



## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 EFFECT OF HUMECTANTS ON $a_w$ AND SENSORY QUALITIES OF CUSTARD CREAM

The effect of added glycerol and fructose (0, 2.5 and 5 %) on  $a_w$  and sensory qualities of custard cream were evaluated (Table 4.1). From ANOVA (Appendix D.1), it was found that all response variables except texture depended on glycerol, fructose and interaction between glycerol and fructose. The  $a_w$  of custard cream was found to decrease with increasing glycerol and fructose because of their excellent water-binding properties. Sweetness and OAA scores increased from 6.06 to 7.39 and 6.16 to 7.29, respectively with an increase in fructose, while the texture was not significant different ( $p>0.05$ ). The minimum  $a_w$  and maximum acceptability of sweetness, texture and OAA were observed in treatment 9, which contained 5% glycerol and 5% fructose. At this level of humectants, the sweetness of the ample may be the most acceptable by the panelists. On the other hand, treatment 3 (contained 5% glycerol and 0% fructose) had minimum sensory scores because of slight bitter taste of glycerol (Cardello *et al.*, 1984) and lack of sweetness from fructose. However, treatment 1 (control), had low sensory scores because of decreasing of sugar from recipe (from 26.2% to 15.1%).

The mathematical models were developed for  $a_w$ , sweetness and OAA from the regression analysis (Equations 4.1-4.3). The R-square values of all responses exceeded 0.80 indicating a high proportion of variability as explained by the data (Filmore, Kramer, and Gerald 1976; Kukreja *et al.*, 2002). The contour plots for these response variables were shown in Figure 4.1. The contour plots were superimposed by chosen  $a_w$  of less than 0.92 because it could inhibit the growth of many pathogenic bacteria, the sensory scores of more than 7.0 which meant that the sample was moderately accepted by panelists. The optimum area was found to locate at the top corner of the plot where glycerol and fructose were about the maximum level (Figure 4.2). Therefore the experiment was repeated by changing the concentration of added glycerol and fructose to 3, 6 and 9% of basic recipe.

**Table 4.1** Effects of humectants on qualities of custard cream at 0, 2.5 and 5 % glycerol and fructose

Treatment no.	Glycerol (%)	Fructose (%)	$a_w$	Average sensory score*		
				Sweetness	Texture <sup>ns</sup>	OAA
1	0	0	0.958±0.001 <sup>e</sup>	6.38±0.42 <sup>ab</sup>	6.51±0.56	6.55±0.47 <sup>abc</sup>
2	2.5	0	0.951±0.004 <sup>de</sup>	6.19±0.17 <sup>ab</sup>	6.06±0.26	6.37±0.04 <sup>ab</sup>
3	5.0	0	0.949±0.015 <sup>de</sup>	6.06±0.04 <sup>a</sup>	5.98±0.10	6.16±0.23 <sup>a</sup>
4	0	2.5	0.942±0.011 <sup>cde</sup>	6.71±0.36 <sup>bc</sup>	6.32±1.14	6.61±0.71 <sup>abc</sup>
5	2.5	2.5	0.929±0.006 <sup>bc</sup>	6.70±0.01 <sup>bc</sup>	6.54±0.37	6.77±0.21 <sup>abc</sup>
6	5.0	2.5	0.918±0.008 <sup>b</sup>	6.54±0.13 <sup>ab</sup>	6.82±0.19	6.66±0.17 <sup>abc</sup>
7	0	5.0	0.939±0.003 <sup>cd</sup>	6.77±0.04 <sup>bcd</sup>	6.58±0.44	6.58±0.03 <sup>abc</sup>
8	2.5	5.0	0.925±0.006 <sup>bc</sup>	7.29±0.07 <sup>cd</sup>	6.82±0.35	7.11±0.16 <sup>bc</sup>
9	5.0	5.0	0.888±0.016 <sup>a</sup>	7.39±0.44 <sup>d</sup>	7.10±0.37	7.29±0.59 <sup>c</sup>

\* 10 point line scale (0 = dislike very much; 10 = like very much)

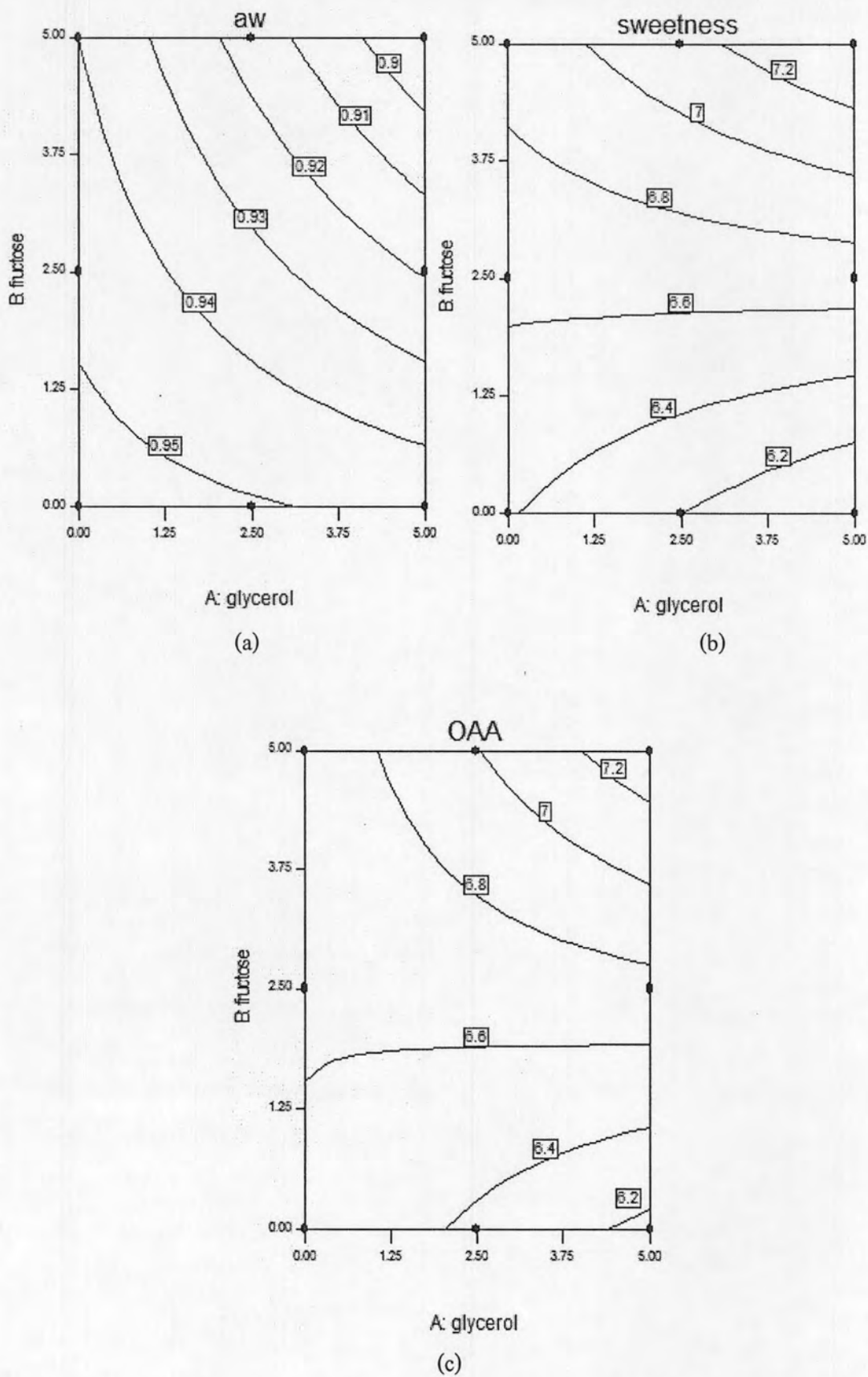
Values are mean ± SD, the means having different superscripts in a column are significantly different ( $p \leq 0.05$ ) and ns is not significantly different ( $p > 0.05$ )

$$a_w = 0.954 - 1.400 \times 10^{-3}G - 2.867 \times 10^{-3}F - 1.680 \times 10^{-3}GF \quad (R^2 = 0.9557) \dots (4.1)$$

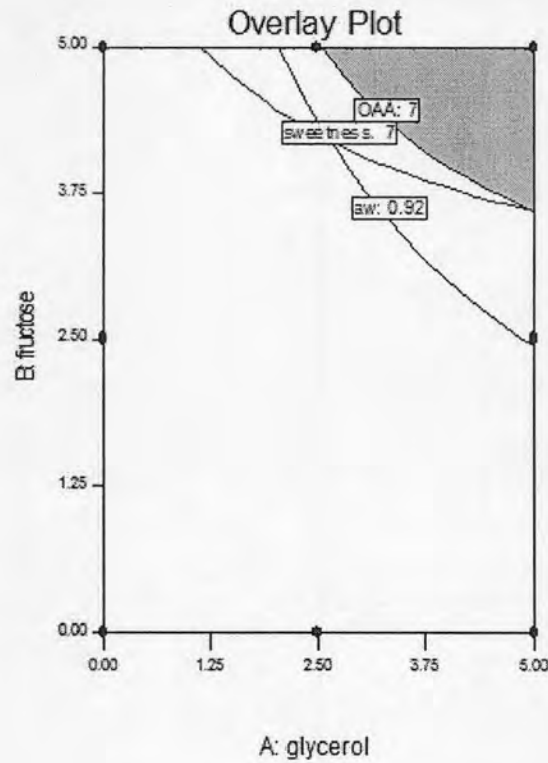
$$\text{Sweetness} = 6.413 - 0.085G + 0.094F + 0.038GF \quad (R^2 = 0.9570) \dots (4.2)$$

$$\text{OAA} = 6.574 - 0.085G + 0.017F + 0.044GF \quad (R^2 = 0.9618) \dots (4.3)$$

Where G is glycerol and F is fructose in % weight of total adjusted recipe



**Figure 4.1** Effects of glycerol and fructose (0, 2.5 and 5%) on (a)  $a_w$ , (b) sweetness, and (c) OAA of custard cream.



**Figure 4.2** Overlay plot of the three response variables of custard cream (0, 2.5 and 5 % glycerol and fructose).

The effects of added glycerol and fructose at 3, 6, and 9 % on the qualities of custard cream are shown in Table 4.2. ANOVA results showed that both  $a_w$  and sensory qualities are significantly different at various glycerol and fructose levels ( $p \leq 0.05$ ). The relationships of each quality and percentage of added glycerol and fructose are presented in Equations 4.4-4.7 with R-square values higher than 0.80. The contour plots for these response variables are shown in Figure 4.3. From the equations and contour plots, it can be observed that all response variables depended on glycerol, fructose, and interaction between glycerol and fructose. The  $a_w$  of custard cream was found to decrease with increasing glycerol and fructose. While the sensory scores increased with increasing glycerol up to 7% at constant fructose level (less than 7.5%) or increasing fructose up to 7.5% at constant glycerol level (less than 7%) (Fig 4.3). This may be because at this level of humectants, the sweetness of the custard was more preferable. From the superimposed plot (Figure 4.4), the optimum performance was found to be 6% of glycerol and fructose each which was located at the center of the optimum area. The predicted  $a_w$  and sensory qualities on sweetness, texture, and OAA were 0.916, 7.51, 7.09 and 7.45, respectively.

**Table 4.2** Effects of humectants on qualities of custard cream at 3, 6 and 9 % glycerol and fructose

Treatment no.	Glycerol (G, %)	Fructose (F, %)	$a_w$	Average sensory score*		
				Sweetness	Texture	OAA
1	3	3	0.947±0.001 <sup>g</sup>	6.43±0.18 <sup>cd</sup>	6.08±0.06 <sup>b</sup>	6.26±0.13 <sup>b</sup>
2	6	3	0.931±0.001 <sup>f</sup>	6.02±0.06 <sup>bc</sup>	6.51±0.15 <sup>cd</sup>	6.48±0.06 <sup>bc</sup>
3	9	3	0.926±0.000 <sup>e</sup>	5.89±0.31 <sup>b</sup>	5.72±0.17 <sup>a</sup>	6.32±0.30 <sup>bc</sup>
4	3	6	0.930±0.002 <sup>f</sup>	6.84±0.08 <sup>d</sup>	6.75±0.11 <sup>de</sup>	6.97±0.06 <sup>e</sup>
5	6	6	0.915±0.002 <sup>e</sup>	7.51±0.21 <sup>e</sup>	7.08±0.15 <sup>f</sup>	7.45±0.06 <sup>f</sup>
6	9	6	0.905±0.002 <sup>b</sup>	6.59±0.11 <sup>d</sup>	6.27±0.16 <sup>bc</sup>	6.56±0.06 <sup>cd</sup>
7	3	9	0.920±0.000 <sup>d</sup>	6.45±0.35 <sup>cd</sup>	5.75±0.12 <sup>a</sup>	6.33±0.20 <sup>bc</sup>
8	6	9	0.903±0.002 <sup>b</sup>	6.61±0.01 <sup>d</sup>	6.92±0.02 <sup>ef</sup>	6.78±0.13 <sup>de</sup>
9	9	9	0.893±0.000 <sup>a</sup>	5.10±0.42 <sup>a</sup>	5.66±0.06 <sup>a</sup>	5.79±0.11 <sup>a</sup>

\* 10 point line scale (0 = dislike very much; 10 = like very much)

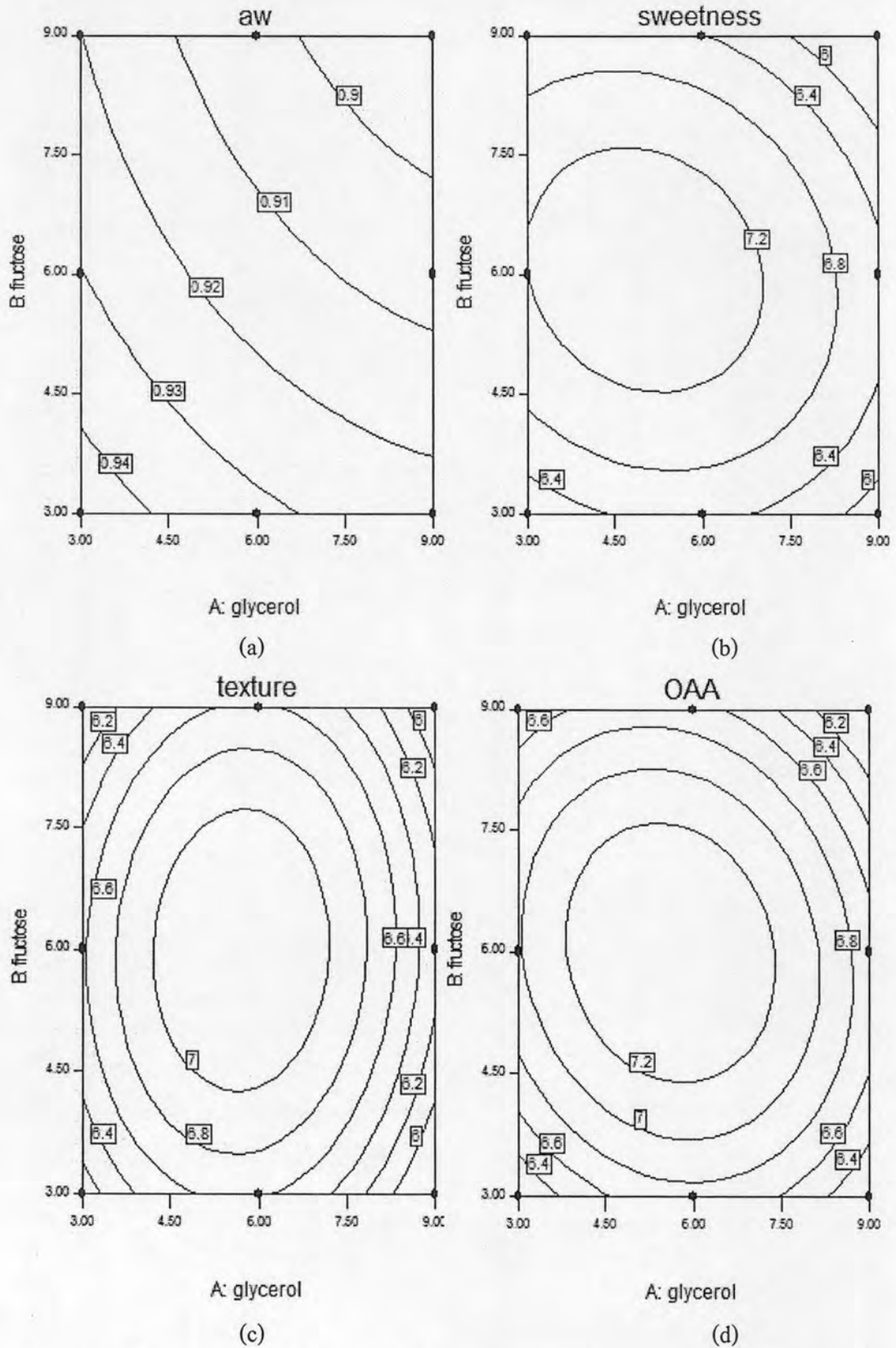
Values are mean ± SD, the means having different superscripts in a column are significantly different ( $p \leq 0.05$ ).

$$a_w = 0.988 - 7.627 \times 10^{-3}G - 7.794 \times 10^{-3}F + 3.810 \times 10^{-4}G^2 + 3.254 \times 10^{-4}F^2 - 1.667 \times 10^{-4}GF \quad (R^2 = 0.9957) \dots (4.4)$$

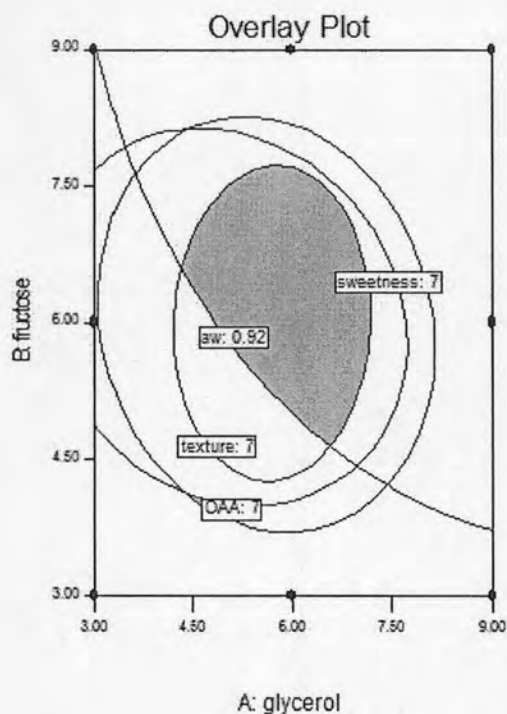
$$\text{Sweetness} = 1.431 + 0.735G + 1.377F - 0.060G^2 - 0.104F^2 - 0.023GF \quad (R^2 = 0.8658) \dots (4.5)$$

$$\text{Texture} = 2.432 + 0.927G + 0.706F - 0.085G^2 - 0.063F^2 + 0.008GF \quad (R^2 = 0.9163) \dots (4.6)$$

$$\text{OAA} = 2.166 + 0.789G + 1.009F - 0.062G^2 - 0.077F^2 - 0.017GF \quad (R^2 = 0.9462) \dots (4.7)$$



**Figure 4.3** Effects of glycerol and fructose (3, 6 and 9%) on (a)  $a_w$ , (b) sweetness, (c) texture, and (d) OAA of custard cream.



**Figure 4.4** Overlay plot of the four response variables of custard cream (3, 6 and 9 % glycerol and fructose).

The optimum level of humectants for custard cream was verified by evaluating the prepared custard cream with 6% glycerol and 6% fructose for  $a_w$  and sensory qualities test. The results were compared with those predicted by the model equations (Table 4.3). The experimental and predicted values were found to be non-significantly different ( $p > 0.05$ ). Therefore, the optimum levels of humectants of 6% glycerol and 6% fructose were used in producing custard cream.

**Table 4.3** Predicted and experimental values of the response variables at optimum level of humectants of custard cream

Response variables	Optimum product	
	Predicted values	Experimental values
$a_w$	0.915	0.918±0.005
Sweetness score	7.38	7.43±0.17
Texture score	7.18	7.25±0.33
Overall acceptance score	7.38	7.57±0.41

## 4.2 EFFECTS OF HUMECTANTS AND LACTIC ACID ON $a_w$ , pH, AND SENSORY QUALITIES OF CHINESE STEAMED BUN

The effects of glycerol (0, 2.5, and 5%) and lactic acid (0, 0.25, and 0.50%) on  $a_w$ , pH, appearance, flavour, texture and OAA of Chinese steamed bun are shown in Table 4.4. From the ANOVA (Appendix D; Table D.4), it was found that only glycerol affected  $a_w$  of the bun while lactic acid affected the pH and there was no interaction effect of glycerol and lactic acid on these qualities. Therefore, the effect of each additive was analyzed separately.

### 4.2.1 Effect of glycerol on $a_w$ , pH and sensory qualities of Chinese steamed bun

As there was no interaction effect of glycerol and lactic acid on  $a_w$ , pH, and sensory qualities, different lactic acid levels were treated as blocks. The effect of glycerol on the qualities of Chinese steamed bun is shown in Table 4.5. The results showed that  $a_w$  of steamed bun decreased significantly from 0.916 to 0.880 as the glycerol level increased. This may be because glycerol substantially lowered  $a_w$ , presumably through lowering the vapor pressure (Lombard *et al.*, 2000) and it forms hydrogen bonds with the available water in the system and makes water unavailable (Charley, 1982). The addition of glycerol also had the advantage in decreasing the amount of water required to form manageable dough, resulting in lower total moisture content (Lombard *et al.*, 2000). On the other hand, the pH, flavour, texture and OAA were not significant different ( $p > 0.05$ ), while the sensory score for appearance decreased as the glycerol level increased which was different from the previous studies on bread (Lombard *et al.*, 2000) and cakes (Cauvain and Cyster, 1996). They found that increasing glycerol resulted in increasing volume and decreasing firmness of bread and softer crumb structure in cake. However, the appearance of the sample containing 2.5% glycerol was not significantly different from control. Therefore, 2.5% glycerol was selected for further study as the sensory qualities were not significantly different from control and  $a_w$  was lower than 0.92.



**Table 4.4** Effects of glycerol and lactic acid on qualities of Chinese steamed bun

Treatment no.	Glycerol (%)	Lactic acid (%)	$a_w$	pH	Average sensory score*			
					Appearance <sup>ns</sup>	Flavour	Texture <sup>ns</sup>	OAA
1	0	0	0.921±0.011 <sup>d</sup>	6.33±0.11 <sup>c</sup>	6.80±0.28	6.72±0.36 <sup>bc</sup>	6.56±0.56	6.91±0.33 <sup>ab</sup>
2	2.5	0	0.901±0.000 <sup>bc</sup>	6.37±0.13 <sup>c</sup>	7.12±0.17	6.51±0.13 <sup>bc</sup>	6.36±0.06	6.76±0.15 <sup>ab</sup>
3	5.0	0	0.879±0.020 <sup>a</sup>	6.36±0.21 <sup>c</sup>	6.05±0.06	6.66±0.19 <sup>bc</sup>	6.78±0.48	6.78±0.35 <sup>ab</sup>
4	0	0.25	0.919±0.003 <sup>d</sup>	5.81±0.10 <sup>b</sup>	7.39±0.18	7.00±0.31 <sup>c</sup>	6.97±0.44	7.19±0.37 <sup>b</sup>
5	2.5	0.25	0.912±0.004 <sup>cd</sup>	5.78±0.09 <sup>b</sup>	6.86±0.01	6.60±0.82 <sup>bc</sup>	6.54±0.17	6.69±0.25 <sup>ab</sup>
6	5.0	0.25	0.874±0.003 <sup>a</sup>	5.89±0.01 <sup>b</sup>	6.16±0.01	5.97±0.02 <sup>abc</sup>	6.47±0.13	6.50±0.01 <sup>ab</sup>
7	0	0.5	0.907±0.003 <sup>cd</sup>	5.36±0.04 <sup>a</sup>	6.22±0.86	5.13±0.61 <sup>a</sup>	6.13±0.69	5.69±0.63 <sup>a</sup>
8	2.5	0.5	0.902±0.008 <sup>bc</sup>	5.34±0.16 <sup>a</sup>	6.53±0.18	5.67±0.58 <sup>ab</sup>	6.67±0.10	6.21±0.33 <sup>ab</sup>
9	5.0	0.5	0.888±0.004 <sup>ab</sup>	5.32±0.08 <sup>a</sup>	6.31±0.19	5.56±0.80 <sup>ab</sup>	6.00±1.47	5.90±1.15 <sup>a</sup>

\* 10 point line scale (0 = dislike very much; 10 = like very much)

Values are mean ± SD, the means having different superscripts in a column are significantly different ( $p \leq 0.05$ ) and ns is not significantly different ( $p > 0.05$ )

**Table 4.5** Effects of glycerol on qualities of Chinese steamed bun

Glycerol (%)	$a_w$	pH <sup>ns</sup>	Average sensory score*			
			Appearance	Flavour <sup>ns</sup>	Texture <sup>ns</sup>	OAA <sup>ns</sup>
0 (control)	0.916±0.008 <sup>c</sup>	5.83±0.44	6.80±0.67 <sup>b</sup>	6.28±0.97	6.55±0.58	6.60±0.80
2.5	0.905±0.007 <sup>b</sup>	5.83±0.47	6.84±0.29 <sup>b</sup>	6.26±0.64	6.52±0.17	6.55±0.33
5.0	0.880±0.011 <sup>a</sup>	5.86±0.47	6.17±0.15 <sup>a</sup>	6.06±0.62	6.42±0.78	6.39±0.67

\* 10 point line scale (0 = dislike very much; 10 = like very much)

Values are mean ± SD, the means having different superscripts in a column are significantly different ( $p \leq 0.05$ ) and ns is not significantly different ( $p > 0.05$ )

#### 4.2.2 Effect of lactic acid on $a_w$ , pH and sensory qualities of Chinese steamed bun

The pH, flavour and OAA of Chinese steamed bun was found to decrease as the lactic acid level increased (Table 4.6). These sensory scores for 0.5% lactic acid sample were significantly different from others. This may due to sharp flavour and slight sour taste of lactic acid (Karthikeyan *et al.*, 2000). On contrary,  $a_w$  as well as the appearance and texture were not significantly different. However, the pH of the Chinese steamed bun with 0.25% lactic acid was reduced to 5.83 without affecting the sensory qualities. Therefore, addition of 0.25% lactic acid was selected for further study.

**Table 4.6** Effects of lactic acid on qualities of Chinese steamed bun

Lactic acid (%)	$a_w$ <sup>ns</sup>	pH	Sensory score*			
			Appearance <sup>ns</sup>	Flavour	Texture <sup>ns</sup>	OAA
0 (control)	0.900±0.021	6.35±0.12 <sup>c</sup>	6.65±0.51	6.63±0.21 <sup>b</sup>	6.57±0.38	6.82±0.24 <sup>b</sup>
0.25	0.902±0.022	5.83±0.08 <sup>b</sup>	6.80±0.56	6.52±0.61 <sup>b</sup>	6.66±0.33	6.79±0.38 <sup>b</sup>
0.50	0.899±0.010	5.34±0.08 <sup>a</sup>	6.35±0.43	5.45±0.58 <sup>a</sup>	6.27±0.79	5.93±0.60 <sup>a</sup>

\* 10 point line scale (0 = dislike very much; 10 = like very much)

Values are mean ± SD, the means having different superscripts in a column are significantly different ( $p \leq 0.05$ ) and ns is not significantly different ( $p > 0.05$ )

#### 4.3 EFFECT OF PRESERVATIVE ON SHELF-LIFE OF CHINESE STEAMED BUN

The effects of preservative on shelf-life of both control (UP) and hurdle treated (HP) Chinese steamed bun during storage at  $30 \pm 2$  °C were investigated. The  $a_w$ , pH, and aerobic plate count (AC) of the samples were analyzed.

From Table 4.7, it was found that the initial  $a_w$  and pH of UP and HP were significantly different ( $p \leq 0.05$ ) as the  $a_w$  and pH of HP were lowered by the addition of glycerol and lactic acid. After storage, the  $a_w$  of both UP and HP samples increased slightly. This may be caused by the high humidity in the surrounding air (Primo-Martin *et al.*, 2006). However, the pH was not noticeable different as that observed in shelf-stable goat meat (Karthikeyan *et al.*, 2000).

From microbial analysis (Table 4.8), the initial AC of both UP and HP samples were not significant different. But after storage the AC of the UP samples were higher than the HP samples at the same level of calcium propionate (CaP). After 10 days storage, AC of the UP samples without CaP and with 0.1% CaP were the highest as it increased rapidly from 1.85 to 6.42 log cfu/g and 1.70 to 6.00 log cfu/g, respectively. According to the standard of TISI, AC of this type of product must not exceed  $1 \times 10^5$  cfu/g. Therefore, the shelf-life of these two samples were about 2-4 days. However, AC of UP with 0.2% CaP increased slowly from 1.85 to 5.99 log cfu/g and its storage life was found to be about 12 days. For the HP samples, it was found that the

level of CaP addition had no significant effect on the AC up to 12 day and the AC of the HP with 0.2% CaP was the lowest. Therefore, lower  $a_w$  and pH used in hurdle treated products created an inappropriate condition for microorganism growth (Leistner, 2000). The levels of preservative had no effect on  $a_w$  of both UP and HP, while pH and AC of the highest level of preservative (0.2% CaP) was significant different from others. This is because calcium propionate is a weak organic acid (Marin *et al.*, 2002). An increase in  $a_w$  during storage may be caused by the bound water released from the food matrix during the microbial growth. Moreover, yeast and mould (YM) were not detected (Table 4.8) which agrees with Hathorn *et al.* (2008) that the mould count for the breads supplemented with sweet potato flour and high-gluten dough enhancers never exceeded 1.0 log cfu/g during the storage period for 8 days. Although both hurdles and preservative affect the growth of microorganisms which is one of the main factors causing food deterioration, there is an increasing pressure to reduce the use of preservative (Sofos and Busta, 1981). Therefore in this study, using only hurdles by lowering  $a_w$  and pH were sufficient to inhibit microbial growth and microbial spoilage in Chinese steamed bun for at least 16 days storage.

The HP samples without preservative were prepared and stored at  $30 \pm 2$  °C. Samples were taken at 0, 4, 8 and 12 days storage for textural measurement and sensory evaluation (Table 4.9). The results show that the storage time had no effect on the springiness of the samples, while the hardness and gumminess increased significantly from 11.20 to 18.51 N and 6.01 to 9.75 N, respectively. The increase in hardness during storage was also found in bread (Caballero, Gomez, and Rosell, 2007). This may be due to staling of starch, especially amylopectin, retrogradation (Knightly, 1988) or the interaction between swollen starch granules and the continuous protein network in bread (Martin and Hosenev, 1991). The hedonic score of the stored sample decreased during prolong storage from 6.18 to 5.20. However, from the pair comparison test, the panelist could not detect the difference between freshly prepared sample and stored sample up to 8 days which may be caused by physical and chemical changes. Therefore, it can be concluded that Chinese steamed bun can be stored for 8 days based on textural measurement and sensory evaluation. The correlations between sensory scores and textural measurement were evaluated (Figure 4.5). The sensory scores (OAA) were found to be linearly correlated with hardness and gumminess with  $R^2$  of 0.85 and 0.96, respectively. But no correlation between sensory scores and springiness and cohesiveness were found.

**Table 4.7** Effects of hurdle and preservative on  $a_w$  and pH of Chinese steamed bun

$a_w$	Storage period in days								
	0	2	4	6	8	10	12	14	16 <sup>ns</sup>
UP	0.925±0.002 <sup>b</sup>	0.930±0.001 <sup>b</sup>	0.937±0.001 <sup>b</sup>	0.941±0.003 <sup>b</sup>	0.944±0.002 <sup>b</sup>	0.946±0.003 <sup>b</sup>	-	-	-
UP + 0.1% CaP	0.922±0.003 <sup>b</sup>	0.930±0.002 <sup>b</sup>	0.935±0.001 <sup>b</sup>	0.943±0.003 <sup>b</sup>	0.946±0.002 <sup>b</sup>	0.947±0.004 <sup>b</sup>	-	-	-
UP + 0.2% CaP	0.925±0.003 <sup>b</sup>	0.929±0.001 <sup>b</sup>	0.936±0.002 <sup>b</sup>	0.943±0.003 <sup>b</sup>	0.945±0.002 <sup>b</sup>	0.948±0.002 <sup>b</sup>	0.950±0.006 <sup>b</sup>	0.951±0.002 <sup>b</sup>	-
HP	0.906±0.003 <sup>a</sup>	0.912±0.001 <sup>a</sup>	0.917±0.002 <sup>a</sup>	0.919±0.003 <sup>a</sup>	0.923±0.002 <sup>a</sup>	0.926±0.003 <sup>a</sup>	0.925±0.004 <sup>a</sup>	0.932±0.007 <sup>a</sup>	0.933±0.005
HP + 0.1% CaP	0.904±0.003 <sup>a</sup>	0.912±0.002 <sup>a</sup>	0.916±0.002 <sup>a</sup>	0.919±0.002 <sup>a</sup>	0.923±0.001 <sup>a</sup>	0.927±0.002 <sup>a</sup>	0.928±0.005 <sup>a</sup>	0.932±0.006 <sup>a</sup>	0.931±0.003
HP + 0.2% CaP	0.905±0.003 <sup>a</sup>	0.913±0.002 <sup>a</sup>	0.915±0.001 <sup>a</sup>	0.920±0.003 <sup>a</sup>	0.924±0.002 <sup>a</sup>	0.926±0.001 <sup>a</sup>	0.926±0.005 <sup>a</sup>	0.931±0.006 <sup>a</sup>	0.930±0.002
pH									
UP	6.25±0.09 <sup>c</sup>	6.32±0.08 <sup>d</sup>	6.29±0.06 <sup>d</sup>	6.36±0.06 <sup>c</sup>	6.36±0.06 <sup>c</sup>	6.40±0.04 <sup>d</sup>	-	-	-
UP + 0.1% CaP	6.28±0.06 <sup>c</sup>	6.33±0.04 <sup>d</sup>	6.29±0.03 <sup>d</sup>	6.34±0.06 <sup>c</sup>	6.32±0.06 <sup>c</sup>	6.39±0.04 <sup>d</sup>	-	-	-
UP + 0.2% CaP	6.21±0.13 <sup>c</sup>	6.24±0.07 <sup>c</sup>	6.19±0.02 <sup>c</sup>	6.22±0.09 <sup>b</sup>	6.20±0.03 <sup>b</sup>	6.26±0.05 <sup>c</sup>	6.29±0.05 <sup>d</sup>	6.28±0.03 <sup>c</sup>	-
HP	5.58±0.20 <sup>b</sup>	5.69±0.11 <sup>b</sup>	5.61±0.03 <sup>b</sup>	5.61±0.08 <sup>a</sup>	5.57±0.07 <sup>a</sup>	5.69±0.04 <sup>b</sup>	5.67±0.06 <sup>c</sup>	5.61±0.10 <sup>b</sup>	5.68±0.10
HP + 0.1% CaP	5.60±0.17 <sup>b</sup>	5.63±0.03 <sup>ab</sup>	5.55±0.04 <sup>a</sup>	5.60±0.10 <sup>a</sup>	5.56±0.08 <sup>a</sup>	5.62±0.05 <sup>a</sup>	5.64±0.04 <sup>b</sup>	5.56±0.09 <sup>ab</sup>	5.66±0.02
HP + 0.2% CaP	5.49±0.09 <sup>a</sup>	5.58±0.09 <sup>a</sup>	5.51±0.01 <sup>a</sup>	5.53±0.04 <sup>a</sup>	5.52±0.04 <sup>a</sup>	5.60±0.03 <sup>a</sup>	5.53±0.03 <sup>a</sup>	5.52±0.09 <sup>a</sup>	5.64±0.06

Values are mean ± SD, the means having different superscripts in a column for each quality are significantly different ( $p \leq 0.05$ )

ns is not significantly different ( $p > 0.05$ ) and – was not analyzed

**Table 4.8** Effects of hurdle and preservative on microbiological properties of Chinese steamed bun

AC (log CFU/g)	Storage period in days								
	0 <sup>ns</sup>	2	4	6	8	10	12	14	16
UP	1.85±0.21	4.56±0.15 <sup>d</sup>	5.04±0.18 <sup>d</sup>	5.43±0.27 <sup>c</sup>	6.56±0.17 <sup>c</sup>	6.42±0.60 <sup>c</sup>	-	-	-
UP + 0.1% CaP	1.70±0.00	4.18±0.19 <sup>c</sup>	5.01±0.14 <sup>d</sup>	5.49±0.45 <sup>c</sup>	6.18±0.08 <sup>c</sup>	6.00±0.21 <sup>c</sup>	-	-	-
UP + 0.2% CaP	1.85±0.21	2.89±0.22 <sup>b</sup>	3.69±0.40 <sup>c</sup>	4.63±0.47 <sup>b</sup>	4.43±0.30 <sup>b</sup>	4.42±0.63 <sup>b</sup>	4.77±0.12 <sup>b</sup>	5.99±0.13 <sup>c</sup>	-
HP	1.70±0.00	1.85±0.21 <sup>a</sup>	2.54±0.48 <sup>b</sup>	2.66±0.19 <sup>a</sup>	2.59±0.26 <sup>a</sup>	2.35±0.07 <sup>a</sup>	2.38±0.59 <sup>a</sup>	2.79±0.68 <sup>b</sup>	3.29±0.17 <sup>ab</sup>
HP + 0.1% CaP	1.85±0.21	1.70±0.00 <sup>a</sup>	1.94±0.34 <sup>a</sup>	2.39±0.55 <sup>a</sup>	2.42±0.33 <sup>a</sup>	2.09±0.13 <sup>a</sup>	2.53±0.46 <sup>a</sup>	3.13±0.09 <sup>b</sup>	3.36±0.08 <sup>b</sup>
HP + 0.2% CaP	1.85±0.21	1.70±0.00 <sup>a</sup>	1.85±0.21 <sup>a</sup>	2.43±0.60 <sup>a</sup>	2.47±0.40 <sup>a</sup>	1.70±0.00 <sup>a</sup>	1.85±0.21 <sup>a</sup>	1.70±0.00 <sup>a</sup>	2.83±0.46 <sup>a</sup>
YM (log CFU/g)									
UP	nd	nd	nd	nd	nd	nd	-	-	-
UP + 0.1% CaP	nd	nd	nd	nd	nd	nd	-	-	-
UP + 0.2% CaP	nd	nd	nd	nd	nd	nd	nd	nd	-
HP	nd	nd	nd	nd	nd	nd	nd	nd	nd
HP + 0.1% CaP	nd	nd	nd	nd	nd	nd	nd	nd	nd
HP + 0.2% CaP	nd	nd	nd	nd	nd	nd	nd	nd	nd

Values are mean ± SD, the means having different superscripts in a column are significantly different ( $p \leq 0.05$ )

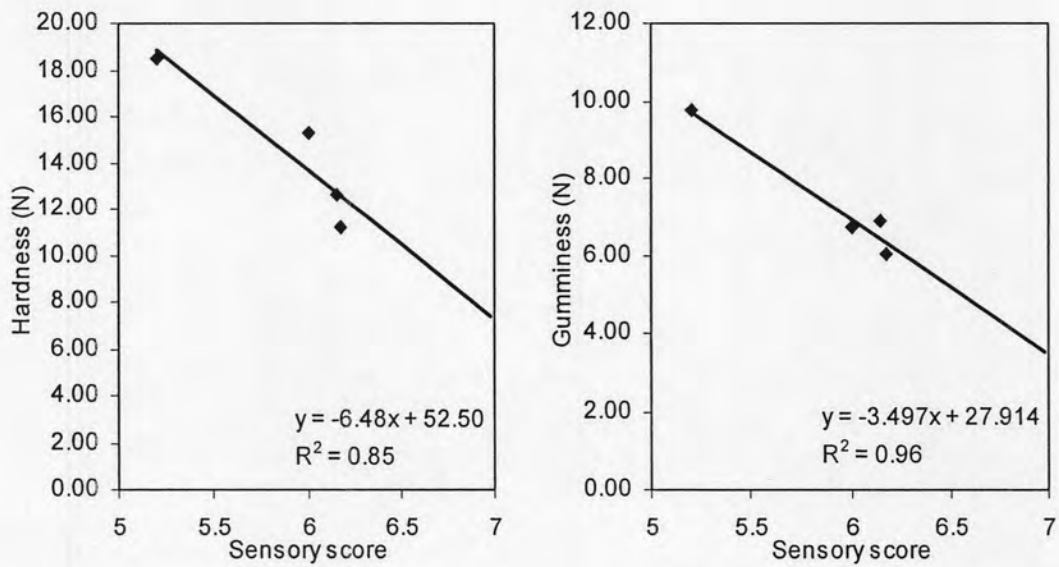
ns in a column of AC is not significantly different ( $p > 0.05$ ), nd was not detected and – was not analyzed

**Table 4.9** Textural properties and sensory score of hurdle treated Chinese steamed bun

	Textural and sensory values at storage period in days			
	0	4	8	12
Hardness (N)	11.20±1.00 <sup>a</sup>	12.59±0.52 <sup>a</sup>	15.32±0.90 <sup>b</sup>	18.51±1.195 <sup>c</sup>
Springiness <sup>ns</sup>	0.90±0.01	0.90±0.01	0.90±0.01	0.90±0.01
Cohesiveness	0.54±0.07 <sup>ab</sup>	0.55±0.05 <sup>b</sup>	0.43±0.07 <sup>a</sup>	0.52±0.05 <sup>ab</sup>
Gumminess (N)	6.01±0.68 <sup>a</sup>	6.89±0.41 <sup>a</sup>	6.70±1.45 <sup>a</sup>	9.75±1.56 <sup>b</sup>
Sensory score (OAA)*	6.18±0.13 <sup>b</sup>	6.15±0.43 <sup>b</sup>	6.01±0.21 <sup>b</sup>	5.20±0.35 <sup>a</sup>

\* 9 point Hedonic scale (1 = dislike extremely; 9 = like extremely)

Values are mean ± SD, the means having different superscripts in a row are significantly different ( $p \leq 0.05$ ) and ns is not significantly different ( $p > 0.05$ )



**Figure 4.5** Relationship of texture analysis and sensory evaluation of hurdle treated Chinese steamed bun.

#### 4.4 EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON SHELF-LIFE OF CCSB

In this experiment the effect of modified atmosphere packaging on shelf-life of CCSB during storage at  $30 \pm 2$  °C were investigated. The  $a_w$ , pH and AC of the hurdle CCSB with and without oxygen absorber (OA) were analyzed.

It was found that there was no significant difference of AC between treatments after 2 day storage but after 4 day storage the AC of various treatments were significant different ( $p \leq 0.05$ ) (Table 4.10). The AC of CCSB without OA increased rapidly and was about 7 log cfu/g on the 8<sup>th</sup> day, while the AC of CCSB with OA was only 4.87 log cfu/g on the 10<sup>th</sup> day. As OA can reduce the oxygen in the package so the growth of aerobic microorganism was inhibited (Leistner, 2000). Therefore the CCSB with and without OA was safe up to 10 days and 4 days storage. Furthermore, yeast and mould could not be detected. The  $a_w$  of bun and cream of CCSB were separately analyzed because initial  $a_w$  was different (Table 4.11). The  $a_w$  of bun and cream of both CCSB with and without OA were not significantly different ( $p > 0.05$ ). After storage, the  $a_w$  of bun increased slightly. While  $a_w$  of cream remained constant up to the 4<sup>th</sup> day storage and then increased slightly. The increase in  $a_w$  may be caused by moisture in the surrounding air or the increase in microorganism resulting in changing the bound water in the food component to free water. For pH, the CCSB with and without OA samples were not significantly different ( $p > 0.05$ ) during storage. Therefore, the treated Chinese steamed bun with custard cream filling packed with oxygen absorber condition, which was safe up to 10 days from microbial spoilage, was chosen for further study.

From the textural measurement of the stored CCSB packed with OA (Table 4.12), it showed that storage time had no effect on the springiness of the sample. The cohesiveness of the stored sample was significantly decreased as found in bakery products (Chiavaro *et al.*, 2008). Hardness and gumminess significantly increased from 9.09 to 16.72 N and 5.12 to 7.28 N, respectively. The sensory score of the stored samples was estimated from Figure 4.5. It was found that the sensory score decreased from 6.70 to 5.52 and 6.52 to 5.90 by hardness and gumminess values, respectively. As the sensory score of the 8<sup>th</sup> day stored sample was about 6 which was the acceptable value, so it could be concluded that the storage life of the treated custard cream filled Chinese steamed bun was extended to 8 days.



**Table 4.10** Effects of hurdle and modified atmosphere on microbiological properties of CCSB

AC (log CFU/g)	storage period in days							
	0	2	4*	6*	8*	10	12	14
HP	1.76±0.08	3.03±0.38	4.62±0.61	6.83±0.35	7.25±0.07	-	-	-
HP + OA	1.83±0.18	2.93±0.34	3.08±0.35	4.16±0.18	4.27±0.07	4.87±0.11	5.63±0.08	5.85±0.07
YM (log CFU/g)								
HP	nd	nd	nd	nd	nd	-	-	-
HP + OA	nd	nd	nd	nd	nd	nd	nd	nd

\* in a column of AC is significantly different ( $p \leq 0.05$ ), nd was not detected and – was not analyzed

**Table 4.11** Effects of hurdle and modified atmosphere on  $a_w$  and pH of CCSB

$a_w$ of cream	storage period in days							
	0	2	4	6	8	10	12	14
HP	0.914±0.001	0.915±0.001	0.916±0.001	0.924±0.001	0.930±0.001	-	-	-
HP + OA	0.914±0.001	0.914±0.001	0.916±0.002	0.924±0.001	0.929±0.002	0.933±0.003	0.933±0.003	0.941±0.002
$a_w$ of bun								
HP	0.903±0.002	0.909±0.001	0.914±0.001	0.922±0.001	0.928±0.002	-	-	-
HP + OA	0.904±0.001	0.910±0.002	0.914±0.001	0.923±0.002	0.929±0.001	0.934±0.002	0.934±0.002	0.941±0.001
pH of bun*								
HP	5.59±0.24	5.61±0.21	5.58±0.08	5.62±0.10	5.64±0.12	-	-	-
HP + OA	5.60±0.15	5.55±0.13	5.54±0.19	5.62±0.18	5.60±0.15	5.54±0.12	5.58±0.09	5.60±0.08

\* pH of bun is not significantly different ( $p > 0.05$ ) and - was not analyzed

**Table 4.12** Textural measurement of hurdle CCSB stored in a package with OA

	Texture values at storage period in days			
	0	4	8	12
Hardness (N)	9.09±0.39 <sup>a</sup>	11.03±0.45 <sup>b</sup>	15.01±0.56 <sup>c</sup>	16.72±1.87 <sup>d</sup>
Springiness <sup>ns</sup>	0.91±0.01	0.90±0.01	0.90±0.01	0.91±0.01
Cohesiveness	0.56±0.05 <sup>c</sup>	0.51±0.02 <sup>bc</sup>	0.49±0.02 <sup>ab</sup>	0.43±0.05 <sup>a</sup>
Gumminess (N)	5.12±0.69 <sup>a</sup>	5.68±0.19 <sup>a</sup>	7.35±0.29 <sup>b</sup>	7.28±1.76 <sup>b</sup>
Sensory score (OAA)* (1)	6.70±0.06 <sup>b</sup>	6.40±0.07 <sup>b</sup>	5.79±0.01 <sup>a</sup>	5.52±0.27 <sup>a</sup>
	(2) <sup>ns</sup>	6.52±0.16	6.36±0.06	5.88±0.04

\* Estimated sensory score from hardness (1) and gumminess (2)

Values are mean ± SD, the means having different superscripts in a row are significantly different ( $p \leq 0.05$ ) and ns is not significantly different ( $p > 0.05$ )

#### 4.5 VERIFICATION

From the optimum conditions (the hurdle treated CCSB by adding 2.5% glycerol and 0.25% lactic acid, without calcium propionate in bun, adding 6% glycerol and fructose each in custard cream and packed in PVDC bag with OA), the CCSB samples were prepared, packaged with OA and stored at  $30 \pm 2$  °C for 8 days and then analyzed for all response variables including,  $a_w$ , pH and textural measurement, microbial analysis and sensory evaluation. The experimental value of each response except sensory evaluation was compared with previous experiment (Table 4.13- 4.14). The experimental values of  $a_w$ , AC, hardness, springiness, cohesiveness and gumminess and previous experiment values were found not to be statistically different at 5% level (Appendix D). While, the sensory scores of appearance, texture of bun and cream, flavour and OAA of CCSB were over 6 in a 9-point structured hedonic scale indicating the product to be acceptable. Thus, the production of hurdle CCSB by adding 2.5% glycerol and 0.25% lactic acid, without calcium propionate in bun, adding 6% glycerol and fructose each in custard cream and packed in PVDC bag with OA can be used to develop safe and acceptable CCSB, stable at ambient temperature for 8 days.

**Table 4.13** AC,  $a_w$  and textural measurement of hurdle CCSB at the 8<sup>th</sup> day

Response variables	Verified values	Experimental results from Tables 4.10-4.12	Difference (%)
Bun $a_w$	0.928±0.001	0.929±0.001	0.108
Cream $a_w$	0.929±0.002	0.929±0.002	0.000
AC (log cfu/g)	4.54±0.31	4.27±0.07	5.95
Hardness	14.86±1.06	15.01±0.56	1.01
Springiness	0.90±0.01	0.90±0.01	0.00
Cohesiveness	0.48±0.04	0.49±0.02	2.08
Gumminess	7.08±0.57	7.35±0.29	3.81

**Table 4.14** Sensory evaluation of hurdle CCSB at the 8<sup>th</sup> day

Response variable	Average sensory score*
Appearance	6.88±0.16
Bun texture	6.80±0.19
Cream texture	6.68±0.31
Flavour	6.90±0.02
OAA	6.62±0.21

\* 9 point Hedonic scale (1 = dislike extremely; 9 = like extremely)

#### 4.6 ESTIMATE COST PER PIECE OF READY-TO-EAT CUSTARD CREAM FILLED CHINESE STEAMED BUN

From the result of process development of CCSB, it was found that cost of product was certainly higher because of addition of hurdle materials including glycerol, fructose, lactic acid, PVDC bag and OA. However, the raised cost is reasonable (Appendix F) to develop in industrial scale.