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Research Article

Role of *Konjac* Flour on Emulsifying Property of Milk and Egg White Proteins and Sensory Acceptance of Gluten-free Cakes

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Abstract: This study was aimed to investigate the effects of three independent variables: *Konjac* concentration (0.1-0.4%), pH level (pH 5-9) and NaCl concentration (0.2-0.6 M) on Emulsifying Activity (EA) of Milk Protein Concentrate (MPC) and Egg White Powder (EWP). Response surface methodology was used to determine the effects and also find out the optimal condition. Both *Konjac* concentration and pH level had greatly significant effects on EA of MPC and EWP, while NaCl concentration had negligible. An increase in *Konjac* concentration showed a positive effect to increase the EA of both MPC and EWP. Moreover, their EA values were found to be more pronounced at pH 6.5-7.5. The interaction of *Konjac* concentration and pH level had a negative effect on EA. The optimal conditions improving the EA of MPC and EWP were applied to the processing of gluten-free rice cakes. Sensory acceptance of gluten-free cakes added with *Konjac* flour was evaluated using a 9-point hedonic scale test. Incorporation of 0.25% *Konjac* flour was appeared to increase sensory scores of the gluten-free cake and showed no significant difference compared to the cake containing 0.4% *Konjac*. Panelists preferred the gluten-free cakes containing *Konjac* and were willing to purchase the products.

Keywords: Emulsifying properties, gluten-free cake, *Konjac* flour, milk and egg white proteins, response surface methodology

INTRODUCTION

In the recent years, an upward trend for hydrocolloid or gum utilization in multi-component food system consisting of polysaccharides, proteins and fat has been observed. Gums have been extensively used in food products for many purposes such as gelling and thickening effects, foaming stabilization, emulsion modification and syneresis reduction during a freeze-thaw cycle (Rosell *et al*., 2007; Kovácová *et al*., 2009). In addition, they have been successfully used to retard staling behavior in bakery products, improve the eating quality of pastas and noodles and extend frozen dough and bread storage (Asghar *et al*., 2007). As seen in the work of Preichardt *et al*. (2011), a neutral, non-gelling polysaccharide named 'galactomannan' can be applied to improve rheological properties of heat-induced whey protein gels. Another study was found with the achievement of carrageenan addition incorporated with adjusting protein solution to pH 6.0 in the presence of 0.2 M NaCl for improving emulsifying properties of milk protein concentrate or sodium caseinate (Mena-Casanova and Totosaus, 2011). The complex formation developed by specific protein-hydrocolloid interactions was responsible for substantially improving emulsifying characteristics, foaming and rheological properties (Mishra *et al*., 2001; Herceg *et al*., 2007).

Konjac flour is a water soluble, non-ionic high polysaccharide containing about 70-85% glucomannan. The flour is extracted from tubers of *Amorphophallus konjac*. Its chemical structure is found to be a linear chain of β-1, 4-linked D-mannose and D-glucose in the ratio of 1.6:1 with a low degree of acetyl groups (Yang *et al*., 2006). Due to its β-linkage structure in which human enzymes do not hydrolyze, the *Konjac* is of interested appreciation as a non-caloric gum for applying in low-calorie food products. *Konjac* flour is one of the most viscous dietary fibers which has been commonly used to thicken the viscosity of the food system (Chua *et al*., 2010). This flour was approved to use as an additive for gelling, thickening, texturizing and binding in various food products (Zhang *et al*., 2005; Akesowan, 2010). Apart from the technological effect, the *Konjac* is also associated with health benefits in terms of controlling weight gained and obesity, improving intestinal activity such as bowel movement and alleviated constipation as well as lowering of blood cholesterol and triglycerides (Takigami, 2000). As a consequence, the application of *Konjac* seems to simultaneously obtain both technological and health promoting effects in the finished products.

Celiac disease, which approximately found one in hundred people, is considered as an important public health issue (Shevakani and Singh, 2014). At the same time, most celiac persons play more attention on alternatives they should consume (Demirkesen *et al*., 2013). Thus, many research studies in gluten-free products have continued to increase. At now, the food product which is healthy, tasty and free of gluten would be a better choice for celiac patients to decrease adverse effects of gluten allergy. Generally, celiac disease causes digestive problems (pain and discomfort in the digestive tract, gas, chronic constipation and diarrhea), inflammation of intestinal cells subjecting to reduce ability to properly absorb nutrients from food, iron deficiency anemia, vitamins and minerals deficiencies as well as a severe skin rash called dermatitis herpetic form is and seizure (Sammu *et al*., 2010). Rice flour, a non-gluten ingredient, is used as a wheat flour alternative for making cakes, cookies and breads. However, it has not similar functional properties as wheat flour; due to it has lower protein and no gluten which contributes to gas retention in batter/dough and protein network formation during baking (Gallagner *et al*., 2004). Unavoidably, it is appeared that diminished viscoelastic properties of dough and lower quality of non-gluten rice products were observed in non-gluten rice products (Maghaydah *et al*., 2013). Various hydrocolloids such as xanthan gum, guar gum and carboxy methyl cellulose have been used to thicken the viscosity of gluten-free batter/dough. A higher batter viscosity, a more tiny air bubbles entrapped in the batter, which expand during baking, thus improving the quality of rice-based bakery products (Sammu *et al*., 2010; Preichardt *et al*., 2011; Ranjbar *et al*., 2012). In addition, a longer shelf life and lower staling behavior were found in the gluten-free muffins and cakes incorporated with xanthan gum (Preichardt *et al*., 2011). To date, the use of *Konjac* flour in the gluten-free rice cake has not yet been investigated.

Response Surface Methodology (RSM) has been extensively used to explore the effects of independent variables on the responses and also to optimize the best condition for the process variables (Anderson and Whitcomb, 2005). At present, there is limited information involving the use of the RSM to study the properties of milk and egg white proteins added with *Konjac* flour. Therefore, this work aimed to understand the influence of various levels of *Konjac*, pH and NaCl on emulsifying property of Milk Protein Concentrate (MPC) and Egg White Powder (EWP) using RSM. The optimal conditions to obtain the maximized values of the properties were investigated. Also, sensory evaluation of gluten-free rice cakes incorporated with optimal levels of *Konjac* flour was studied.

MATERIALS AND METHODS

Materials: *Konjac* flour (Chengdu Newstar Chengming Bio-Tech Co., Ltd., Chengdu, Sichuan, China), milk protein concentrate (MPC 80, Vitalus Nutrition, Inc., Abbotsford, Canada) and egg white powder (egg albumen) (Michael Foods, Inc., Minnesota, USA) were used. Refined corn oil and cake ingredients included wheat and cake flour, sugar, salt, fresh eggs, soybean oil, evaporated milk and baking powder were purchased from a local supermarket.

Emulsifying activity: The emulsion capacity was determined according to the method of Yasumatsu *et al*. (1992) with some modification. To prepare emulsion, 25 mL of corn oil and 25 mL of protein solution with different levels of variables were mixing by a blender at speed 2 for 1 min. After centrifuging at 2000×g for 15 min, the mixture was poured into a 100-mL cylinder and then the height of emulsified layer was measured. All analyses were performed in 7 replications. Emulsifying Activity (EA) was expressed as a percentage of the height of emulsified layer in relation to total liquid content.

Experimental design: The protein was employed at 1% (w/v). Five levels (-1.68, -1, 0, 1 and 1.68, respectively) containing twenty experimental treatments were run using a Central Composite Rotatable Design (CCRD) with 3 independent variables including *Konjac*, pH and NaCl. The coded and actual values used for this experimental design are shown in Table 1. The predictive models for EA of MPC and EWP are proposed in the equation below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where, Y is the predictive response; X_1, X_2 and X_3 are independent variables such as *Konjac* concentration (%), pH level and NaCl concentration (M); b_0 is the intercept and b_1, b_2 and b_3 are linear, b_{11}, b_{22} and b_{33} are quadratic and b_{12}, b_{13} and b_{23} are interaction coefficient, respectively.

Cake preparation: The standard cake was prepared based on flour weight basis. Initially, 100 g wheat flour, 112.4 g sugar, 3 g salt and 3 g baking powder were thoroughly mixed in a bowl by hand. The 55.5 g egg yolk, 30 g soybean oil, 30 g water, 120 g orange juice and 3 g vanilla flavor were well mixed in another bowl. The liquid mixes were poured into the dry ingredients bowl and then well mixed with an eggbeater until smooth (about 2 min). The whipped egg white was prepared by whipping 100 g egg white, 0.7 g salt and 0.5 g cream of tartar until the soft peak was formed. The 24 g sugar was added and whipped to form firm, moist peaks. The whipped egg white was folded into the flour-liquid mixture, gently mixed and immediately deposited into cake pans. The batter was baked at 170-180°C for 25-30 min. The cake was allowed to cool for 1 h, packed in low density polyethylene bags and kept at room temperature (29-30°C) for the next day

Table 1: Three dependent variables with coded and actual values and results of EA of MPC and EWP under various levels of *Konjac* concentration, pH level and NaCl concentration

	Coded (actual) values			EA (%)	
Experimental run	*Konjac* concentration (%)	pH level	NaCl concentration (M)	MPC	EWP
1	-1 (0.10)	-1 (5)	-1 (0.2)	67.4	81.2
2	+1 (0.40)	-1 (5)	-1 (0.2)	94.3	93.1
3	-1 (0.10)	+1 (9)	-1 (0.2)	43.6	41.2
4	+1 (0.40)	+1 (9)	-1 (0.2)	57.9	46.4
5	-1 (0.10)	-1 (5)	+1 (0.6)	61.1	74.4
6	+1 (0.40)	-1 (5)	+1 (0.6)	97.8	92.7
7	-1 (0.10)	+1 (9)	+1 (0.6)	49.3	49.7
8	+1 (0.40)	+1 (9)	+1 (0.6)	64.8	54.4
9	-1.68 (0.00)	0 (7)	0 (0.4)	48.9	48.8
10	+1.68 (0.50)	0 (7)	0 (0.4)	96.5	72.4
11	0 (0.25)	-1.68 (3.6)	0 (0.4)	55.9	90.5
12	0 (0.25)	+1.68 (10.4)	0 (0.4)	31.9	21.4
13	0 (0.25)	0 (7)	-1.68 (0.06)	92.4	89.9
14	0 (0.25)	0 (7)	+1.68 (0.74)	98.2	93.2
15	0 (0.25)	0 (7)	0 (0.4)	92.0	92.7
16	0 (0.25)	0 (7)	0 (0.4)	96.8	93.6
17	0 (0.25)	0 (7)	0 (0.4)	93.2	91.1
18	0 (0.25)	0 (7)	0 (0.4)	92.1	93.5
19	0 (0.25)	0 (7)	0 (0.4)	97.5	90.4
20	0 (0.25)	0 (7)	0 (0.4)	97.9	93.9

MPC: Milk protein concentrate; EWP: Egg white powder; EA: Emulsifying activity (%)

analysis. The gluten-free cakes were made by replacing wheat flour with rice flour in the absence and presence of *Konjac* flour at optimal levels.

Sensory evaluation: Initially, the sensory acceptance of wheat and gluten-free rice cakes containing 0, 0.25 and 0.4%, respectively *Konjac* flour was evaluated using 9-point hedonic scale test (1 = extremely dislike, 9 = extremely like). Panelists were invited to assess theses sensory attributes including appearance, taste, texture and overall acceptability. In addition, a 5-point scale test (1 = certainly would not buy, 5 = certainly would buy) was used to assess for product purchase. All sensory tests were conducted by 100 untrained panelists from the University of the Thai Chamber of Commerce, Thailand. Panelists were invited to rinse their palates before testing each sample.

Statistical analysis: All experiments were carried out in triplicate. Data were analyzed for regression and Analysis of Variance (ANOVA) using the Design-Expert® Trial Educational version 8.0.2 software (State-Ease Inc., Minneapolis, Minnesota, USA). For gluten-free cake study, sensory data were conducted on a Randomized Complete Block Design (RCBD). Means with a significant difference ($p<0.05$) were compared using Duncan's new multiple range test (Cochran and Cox, 1992).

RESULTS AND DISCUSSION

Statistical analysis and model fitting: Results of different levels of *Konjac* concentration, pH and NaCl concentration on emulsifying activities of MPC and EWP determined by a CCRD design are presented in Table 1. The EA of MPC ranged from 31.9 to 98.2%, while that of EWP ranged from 21.4 to 93.9%, respectively. The statistical analysis for regression coefficients, sum of squares and p-values of responses of MPC and EWP is shown in Table 2. There were linear, quadratic and interaction effects on each model. The EA model of MPC was significant and its determination coefficients (R^2) was found to be 0.9793, while that of EWP was also effective with R^2 of 0.9951. The results indicated that the fitted model accounted for more than 97% of the variance in the experimental data. Moreover, their high R^2 values confirmed the good correlation between the experimental and predicted values (Koocheki *et al*., 2010). Here, both models also displayed no significant ($p>0.05$) lack of fit values (Table 2), consequently advocating the reliability and accuracy of the EA models of MPC and EWP. The fitted EA models with significant actual factors were described in the following Eq. (2) to (3):

$$MPC = -149.19 + 349.49X_1 + 60.98X_2 - 14.08X_1X_2 - 369.13X_1^2 - 4.60X_2^2 \quad (2)$$

$$EWP = -33.94 + 349.73X_1 + 33.45X_2 - 8.46X_1X_2 + 7.41X_2X_3 - 552.82X_1^2 - 3.12X_2^2 \quad (3)$$

Analysis of response surface plots: From Table 2, EA of both MPC and EWP were significantly affected by linear and quadratic terms of *Konjac* concentration and pH level at $p<0.001$, while NaCl concentration was not found to be significant at $p<0.05$. The interaction of "*Konjac* concentration and NaCl concentration" was found to be significant at $p<0.05$ and $p<0.01$ on EA of MPC and EWP, respectively. In addition, the EA of

Table 2: Coefficients of responses, sum of squares and p-values of EA of MPC and EWP

	MPC			EWP		
Term	Coef.	S.S.	p-value*	Coef.	S.S.	p-value*
Model	-	9565.7700	<0.0001	-	9871.6300	<0.0001
Intercept	94.99	-	-	92.58	-	-
X_1: *Konjac* concentration	12.70	2203.0000	<0.0001	5.84	466.1800	<0.0001
X_2: pH level	-10.64	1547.2400	<0.0001	-19.47	5177.5600	<0.0001
X_3: NaCl concentration	1.43	28.0000	0.2667	1.09	16.1500	0.0924
X_1X_2	-4.22	142.8000	0.0240	-2.54	51.5100	0.0077
X_1X_3	1.38	15.1200	0.4075	0.74	4.3500	0.3569
X_2X_3	1.93	29.6400	0.2540	2.96	70.2100	0.0031
X_1^2	-8.31	994.0800	<0.0001	-11.57	1929.0000	<0.0001
X_2^2	-18.49	4925.7200	<0.0001	-13.21	2516.1700	<0.0001
X_3^2	-0.32	1.4300	0.7957	-0.63	5.6700	0.2962
Lack of fit	-	163.8400	0.0690	-	36.0700	0.1021
R-square		0.9793			0.9951	

*: Significant at the 99.9% level if p<0.001, 99% level if p<0.01 and 95% if p<0.05; Coef.: Correlation regression; S.S.: Sum of squares; EA: Emulsifying activity (%); X_1: *Konjac* concentration (%, w/v); X_2: pH level; X_3: NaCl concentration (M)

EWP was significantly affected (p<0.01) by the interaction of "pH level and NaCl concentration". The effect of *Konjac* concentration, pH level and NaCl concentration on EA of MPC is given in Fig. 1a to c and that of EWP is in Fig. 1d to f. Response surface graphs were plotted between the independent variables while keeping another independent variable at the zero coded level. It was seen that the graphs of both MPC and EWP demonstrated similar manners. In this study, the maximum amount of oil dispersing in an aqueous solution was measured and defined as EA. The EA is the result of soluble and insoluble protein fractions and other components such as polysaccharides. Regarding the results in Fig. 1a and d, at 0.4 M NaCl concentration, it was observed that both MPC and EWP exhibited the most EA at the optimal pH ranged from 6.5 to 7.5 and their EA values tended to decrease as the pH was either lower or higher than the optimal pH level. An increase in *Konjac* concentration from 0.1 to 0.4% increased the emulsified layer phase or EA of MPC and EWP. This indicated that the *Konjac* flour has an ability to enhance EA of the both proteins, which was attributed to it can increase the viscosity of continuous aqueous phase to prevent or retard oil droplets come to flocculate or coalesce, consequently the droplets still disperse individually in the aqueous medium contributing to the good emulsion system (Alleoni, 2006). This was confirmed by the study of Mena-Casanova and Totosaus (2011), which improved emulsifying properties of milk proteins with kappa and lambda carrageenans. They revealed the increase of emulsifying properties as a result of functional properties of the carrageenans to greater the oil incorporated into the emulsion before breaking down. Farshchi *et al*. (2013) stated that the interaction between protein-polysaccharide (milk protein-locust bean gum) molecules promoted strengthen and thicker interfacial layer, which was associated with the steric repulsion on oil droplets and protected the droplets against the flocculation sand coalescence. This can stabilize and enhance kinetic stability of the oil-water emulsion. Nevertheless, the interaction of "*Konjac* concentration and pH level" had a negative effect (Table 2), which caused a rapid decrease in EA of MPC and EWP when 0.4% *Konjac* concentration and pH at 9 were applied.

Figure 1b and e show that, at pH 7, the variation of NaCl concentration (0.2-0.6 M) did not affected EA of MPC and EWP. Increasing of *Konjac* concentration promoted the increment of EA of the proteins, indicating that the *Konjac* would be potential to interact with MPC and EWP to generate the proteins-*Konjac* complexes with strong interactions as the pH system was at pH 7. This finding was not consistent with the work of Mena-Casanova and Totosaus (2011) who presented that both higher pH values (pH>6) and ionic strength (NaCl>0.2 M) were parameters to decrease the emulsifying capacity of the protein-carrageenan mixtures. It was probably due to the *Konjac* is a non-ionic polysaccharide, not as anionic polysaccharides like kappa carrageenan, where sodium salts affect the sulfated ester groups and inhibit the helix formation of kappa carrageenan (Nussinovitch, 1997).

The effects of pH level and NaCl concentration on EA of MPC and EWP, where the *Konjac* concentration was at 0.4%, are presented in Fig. 1c and f. An increase in NaCl concentration had no effect on EA, while the EA of the both proteins tended to decrease at a high pH level (pH 8-9). The reduction of electrostatic interactions between polysaccharides and protein micelles considered to be a factor causing the instability of emulsion (Spagnuolo *et al*., 2005). It was also found that the interaction of "pH level and NaCl concentration" had a negative effect on the EA of EWP (Fig. 1f), showing a rapid decrease in EA at higher levels of pH (pH 8-9) and NaCl concentration (0.5-0.6 M).

Optimization and validation: The optimal levels of *Konjac* concentration, pH level and NaCl concentration to achieve the best emulsifying properties of MPC and EWP were determined using the Design-

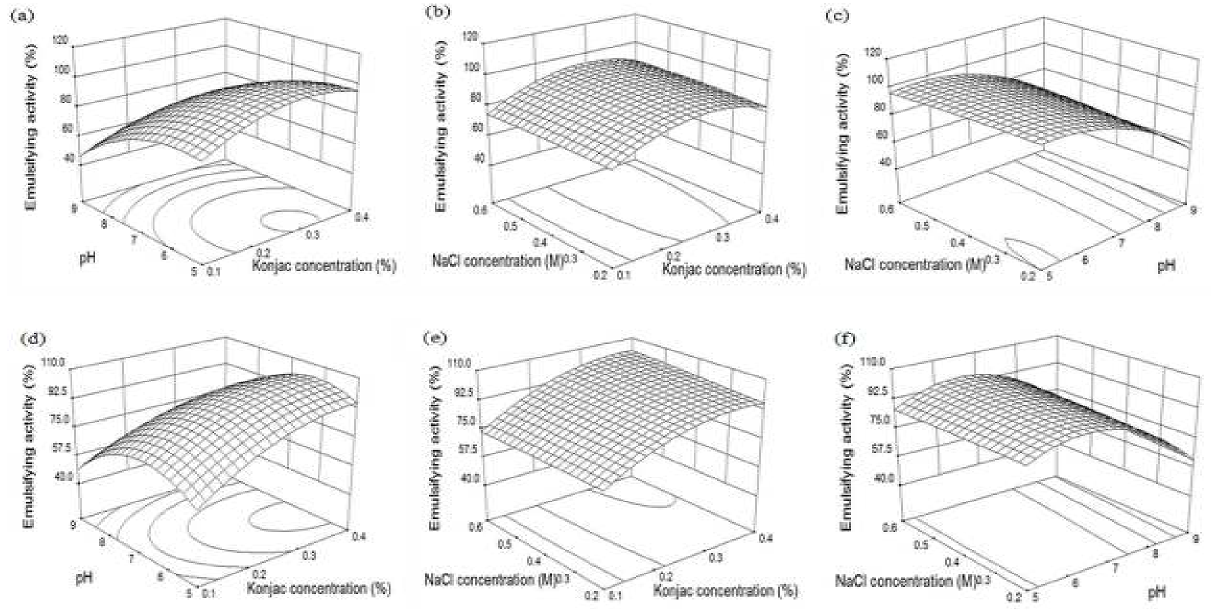

Fig. 1: Response surface graphs for EA of MPC (a-c) and EWP (d-f) under various levels of *Konjac* concentration, pH level and NaCl concentration

Table 3: Sensory scores of gluten-free rice cakes with *Konjac* flour

Formulation*	Sensory scores** Appearance	Taste	Texture	Overall acceptability
C	7.2±0.4^{b}	6.7±0.6^{a}	7.0±0.8^{b}	7.0±0.5^{b}
T1	6.4±0.6^{a}	6.3±0.5^{a}	5.6±0.7^{a}	6.1±0.7^{a}
T2	7.6±0.7^{b}	6.6±0.8^{a}	7.3±1.0^{bc}	7.3±0.9^{b}
T3	7.7±0.3^{b}	6.6±0.4^{a}	7.6±0.6^{c}	7.4±0.7^{b}

Means in the same column with different superscripts are different (p<0.05); *: Formulation included: C: Wheat cake; T1: Gluten-free rice cake; T2: Gluten-free rice cake containing 0.25% *Konjac* flour; T3: Gluten-free rice cake containing 0.4% *Konjac* flour; **: Based on a 9-point hedonic scale test (1 = extremely dislike, 9 = extremely like)

Expert®software. The optimized condition obtaining the maximized EA (98.2%) for MPC was found to be 0.4% *Konjac* concentration, pH 6.81 and 0.23 M NaCl concentration, which had a desirability value of 0.961. For EWP (EA = 97.6%), the condition with 0.25% *Konjac* concentration, pH 7.12 and 0.3M NaCl concentration was found to be optimal (desirability value = 0.997).

The suitability of the models was carried out using the recommended optimal conditions. The experimental results (n = 3) of EA determined in MPC and EWP were 97.5 and 96.2%, respectively, which were close to the predicted values, indicating that the models are quite accurate in prediction.

Sensory evaluation of gluten-free rice cakes added with *Konjac* flour: Table 3 presents the results of sensory evaluation of gluten-free rice cakes added with *Konjac* flour. It can be seen that the gluten-free cake (T1) had significantly lower (p<0.05) scores for all sensory attributes, in the exception of taste as compared with the wheat Cake (C). This indicated that the total wheat replacement with rice flour alone caused the product decreased in appreciated characteristics. It is possibly because the difference in functional properties between wheat and rice flour. Wheat flour contains gluten which is predominant for water holding capacity; consequently, it promotes a viscous batter that potentially entraps air bubbles occurred during a mixing process. When baking, the high temperature causes these bubbles to expand the stretchy gluten from the flour, resulting in a risen batter, loss of gluten elasticity and final formation of permanent cake structure. This specific property cannot found in the rice flour (Preichardt *et al*., 2011). The addition of 0.25% *Konjac* flour significantly increased (p<0.05) all sensory attributes of the T2 cake, in exception of taste with respect to the T1 cake. The T2 cake had better product characteristics, namely higher in volume and height, small air cells dispersion in crumb and softer texture, leading to increase in consumers' palatability. Again in Table 3, the overall acceptability score of the T2 cake was found to be 7.3, indicating as well accepted in a level of moderate preference, which was influenced by increasing texture quality as a result of 0.25% *Konjac* addition. Although the use of 0.4% *Konjac* tended to

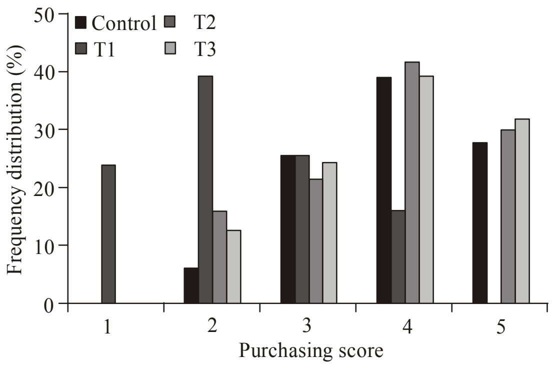

Fig. 2: Frequency distribution of purchasing decision of gluten-free rice cakes with *Konjac*
Flour: C = control wheat cake and T1 to T3 are gluten-free rice cakes with 0, 0.25 and 0.4% *Konjac* flour, respectively; Based on a 5-point structured scale test (1 = certainly would not buy, 5 = certainly would buy)

effectively and greatly aid in cake characteristics, there was no significant sensory differences ($p>0.05$) compared to the cake with 0.25% *Konjac*. Thus, the use of 0.25% *Konjac* flour seems to be sufficient for improving characteristics and sensory acceptance of the gluten-free cake.

The results of consumer purchase of gluten-free cakes, with and without *Konjac*, compared to the wheat cake are given in Fig. 2. The frequency distribution of purchasing decision at score of 5 and 4 (would certainly or possibly buy the product) found in the wheat control, T1, T2 and T3 cakes were approximately 68, 10, 72 and 72%, respectively. At the same time, the T1 cake was mostly chosen at score of 2 and 1 (would certainly or possibly not buy the product), revealing that 64% panelists were unlikely to the product characteristics. They also comment that the T1 cake (no added *Konjac*) had more dense and compact crumb texture. This finding was in agreement with the acceptance test in Table 3, showing that significantly lower scores of appearance, texture and overall acceptability were found in the T1 cake.

CONCLUSION

The RSM was effective to describe how *Konjac* concentration, pH level and NaCl concentration influencing on EA of MPC and EWP. The higher EA was more evident by increasing *Konjac* concentration, especially at the pH of 6.5-7.5. At the lowest *Konjac* concentration (0.1%) and pH 9, the EA of MPC and EWP was found to be the lowest. The NaCl concentration had a slight effect on EA. The maximum EA of MPC was achieved by using 0.4% *Konjac* concentration, pH 6.81 and 0.23 M NaCl concentration, whereas the condition of 0.25% *Konjac* concentration, pH 7.12 and 0.3 M NaCl concentration was optimal for EWP. The gluten-free rice cake containing 0.25% *Konjac* flour had significantly higher ($p<0.05$) scores for appearance, texture and overall acceptability compared to the no added *Konjac*, gluten-free cake. The panelists were more likely to purchase the wheat cake equal to gluten-free cakes with 0.25 and 0.4% *Konjac* flour.

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