Optimization of Formulation of CMC-Na, Xanthan Gum and Carrageenan Affecting the Physicochemical Properties of Papaya-Wolfberry Beverage using Response Surface Methodology

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Summary: CMC-Na, xanthan gum and carrageenan are widely employed in food industry. They were used for its thickening properties of aqueous solutions or emulsifying abilities. The present work aims to optimize the formula of the three stabilizers in the process of papaya-wolfberry beverage by response surface methodology (RSM). The results showed that the models were significantly ($p < 0.05$) fitted for describing the viscosity and cloudiness of papaya-wolfberry beverage. The results also indicated that the linear terms of CMC-Na and xanthan gum were the most significant ($p < 0.05$) variables affecting the viscosity, while xanthan gum and carrageenan were the most significant ($p < 0.05$) variable affecting the cloudiness. The interaction of CMC-Na and xanthan gum behaved extremely significant for viscosity. From the optimization procedure, the best formula for viscosity was obtained at the combined level of 0.0652% (w/w) CMC-Na, 0.1070% (w/w) xanthan gum and 0.1485% (w/w) carrageenan, and the other group of 0.0623% (w/w) CMC-Na, 0.1375% (w/w) xanthan gum and 0.1461% (w/w) carrageenan for cloudiness. The results of our study would be used to improve the quality of papaya-wolfberry beverage and increase its economic efficiency.

Keywords: CMC-Na; Xanthan gum; Carrageenan; Papaya; Wolfberry, Beverage.

Introduction

The papaya is a kind of tropical fruit that is widely cultivated and consumed [1]. Papayas are not only valued for the flavor and taste of their soft red pulp, but also a good dietary source of nutrients, and beneficial bioactive compounds, such as carotenoids and glucosinolates [2-3]. The green life of papayas was very short because of their rapid softening as well as their susceptibility to physical injury and mold growth [4]. So how to reduce the postharvest losses is an important topic which researchers are interested in.

Wolfberry fruit is a red–orange berry of the Solanaceae family [5]. Wolfberry is a popular Chinese traditional herbal medicine [6]. Wolfberry fruit are rich in carotenoids, which are beneficial to eye health and vision, so they have been traditionally used as functional food [7]. Recently, the growing interest in their nutritional role and chemotherapeutic properties has prompted the identification of specific dietary constituents of wolfberry fruits [8].

CMC-Na is used as a thickener, binder, stabilizer, suspending and water-retaining agent in pharmaceutical, food and other major industries [9]. Xanthan gum is widely used in the food industry for its great solubility in cold water and its high thickening properties at very low concentration [10]. Carrageenans are sulphated, anionic polysaccharides extracted from red seaweeds. Carrageenan is known for its interactions with casein micelles, which is added as a thickening agent [11-12].

In this paper, papaya and wolfberry were used as the raw materials to process a kind of papaya-wolfberry composite beverage. CMC-Na, xanthan gum and carrageenan were used as the stabilizer for the beverage. The present work was conducted to investigate the influence of the three stabilizers on viscosity and cloudiness of the papaya-wolfberry beverage and determine an optimal formula by Response Surface Methodology (RSM).

Results and Discussion

The Ratio of Papaya Juice to Wolfberry Juice

The ratio of papaya/wolfberry juice played an important role in the quality of the finished beverage. Papaya showed pale yellow, refreshing flavor and fragrance, while wolfberry juice showed orange color, slightly sour and light flavor. The ratio of papaya/wolfberry juice significantly affected the color, taste, flavor and structural state of the final product. The sensory evaluation results were shown in Fig. 1.

It was found that papaya juice was viscous and rich in papain [13], so it was easily foaming during mixing process. Wolfberry juice showed deep orange yellow and was difficult to muddy. But after

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they were mixed, the color, taste, structural state of the compound juice would be affected. As can be seen from Fig. 1, when the proportion of papaya/wolfberry juice was 4:3 (w/w), the sensory score was higher than that of other samples. Therefore, in following studies, the ratio of papaya/wolfberry juice was fixed as 4:3 (w/w).

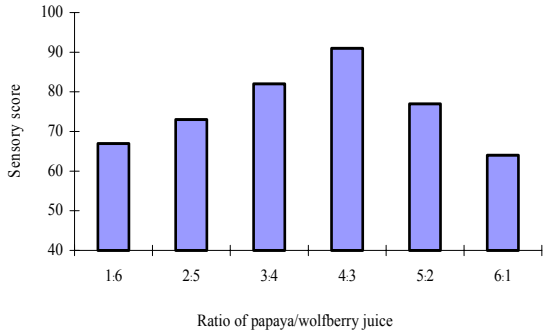


Fig. 1: Ratio of papaya juice and wolfberry juice

Fitting the Response Surface Models

The effect of three independent variables, CMC-Na content, xanthan gum content and carrageenan content, on two response variables (viscosity and cloudiness) was evaluated by using the response surface methodology (RSM). Each of variables to be optimized was coded at three levels: -1, 0 and 1. Table-1 shows the variables, their symbols and levels. The selection of variable levels was based on the study of single factor experiment. As shown in **Table 2**, three-factor central composite design (CCD) was used to investigate the main and combined effects of the three variables on the physical properties of papaya-wolfberry beverage and create models between the variables. If the proposed model is adequate, as revealed by the diagnostic checking provide by an analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response surface and located the optimum. The center point was repeated six times to calculate the repeatability of the method.

Table-1: Variables and their levels for central composite design.

Variable	Symbol	Coded-variable level				
		-α	-1	0	1	+α
CMC-Na (% w/w)	X_1	0.046	0.06	0.08	0.10	0.114
Xanthan gum (% w/w)	X_2	0.016	0.05	0.1	0.15	0.184
Carrageenan (% w/w)	X_3	0.016	0.05	0.1	0.15	0.184

Table-2: Central composite design: independent (Xi) and response variables (Yj) (mean±SD).

Run	Independent variable (%)			Response variable	
	CMC-Na (X_1)	Xanthan gum (X_2)	Carrageenan (X_3)	Viscosity cP	Cloudiness
1	0.100	0.150	0.150	14±2	0.062±0.0002
2 (C)	0.080	0.100	0.100	25±3	0.045±0.0000
3	0.060	0.050	0.050	21±5	0.042±0.0007
4	0.080	0.184	0.100	65±7	0.078±0.0004
5(C)	0.080	0.100	0.100	42±3	0.065±0.0002
6	0.080	0.100	0.016	26±7	0.044±0.0003
7	0.100	0.050	0.050	45±4	0.046±0.0004
8	0.100	0.150	0.050	28±3	0.056±0.0001
9(C)	0.080	0.100	0.100	48±4	0.055±0.0000
10(C)	0.080	0.100	0.100	38±2	0.058±0.0005
11	0.060	0.150	0.150	72±5	0.088±0.0005
12	0.114	0.100	0.100	35±1	0.055±0.0004
13	0.080	0.100	0.184	58±3	0.076±0.0006
14	0.100	0.050	0.150	38±5	0.060±0.0001
15	0.060	0.050	0.150	44±3	0.056±0.0000
16(C)	0.080	0.100	0.100	32±3	0.051±0.0000
17(C)	0.080	0.100	0.100	29±2	0.032±0.0002
18	0.060	0.150	0.050	55±2	0.067±0.0003
19	0.046	0.100	0.100	60±6	0.038±0.0005
20	0.080	0.016	0.100	21±5	0.039±0.0004

(C), Center point.

In our work, response surface analysis were carried out to fit mathematical models based on the experimental data. According to the experiment design, ANOVA for the regression was performed to assess the "goodness of fit". Statistically significant ($p < 0.05$ and $p < 0.01$) were included in the reduced model. The models were shown in equations (1)–(2).

The model for Y_1 (Viscosity) was:

$$Y_1(\text{Viscosity/cP}) = -66.65362 + 404.16579x_1 + 1112.08125x_2 + 687.88144x_3 - 12875x_1x_2 - 7625x_1x_3 - 650x_2x_3 + 7791.26939x_1^2 + 610.207x_2^2 + 468.78564x_3^2 \quad (1)$$

The model for Y_2 (Cloudiness) was:

$$Y_2(\text{Cloudiness}) = -0.025094 + 1.01498x_1 + 0.36599x_2 - 0.014182x_3 - 5.625x_1x_2 - 1.875x_1x_3 - 0.05x_2x_3 - .66552x_1^2 + 1.43057x_2^2 + 1.64271x_3^2 \quad (2)$$

The result of ANOVA was shown in Table-3. The results indicated that the response surface models with high coefficient of determination (R^2) 0.7984 and 0.7337 and the p-value of 0.015 and 0.0481 implies the models for Viscosity and Cloudiness were significant ($p < 0.05$). The lack of fit, which measures the fitness of models, resulted in no significant ($p > 0.05$) in terms of the response variables studied, indicating that models were sufficiently accurate for predicting those response variations.

Table-3: ANOVA and regression coefficients of the first- and second-order polynomial regression models.

Source		*Y_1 (Viscosity)*		*Y_2 (Cloudiness)*	
		F-ratio	p-value	F-ratio	p-value
Model(Regression)		4.40039	0.015*	3.061284	0.0481*
linear term	X_1	8.837139	0.014*	0.000117	0.9916
	X_2	6.707159	0.027*	12.63995	0.0052**
	X_3	3.940684	0.0752	8.262603	0.0165*
Interaction term	X_1X_2	13.45974	0.0043**	2.41216	0.1514
	X_1X_3	4.720869	0.0549	0.268018	0.6159
	X_2X_3	0.214412	0.6532	0.001191	0.9731
Quadratic term	X_1^2	1.420665	0.2608	0.060953	0.81
	X_2^2	0.3404	0.5725	1.756606	0.2145
	X_3^2	0.200902	0.6635	2.316186	0.159
Lack of Fit		1.667652	0.2941	0.594788	0.7088

* Significant at $p < 0.05$; ** Significant at $p < 0.01$.

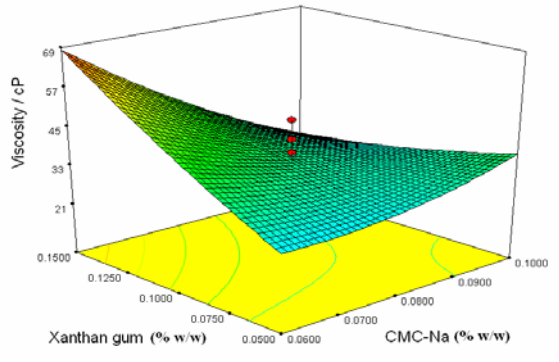
It was found that the linear effects of independent variables X_1 (CMC-Na) and X_2 (xanthan gum) were significant for viscosity, while the linear effects of independent variables X_2 (xanthan gum) and X_3 (carrageenan) were significant for cloudiness. As shown in **Table 3**, the interaction term of X_1X_2 (CMC-Na and xanthan gum) was extremely significant for viscosity response. These significant terms should be considered as the primary factors for determining the variation of viscosity and cloudiness.

Response Surface Plots for Viscosity

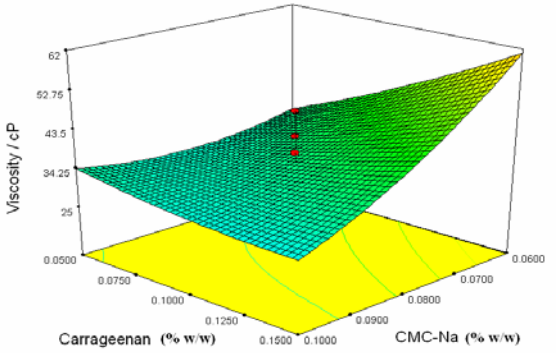
Response surface contour plots of the response surface methodology as a function of two factors at a time, holding all other factors at fixed levels, are more helpful in understanding both the main and the interaction effects of these two factors. These plots can be easily obtained by calculating from the model, the values taken by one factor while the second varies (from -1 to +1, step 1 for instance) with constraint of a given Y value.

As shown in Fig. 2.(a,b), the viscosity of papaya-wolfberry beverage significantly ($p < 0.05$) increased as the CMC-Na and Xanthan gum contents increased. Moreover, the viscosity of papaya-wolfberry beverage was significantly ($p < 0.05$) influenced by interaction effect of CMC-Na and xanthan gum. It is well known that CMC-Na is an extensively studied anionic polysaccharide and it is usually used as a thickener, binder, stabilizer, suspending and water-retaining agent. Xanthan gum is a natural polysaccharide and an important industrial biopolymer. Xanthan gum has been used in a wide variety of foods for a number of important reasons, including emulsion stabilization, temperature stability, compatibility with food ingredients, and its pseudoplastic rheological properties. The results of above demonstrated CMC-Na and Xanthan gum do obviously can increase the viscosity of beverage.

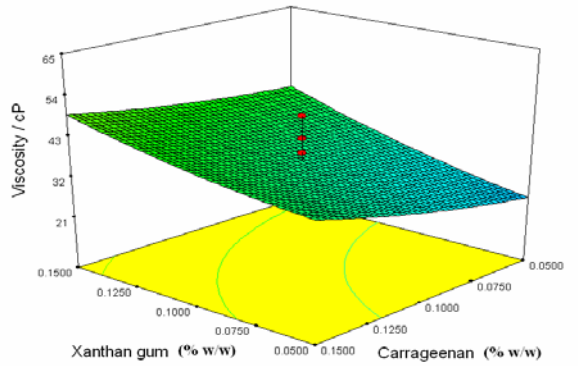
From Fig. 2 (c), it was shown that the viscosity of papaya-wolfberry beverage increased as the carrageenan content increased, but this effect was not significant ($p > 0.05$).



(a) Hold values: Carrageenan 0.1.



(b) Hold values: Xanthan gum 0.1



(c) Hold values: CMC-Na 0.08

Fig. 2: Response surface plots for viscosity as a function of (a) CMC-Na and xanthan gum contents, (b) CMC-Na and carrageenan contents and (c) xanthan gum and carrageenan contents.

Response Surface Plots for Cloudiness

Fig. 3 exhibited that the cloudiness increased as the concentration of X_2 (xanthan gum) and X_3 (carrageenan) in the formulation increased. High concentration of main emulsion components provided the high cloudiness value. Because of the positive effects of both hydrocolloids on the droplet concentration thereby increasing the cloudiness, theoretically, the emulsion cloudiness is due largely to the suspended solid particles in the emulsion and dispersion system.

Optimization Procedure for Predicting a Desirable Formulation

A numerical optimization was also carried out for simultaneous multiple optimization of response variables and determining the overall optimal condition. The desired goals for each response variable were chosen. The optimization procedures showed the optimum formulation for viscosity to be at 0.0652% (w/w) CMC-Na, 0.1070% (w/w) xanthan gum and 0.1485% (w/w) carrageenan. Under the optimum condition, the corresponding predicted response value for viscosity was 57.307 cP. The optimization procedures showed the optimum formulation for cloudiness to be at 0.0623% (w/w) CMC-Na, 0.1375% (w/w) xanthan gum and 0.1461% (w/w) carrageenan. Under the optimum condition, the corresponding predicted response value for cloudiness was 0.0758. Verification experiment at the optimum condition, consisting of 3 runs was performed, and the viscosity and cloudiness of 64.32±3.24 cP and 0.084 ± 0.006 were obtained.

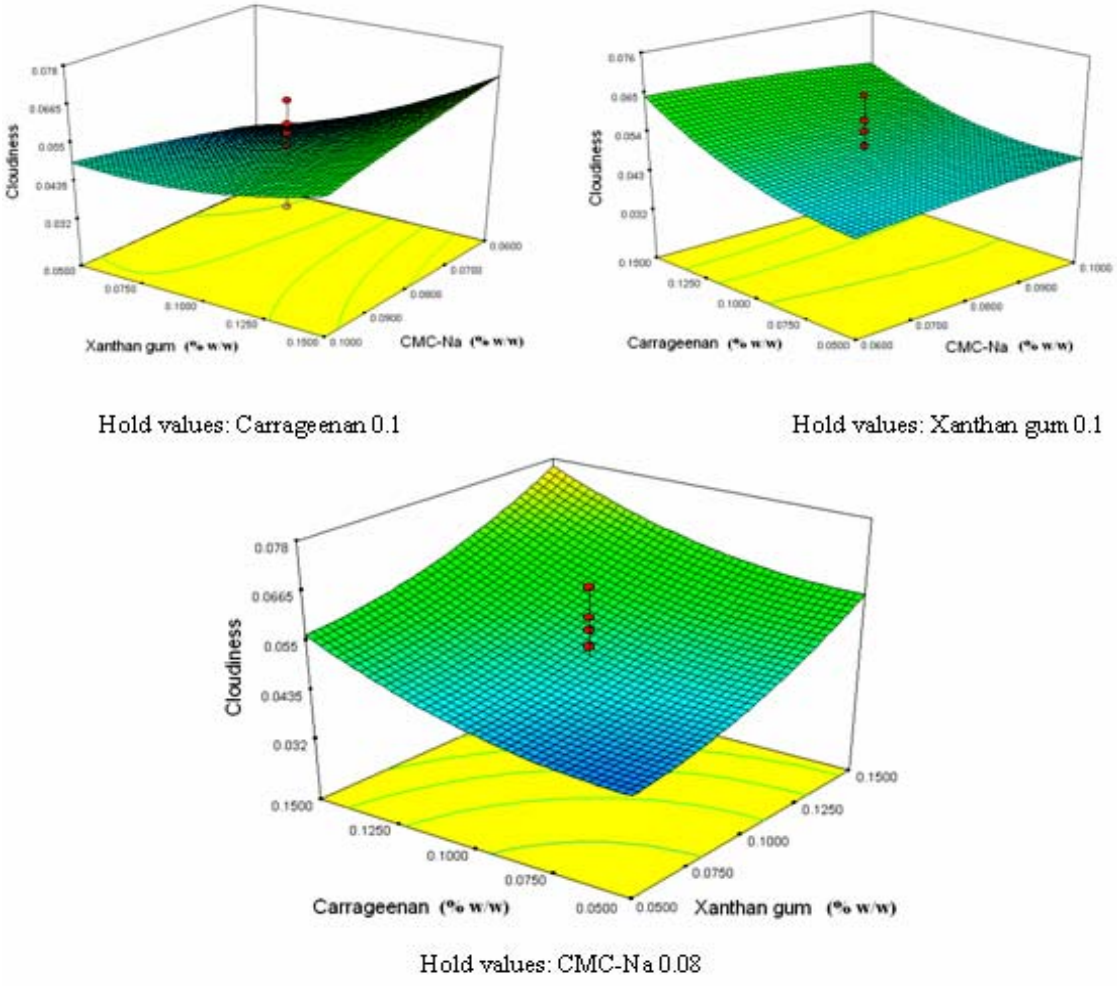


Fig. 3: Response surface plots for cloudiness as a function of (a) CMC-Na and xanthan gum contents, (b) CMC-Na and carrageenan contents and (c) xanthan gum and carrageenan contents.

Experimental

Materials

CMC-Na was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd; Xanthan gum and carrageenan were purchased from Hebeidadi Biological Technology Co., Ltd; Papaya and wolfberry were purchased in local market.

CMC-Na, xanthangum and carrageenan were dissolved with deionized water respectively. Xanthangum and carrageenan solutions were kept over night at room temperature for full hydratation.

Preparation of Juice

Preparation of papaya juice: papaya → selected → cleaned → peeled and seeded → adding four fold water → expressed juice → filtered → obtained papaya juice.

Preparation of wolfberry juice: wolfberry → selected → cleaned → soaked → took out → adding eight fold water → expressed juice → filtered → centrifuged → supernatant → obtained wolfberry juice

Preparation of Beverage

The main process flowchart of papaya-wolfberry beverage was as follows:

Papaya juice and wolfberry juice → mixed → adding the three stabilizers, sugar and citric acid → homogenization → filling → sealing → sterilization → final product.

Sensory Analysis

The final beverages were evaluated by a panel of ten well-trained flavorists (five females and five males, students and staff of Henan Institute of Science and Technology, China). Refrigerated (10 °C) samples of 10 mL were presented in clear glasses with a volume of 50 mL. The total score is 100 points. Tasters were asked to indicate how much they liked or disliked each product based on taste (40 points), odor (30 points) and appearance (30 points) of the beverages.

Analysis of Viscosity

The viscosity of papaya-wolfberry beverage was measured immediately after the sample preparation by Rapid Visco-Analyzer (RVA-Series 4, Newport Scientific Pty. Ltd., Warriewood, Australia). The viscosity measurement range of a RVA appears in the unit of cP. For each sample, the viscosity was reported as an average of two individual measurements. A fixed volume (30mL) of beverage was measured each time and the paddle speed was kept 160 rpm throughout the measurements for 3 minutes. The measurement of viscosity was carried out in triplicate.

Analysis of Cloudiness

The measurement of cloudiness was carried out using a UV–visible spectrophotometer (UV-5800, Shanghai Metash Instruments Co., Ltd., Shanghai, China). Spectra were obtained over the wavelength range of 200–800 nm. The beverage was transferred to a quartz cuvette with a 1 cm path length for the measurement of cloudiness. Cloudiness was then calculated from the absorbance reading at 640 nm. High absorbance reading values correspond to the high cloudiness. Distilled water was used as a reference. The measurement of cloudiness was carried out in triplicate.

Experimental

Response surface methodology (RSM) was used to determine the optimum levels of CMC-Na content, xanthan gum content and carrageenan content to improve the quality of papaya-wolfberry beverage. The coded and uncoded independent variables used in RSM design were shown in Table 1 and Table-2.

Statistical Analysis

A software package (Design Expert 7.0) was used to fit the second-order models and generate response surface plots. The model proposed for the response (Y) was:

$$Y = b_0 + \sum_{n=1}^{4} b_n x_n + \sum_{n=1}^{4} b_{nn} x_n^2 + \sum_{n \neq m-1}^{4} b_{lm} x_n x_m \qquad (3)$$

where b_0 is the value of the fitted response at the center point of the design, which is point (0, 0). b_n, b_{nn} and b_{nm} are the linear, quadratic and cross-product regression terms, respectively.

Conclusions

In the present work, the dependence of viscosity and cloudiness of papaya-wolfberry beverage on the content of main emulsion components was studied by the RSM. The results indicated that the variables X_1 (CMC-Na) and X_2 (xanthan gum) were significant for viscosity and variables X_2 (xanthan gum) and X_3 (carrageenan) were significant for cloudiness, and the interaction of CMC-Na and xanthan gum behaved extremely significant for viscosity. ANOVA analysis offered the significant ($p < 0.05$) models thus ensuring reliable adjustment of experimental data to the independent variables studied. Optimum production conditions also could be achieved by Response surface methodology.

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