

## Improvement of texture and gel stability of restructured frozen mango pulp by using xanthan gum and locust bean gum

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### ABSTRACT

Frozen restructured mango pulp (FRMP) is a form of preserved mango with a consistent texture and the ability to hold a definite shape. The objective of this study was to investigate the effect of xanthan gum (XG) combined with locust bean gum (LBG) on the textural properties, syneresis and sensory acceptance for FRMP from Nam Dok Mai variety and Ok Rong variety. Response surface methodology was employed for investigating the effect of XG (0.3-0.6% w/w) and LBG (0.2-0.6% w/w) on penetration force and syneresis of the FRMP samples. It was found that the relationship between these parameters could be fitted well to a first order linear model. Whilst both XG and LBG had a positive effect on the penetration force of the samples, a negative effect was observed for syneresis. Sensory evaluation using a 9-point hedonic scale indicated that the frozen restructured mango pulp containing 0.6% w/w XG and LBG received a significantly higher overall liking score than the sample containing 0.3% w/w XG and 0.2% w/w LBG, regardless of mango variety. Thus, there is a high potential for the application of XG-LBG blends to the preservation of fresh mango pulp and other fruit pulps.

**Keywords:** mango; restructured fruit pulp; frozen fruit; xanthan gum; locust bean gum

### 1. INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical fruit having a unique taste and flavor depending on its species. Thailand is one of main producers and exporters of fresh mango (Ledeker et al., 2014). Nam Dok Mai variety and Ok Rong variety are popular Thai mangoes, with the former being the main variety for export and the latter mostly locally consumed. Both varieties have strong aroma and flavor, especially sweetness (Suwonsichon et al., 2012; Ledeker et al., 2014). In Thailand, these ripe mango fruits are usually served with steamed sticky rice enriched with coconut milk and considered to be a typical Thai dessert (Moore, 2015). However, fresh mango fruits are

seasonal commodities, which are not available all year round. Chilling and freezing are common methods employed for preserving fresh agricultural products including mango fruits. However, chilling injury, freezing burn and drip loss usually occur during storage under low temperature (Ledeker et al., 2014). The restructuring of fruit pulp before freeze storage, is an alternative way of preserving fruit pulp for later applications that include, as an ingredient in baked goods, ice cream desserts and so on. The restructuring of fruit pulp can be achieved using hydrocolloids as a binding agent, resulting in textural characteristics and the ability to hold a definite shape, similar to those of fresh fruit. However, the extent of each hydrocolloid's

effect depends on its type and quality as well as the fruit pulp employed and its interaction with other components (Grizotto et al., 2007).

Amongst various types and properties of hydrocolloids, alginate and pectin are often used in several restructured fruit pulps to increase firmness and rigidity of the fruit pulp (Mouquet et al., 1992). However, these hydrocolloids provide too brittle and rigid a texture, which would not be desirable in some soft fruit pulp such as ripe mango (Mouquet et al., 1992; Suwonsichon et al., 2012). The combined addition of xanthan gum (XG) and locust bean gum (LBG) to jelly were found to lower the syneresis with satisfactory sensory attributes. In addition, the texture of the product obtained from adding XG and LBG was found to be elastic and non-brittle (Khouryieh et al., 2005, Suwonsichon et al., 2012, Nussinovitch and Hirashima, 2014). Therefore, it is possible to use these combined hydrocolloids for improving the texture of restructured mango to achieve satisfactory product properties. However, there is no study reporting on the application of these hydrocolloids to the production of restructured fruits, including mango fruit. There is also no study reporting on the attributes and stability of freeze stored restructured fruits containing these hydrocolloids.

Xanthan gum is a heteropolysaccharide produced from exocellular of *Xanthomonas campestris*. Xanthan gum has an  $\alpha$ -1,4-linked  $\beta$ -D-glucose backbone with mannose and glucuronic acid side chains. The residue mannose is linked to an acetyl group and another mannose is linked to pyruvic acid, forming a helical structure. Xanthan gum is a thickening agent and a shear thinning fluid with a high nominal viscosity, being stable over a wide range of temperatures and pH values (Morris, 2009, Chivero et al., 2015). Locust bean gum is a plant seed glucomannan containing  $\beta$ -1,4-D-mannopyranosyl and  $\beta$ -1,6-D-galactose which is dissolved in hot water to form viscous macromolecule solution (Dunstan et al., 2001;

Sousa and Gonçalves, 2015).

Both xanthan gum and locust bean gum form viscous aqueous solutions. When xanthan gum and locust bean gum are mixed in a food matrix, gelling formation and changes to textural properties can be anticipated. According to the rheological study of xanthan gum and locust bean gum interaction in dilute solution by Higiroy et al. (2006), it was found that xanthan gum-locust bean gum blends are viscoelastic and lead to gels forming. The interaction and synergistic effect of xanthan gum and locust bean gum on gel formation occurs between the side chains of xanthan gum and the backbone of locust bean gum, according to the lock and key model (Higiroy et al., 2006). The textural properties of mixed hydrocolloid aqueous solutions depend on their concentration, hydrocolloid ratio and the mixing temperature. The sample containing locust bean gum and xanthan gum with high mixing temperature was found to be of high viscosity and high elasticity, due to crosslinking between the locust bean gum and the xanthan gum, leading to syneresis reduction. Moreover, it has been reported that mixed xanthan gum and locust bean gum solutions have more rigid textural attributes as indicated by a higher storage modulus than either individual xanthan gum or locust bean gum solutions (Copetti et al., 1997, Khouryieh et al., 2015, Renou et al., 2013, Zhan et al., 1993). However, this also depends on the acetate and pyruvate compositions. A low amount of acetate with a high amount of pyruvate facilitates the cooperative effect of xanthan gum and locust bean gum (Morris, 1990; Sandolo et al., 2010; Williams and Phillips, 2010).

Response surface methodology (RSM) is a tool using mathematical and statistical techniques to indicate combined levels of variables and optimize the response in a given situation (Grizotto et al., 2007; Mirhosseini et al., 2009). According to previously published literature, there exists no report on the combined effect of xanthan gum and locust bean gum

on the properties of food matrices, which also includes an analysis using a developed mathematical model.

The objective of this study was to investigate the effect of xanthan gum and locust bean gum on texture and syneresis of frozen restructured mango pulp prepared from Nam Dok Mai variety and Ok Rong variety. Mango varieties were selected based on their local popularity and allowing comparison for their influence on textural properties, syneresis and sensory acceptance in response to the combined effect of xanthan gum and locust bean gum. RSM was used for generating experimental formulations of xanthan gum and locust bean gum to be added to restructured mango, enabling the development of a regression model. The optimized xanthan gum and locust bean gum concentrations were consequently determined. Subsequently, selected xanthan gum and locust bean gum formulations were subjected to sensory evaluations to verify and confirm suitable formulations.

## **2. MATERIALS AND METHODS**

### **2.1 Preparation of mango pulp**

Green mango fruits (cv. Nam Dok Mai and cv. Ok-Rong), were ripened for 96 h at ambient temperature (30±1°C). Each ripe mango fruit (pH 4.8, total soluble solids 18°Brix for cv. Nam Dok Mai and pH 5.5, total soluble solids 26°Brix for cv. Ok Rong) was peeled and its pulp was separated, soaked in 1% w/w ascorbic acid (Foodchem International Co. Ltd, China), crushed using a high power blender (Buono, BUO-127799T, Taiwan, 10 speed levels, maximum speed of 38,000 rpm) at a fixed speed level 4 for 10 s to obtain medium particle size mango pulp (2.36-4.74 mm, as determined by sieve analysis).

### **2.2 Preparation of restructured mango pulp**

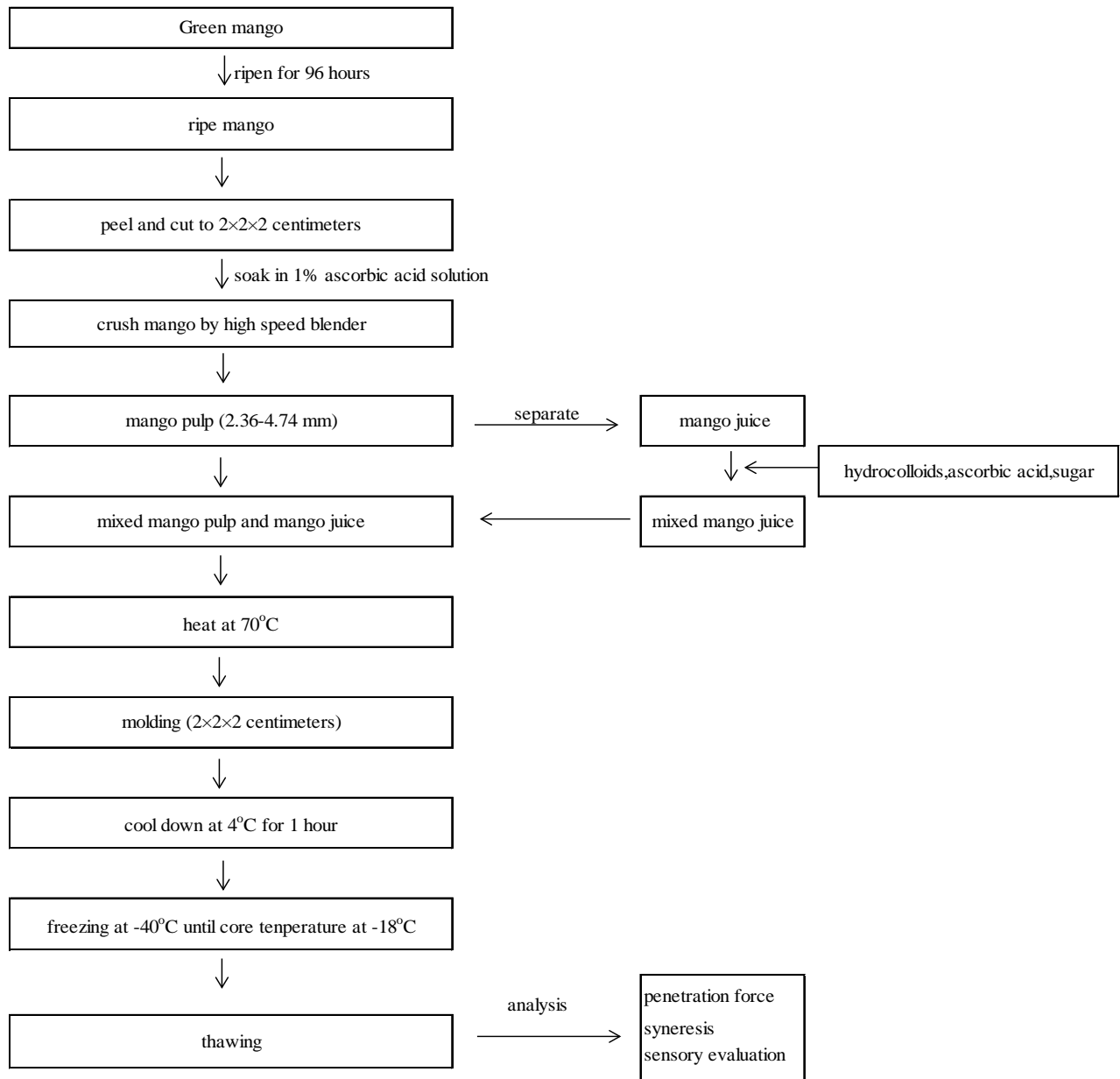
Xanthan gum (XG) (Jungbunzlauer Austria AG, Wein, Austria), locust bean gum (LBG) (Ingredient Center Co., Ltd., Bangkok, Thailand), 1% w/w commercial refined sugar (Lin, Thailand) and 0.1%

w/w ascorbic acid were mixed as dry ingredients. This dry mixture was subsequently mixed with mango pulp, followed by heating at 70°C for 1 min and then poured into a 2-cm<sup>3</sup> mold to be stored at 4°C for 1 h before freezing at -40°C for 24 h, whilst finally being stored at -18°C for 1 week. The frozen restructured mango pulp (FRMP) was thawed at 30°C for 2 h before analysis of penetration force and syneresis. The process flow diagram of restructured mango preparation is shown in Figure 1.

### **2.3 Experimental design and optimization**

A commercial statistical package, Design Expert, version 6.0.8 (Stat-Ease Inc., MI, USA) was employed for designing of the experiments following RSM. D-optimal with an assumed quadratic model was chosen for generating combinations of the two independent variables, XG and LBG. According to previously published literature (Sousa and Gonçalves, 2015, Chivero et al., 2015) and preliminary experiments, XG and LBG were each set at concentration ranges of 0.3-0.6% w/w and 0.2-0.6% w/w, respectively. At concentrations beyond the experimental range, undesirable product characteristics were obtained. At concentrations below this range, the restructured mango became very soft without holding a definite shape, whereas a viscous mango pulp slurry was prone to burning during heating process at concentration above the experimental range. The design comprised of 12 runs for each mango variety. The suitable model was evaluated using least square linear regression in order to identify any significant effect of hydrocolloid concentration on attributes of the frozen restructured mango, including the effect on penetration force and syneresis. The selected results based on significant model fitting (F-test,  $p < 0.05$ ) with non-significant lack-of-fit ( $p > 0.05$ ) are illustrated in three dimensional graphs. Sensory score was a response variable excluded from the RSM to minimize variation and error from large tasting samples that would bring about panelists'

fatigue (Heymann and Lawless, 2013)



**Figure 1** Process flow diagram for production of frozen restructured mango pulp

Following the RSM, a desirability function was employed for the optimization of XG and LBG concentrations to achieve desirable responses including penetration force and syneresis. Because this study involved the optimization of multiple responses, an overall desirability value was calculated to determine

the optimum concentrations of XG and LBG. The overall desirability can be defined as the geometric mean of the desirability for each response (Anderson and Whitcomb, 2016). In this study, the desirability function was defined for each response, i.e., maximum penetration force and minimum syneresis. Thus, the

optimum XG and LBG concentrations were determined at the highest penetration force and the lowest syneresis of FRMP after thawing. The optimized concentration of XG and LBG was selected for FRMP preparation for sensory evaluation and compared with samples containing lower concentration limits of each hydrocolloid (0.2% w/w LBG and 0.3% w/w XG) set as a control to confirm the result.

#### 2.4 Penetration force measurement

A piece of FRMP (2×2×2 cm), after thawing at 30°C for 1 h, was evaluated for penetration force by using a texture analyzer TA-XT 2i (Stable Micro System, England). The test was conducted with a 10-mm diameter cylinder probe at a velocity of 1 mm/s and 50% strain. The data was reported as a penetration force (N). The measurement of each trial sample was repeated 10 times of which a mean value was calculated and reported.

#### 2.5 Syneresis determination

Syneresis of FRMP was evaluated after thawing following a method modified by Arocas et al. (2009). FRMP was weighed before placing on filter paper and left at 30°C for 2 h. The thawed restructured mango pulp was taken out from the filter paper, and the change in weight of the filter paper was measured and calculated. Syneresis was calculated using Equation (1). The measurement of each trial sample was triplicated.

$$\text{Syneresis (\%)} = \frac{W_a - W_b}{W_s} \quad (1)$$

where  $W_a$  = weight of filter paper after thawing  
 $W_b$  = weight of blank filter paper  
 $W_s$  = weight of sample.

#### 2.6 Sensory evaluation and data analysis

Selected FRMP samples justified from RSM optimization with regard to penetration force and syneresis were subjected to sensory evaluation. The FRMP samples (2×2×2 cm) after thawing were

simultaneously served with steamed sticky rice enriched with coconut milk with random order to each untrained panelist of 50 persons who like eating mango. They were students and staff of Silpakorn University, Sanamchandra Palace Campus, Thailand who were 18-30 years old (male 30% and female 70%). Panelists were asked to taste each sample and evaluate according to their overall perception of the samples. A 9-point hedonic scale was employed, with 1 being “dislike extremely”, 5 being “neither like nor dislike” and 9 being “like extremely” was employed.

The sensory data were collected and analyzed using SPSS statistics version 19.0.0 (IBM, USA), following randomized complete block design, where panelists were treated as blocks. Mean values were compared by Duncan’s new multiple ranges test. Statistical significance was determined at 95% confidence ( $p < 0.05$ ).

### 3. RESULTS AND DISCUSSION

#### 3.1 Model fitting and statistical analysis

The effects of independent variables (XG and LBG) on physical properties of FRMP including penetration force and syneresis were investigated using RSM. Penetration force and syneresis of FRMP from cv. Nam Dok Mai and cv. Ok Rong after thawing are shown in Table 1 and Table 2, respectively. The estimated regression coefficients of the response surface models, including the corresponding adjusted  $r^2$  of FRMP cv. Nam Dok Mai and cv. Ok Rong are shown in Table 3 and Table 4, respectively. The adjusted  $r^2$  was estimated to indicate the model suitability. The adjusted  $r^2$  values of the model for penetration force and syneresis of FRMP cv. Nam Dok Mai are 0.89 and 0.98, respectively, while those of cv. Ok Rong are 0.92 and 0.97, respectively. Regression models with  $r^2$  values higher than 0.8 are considered as valid ones (Caporaso et al., 2016). However, the non-significant lack-of-fit is an indication of the adequacy of a model to describe the experimental factors. According to the

analysis, the lack-of-fit of the model was not significant ( $p>0.05$ ). The results indicated that the effect of XG and LBG on penetration force and syneresis of frozen restructured mango pulp could be well explained by the first order linear model ( $p<0.05$ ) as following.

$$Y_i = \beta_0 + \sum \beta_i X_i + \varepsilon \quad (2)$$

where  $Y_i$  = response variables ( $Y_1$  = penetration force (N),  $Y_2$  = syneresis (%))

$X_i$  = independent variables ( $X_1$  = XG concentration (%w/w),

$X_2$  = LBG concentration (%w/w)),  $\beta_0 = Y_i$  value when  $X_i$  equals 0,  $\beta_i$  = the change in  $Y_i$  for a unit change in  $X_i$ ,  $\varepsilon$  = random error.

The significance of the linear model suggested no interaction or curvature effect between XG and LBG on penetration force and syneresis of FRMP. Considering the coefficient of XG and LBG in the linear model ( $\beta_1$  and  $\beta_2$ ), XG seemed to have more impact on the penetration force and syneresis than LBG. However, the effects of these two variables were significant in the linear regression model ( $p<0.05$ ) for mango pulp samples from both cv. Nam Dok Mai and

cv. Ok Rong. The effect of XG and LBG on the physical properties measured in this study was found to be independent of mango variety.

### 3.2 Penetration force

As presented in Table 3, the coefficients of the regression model for penetration force of FRMP cv. Nam Dok Mai was positively associated with XG and LBG concentrations. As concentrations of XG and LBG increased, the penetration force also increased. An increase in penetration force indicated improvement in rigidity and firmness of the sample. This strong structure of sample could endure conformational change during freezing. Similar results were found for FRMP cv. Ok Rong (Table 4). This result was in accordance with the previous study by Copetti et al. (1997) who reported that mixed XG and LBG solutions resulted in higher storage moduli than for individual XG or LBG solutions. Storage modulus expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation (solid-like) (Rao, 1999; Harris, 1990). Thus, an increase in gel elasticity and product rigidity could be anticipated when XG and LBG are combined in the food matrix.

**Table 1** Experimental design of FRMP cv. Nam Dok Mai showing varied concentrations of xanthan gum and locust bean gum according to D-optimal design

Runs	Independent variables		Physical properties	
	Xanthan gum (% w/w)	Locust bean gum (% w/w)	Penetration force (N)	Syneresis (%)
1 (control)	0.3(0)	0.2(-1)	1.05	10.31
2	0.3(-1)	0.6(1)	1.05	6.09
3	0.45(0)	0.6(1)	1.29	4.04
4	0.6(1)	0.2(-1)	1.27	6.89
5	0.3(-1)	0.4(0)	1.07	7.73
6	0.6(1)	0.4(0)	1.32	5.17
7	0.3(-1)	0.2(-1)	1.00	10.00
8	0.45(0)	0.2(-1)	1.11	8.03
9	0.6(1)	0.2(-1)	1.24	6.97
10	0.6(1)	0.6(1)	1.42	2.39
11	0.3(-1)	0.6(1)	1.10	6.16
12	0.45(0)	0.4(0)	1.12	6.67

**Table 2** Physical properties of FRMP cv. Ok Rong at varied concentrations of xanthan gum and locust bean gum according to D-optimal design

Runs	Independent variables		Physical properties	
	Xanthan gum (% w/w)	Locust bean gum (% w/w)	Penetration force (N)	Syneresis (%)
1 (control)	0.3(-1)	0.2(-1)	1.29	19.09
2	0.3(-1)	0.6(1)	1.59	10.69
3	0.45(0)	0.6(1)	1.61	8.39
4	0.6(1)	0.2(-1)	1.47	13.83
5	0.3(-1)	0.4(0)	1.41	15.25
6	0.6(1)	0.4(0)	1.58	8.06
7	0.3(-1)	0.2(-1)	1.33	18.99
8	0.45(0)	0.2(-1)	1.35	16.11
9	0.6(1)	0.2(-1)	1.49	13.22
10	0.6(1)	0.6(1)	1.81	6.38
11	0.3(-1)	0.6(1)	1.55	10.63
12	0.45(0)	0.4(0)	1.59	9.79

**Table 3** Regression analysis of independent ( $X_1$ ,  $X_2$ ) and dependent variables (penetration force and syneresis) for FRMP cv. Nam Dok Mai

Variable	Estimated coefficients response		F-value	
	Penetration force	Syneresis	Penetration force	Syneresis
Model	Linear	Linear	-	-
constant	1.186	6.396	47.97**	299.27**
$X_1$	0.137	-1.618	87.54**	251.75**
$X_2$	0.059	-2.081	16.10*	416.66**
Lack of fit	-	-	2.276	6.897
$r^2$	0.914	0.985	-	-
$r^2$ (adjusted)	0.895	0.982	-	-
C.V. (%)	3.71	4.51	-	-

$X_1$  = xanthan gum,  $X_2$  = locust bean gum

\* Significant at  $p < 0.05$

\*\* Significant at  $p < 0.001$

**Table 4** Regression analysis of independent ( $X_1$ ,  $X_2$ ) and dependent variables (penetration force and syneresis) for FRMP cv. Ok Rong

Variable	Estimated coefficients response		F-value	
	Penetration force	Syneresis	Penetration force	Syneresis
Model	Linear	Linear	-	-
constant	1.516	12.222	55.33**	168.46**
$X_1$	0.092	-2.769	37.91**	118.76**
$X_2$	0.136	-3.982	84.09**	252.99**
Lack of fit	-	-	4.46	12.76*
$r^2$	0.925	0.974	-	-
$r^2$ (adjusted)	0.908	0.968	-	-
C.V. (%)	2.96	6.01	-	-

$X_1$  = xanthan gum,  $X_2$  = locust bean gum

\* Significant at  $p < 0.05$

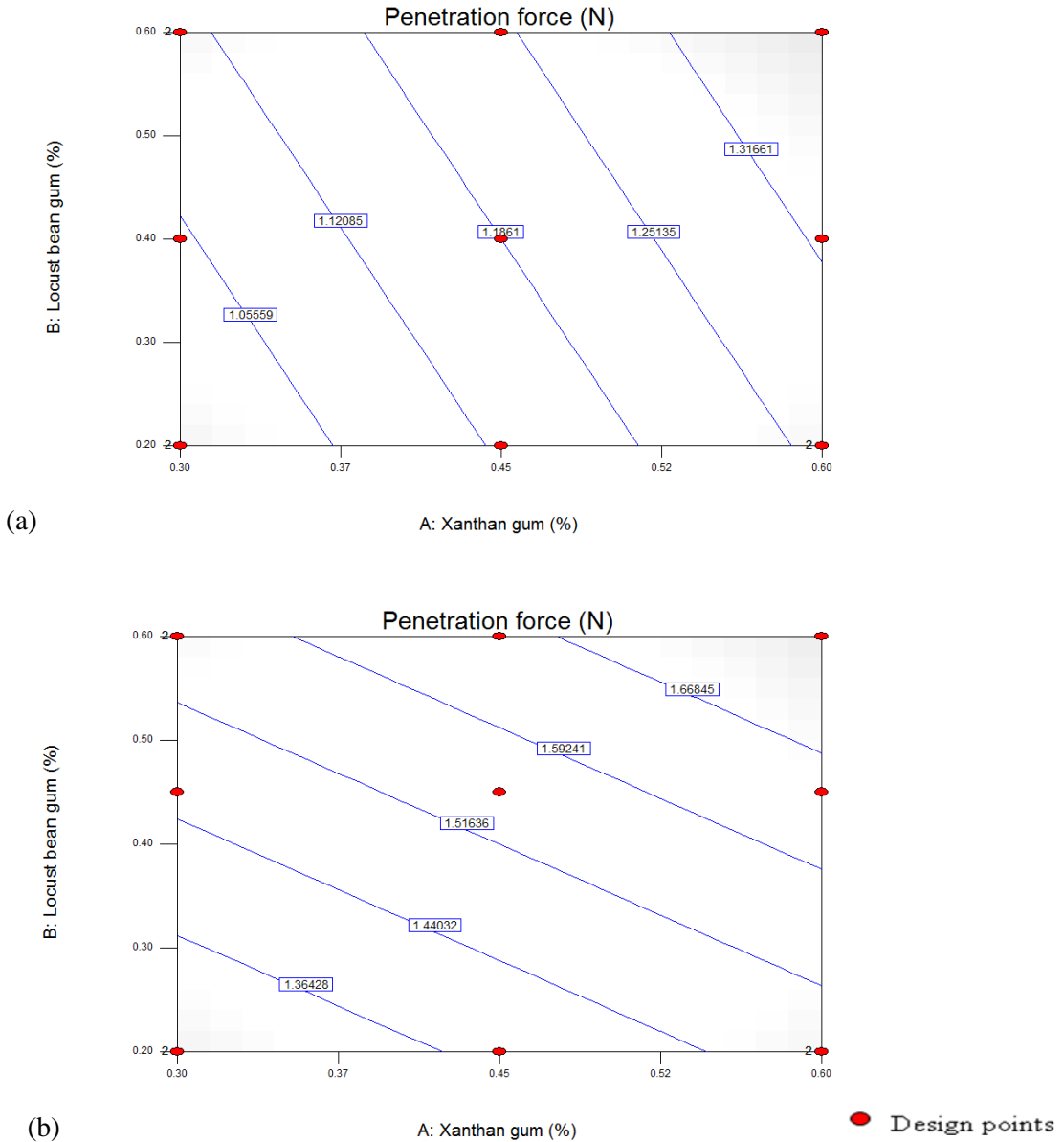
\*\* Significant at  $p < 0.001$

Figure 2a and Figure 2b show the response surface plots of FRMP cv. Nam Dok Mai and cv. Ok Rong, respectively, for the penetration force. The highest penetration force was obtained for the

highest concentrations of XG and LBG. At high concentrations of XG and LBG (0.6% w/w), the strength of the gel network increases due to the formation of more junction zones between the mannose



backbone (smooth region) of LBG and the ordered xanthan gum helices (Nussinovitch and Hirashima, 2014; Dumitriu, 1998; Phillips and Williams, 2009).



**Figure 2** Response surface plots for penetration force (N) after thawing of FRMP (a) cv. Nam Dok Mai and (b) cv. Ok Rong, showing the effects of xanthan gum and locust bean gum

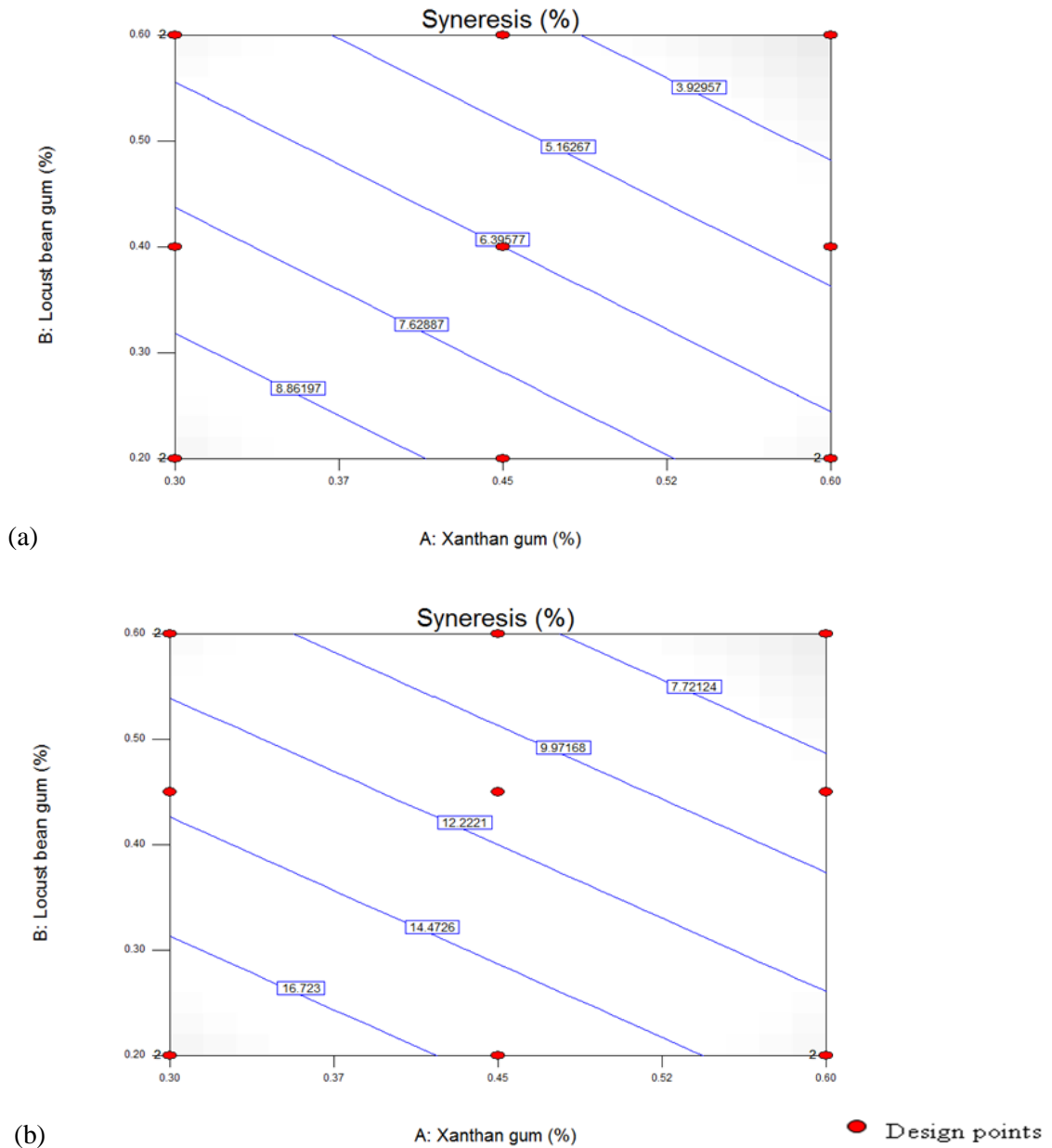
### 3.3 Syneresis

The coefficient values obtained from the regression model for syneresis of FRMP cv. Nam Dok Mai and cv. Ok Rong are presented in Tables 3 and 4. It was found that syneresis of FRMP was negatively associated with concentrations of XG and LBG. As the

concentration of both hydrocolloids increased, the syneresis of the frozen sample decreased. Thus, the lowest syneresis effect was found at the upper range of hydrocolloid concentrations tested (0.6% w/w). This result could be explained by increase in porosity due to an increasing amount of XG and LBG inside which

water was trapped. This result was similar to the reduction of syneresis found in sugar free jelly, resulting from the combination of XG and LBG as previously reported by Khouryieh et al. (2005). The response surface plot of FRMP cv. Nam Dok Mai and

cv. Ok Rong for syneresis is shown in Figure 3a and Figure 3b, respectively. The lowest syneresis was obtained at the upper limit of the XG and LBG concentration range (0.6% w/w).

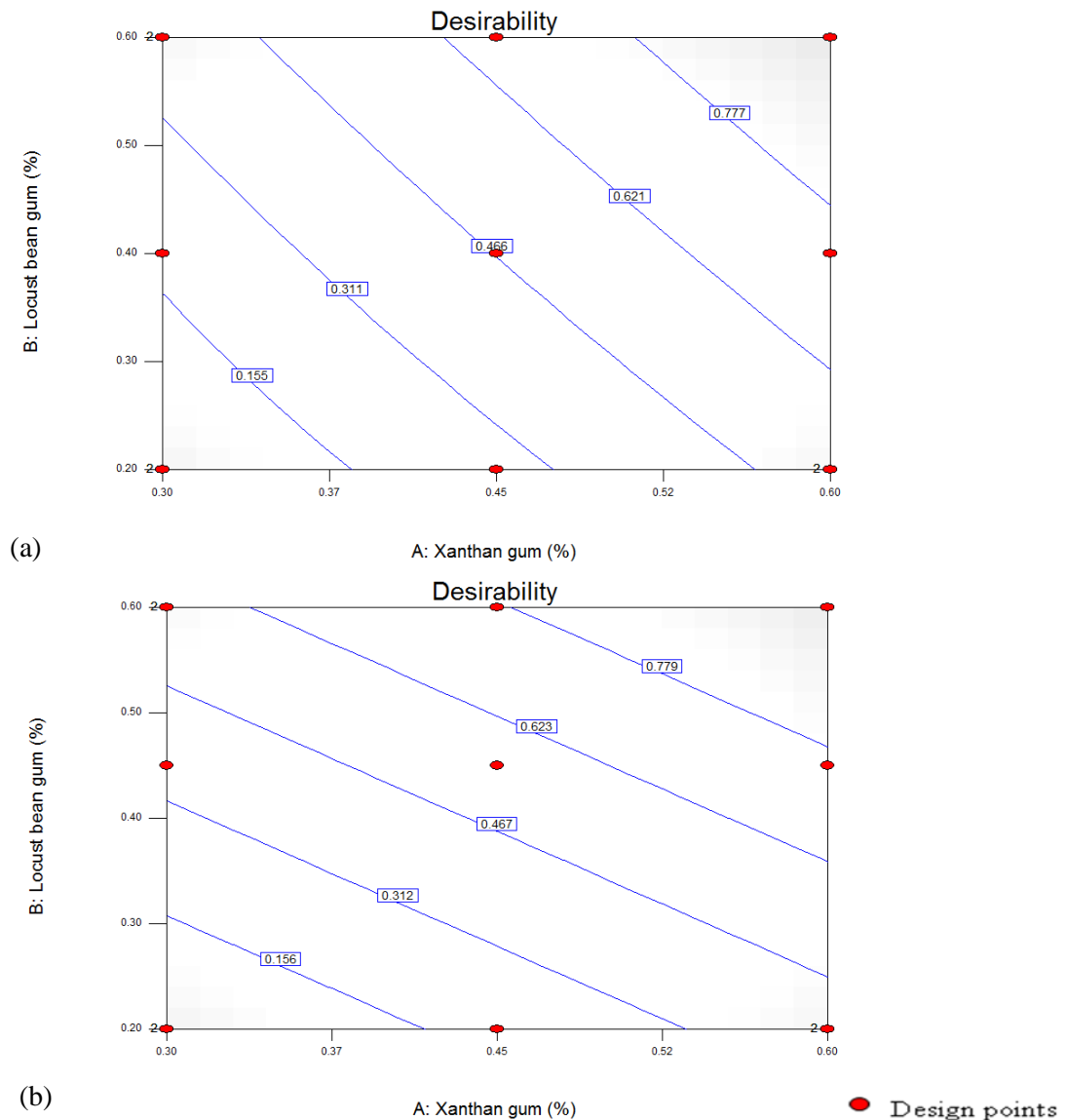


**Figure 3** Response surface plots for syneresis (%) after thawing of FRMP (a) cv. Nam Dok Mai and (b) cv. Ok Rong, showing the effects of xanthan gum and locust bean gum

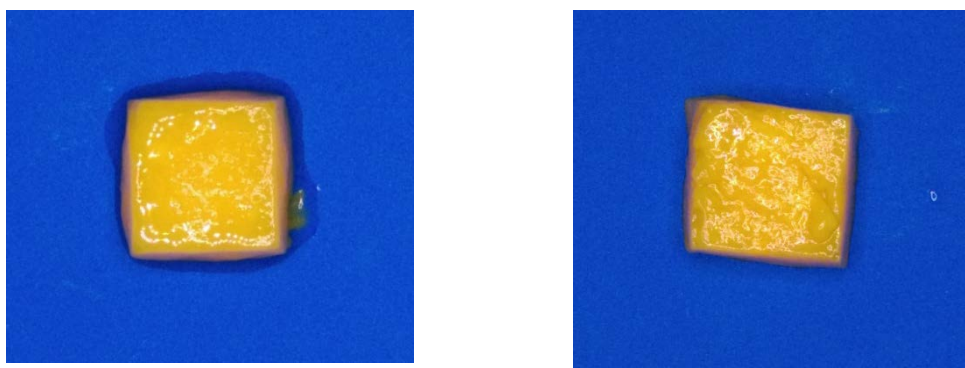
### 3.4 Optimization of XG and LBG

Optimum XG and LBG concentrations were predicted for producing FRMP with the highest penetration force and the lowest syneresis after thawing. As illustrated in Figure 4a and Figure 4b for FRMP cv. Nam Dok Mai and cv. Ok Rong, respectively, the highest overall desirability obtained from the maximized penetration force and the minimized syneresis, indicated that XG and LBG concentrations

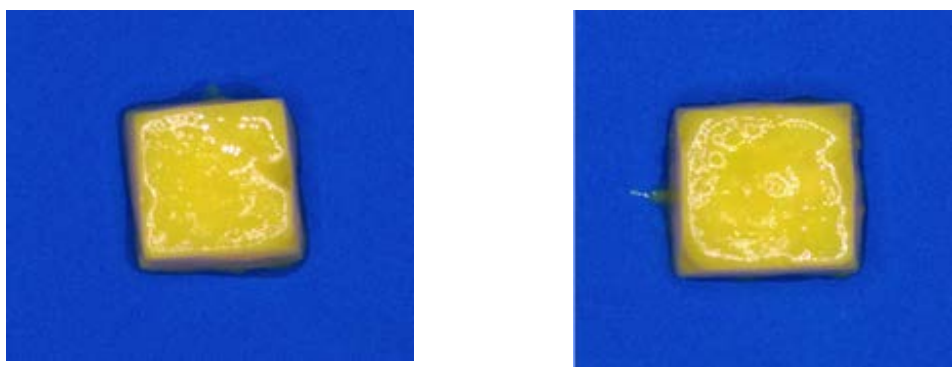
0.6% w/w, are most suitable. The highest amount of hydrocolloids within the experimental range were found to be optimum because both XG and LBG had a linear effect on the response variables set in this study as described earlier. Images of FRMP prepared from different concentrations of XG and LBG are presented in Figure 5 for cv. Nam Dok Mai and Figure 6 for cv. Ok Rong.



**Figure 4** Optimization of xanthan gum and locust bean gum concentrations providing the highest desirability value where maximum penetration force and minimum syneresis are expected for FRMP (a) cv. Nam Dok Mai and (b) cv. Ok Rong



**Figure 5** FRMP cv. Nam Dok Mai; (a) 0.3% w/w xanthan gum and 0.2% w/w locust bean gum added, and (b) 0.6% w/w xanthan gum and 0.6% w/w locust bean gum added



**Figure 6** FRMP cv. Ok Rong; (a) 0.3% w/w xanthan gum and 0.2% w/w locust bean gum added, and (b) 0.6% w/w xanthan gum and 0.6% w/w locust bean gum added

### 3.5 Sensory evaluation

Desirable FRMP was expected to be high in penetration force and low in syneresis. According to the RSM experiment, XG at a concentration of 0.6%w/w and LBG at a concentration of 0.6% w/w gave the maximum penetration force and minimum syneresis, for FRMP made from either cv. Nam Dok Mai or cv. Ok Rong. However, these physical properties would not be sufficient to ensure product quality in terms of consumer acceptability. In order to confirm the results, a sensory evaluation using a 9-point hedonic scale for overall liking was performed. Without hydrocolloid addition, the mango pulp could not maintain a definite shape.

Therefore, the FRMP containing XG and LBG at the lower limit of the experimental concentration range (0.3% w/w for XG and 0.2% w/w for LBG) was set as a control. As presented in Table 5, it was found that frozen restructured mango pulp with an XG and LBG concentration of 0.6% w/w, either for cv. Nam Dok Mai or cv. Ok Rong, received a significantly higher overall liking score than the control sample ( $p < 0.05$ ). Therefore, XG and LBG addition resulted not only in a stable texture whilst maintaining a definite shape and reduced syneresis of FRMP, but it also resulted in a satisfactory sensory perception of the consumed product.

**Table 5** Overall liking scores of FRMP cv. Nam Dok Mai and cv. Ok Rong, comparing between control sample and optimized sample

Sample	Overall liking score	
	cv. Nam Dok Mai	cv. Ok Rong
Xanthan gum 0.3% w/w + Locust bean gum 0.2% w/w (control)	5.84±1.08 <sup>a</sup>	6.31±0.98 <sup>a</sup>
Xanthan gum 0.6% w/w + Locust bean gum 0.6% w/w (optimized sample)	7.24±0.87 <sup>b</sup>	7.43±1.13 <sup>b</sup>

Note: Means and standard deviation followed by different superscript letters in the column are significantly different ( $p < 0.05$ )

#### 4. CONCLUSION

Combined addition of XG and LBG resulted in the improved characteristics of FRMP by stabilizing the texture and decreasing syneresis after thawing. Regression analysis indicated that both hydrocolloids are positively associated with penetration force but negatively associated with syneresis. Moreover, increasing XG and LBG concentrations resulted in a significantly improved overall liking score of the FRMP, regardless of the mango variety studied. The optimum concentrations of XG and LBG were found to be equivalent to 0.6% w/w.

#### ACKNOWLEDGEMENTS

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