Quality characteristics and storage stability of reduced-calorie mung bean marzipan incorporated with konjac flour and pumpkin

Akesowan, A. and Choonhahirun, A.

Department of Food Science and Technology, School of Science and Technology, University of the Thai Chamber of Commerce, 126/1 Vibhavadee-Rangsit Rd., Dindaeng, Bangkok 10400, Thailand

Abstract
This study was aimed to develop the quality of reduced-calorie mung bean marzipan by konjac flour (0.1-0.3%, w/w) and pumpkin (15-30% by mung bean weight). Response surface methodology was used to optimize the product formulation. Incorporation of konjac flour greatly improved sensorial texture of reduced-calorie marzipan, while increasing pumpkin level mostly influenced product color. Optimized marzipan was achieved by adding 0.3% konjac flour and 25.16% pumpkin. In comparison to the regular, optimized marzipan had significantly higher (p<0.05) pH, $L^*$ and $b^*$ values, but lower (p<0.05) consistency. Consumers gave higher (p<0.05) scores for taste, texture and overall acceptability of the regular marzipan; however, they preferred (p<0.05) color of optimized sample. Both samples had the decrease in physical and sensory properties after 7 days of refrigerated storage, but they were microbiologically safe. Optimized marzipan had lowered fat content and caloric value in relation to the regular.

Keywords
Konjac flour
Low-calorie dessert
Marzipan
Pumpkin
Response surface methodology

Introduction
Desserts, generally contain high amount of sugar and fat, are preferably consumed by all aged people. A high intake of these products leads to health-problems such as coronary disease, diabetes, high blood pressure, obesity, high cholesterol and some cancers (Newsome, 1993). Functional desserts which formulated to contain low sugar/fat content or make them healthier without adverse effect on eating quality would be required for health-conscious consumers. Sugar is a primary ingredient for replacement. A variety of sugar substitutes; both synthetic sweeteners such as saccharin, aspartame, acesulfame-K, and sucralose, and also plant-derived sweeteners such as stevioside are currently used (Hosseini et al., 2015). Whey and soy proteins are generally used as fat replacers, primarily through technological properties and health benefits. They contain high quality proteins and essential amino acids and provide functional health advantages for lowering cholesterol, blood pressure, and risk of cardiovascular disease and cancer, meanwhile promoting immunity. Isoflavones, one of phytochemicals found in these proteins, are natural antioxidants that promote human disease prevention (Conforti and Davis, 2006; Nordqvist, 2015). The success for the manufacture of functional desserts is rely on various factors such as characteristics of food product, method of processing, level of sugar/fat reduction as well as types and properties of sugar or fat substitutes. However, a concern for consumers’ sensorial satisfaction of the products should not be negligible.

A most popular Thai mung bean marzipan namely ‘Look Choop’, which usually shaped into small fruits and vegetables and coated with agar, has been studied for energy reduction (Akesowan and Choonhahirun, 2013). The optimal reduced-calorie marzipan, achieved by substituting 75% sugar by erythritol-sucralose (98.6:1.4) blend and 25% coconut milk by soy milk, had lowered fat content and total caloric value approximately 16.1% and 16.4% in relation to the regular marzipan. However, the softer and less cohesive texture does not meet the product quality, and it also found to negatively affect eating quality and consumer preference. Many studies have attempted to modify the properties of food products using hydrocolloids through their diverse functional properties such as gelling, thickening, stabilizing, water retention, and textural enhancing properties (Rosell et al., 2007; Sim et al., 2011). Konjac flour, a non-ionic hydrocolloid consisting of D-glucose and D-mannose (1:1.6) units with β-1,4-linkages, was selected in this study (Yeh et al., 2010). It has been widely used for modifying textural characteristics, water retention, and foam and emulsion stability in...
food products (Takigami, 2010; Akesowan, 2015). In view of health benefits, the konjac has been used for reducing weight, obesity, cholesterol, triglycerides, constipation and the risk of tumors and some cancers. It can be used as a good source of dietary fiber as well (Luo et al., 2013; Zhang et al., 2005).

Cooked mung bean seed is a good source of protein (22-25%) and dietary fiber (7-9%) but low in fat (2-5%), cholesterol, and sodium. The seed is highly nutritious because it is reported to be rich in folate, thiamine, magnesium and manganese, but deficient in essential vitamins such as vitamin A, C, and E (Mubarak, 2005). The fortification with nutritionally natural ingredients could not only make mung bean marzipan healthier, but also create diversified dessert products. Pumpkin is considered to be a healthy ingredient because it is high in vitamin A, C, and E, and beta-carotene besides being low in calorie, fat, and cholesterol. It also contains high amount of dietary fiber, riboflavin, potassium, copper and manganese (Dhiman et al., 2009). The consumption of food containing pumpkin has been found to support health and immunity, while reducing the risk of diabetes, obesity, heart disease and certain types of cancers. Beta-carotene, a powerful antioxidant which is converted to vitamin A in the body, promotes eye health (good vision) and prevents a degenerative eye disease (Chandrasekhar and Kowsalya, 2002). Kwon et al. (2007) also reported that phenolic phytochemicals found in pumpkin were beneficial to manage for hyperglycemia and hypertension.

To the best of our knowledge, this study was aimed to assess the effects of konjac flour and pumpkin on sensory characteristics of reduced-calorie mung bean marzipan and to optimize the product formulation using response surface methodology (RSM). The optimal formulation was investigated for proximate analysis, consumer acceptance, and storage stability at refrigerated temperature (10-12°C).

**Materials and Methods**

**Materials**

Peeled mung beans (Raithip®, Rai Thanee, Thailand), fresh pumpkin (Cucurbita maxima), erythritol-sucralose (98:6:1.4) blend (D–et®, U-Sing Co., Ltd., Thailand), soy milk (Silk® (unsweet), White Wave Foods, USA), coconut milk (Aroidee®, Thai Agri Foods, Thailand), sugar and agar powder were purchased from a local supermarket. Konjac flour (Chengdu Newstar Chengming Bio-Tech Co., Ltd., China) was used in this study.

**Experimental design**

Two independent variables, konjac flour and pumpkin, were studied for their effects on sensory properties of reduced-calorie marzipan using a central composite rotatable design (CCRD). The experimental design consisting of five coded levels, -1.41, -1, 0, 1, 1.41, and actual values is presented in Table 1. The mathematical model used for predicting the responses is in the equation below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

Where Y is the observed response; $X_1$ and $X_2$ represent the konjac flour and pumpkin, respectively; $b_0$ is the intercept, $b_1$ and $b_2$ are linear, $b_{11}$ and $b_{22}$ are quadratic, and $b_{12}$ is the interaction coefficients, respectively.

**Preparation of mung bean marzipan**

Regular mung bean marzipan (% by total weight) contained 40% mung bean, 40% coconut milk and 20% sugar. Reduced-calorie formulation was substituted for 75% sugar by erythritol-sucralose blend (based on equivalent sweetness) and for 25% (w/w) coconut milk by soy milk. Mung beans, cleaned and soaked in water overnight, were steamed approximately 30 min or until soft. The beans were ground with sugar and coconut milk using a Waring blender® (Model 7011S, Waring Commercial, CT, USA) for 1 min before pouring into a brass wok. The mixture was heated at low temperature (55-60°C) for 20-30 min with continuous mixing by a wooden paddle until the paste was smooth and thickened. Upon cooling, the paste was kneaded once, and rolled into a ball size (2-cm diameter), followed by painting with a food color. Each marzipan was dipped in a hot agar-sugar (2:3) solution (12% w/v), allowed agar to set before dipping again, and then kept in a plastic box before analysis. In the RSM study, reduced-calorie marzipan was prepared by incorporating with konjac flour (0.1-0.3% w/w) and steamed pumpkin (15-30% by mung bean weight) followed the experimental design.

**pH measurement**

A 5 g of sample was blended with 50 mL of distilled water for 1 min. The pH was measured using a pH meter (Model 320, Metler-Toledo Ltd, Essex, UK).

**Consistency**

Consistency, expressed as the peak force used to penetrate into a sample, was determined using a Lloyd texture analyzer (Model LRX, Lloyd Instruments,
Hampshire, UK). The sample was filled into cylinder cups (4 cm diameter x 6 cm height) to reach 4 cm height. The puncture probe (1.25-cm diameter) working with a crosshead speed at 250 mm/min was used to compress the sample up to the 50% original height. Peak force (N) was recorded. All analyses were performed in five replications.

Color measurement
Hunter color scales; L* (lightness), a* (red/green) and b* (yellow/blue) values, were measured by a HunterLab digital colorimeter (Model ColorFlex, Hunter Associates Laboratory, Reston, VA). All analyses were performed in five replications.

Proximate analysis and caloric value.
Chemical compositions (moisture, protein, fat, ash and total carbohydrate) of regular and optimal marzipan were determined according to AOAC (1990) methods. The energy value was calculated based on the content of fat, protein, and carbohydrate.

Sensory evaluation
Twenty-four people who occasionally consumed mung bean marzipan were chosen as panelists for product formulation. Color, taste, texture and overall acceptability were evaluated using a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like). Optimized marzipan was conducted on consumer acceptance testing using a 9-point hedonic scale and purchasing decision using a 5-point scale test (1 = certainly would not buy, 5 = certainly would buy). A total of 80 consumers drawn from the University of the Thai Chamber of Commerce in Thailand was used. Panelists were instructed to rinse their palates before testing each sample.

Microbiological determination
Samples of 10 g (1, 3, 5 and 7 days) were aseptically weighed and placed in a stomacher bag containing 90 mL of sterile 0.1% peptone (Difco) diluent and pummeled for 1 min with a Stomacher-400 (Tekmar Company, Cincinnati, OH). Appropriate dilutions of samples were prepared in sterile 0.1% peptone and plated in duplicate on the following media and incubation conditions used: (a) Petrifilm™ Aerobic Plate Count: 35-37°C for 1-2 days and (b) Petrifilm™ Yeast and Mold Count: 25-27°C for 3-5 days. The microbial counts were expressed as log CFU/g.

Statistical analysis
All analyses were carried out in triplicate unless otherwise indicated. In case of formulation optimization, data were analyzed for regression and analysis of variance (ANOVA) using Design–Expert® Trial Educational version 8.0.2 software (State-Ease Inc., Minneapolis, Minnesota, USA). In other studies, physical and sensory data were analyzed by ANOVA at the 95% confidence level. Means with a significant difference (p<0.05) were compared using t-test method (Cochran and Cox, 1992).

Results and Discussion

Model evaluation
Sensory results of reduced-calorie marzipan added with konjac flour and pumpkin are presented in Table 1. All formulations showed mean values ranging from ‘moderately like (6.9)’ to ‘highly like (8.2)’. This implied that incorporation of konjac flour and pumpkin generated favorable sensory scores in reduced-calorie marzipan. There seems to be an advantage as the food product with high acceptance is likely to grow in the market. The ANOVA and regression analysis in Table 2 show that sensory models such as color, taste, texture and overall acceptability were significant (p<0.01). The models had coefficients of determination (R²) ranged between 0.9005-0.9561, in accordance with insignificant lack of fit values (p>0.05), indicating that all models were fitted well and reliable. Among responses, the texture model showed the highest R²; hence, it could better explain the variability of data.

Sensory quality of reduced-calorie marzipan added with konjac flour and pumpkin
Color. The variation of pumpkin had a positive linear effect (p<0.001) on color of reduced-calorie marzipan, while the addition of konjac flour was negligible (Table 2). Addition of pumpkin (15-30%) was effective in increasing color preference (Figure 1a). In this study, the reduced-calorie marzipan was made by the substitution of 75% sugar with erythritol-sucralose blend and of 25% coconut milk with soymilk; hence, the product color was light or pale compared to the regular formulation. This is because the amount of sugar (sucrose) participating in the Maillard browning reaction is decreased, as sucrose, thermally degrades to glucose and fructose which are active reducing sugars to react with amino acids, resulting in the formation of brown pigments (melanoidin) (Alais and Linden, 1991). The low concentration the sugar is, the less brown formation the product occurs. Under this condition, reduced-calorie marzipan showed a higher lightness, a major change led to low consumer acceptance. Incorporation of pumpkin exhibited a good point to
enhance product color acceptance as confirmed by a rising color score. The result was possibly due to the yellow color of the pumpkin. Additionally, the increase in glucose and fructose, as a consequence of the addition of pumpkin which has a sweet taste, was subjected to brown pigment formation through the Maillard reaction. Most panelists admired color appearance of marzipan containing konjac and pumpkin, although it seemed different from the traditional marzipan.

Taste. According to Table 2, the quadratic term of konjac flour (p<0.001), followed by linear (p<0.01) and quadratic (p<0.001) terms of pumpkin had effects on taste of reduced-calorie marzipan. No interaction effect of konjac flour and pumpkin was found. The addition of pumpkin from 15 to 25% increased taste liking scores (Figure 1b). It is generally known that pumpkin has sweet taste and unique flavor, which may be supportive in sweet attribute of marzipan. However, panelists gave lower scores if marzipan contained high amounts of pumpkin (>27%). The stronger pumpkin odor and aftertaste was found to mask original taste and flavor of mung bean. This was in accordance with other studies which showed the limitation for pumpkin fortification depending on food products. Wongsagonsub et al. (2015) reported that the maximum level of 20% pumpkin flour could be incorporated into wheat flour for bread production. A similar work revealed that biscuits developed by replacing wheat flour with pumpkin powder (<2.5% level) was more desirable than those with higher pumpkin powder (Kulkarni and Joshi, 2013).

Table 1. Experimental conditions and sensory results of reduced-calorie marzipan with various levels of konjac flour and pumpkin

<table>
<thead>
<tr>
<th>Run</th>
<th>Coded (actual) values</th>
<th>Sensory scores</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>X2</td>
<td>Color</td>
<td>Taste</td>
</tr>
<tr>
<td>1</td>
<td>-1 (0.1)</td>
<td>7.41±0.87</td>
<td>7.3±0.92</td>
</tr>
<tr>
<td>2</td>
<td>+1 (0.3)</td>
<td>7.35±0.86</td>
<td>7.9±0.69</td>
</tr>
<tr>
<td>3</td>
<td>-1 (0.1)</td>
<td>7.9±0.76</td>
<td>6.9±0.46</td>
</tr>
<tr>
<td>4</td>
<td>+1 (0.3)</td>
<td>8.1±0.74</td>
<td>8.6±0.71</td>
</tr>
<tr>
<td>5</td>
<td>-1.41 (0.06)</td>
<td>7.7±0.83</td>
<td>6.7±0.60</td>
</tr>
<tr>
<td>6</td>
<td>+1.41 (0.34)</td>
<td>7.7±0.44</td>
<td>7.9±0.78</td>
</tr>
<tr>
<td>7</td>
<td>0 (0.2)</td>
<td>7.9±0.64</td>
<td>7.3±0.94</td>
</tr>
<tr>
<td>8</td>
<td>0 (0.2)</td>
<td>8.2±0.86</td>
<td>7.9±0.39</td>
</tr>
<tr>
<td>9</td>
<td>0 (0.2)</td>
<td>7.9±0.40</td>
<td>7.6±0.42</td>
</tr>
<tr>
<td>10</td>
<td>0 (0.2)</td>
<td>7.7±0.37</td>
<td>7.6±0.66</td>
</tr>
<tr>
<td>11</td>
<td>0 (0.2)</td>
<td>7.6±0.86</td>
<td>7.7±0.66</td>
</tr>
<tr>
<td>12</td>
<td>0 (0.2)</td>
<td>7.7±0.71</td>
<td>7.8±0.73</td>
</tr>
<tr>
<td>13</td>
<td>0 (0.2)</td>
<td>7.6±0.65</td>
<td>7.8±0.60</td>
</tr>
</tbody>
</table>

1Code values: X1 = % (w/w) konjac flour and X2 = % (w/w) pumpkin proportion in a mung bean/pumpkin mixture.

Figure 1. Sensory properties of reduced-calorie marzipan with various levels of konjac flour and pumpkin: (a) color, (b) taste, (c) texture and (d) overall acceptability

Texture. Konjac flour showed linear (p<0.001) and quadratic (p<0.05) effects on texture scores, while pumpkin had an insignificant effects (p>0.05) (Table 2). There was no interaction effect between the two variables. The soft texture resulting from the substitution of sugar and coconut milk in reduced-calorie marzipan seemed to be a defect that decreased consumer preference (Akesowan and Choonhahirun, 2013). The mixing process was also found more difficult as compared to that of the regular, as the
reduction of coconut milk caused low lubricated oil portion in the mixture. During heat processing, it needed more effort to mix the mixture, and usually found some mung bean paste stuck on a brass wok. Konjac addition could overcome the negative texture of reduced-calorie marzipan, as evident by panelists gave higher texture scores when increasing of konjac levels from 0.1% to 0.3% (Figure 1c). It might be due to konjac flour has good thickening ability and water holding capacity, thus it thickens the mung bean-pumpkin paste and aids in combining all ingredients together. The finished paste was more consistent, cohesive and smooth. Konjac addition also facilitated to the mixing process, thus it seemed to reduce residual paste sticking on the wok.

Overall acceptability. As shown in Table 2, overall acceptability was affected by linear and quadratic terms of konjac flour (p<0.01), followed by linear (p<0.01) and quadratic (p<0.05) terms of pumpkin. There was no interaction effect between the variables. Increases in konjac flour range of 0.1-0.3% and pumpkin range of 15-30% increased overall acceptability scores (Figure 1d). It noted that, at higher level of pumpkin (>27%), panelists perceived stronger odor and taste of pumpkin which masked the original taste of the product. This is why lower overall acceptability score was found at high pumpkin addition.

Formulation optimization
The optimal reduced-calorie marzipan incorporated with konjac flour and pumpkin was designed to maximum consumer preference using the Design-Expert® software. The optimized formulation was achieved with 0.3% konjac flour and 25.16% pumpkin, which had a desirability value of 0.859. Predicted scores of color, taste, texture and overall acceptability were 7.8, 7.7, 7.9 and 7.9 respectively. The validation of the optimal condition was checked by an experimental run. As compared with sensory testing, scores obtained for color, taste, texture and overall acceptability were 7.7, 7.4, 8.1 and 7.6, respectively, which revealed a percentage of relative error of 1.3%, 4.1%, 2.5% and 3.9%, respectively. The results exhibited that the model of optimized formulation was reliable.

Proximate composition
Chemical compositions of regular and optimized marzipan are presented in Table 3. Lower fat and carbohydrate found in the optimized formulation (p<0.05) were possibly caused by the 75% sugar
substitution with erythritol-sucralose and 25% coconut milk substitution with soymilk. An erythritol-sucralose blend provides approximately 8 times of sucrose sweetness and caloric value about 0.18 kcal/g (U-sing, Co., 2012). In case of soymilk, it contains only 2-3% fat in comparison with canned coconut milk (12-15% fat) (Seow and Gwee, 1997; Ikya et al., 2013). Optimized marzipan showed the decrease in fat from 5.44% to 3.78% or a 31% fat reduction and caloric value from 216.16 to 159.26 kcal/g or a 26% calorie reduction, in relation to the regular. Protein content was not different (p>0.05) in both samples. The increase in ash content implied that there was more minerals in optimized sample as agreed with Kulkarni and Joshi (2013) who found higher ash content indicating more minerals such as calcium, phosphorus and iron in biscuits incorporated with pumpkin powder. Higher moisture content was found in optimized marzipan which could be predicted that it was susceptible to microbial spoilage. All chemical compositions of both samples were consistent (p>0.05) after refrigerated storage for 7 days, except for moisture content that significantly decreased (p<0.05).

Storage stability

Physical properties

On the day of processing (day 0), pH value of the optimized marzipan was higher (p<0.05) than the regular (Table 3). Both regular and optimized samples had lowered pH by the 7th day of storage. This was possibly due to the microbiological deterioration caused by *Staphylococcus aureus*, *Escherichia coli*, yeast and mold. In particular, *S. aureus* utilizes the food as a nutrient source and produces lactic acids which reduced the pH of the samples. The consistency of optimized marzipan was found to be 8.84 N (Table 3) which was raised from that of original reduced-calorie marzipan (7.56 N, data not shown). It seemed that the higher consistency was improved by the addition of konjac flour, which would be related to its water holding capacity. Nevertheless, it was relatively lower (p<0.05) than the regular. Optimized marzipan had higher \( L^* \) and \( b^* \) values than the regular, indicating a more bright and yellowish color which was attributed to pumpkin addition. Significant decreases in \( L^* \) and \( b^* \) values in both samples after storage were possibly attributed to various reasons such as brown formation via the Maillard reaction,
darker pigment products from lipid oxidation, and pigment degradation by oxidative reaction (Alais and Linden, 1991).

Sensory evaluation

Results of consumer acceptance evaluated for regular and optimized marzipan are shown in Table 3. On day 0, average sensory score of the regular was ranged from 7.1 to 8.1, while that for optimized formulation was 7.2 to 7.5. Most consumers preferred the regular as noticed by significantly higher (p<0.05) scores for taste, texture, and overall acceptability. Optimized marzipan showed (p<0.05) higher color liking score as compared with the regular. This finding suggested that pumpkin addition was likely to promote product color preference. When considering the results of purchasing decision (Figure 2), a percentage of consumers that rated in scores of 5 and 4 (would certainly or possibly buy the product) for regular and optimized samples was approximately 66% and 57%, respectively. These consumers who are willing to buy optimized marzipan would be motivated by the importance of health and wellness.

On day 7, the regular marzipan was scored in range of 7.0-7.7 and the optimized marzipan was 7.0-7.4 (Table 3). Although the scores decreased from the day 0, both samples were still accepted in the moderate preference. A color liking score remained consistent (p>0.05) in both samples after storage, while other attributes such as taste, texture, and overall acceptability were scored lowered (p<0.05). A taste liking score was markedly decreased in the regular compared to optimized marzipan, which might be due to the regular contained higher amount of coconut milk. Lipid oxidation is a major chemical deterioration restricting the shelf life of food products. In this study, rich fat-containing coconut milk is susceptible to the oxidative reactions, thereby the release of short-chain fatty acids (butyric, caproic, caprylic and capric acids) is of major concern to off-odors while the release of medium-chain fatty acids (lauric and myristic acids) produces a soapy taste (Seow and Gwee, 1997). Unpleasant tastes and odors occurred would affect product taste perception. Decreasing of texture scores might be due to both samples became more dry, hard and crumbly. This in turn may start to lose product freshness and succulence. The decrease in overall acceptability might be influenced by the lower consumer perception in taste and texture of the samples.

Microbiological count

The total aerobic count of the regular was ranged from 2.57 log CFU/g on day 1 to 2.41 log CFU/g on day 7, while in optimized marzipan it ranged from 3.05 to 3.51 log CFU/g (data not shown). A higher growth of total aerobic count in optimized marzipan was possibly due to it contained higher moisture content which support the growth of microorganisms. After 7 days of storage, total aerobic bacteria counts in regular and optimized samples were 2.5 and 3.5 log CFU/g which were below the standard level of 4 log CFU/g for Thai desserts (Jariyawaranugoon and Akesowan, 2010). This indicated both samples were safe for consumption. Although a growth of yeast was found in both samples (data not shown), it was slight and negligible. However, the growth of mold was not detectable.

Conclusion

In incorporation of konjac flour effectively improved texture of reduced-calorie mung bean paste, thereby the marzipan had better eating quality. Visual paste color was notably advantaged by pumpkin addition which enhanced consumer acceptance. Optimized marzipan, achieved by the addition of 0.3% konjac flour and 25.16% pumpkin, had better physical and sensory properties, and was considered healthier as the reduction of 31% fat content and 26% total caloric value based on the regular. The marzipan was microbiologically safe when stored at 10-12°C for 7 days.

Acknowledgement

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