Studies on the Optimum Models of the Dairy Product Kou Woan Lao Using Response Surface Methodology

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ABSTRACT : Kou Woan Lao is an oriental-style dairy product, which is coagulated by milk-clotting enzyme from the culture filtrate of lao-chao. The product appears smooth, sweet, slightly wine flavour, and the flavour differs from yogurt. The aim of this study was to search for the optimum model to shorten the period of manufacture, and to improve the quality of Kou Woan Lao. A response surface design was used for studying the effects of addition of carrageenan, locust bean gum, and culture filtrate from lao-chao on the curd firmness, viscosity, and syneresis. Results indicated that the best rheological property, preservative quality and acceptability of Kou Woan Lao could be obtained by the combination of 0.22% carrageenan, 0.21% locust bean gum and 12% culture filtrate from lao-chao. The curd firmness, viscosity and syneresis of resultant product were 29.3 g, 21,347.7 cps, and 8.92%, respectively and the microstructure of the curd revealed a relatively complete three-dimensional spider web-like structure. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 10 : 1470-1476)***

Key Words : Kou Woan Lao, Lao-chao, Curd Firmness, Viscosity, Syneresis, Response Surface Methodology

INTRODUCTION

In order to develop new yogurt-like dairy products satisfying consumer preferences for a low-acid or non-sour product, culture filtrates from a fermented rice product (lao-chao) have been used as both milk-clotting and flavoring additives (Onyeneho et al., 1987; Kuo et al., 1996; Lin and Chen, 1996). Well-known in China, lao-chao has traditionally been produced by inoculating steamed glutinous rice with commercial starter called Chinese yeast-ball (chiu-yao), followed by fermentation at room temperature for 2-3 days (Wang and Hesseltine, 1970). Actual fermentation time depends on temperature and personal preferences, with a longer fermentation producing a drink more like wine in flavor. Unlike other fermented foods that are usually salty in flavor, lao-chao has a sweet taste. As the amylopectin content for glutinous rice is relatively high, the amylolytic digestion of starch results in a sweet liquefied pasty product (Fennema, 1985) which can be consumed on its own, or cooked with eggs or other foods as a dessert (e.g. Kou Woan Lao). It can also be used for other dishes, such as seafoods (Wang and Hesseltine, 1970).

There were several kinds of organisms in commercial chiu-yao, consisting of *Rhizopus oryzae, Rhi. chinensis, Rhi. javanicus, Aspergillus oryzae, Mucor racemosus, Amylomyces rouxii, Saccharomycopsis fibuligera,* etc. (Ellis et al., 1976; Hesseltine, 1983; Wang and Hesseltine, 1970). The starter in a dry state was prepared under relatively poor microbiological conditions by persons untrained or poorly trained in microbiology. It was very difficult to control the microflora of commercial chiu-yao for the fermentation. Therefore, some selected molds and yeasts were used to prepare lao-chao from steamed glutinous rice (Wei and Jong, 1983; Lin and Chen, 1996). For example, Lin and Chen (1996) had attempted to screen appropriate pure cultures for manufacturing lao-chao, with *Rhi. javanicus* and *Sac. cerevisiae* chosen as the mold and yeast starter, respectively. *Rhi. javanicus*, could produce milk-clotting enzyme with good milk-clotting activity of culture filtrate from lao-chao.

Kou Woan Lao was coagulated by the culture filtrate of lao-chao and appeared soft and aqueous in texture. It was considered necessary to improve the texture to produce a highly acceptable dairy product. Labropoulos et al. (1984) indicated that increasing viscosity by increasing total solids and addition of stabilizers could improve the texture. For example, carrageenan and locust bean gum (LBG) mixed gels had been studied principally as texturing agents for food products (Arnaud et al., 1989). Duran et al. (1985) reported compressional testing on 1% total hydrocolloid gels showing the increase in maximum deformation in the presence of LBG. Ainsworth and Blanshard (1980) proposed that the increase of flexibility was obtained by the addition of 1% carrageenan and 1% LBG.

In this paper we discuss the optimum models of Kou Woan Lao by the addition of carrageenan, LBG and culture filtrate from lao-chao using RSM for systematic production. Further, for simple in-home preparation and quantity production, the possibility of gum taking the place of milk-clotting enzyme was studied.

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MATERIALS AND METHODS

Preparation of inocula

The mold, *Rhizopus javanicus* (CCRC 30288), and yeast starter, *Saccharomyces cerevisiae* (CCRC 21685), were obtained from the Culture Collection and Research Center (Taiwan) and used to inoculate steamed glutinous rice. Before use, both mold and yeast strain were transferred to slants and incubated at 30±1°C for 3 days. Spore suspensions for inoculation were prepared by adding sterilized distilled water containing Tween 80 (0.1 g/l) to the slants and shaking the cultures vigorously for 1 min.

Preparation of fermented rice (lao-chao) and culture filtrate

Glutinous rice was purchased from a local market in Taipei. Glutinous rice (100 g), which had been washed with distilled water and drained, was soaked with 75 ml distilled water at 20-25°C for 12 h, sterilized at 121°C for 15 min, and then cooled to 35°C. The steamed glutinous rice was inoculated with 0.5 ml of the spore suspensions containing 5×10^6 spores of each strain, followed by static incubation at 30±1°C in a 250 ml beaker and covered with aluminum foil. After fermentation for 6 days, the culture filtrate was obtained by filtration through four layers of cheesecloth. The sediment in the culture filtrate was eliminated by centrifuging at 5,000×*g* for 30 min (Kubota, KR-20000T, Tokyo 113, Japan). The culture filtrate was stored at 4°C for further analysis.

Preparation of Kou Woan Lao

Kou Woan Lao was prepared by adding culture filtrate, carrageenan, and LBG to pasteurised (at 65°C for 30 min) skim milk containing 12% nonfat milk powder (Anchor, Wellington, New Zealand) and 4% sucrose, then incubating at 37±1°C for 2.5 h. Culture filtrate concentration was varied over the range 8-12%, the concentrations of carrageenan and LBG were both 0.15-0.25%. The product was stored at 4°C for further analysis.

Determination of curd firmness, viscosity, syneresis and microstructure

Curd firmness, as measured by the breaking force of the milk coagulum, was determined using a rheometer (Fudoh, NRM-2011J-CW, Japan) with a rheoplotter (Rikadenki Kogyo, FR 801, Japan). The rheometer adaptor No.4 (20 mm dia) was used and the table speed was 50 mm/min. Viscosity was determined using a viscometer (Brookfield, LVDV-II + Viscometer, U.S.A.) with a No.3 disc spindle and a speed of 0.3 rev./min.

Analysis of syneresis was performed according to the method of Marshall (1982), with slight modification. Curds were placed in 100 ml beaker and maintained at 38°C in a water bath. After 30 min, curds were cut crosswise with a scalpel. After 30 more min in a water bath, curds were poured into another beaker, stainless steel wire grids (9 mesh/cm) were placed gently on the surface of curds to prevent them from falling out, and the whey was drained and weighed.

The microstructure of the curd was investigated by scanning electron microscopy (SEM). Samples were taken from the center of the curds, cut into 2×5×10 mm pieces, and fixed for 4 h in 0.7% glutardialdehyde. Specimens were washed twice with deionized water and dehydrated in 20, 40, 60, 80, 95 and 99.5% absolute alcohol at 1 h intervals. Samples were critical-point-dried from CO_2 using a Critical Point Dryer Samdri-PVT-3B (Tousimis, Rockville, US), mounted on aluminium stubs and coated with gold using an Ion Coater JFC1100E (Jeol, Tokyo, Japan). The preparations were observed with a Scanning Electron Microscope JSM-6300 (Jeol, Tokyo, Japan) (Lin et al., 1994).

Sensory evaluation of Kou Woan Lao

Kou Woan Lao was presented to a panel composed of volunteer graduate and undergraduate students from the National Taiwan University, Department of Animal Science. The panelists were fifteen women and fifteen men, aged 21-32 years. A nine-point hedonic scale was used to evaluate the curd's flavor, texture and overall acceptability (one - extreme dislike, three - moderate dislike, five - neither like nor dislike, seven - like moderately and nine - like extremely) (Meiselman, 1984).

Experimental design and data analysis

According to Box and Behnken (1960), a three-variable and three-level design method was performed to study the effect of the independent variables, e.g. the addition of carrageenan concentration (X_1), locust bean gum concentration (X_2), and culture filtrate concentration (X_3) on process responses, e.g. curd firmness (Y_1), viscosity (Y_2), and syneresis (Y_3). So that:

$$Y_i=a_0+a_1X_1+a_2X_2+a_3X_3+a_{12}X_1X_2+a_{13}X_1X_3+a_{23}X_2X_3+a_{11}X_1^2+a_{22}X_2^2+a_{33}X_3^2$$

where a_i is the coefficient for the quadratic model. The range of independent variables was recorded in table 1. In the optimization study, acceptability data were fitted to a second order model equation provided by the Design-Expert® software package (Stat-Ease, Inc., 2000).

All results were analyzed using the general linear model procedure from the SAS software package (SAS Institute, Inc., 1987), with Duncan's multiple range test for significance (Montgomery, 1991) used to detect differences between treatment means. All experiments were replicated three times.

RESULTS AND DISCUSSION

The physical characters of the curd of Kou Woan Lao are of primary importance with reference to the quality, texture, and body of cultured dairy products. In general, any natural or processing influence that affects chemical or physical milk composition and constituents may be reflected in the strength of the curd formed on coagulation (Labropoulos et al., 1984). Our current objective was to obtain higher curd firmness, viscosity, and lower syneresis of Kou Woan Lao by the addition of carrageenan, LBG and culture filtrate from lao-chao. Samples were selected according to the three variables- three levels response surface design (table 1). The design comprised 17 points: twelve factorial and five central points.

The models of RSM

Curd firmness (Y_1) assigned to experimental samples prepared with addition of carrageenan (X_1), LBG (X_2) and culture filtrate (X_3) ranged between 22.6 g and 29.3 g, whereas viscosity (Y_2) ranged from 19,482 to 22,029 cps, and syneresis (Y_3) ranged from 10.07 to 13.63% (table 1). Curd firmness, viscosity, and syneresis were related to concentration of ingredients by below quadratic model equations:

$$Y_1=28.7+1.01X_1+1.16X_2+1.55X_3+0.57X_1X_2-0.25X_1X_3-0.05X_2X_3-1.61X_1^2-0.76X_2^2-1.34X_3^2$$

$$Y_2=21093.4+635.38X_1+653.25X_2+184.62X_3+98X_1X_2-29.75X_1X_3-6X_2X_3-286.58X_1^2-167.33X_2^2-265.57X_3^2$$

$$Y_3=10.12+0.04X_1-0.19X_2-0.38X_3+0.14X_1X_2-0.28X_1X_3+0.23X_2X_3+1.59X_1^2+1.71X_2^2-1.06X_3^2$$

The "Lack of Fit Tests" compared the residual error to the "Pure Error" from replicated design points. If there was significant lack of fit, there must be care about using the model as a response predictor. As shown in table 2, the quadratic model did not show significant lack of fit ($p>0.05$); it revealed that this model was suited for the analysis of these responses. Besides, high R-squared value (curd firmness was 98.22%, viscosity was 97.97%, and syneresis was 97.56%) of the response variation was explained by the regression model which showed no significant lack of fit, also.

The optimum models of Kou Woan Lao

In addition to test the significance of regression model, the contour plot of conversion as a function of two factors *via* the fixation of other factors was applied to show how the response changed. For response surface designs the carrageenan versus LBG plot of conversion was set, with the addition of culture filtrate held as a constant. Contour plots of curd firmness, viscosity, and syneresis of Kou Woan Lao for culture filtrate at the three added levels (8%, 10%, and 12%) are presented in fig. 1, 2, and 3. The highest curd firmness 29.6 g was obtained when carrageenan was 0.22%, LBG was 0.24%, and culture filtrate was 12%. The highest viscosity 21,927cps was obtained when carrageenan was 0.25%, LBG was 0.25%, and culture filtrate was 12%. The lowest syneresis 8.92% was obtained when carrageenan was 0.20%, LBG was 0.20%, and culture filtrate was 12%.

Table 1. Experimental data of curd firmness, viscosity and syneresis of Kou Woan Lao (n=5)

Ex. runs	Factors						Responses		
	Carrageenan (%) X_1		Locust bean gum (%) X_2		Culture filtrate (%) X_3		Curd firmness (g)	Viscosity (cps)	Syneresis (%)
	Code	Uncode	Code	Uncode	Code	Uncode			
1	+1	0.25	+1	0.25	0	10.0	28.7	22,029	13.63
2	+1	0.25	-1	0.15	0	10.0	25.4	20,518	13.16
3	-1	0.15	+1	0.25	0	10.0	26.1	20,601	13.39
4	-1	0.15	-1	0.15	0	10.0	25.1	19,482	13.48
5	+1	0.25	0	0.20	+1	12.0	28.4	21,375	10.19
6	+1	0.25	0	0.20	-1	8.0	25.7	21,053	11.33
7	-1	0.15	0	0.20	+1	12.0	26.3	20,125	10.53
8	-1	0.15	0	0.20	-1	8.0	22.6	19,684	10.56
9	0	0.20	+1	0.25	+1	12.0	29.3	21,482	10.07
10	0	0.20	+1	0.25	-1	8.0	26.4	21,137	10.53
11	0	0.20	-1	0.15	+1	12.0	26.9	20,196	10.56
12	0	0.20	-1	0.15	-1	8.0	23.8	19,827	11.93
13	0	0.20	0	0.20	0	10.0	28.9	21,120	10.11
14	0	0.20	0	0.20	0	10.0	28.7	21,066	10.10
15	0	0.20	0	0.20	0	10.0	28.3	20,903	10.14
16	0	0.20	0	0.20	0	10.0	29.1	20,964	10.12
17	0	0.20	0	0.20	0	10.0	28.5	21,414	10.15

Table 2. Analysis of variance for various responses

Source	Degree of freedom	Sum of squares		
		Curd firmness	Viscosity	Syneresis
Model	9	62.94	7.76×10^{6}	2.96×10
Linear	3	38.23	6.92×10^{6}	1.43
Quadratic	6	24.71	8.41×10^{5}	2.79×10
Residual	7	1.14	1.61×10^{5}	0.73
Lack of fit	3	0.74	3.45×10^{3}	0.73
Pure error	4	0.40	1.57×10^{5}	1.72×10^{-3}
R^2 (%)		98.22	97.97	97.56
Source		F value		
		Curd firmness	Viscosity	Syneresis
Model		42.85**	37.55*	31.15**
Linear		6.41**	29.93**	0.22
Quadratic		25.23**	6.10*	44.46**
Lack of fit		2.48	0.029	566.72

* Significant at 5% level, ** Significant at 1% level.

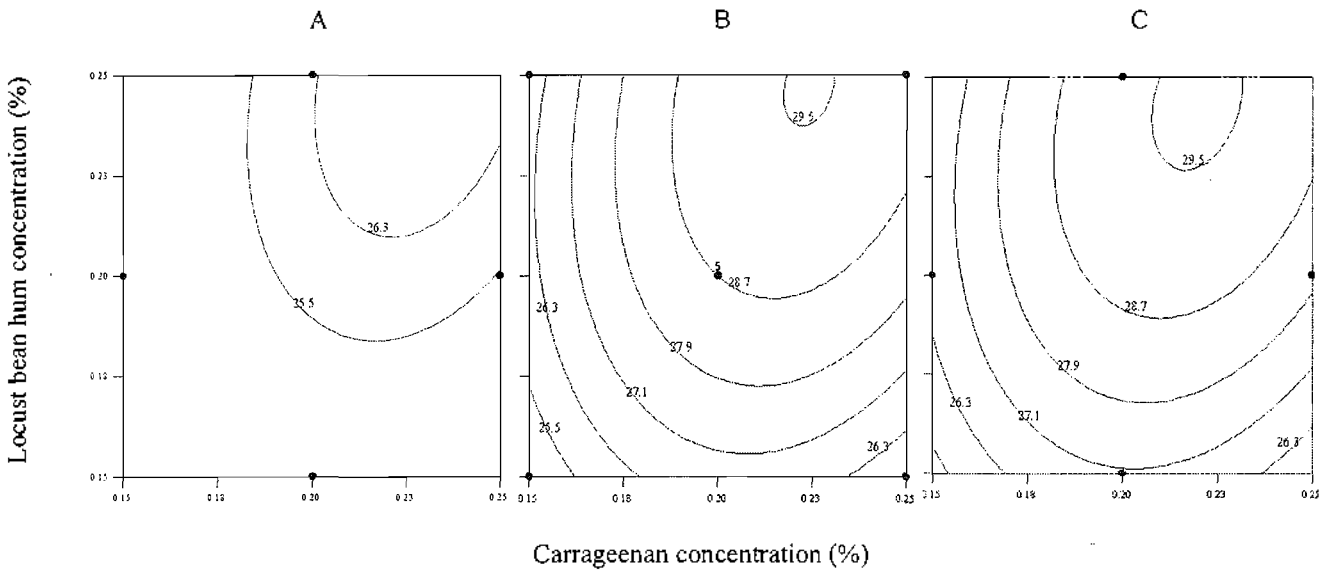

Figure 1. Contour plots of curd firmness of Kou Woan Lao for culture filtrate at the three added levels. (A: 8%, B: 10%, C: 12%.)

Furthermore, Design-Expert software provided four optimum process models of Kou Woan Lao to obtain highest curd firmness and viscosity, but syneresis was the lowest simultaneously (table 3). The best model was curd firmness 29.3 g, viscosity 21,347.7 cps, and syneresis 8.92% when carrageenan was 0.22%, LBG was 0.21%, and culture filtrate was 12%.

Storage effects on curd firmness, viscosity, and syneresis of Kou Woan Lao

Considering what was mentioned above, the most optimum model of Kou Woan Lao by the addition of carrageenan, LBG and culture was selected and the curd only with 12% culture filtrate was used as control. Changes of curd firmness, viscosity and syneresis of Kou Woan Lao during 0-12 d storage are presented in table 4. Curd firmness and viscosity of Kou Woan Lao with edible gum added decreased throughout the storage period, curd firmness decreased from 27.6 to 22.9 g and viscosity decreased from 20,465 to 15,263 cps. Those without edible gum added also decreased throughout the storage period, curd firmness decreased from 25.7 to 15.3 g and viscosity decreased from 19,609 to 12,834 cps. The results showed that changes on curd firmness and viscosity of Kou Woan Lao with edible gum added were smaller than those without edible gum added. The quality of the former might be more

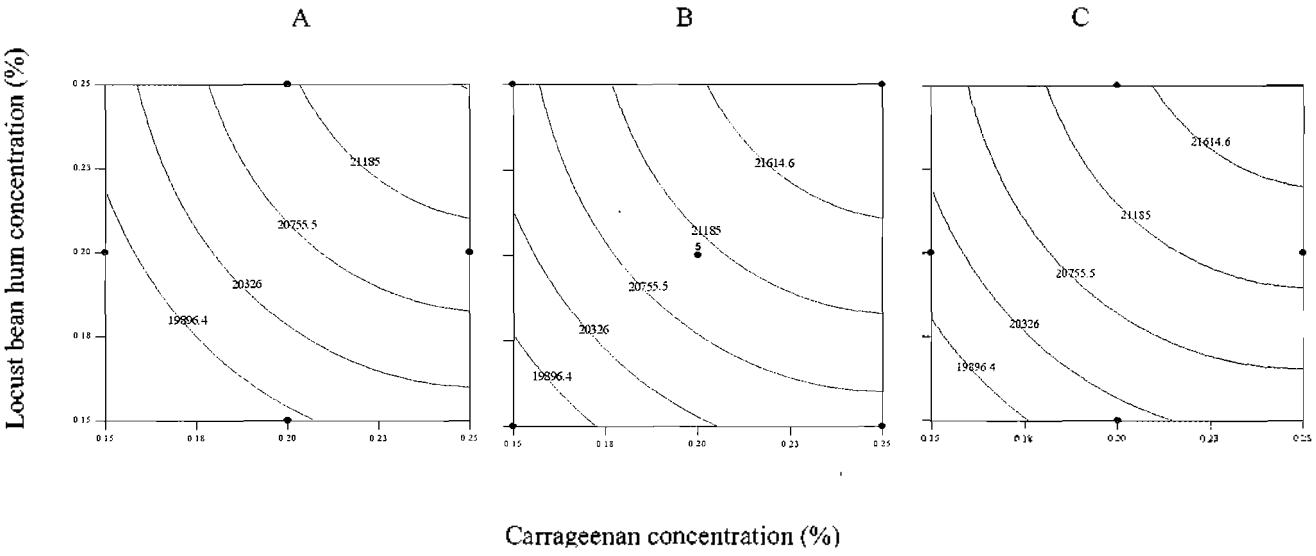

Figure 2. Contour plots of viscosity of Kou Woan Lao for culture filtrate at the three added levels. (A: 8%, B: 10%, C: 12%.)

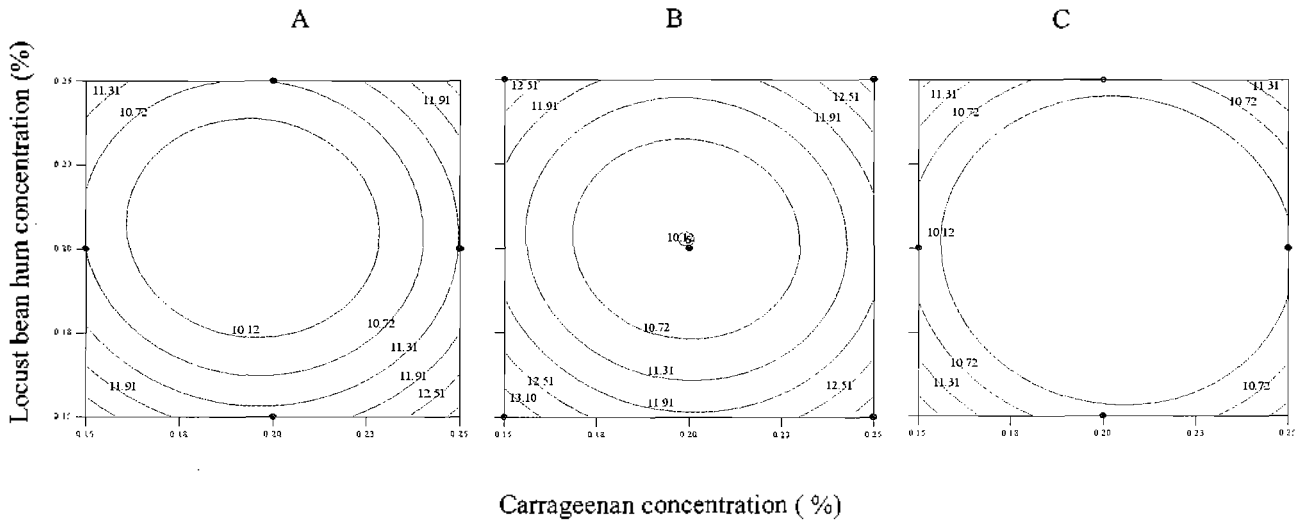

Figure 3. Contour plots of syneresis of Kou Woan Lao for culture filtrate at the three added levels. (A: 8%, B: 10%, C: 12%.)

stable than the latter during the storage period. On the other hand, there was no significant difference ($p>0.05$) in the increment of syneresis throughout the storage period whether adding gum or not. But it was noticeable that syneresis of Kou Woan Lao with edible gum added was less volume than those without edible gum added.

Microstructure of curd

SEM observations of the microstructure of curds showed that the casein joined together chained by chain-like

Table 3. The optimum models of Kou Woan Lao

No.	Carrageenan (%)	Locust bean gum (%)	Culture filtrate (%)	Curd firmness (g)	Viscosity (cps)	Syneresis (%)	Desirability
1	0.22	0.21	12	29.3	21,347.7	8.92	0.770
2	0.22	0.20	12	29.0	21,239.0	8.86	0.759
3	0.22	0.20	12	29.0	21,294.8	8.98	0.758
4	0.21	0.22	8	26.4	20,993.0	9.66	0.658

Table 4. Changes of curd firmness, viscosity, and syneresis of Kou Woan Lao during storage*

Storage days	Curd firmness (g)		Viscosity (cps)		Syneresis (%)	
	Edible gum added	Without edible gum	Edible gum added	Without edible gum	Edible gum added	Without edible gum
0	27.6±0.5^{a}	25.7±0.8^{a}	20,465±1,217^{a}	18,609±583^{a}	8.67±0.56^{a}	13.38±0.62^{a}
2	27.4±0.5^{a}	25.2±1.0^{a}	20,056±832^{a}	18,345±675^{a}	8.96±0.79^{a}	13.05±0.96^{a}
4	27.1±0.7^{a,b}	24.1±0.8^{a,b}	19,891±1,169^{a}	17,996±563^{a}	9.74±0.53^{a}	14.23±1.25^{a}
6	26.9±0.9^{a,b}	22.8±1.5^{b}	18,977±667^{a}	16,543±693^{b}	10.23±0.82^{a}	15.06±1.40^{a,b}
8	25.5±1.1^{b,c}	18.6±1.6^{c}	17,357±580^{b}	16,058±751^{b}	12.35±1.03^{b}	17.45±1.40^{b,c}
10	24.2±0.8^{c,d}	16.8±1.5^{c,d}	16,734±896^{b,c}	14,064±552^{c}	13.12±0.93^{b,c}	18.08±1.83^{c}
12	22.9±1.7^{d}	15.3±1.2^{d}	15,263±609^{c}	12,834±776^{d}	14.82±1.80^{c}	19.82±1.80^{c}

*N=5.

^{a,b,c,d}: Means with different superscripts in the same column differ significantly ($p<0.05$).

fibers which created a three-dimensional spider web-like structure. Comparison of with and without edible gum added (fig. 4- A, B, C, D) on the microstructure of curd, shows the holes were smaller in curd with edible gum added. Herrtje (1993) proposed that under the influence of carrageenan, casein micelles were partly disintegrated into smaller units that partly adhere to the carrageenan threads and form chains. Further, by contrast with table 4, the curd with edible gum added had higher firmness and lower syneresis. Comparison of more edible gum added (fig. 4- E, F), shows the holes in the structure of curd were relatively smaller and didn't increase the firmness; it would reduce the space of microstructure and resulted in higher syneresis.

Sensory evaluation

Sensory evaluation scores (table 5) clearly demonstrated that texture and overall acceptance of Kou Woan Lao with edible gum added were higher than without edible gum added ($p<0.05$). There was no significant difference in the flavor of two groups ($p>0.05$). On the whole, Kou Woan Lao with edible gum added had better texture and both of them exhibited similar preference in flavor.

We have achieved our purpose to improve the texture of Kou Woan Lao. As mentioned above, the best rheological property, preservative quality and acceptability of Kou Woan Lao could be obtained by the combination of 0.22% carrageenan, 0.21% LBG and 12% culture filtrate from lao-chao. The curd firmness, viscosity and syneresis of resultant product were 29.3 g, 21,347.7 cps, and 8.92%, respectively. Furthermore, as contrasted with yogurt which had stronger flavor and higher curd firmness than Kou Woan Lao, it needs to be considered how to enhance the flavor of Kou Woan Lao to increase the acceptance by consumers.

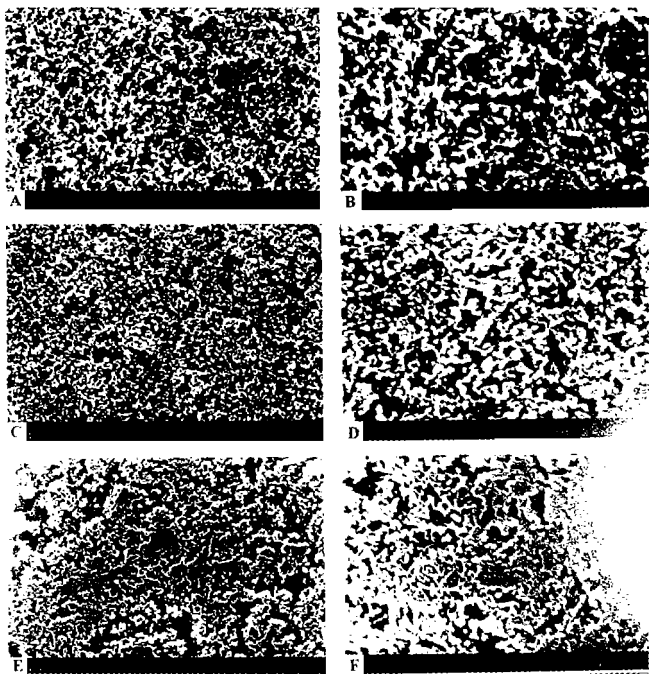

Figure 4. Effect of edible gum added on the microstructure of curd

A (×7,500), B (×15,000): without edible gum.

C (×7,500), D (×15,000): 0.22% carrageenan and 0.21% locust bean gum added.

E (×7,500), F (×15,000): 0.25% carrageenan and 0.25% locust bean gum added.

Table 5. Sensory evaluation scores of Kou Woan Lao* (n=30)

Sensory evaluation	Edible gum added	Without edible gum
Flavor	6.25±1.16^{a}	6.70±1.22^{a}
Texture	6.80±1.40^{a}	5.00±1.21^{b}
Overall acceptability	6.58±1.30^{a}	5.68±1.16^{b}

* Nine-point hedonic scale test.

^{a,b} Means with different superscripts in the same row differ significantly. ($p<0.05$)

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