to raw soybeans, 2. After 0.25 % (w/v) bittern and 5 % (w/v) yellow soft sugar to soymilk, it heated about 5 min at 80 °C, 3. It could enhance the quality of food with the addition of 10 % (w/v) mashed pumpkin as a secondary ingredient. It showed great sensory potential and has high proteins, low fats and carbohydrates, and low calorie. Moreover, it was β -carotene-rich pudding-like food.

Conclusion: It could develop a smooth, readily meltable in the mouth, and delicious pudding-like food with satisfactory appearance and high nutritive value using soybeans instead of eggs and milk.

Keywords: Development; non-use of eggs; pudding-like food; pumpkin; soybean.

1. INTRODUCTION

Nowadays trends of eating habits have changed more and more. Consumers need to maintain a healthy dietary life and therefore tend to require foods with high nutritional values and functionalities for health. On the contrary, about one in two people of the total population suffers from some kind of allergic diseases in Japan [1]. The number of these patients has rapidly increased in the recent year and it is in an extremely serious condition. According to current estimates [1], prevalence of food allergy among allergic diseases tends to be high at an early age as follows: about 5-10 % (baby), about 5 % (infant), about 1.5-3 % (after later childhood). Particularly, egg, milk, and dairy products are main foods causing allergy on people under 19 years old [1]. At present, a duty of display to the packing of processed foods is imposed about items specified as seven raw materials that induce critical allergies, such as egg, milk, wheat, buckwheat, peanut, shrimp, and crab [2]. As food allergy is a cause of anaphylaxis [3], which is a life-threatening generalised or systemic hypersensitivity reaction [4], it take a growing interest in food allergy all over the world to assist in allergy and allergen risk management and to decrease frequency and intensity of anaphylaxis to improve the quality of life for food allergic people and to treat patients [5,6].

Soybeans [*Glycine max* (L.) Merr.] are one of plant that belongs to Fabaceae. About 90 % of world consumption of fully ripened beans is used for oil pressing as oilseeds and its strained lees are utilised for feeds. In Japan, soybeans have been used for a long time as foods for production of tofu (soybean curd), natto (fermented soybeans), miso (fermented soybean paste), soy sauce, and boiled beans and so on. It is known that soybeans are food with high nutritive value.

It is rich in high quality of proteins (amino acid score = 100), polyunsaturated fatty acids such as linoleic acid, oleic acid, and α -linolenic acid, oligosaccharides such as stachyose and raffinose, and dietary fibers [7]. In addition, soybeans contain some phytochemicals such as isoflavones (daidzein, genistein, and glycitein), lecithins, phytosterols, and saponins [8,9]. Moreover, soybeans are recognised as food with health benefits that soy protein reduced risk of cardiovascular disease by cholesterol-lowering effect [10] and soy protein peptic hydrolysate and soy protein peptic hydrolysate with bound phospholipids lowered the serum cholesterol levels [11]. On the other hand, soy has been used more than 100 years ago as one of ingredient of soy-based infant formulas, which begin to be used as substitutes for milk in children with cow's milk protein allergies [12,13].

Pudding is a food that ingredients such as wheat flour, rice, egg, milk, and butter, sugar, and salt, etc are mixed, and then are heated with steam or baked. It is very popular food with people of all ages, from children to the elderly. However, it is generally used items specified as seven raw materials such as wheat flour, egg, and milk as main ingredients. Therefore, it is impossible to eat these puddings for food allergic people and its patients. If smooth, creamy, and delicious pudding-like food can produce to use soybeans with high nutritive value, it makes a huge contribution and an improvement to the quality of everyday life for peoples suffering from these allergies. In the present study, we tried to develop an enhanced value-added pudding-like food with soybeans instead of eggs and milk. Moreover, it was investigated proximate composition, physicochemical and sensory properties of these foods and compared to these properties of commercially available puddings.

2. MATERIALS AND METHODS

2.1 Materials

Soybeans *Toyomasari* (a brand variety of the growing district of Hokkaido, Japan) produced in Hokkaido, Japan were purchased from Suzuya kokumotsu Co., Ltd. (Miyagi, Japan). Bittern for industrial use as a coagulant was from Sizenkenkou Co., Ltd. (Nara, Japan). Granulated sugar, white sugar, and yellow soft sugar were obtained from Itochu sugar Co., Ltd. (Aichi, Japan). Western pumpkin (*Cucurbita maxima*) and commercially available puddings (namely pudding A and pudding B), which chicken eggs were used as one of ingredients, were purchased from a local supermarket, Yamagata, Japan. Particularly, pudding A was used with caramel sauce removed in the experiment.

2.2 Preparation of Pudding-like Food

After raw soybeans were selected and removed contaminants, these were added three volumes of distilled water, and then were soaked for 18 h at room temperature. After draining, these were added distilled water, and then were ground using a mixer (MX-V100; SANYO Electric Co., Ltd., Osaka, Japan) for 1 min (raw ground macerated soybean; raw go). These were cooked over a medium heat with stirring until boiling, and then were cooked over a low heat for 5 min. After cooling at room temperature, these were centrifuged at 15,000 x g for 30 min at 20 °C. The supernatants were used as soymilk. These were put into a glass bottle with sugar and bittern, and then were heated in a water bath at 80°C. After cooling, physicochemical properties of pudding-like food were investigated.

2.3 Physicochemical Property

Solid soybean content of soymilk was measured using a digital soymilk densitometer (PAL-27S; Atago Co., Ltd., Tokyo, Japan). Viscosity of soymilk was determined at 23 °C using a viscometer (TVC-7; Toki sangyo Co., Ltd., Tokyo, Japan). Total soluble solids (TSS) were determined using a digital saccharimeter (PAL-Pâtissier; Atago Co., Ltd., Tokyo, Japan). Color analysis was performed using a colorimeter (NR-11A; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. CIE $L^*a^*b^*$ system

was used as the relation to human eye response to color. The color was measured on three different spots. Color difference (ΔE^*ab) was also calculated as follows: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. 0-0.5: trace; 0.5-1.5: slight; 1.5-3.0: noticeable; 3.0-6.0: appreciable; 6.0-12: much; more than 12: very much. Breaking strength was analysed using a rheometer (TPU-2; Yamaden Co., Ltd., Tokyo, Japan). Samples were compressed at a compression speed of 2.5 mm/s to a clearance of 10 mm with a cylindrical plunger No. 3 (16 mm diameter).

2.4 Sensory Evaluation

Pudding-like food and commercially available puddings were used for sensory evaluation [14]. A five-point hedonic scale was used with 1 = dislike extremely, 3 = neither like nor dislike, and 5 = like extremely. Panel of four trained panelists was questioned to evaluate appearance, colour (yellowness), softness, smoothness, melt-in-the-mouth feeling, sweetness, deliciousness, and overall acceptance of puddings. Panel was instructed to rinse mouths with water for each sample, and was not to make comments to prevent influencing other panelists during analysis. The ethical issues of sensory analysis were approved by the Institutional Ethics Committee of Yamagata University.

2.5 Proximate Composition

Moisture content was measured using a Moisture Determination Balance (FD-600; Kett Electric Laboratory, Tokyo, Japan). Crude proteins were determined by the Kjeldahl method using a conversion factor of 6.25. Crude lipids were analysed by chloroform-methanol extraction. Measurements of crude ashes were used an electric furnace (AMI-II; Nitto Kagaku Co., Ltd., Aichi, Japan). Carbohydrates were calculated by difference. Salts were determined using a digital salinity concentration meter (EB-158P; Eishin Co., Ltd., Hiroshima, Japan). The energy was calculated using the Atwater's calorie factors [15]. β -Carotene content was measured using the acetone-hexane extraction method [16].

2.6 Statistical Analysis

Each assay was repeated 3 times independently, and the data were analysed using Excel software and the results were reported as means \pm standard deviation.

3. RESULTS AND DISCUSSION

3.1 Adding Water Rate to Soybeans

In the preparation of soymilk, adding water contents to raw soybeans have effects on the physicochemical properties of pudding-like food [17]. Soymilk was prepared by addition of different volumes of distilled water to soybeans after soaking and draining. As a result, in case of the addition of three volumes of water, solid soybean content of soymilk and its viscosity were about 12.2 % and 29.1 mPa · s, respectively (Fig. 1). The content linearly decreased with increasing content of adding water. In case of the addition of six volumes of water, the content was about 8.6 %. The viscosity in addition of four volumes of water slightly decreased compared to that of three volumes of water. Moreover, the addition of five volumes of water drastically decreased the viscosity to about 16.8 mPa · s and it in addition of six volumes of water was almost as same as that in addition of five volumes of water. It suggested that it was most suitable for soymilk preparation to add four volumes of water to raw soybeans.

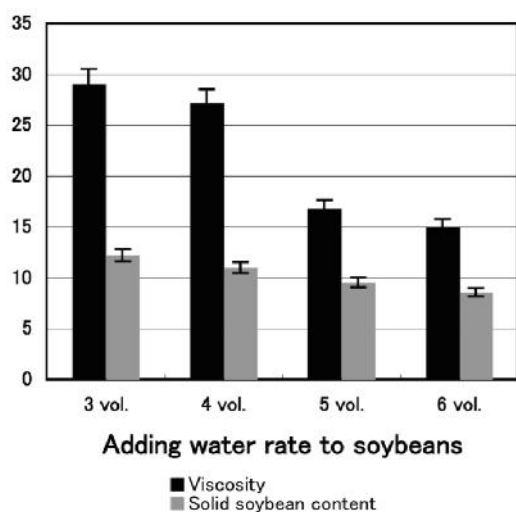


Fig. 1. Effects of adding water rates to raw soybeans on viscosity and solid soybean content of soymilk

3.2 Optimum Bittern Content

Different concentrations of bittern were used to gelatinise soymilk of non-use of eggs. On 0.1 and 0.2 % (w/v) bitterns, soymilks did not entirely gelatinise and were mostly in viscous and liquid

state. However, soymilk gelatinised by addition of 0.25 % (w/v) bittern and its breaking strength was about 0.72 N (Fig. 2). Moreover, breaking strength linearly increased with increasing concentration of bittern, and it amount to maximum value about 1.30 N in addition of 0.5 % (w/v) bittern. On the other hand, it decreased in addition of over 1.0 % (w/v) bittern and breaking strength on 1.5 % (w/v) bittern was about 0.92 N. By sensory analysis, pudding-like food added 0.25 % (w/v) bittern had tender and felt smooth. Addition of over 1.0 % (w/v) bittern gave intensely bitter taste and poor eating-quality characteristics. Salt coagulants such as bittern has the property that decrease of breaking strength is slower when it excess of the maximum [18].

3.3 Heating Time and Temperature of Soymilk

Heating time and temperature of soymilk has an impact on physical properties of pudding-like food. After the addition of 0.25 % (w/v) bittern, it was investigated the effects of heating time on breaking strength of food. Breaking strength before heating was about 0.57 N. It increased to about 0.79 N by heating for 5 min and was almost no change till after 10 min. However, it linearly increased to about 1.14 N again till 20 min, and then was not shown the change till 30 min (about 1.16 N).

Soymilk was heated for 5 min at each temperature after addition of 0.25 % (w/v) bittern. It was investigated the effects of heating temperature on breaking strength of food. It was the highest about 0.55 N at 80 °C heating, followed by about 0.50 N at 90 °C and about 0.48 N at 70 °C. By sensory analysis, it found that heating time and temperature after addition of bittern were optimally about 5 min and at 80 °C, respectively.

3.4 Addition of Sugars

It found that it was necessary to add 5 % (w/v) sugar to improve the taste of pudding-like food by preliminary experiments. Therefore, it was investigated the effects of kinds of sugars (granulated sugar, white sugar, and yellow soft sugar) on physicochemical properties of pudding-like foods. Sugar additions were performed simultaneously with the addition of bittern. As a result, TSS of food

added granulated sugar was the highest about 9.7 % compared to those of foods added white sugar (about 8.6 %) and yellow soft sugar (about 8.8 %) (Table 1). All of them were extremely lower than those of commercially available puddings (pudding A: 20.1 %, pudding B: 24.3 %).

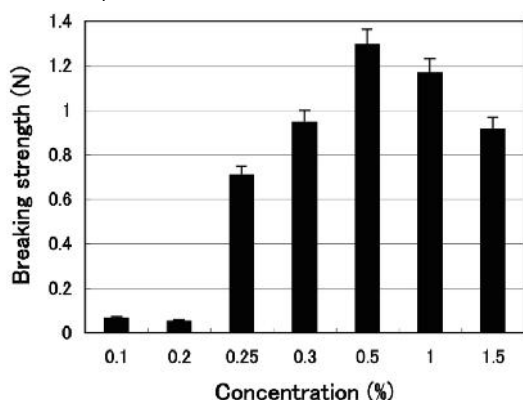


Fig. 2. Effects of adding bitter rate to soymilk on breaking strength of pudding-like food

Color characteristics of pudding-like foods were investigated. Food added white sugar had higher L^* value among them. The value was higher than that of pudding A but was lower than that of pudding B. There was not much difference on a^* values among these foods, and these values were remarkably lower than those of pudding A and pudding B. Foods added sugars had lower b^* values in comparison to pudding B. Particularly, the values were significantly lower than that of pudding A. The ΔE^*ab values were calculated, and color difference was evaluated to pudding A as very much. On the other hand, it was also evaluated to pudding B as follows: granulated sugar and white sugar (appreciable) and yellow soft sugar (much), respectively.

Breaking strength of pudding-like foods was measured. As a result, lower breaking strengths were observed about 0.420-0.484 N. These values were higher than that for pudding B, but were considerably lower than that for pudding A. By sensory evaluation, it found that it could mask the unpleasant odour of soymilk (green smell peculiar to soybeans and unripe-smelling taste) and prepare pudding-like food with mild and soft feelings by addition of yellow soft sugar, in comparison to addition of granulated sugar and white sugar. It might be due to caramel components.

3.5 Addition of Pumpkin as a Secondary Ingredient

Pumpkin is any plant of the genus pumpkin of the family Cucurbitaceae and is a vegetable native to North and South America. Pumpkin has various uses and is used not only ingredients of dishes such as cooked dishes, croquettes, salads, soups, tempuras, but also secondary ingredients of breads, Japanese traditional confectionery as *manjyu*, and Western confectioneries as cakes and pies. It is one of green and yellow vegetables that are especially rich in vitamins such as carotene, B₁, B₂, and C, minerals as calcium, iron, magnesium, and potassium, and dietary fibers [15]. β -Carotene has multiple biological effects such as anticancer and immunostimulatory effects, eyesight keeping effect, and health maintenance effects of mucous membranes and skins [19]. Vitamin C possess protective properties against life style-related diseases such as arteriosclerosis, arthritis, brain dysfunction, cardiovascular diseases, cancer, heart disease, hypertension, inflammation, and liver disease [20]. Moreover, intake of dietary fibers effectively accelerates defecation for preventing and curing constipation [21] and alleviates type 2 diabetes [22].

Table 1. Physicochemical properties of pudding-like foods prepared by addition of sugars

	TSS (%)	Color			ΔE^*ab	Breaking strength (N)	
		L^*	a^*	b^*			
Granulated sugar	9.7±0.5	78.70±2.34	-1.22±0.12	9.90±0.62	17.41	6.30	0.484±0.033
White sugar	8.6±0.4	79.49±2.11	-1.32±0.17	10.33±0.55	17.10	5.59	0.420±0.048
Yellow soft sugar	8.8±0.4	77.18±1.98	-1.22±0.15	10.84±0.68	16.44	6.70	0.453±0.059
Pudding A	20.1±1.0	77.30±2.03	2.54±0.23	26.84±2.01	-	13.26	0.676±0.094
Pudding B	24.3±1.2	82.27±3.51	0.86±0.11	14.66±1.73	13.26	-	0.399±0.080

Table 2. Physicochemical properties of pudding-like foods prepared by addition of different amounts of mashed pumpkin

Additive amounts of mashed pumpkin (%)	TSS (%)	Color			ΔE^*ab		Breaking strength (N)
		L^*	a^*	b^*			
5.0	4.9±0.3	75.18±1.99	-0.39±0.05	19.82±1.94	7.90	8.86	0.274±0.017
10.0	5.6±0.4	73.43±1.65	1.11±0.09	28.00±2.16	4.29	16.01	0.315±0.021
20.0	5.9±0.5	71.89±1.23	2.51±0.15	35.52±2.57	10.23	23.36	0.328±0.023
Pudding A	20.1±1.0	77.30±2.03	2.54±0.23	26.84±2.01	-	13.26	0.676±0.094
Pudding B	24.3±1.2	82.27±3.51	0.86±0.11	14.66±1.73	13.26	-	0.399±0.080

**Fig. 3. Pudding-like foods prepared by addition of different amounts of mashed pumpkin**

Left: 5 % (w/v) pumpkin, center: 10 % (w/v) pumpkin, right: 20 % (w/v) pumpkin

Pumpkin was cut and removed skins, and then was heated for 2 min at 600W using a microwave (MTO-16E6-W, Yamazen Co., Ltd., Osaka, Japan). These were mashed and pudding-like foods without addition of sugar were prepared by addition of different amounts of mashed pumpkin (Fig. 3). As a result, TSS of pudding-like food increased with increase of additive amounts of pumpkin, although these values (about 4.9-5.9 %) were fairly low compared to those of commercially available puddings (Table 2).

Next, color characteristics of pudding-like foods were investigated. The L^* value of food added 5 % (w/v) pumpkin was about 75.18, and it was lower than those of commercially available puddings. The value was lowered with increase of additive amounts of pumpkin. On the other hand, a^* and b^* values significantly increased with increase of additive amounts of pumpkin. The a^* values of foods added 10 or 20 % (w/v) pumpkin were the same or higher than those of commercially available puddings. Also, the b^* values of foods added pumpkin were considerably higher than those of commercially available puddings in all cases. Particularly, pudding-like food added 20 % (w/v) pumpkin had the highest b^* value about 35.52. The ΔE^*ab values were calculated, and color difference was evaluated to commercially available puddings. It

was evaluated to pudding A as follows: foods added 5 or 20 % (w/v) pumpkin (much) and food added 10 % (w/v) one (appreciable), respectively. Moreover, it was also evaluated to pudding B as follows: food added 5 % (w/v) pumpkin (much) and foods added 10 or 20 % (w/v) ones (very much), respectively.

Breaking strengths of pudding-like foods were measured. These values were remarkably low about 0.274-0.328 N compared to those of commercially available puddings, although these increased with increase of additive amounts of pumpkin. By sensory evaluation, it found that it could enhance the quality of pudding-like food with addition of 10 % (w/v) mashed pumpkin. However, it was necessary to supplementing a disadvantage that was unsatisfied with sweetness compared to commercially available puddings. In addition, pudding-like food was prepared to add 5 % (w/v) yellow soft sugar in above conditions. TSS value increased to about 10.8 % and color parameters were as follows: $L^* = 72.56$, $a^* = 3.82$, and $b^* = 27.49$, respectively. The ΔE^*ab values and color difference to commercially available puddings were 4.95 (appreciable) to pudding A and 13.52 (very much) to pudding B, respectively. Breaking strength was fairly high about 0.840 N in comparison to those of pudding A (0.676) and pudding B (0.399).

3.6 Sensory Evaluation

Sensory analysis of pudding-like food was performed and was compared to those of commercially available pudding A and pudding B. As a result, pudding-like food had good appearance as well as commercially available puddings (Fig. 4). Color (yellowness) on pudding A was better among these puddings, followed by pudding-like food. On the other hand, pudding B showed low score because of high L^* value (Table 2), suggesting low content of β -carotene. Pudding-like food had softness, smoothness, and melt-in-the-mouth feeling with high scores as well as pudding B, while pudding A showed low scores. Among them, puddings A and B had good sweetness: these were sweet pudding. On the contrary, pudding-like food showed low score unlike in the case of commercially available puddings, indicating pudding with less sugar. Each pudding had the highest score in terms of deliciousness. The sensory scores for overall acceptance were 4.7 (pudding-like food), 4.5 (pudding A), and 5 (pudding B), respectively. From these results, it was suggested that it could be produced pudding-like food with good sensory acceptability by addition of pumpkin.

3.7 Proximate Composition

Proximate composition of pudding-like food was investigated. As a result, water, crude proteins, crude lipids, carbohydrates, and ashes contents were as follows: 86.2 ± 0.1 , 4.7 ± 0.1 , 2.4 ± 0.2 , 6.2 ± 0.1 , and 0.5 ± 0.1 g/100 g, respectively. Salts were contained about 0.2 g/100 g. Energy was calculated about 65.2 kcal/100 g. On the other hand, composition of commercially available pudding A except caramel sauce and pudding B was as follows: water (75.8 and 73.3 g/100 g), crude proteins (1.7 and 3.6 g/100 g), crude lipids (7.4 and 11.6 g/100 g), carbohydrates (14.7 and 11.0 g/100 g), and ashes (0.4 and 0.5 g/100 g), respectively. Salts of both puddings were 0.1 g/100 g. Energies were calculated about 132.2 and 162.8 kcal/100 g, respectively. Composition of cream caramel is reported in Standard Tables of Food Composition in Japan 2018 as follows: water (74.1 g/100 g), crude proteins (5.5 g/100 g), crude lipids (5.0 g/100 g), carbohydrates (14.7 g/100 g), and ashes (0.7 g/100 g), respectively. Salts and energy are 0.2 g/100 g and 126 kcal/100 g, respectively [15]. The contents of carbohydrates are significantly high in comparison to those of pudding-like food and pudding B, because of including caramel sauce. β -Carotene content of pudding-like food was

measured about 0.344 mg/100 g. On the other hand, contents on commercially available puddings A and B were about 0.587 and 0.196 mg/100 g, respectively. It was assumed that contents were due largely to ingredients such as chicken egg powders and carotene pigments for pudding A and as chicken eggs and vegetable oils for pudding B. It suggested that content on pudding-like food almost depended on pumpkin used for its preparation.

Generally, soymilk coagulates by addition of a coagulant such as bittern. Ono et al. [23] investigated properties of soluble and particulate proteins in soymilk. As a result, they clarified that particulate proteins was sensitive to Ca^{2+} and H^+ , therefore these aggregated at low concentration of coagulant compared to soluble proteins. Guo et al. [24,25] reported that soluble proteins during soymilk curdling induced by coagulant formed particles and then aggregated. On the contrary, protein particles conjugated with lipid droplets earlier than soluble proteins. That is, Ono et al. [25] drew the following conclusion: soymilk proteins first were denatured by heat treatment and rearranged to soluble and particulate proteins. Next, surface charge of protein particles was neutralised by addition of coagulant and hydrophobic interaction occurred among protein particles. Moreover, three dimensionally network structure was produced between protein particles and lipid droplets (tofu curd formation). They also clarified that breaking stress of tofu curd was hard with increase of content of protein particles. Main components of soybean proteins are glycinin (11S globulin) and β -conglycinin (7S globulin) that account for about 60 % of its proteins. Hirotsuka [26] reported that tofu prepared from these protein fractions had completely different properties; that is, tofu made from glycinin fractions was extremely hard and brittle, whereas it from β -conglycinin fractions had stickiness and clear sense. In the present study, we developed a pudding-like food with soybeans instead of eggs and milk for contribution and improvement to the quality of everyday life for peoples suffering from egg and milk allergies. It was new type of pudding-like food that has high proteins, low fats and carbohydrates, and low calorie. Moreover, it was β -carotene-rich pudding-like food. According to the above findings, it might be able to produce pudding-like foods with different texture. Further work is ongoing to develop high quality of pudding-like food so as to make the best use of characteristics of these fractions.

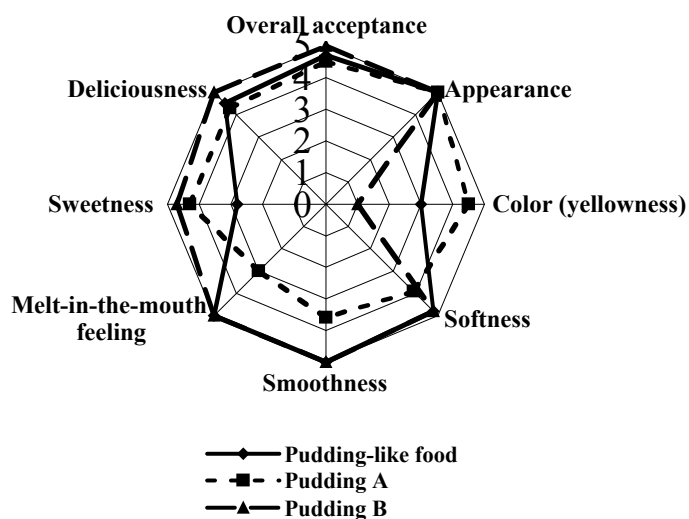


Fig. 4. Comparison of sensory evaluation between pudding-like food and commercially available puddings

4. CONCLUSION

It could develop a smooth, readily meltable in the mouth, and delicious pumpkin pudding-like food with satisfactory appearance and high nutritive value using soybeans instead of eggs and milk. It might also be used as a substitute of commercially available puddings to improve the quality of everyday life for not only peoples suffering from egg and milk allergies but also vegans.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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