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Optimization of Processing Parameters for Clarification of Blended Carrot-orange Juice and Improvement of its Carotene Content

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Abstract: The aim of this work was to optimize the processing parameters for the clarification of blended carrot-orange juice using Response Surface Methodology (RSM) and to improve the carotene content. The blended carrot-orange juice was treated with Pectinex Ultra SP-L enzyme at different concentrations (0.04-0.12%), pH (2.5-5), reaction temperature (40-60ºC) and time (70-110 min). The effect of these independent variables on clarity, turbidity, and viscosity of the carrot-orange juice was evaluated (coefficient of determination (R^2) greater than 0.9). From the RSM analysis, the optimum processing conditions were found as; 0.06% (w/v) enzyme concentration, 3.6 pH, 49ºC temperature, 91 min reaction time. The estimated carotene content in raw juice was higher (>5 mg/mL) than that of clear juice (<5 mg/mL) following enzymatic clarification. None the less, the combination of blended carrot-orange juice with 2-Hydroxypropyl-β-Cyclodextrin prior to clarification improved carotene content by threefold compared to clear juice. Hence, the clear carrot-orange juice favorably improves the nutritional content and consumer acceptance.

Key words: Blended carrot-orange juice, carotene content, enzyme clarification, 2-hydroxypropyl-β and γ-Cyclodextrin, response surface methodology

INTRODUCTION

Fruits and vegetables are critical to good health, and certainly good for all age categories as it forms an important portion of a healthy diet. Carrot (*Daucus carota*) is a worldwide root vegetable that is highly nutritional, and an important source of β-carotene besides its appreciable amount of vitamins and minerals often used for juice production (Walde *et al*., 1992; Demir *et al*., 2004). In recent years, a steady increase of carrot juice consumption has been reported in many countries (Schieber *et al*., 2001). Orange (*Citrus cinensis*) on the other hand, is a distinguished, widely consumed fruit, particularly appreciated for its fresh flavor, vitamin C, and its natural antioxidants source having health benefits (Campos *et al*., 2010; Gardner *et al*., 2000). Orange is extensively produced in Brazil, United States, Mexico, and China.

Juice blending is one of the best methods to improve the nutritional quality of the juice. It can improve the vitamin and mineral content depending on the kind and quality of fruits and vegetables used (De Carvalho *et al*., 2007). Apart from nutritional quality improvement, blended juice can be improved in its sensory and flavor characteristics according to their raw materials (Akinwale, 2000; Jain and Khurdiya, 2004).

Different methods for processing of clear juice have been reported with the aim of improving consumer acceptance in the market. Clarification is a beneficial step in the processing of juices, it is often achieved through enzymatic treatment, membrane filtration, or using clarifying aids such as chitosan, gelatin bentonite, polyvinyl pyrrolidone or synergistically combining two clarifying aids compounds (Gainvors *et al*., 1994). The use of commercial pectic enzymes is common in fruit juice processing. The technological advantages of pectic enzyme use have been shown in many studies; they give a more rapid flow of juice, improve juice yields, facilitate filtration, and gave greater clarity (Alkorta *et al*., 1998; Naidu and Panda, 1998). The enzymatic hydrolysis of pectin depends on several physicochemical factors such as incubation time, temperature, pH and enzyme concentration (Lee *et al*., 2006; Rai *et al*., 2004; Sin *et al*., 2006).

The determination of the optimal operating conditions by varying one parameter while keeping the others at constant level was used so far in practice but this method presented many disadvantages such as no interactive

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effects among the variables, thus it cannot depict the net effects of various parameters on the reaction rate. The Response Surface Methodology (RSM) was developed to overcome those disadvantages by reduction of the number of experimental trials needed to evaluate multiple parameters and their interactions, thus, less time consuming compared to other approaches. RSM has been widely applied in optimization processes in food industries (Lee *et al*., 2006; Sin *et al*., 2006; Wong *et al*., 2003; Yusof and Nuzarina, 1994).

Hydroxypropyl beta cyclodextrin is modified β-CDs having a higher aqueous solubility (>60%) and a proven safe profile, especially for parenteral uses. 2-hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative, is an alternative to α-, β- and γ-cyclodextrin, with improved water solubility properties (Sarah and Robert, 2005). γ-Cyclodextrin is a cyclic polymer consisting of eight glucose units linked by α-1,4 bonds. It is produced enzymatically from liquefied starch. γ-Cyclodextrin can form complexes with natural colours, flavours and vitamins allowing its use as a carrier and stabilizer for these additives. Fruits and vegetable juices are also treated with CD to encapsulate phenolic compounds, which cause enzymatic browning by removing polyphenoloxidase from juices by complexation (Mamata *et al*., 2002). The formation of an inclusion complex between bixin (α carotenoid) and α-cyclodextrin improved the solubility of the carotenoid in water and protected it against degradation in the presence of oxygen and light (Lyng *et al*., 2005).

Though, many works have been done so far on clarification of juice using enzymes and optimization of processing parameters using RSM but usually researchers have not focused on the on juice quality after clarification process. Furthermore, there has been no detailed report on the clarification of blended carrot-orange juice using enzyme and optimization of processing parameters using RSM. Therefore the purpose of this study was to (1) establish the optimum conditions for clarification of blended carrot-orange juices using RSM, (2) evaluate the carotene content of the clear juice compared to the raw juice.

MATERIALS AND METHODS

Carrots and oranges were obtained from fresh fruits market (*Da Runfa*) in Wuxi, Jiangsu, P.R. China. Pectinex Ultra SP-L from *Aspergillus aculeatus,* Commercial pectinolytic enzyme, was obtained from Novozyme, (Beijing, and P.R. China). 2-Hydroxypropyl-β-Cyclodextrin, 98% (2-HP-β-CD) was purchased from Zibo Qianhui Fine Chemical Co., Ltd., (Shandong, P.R. China) and γ-Cyclodextrin, 98% (γ-CD) was purchased from Jiangsu Fengyuan Biotechnology Co., Ltd., P.R. China. Other chemicals used were of analytical grade purchased from Shanghai Chemical Reagent Co. Ltd, P.R. China. This study was conducted in the Food Science and Technology Lab of Jiangnan University, Wuxi, Jiangsu Province, China during the period May-July, 2010.

Experimental design: In this study, a 2^4 full factorial central composite design comprising 30 experimental runs was employed and experiments were performed in randomized order according to the run number as arranged by the software. The parameters considered during enzymatic optimization were as follows: Enzyme concentration, 0.04-0.12% (w/v); temperature, 40-60ºC; pH, 2.5-4.5 and time, 70-110 min. The range of independent variables was based on the values obtained in preliminary experiments. The responses obtained were the clarity, turbidity and viscosity.

Data from the central composite design were analyzed by multiple regressions to fit the following second-order polynomial model:

$$Y = f(X_1, X_2, X_3, X_4) = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} bi_j X_i X_j \quad (1)$$

Where; b_0 is an intercept, b_i the linear coefficient, b_{ii} the quadratic coefficient and b_{ij} the interactive terms and subscript 1 for incubation temperature, 2 pH, 3 enzyme concentration and 4 incubation temperature. The coded independent variables were represented by X_i, X_i^2 and X_iX_j respectively. The coded levels of independent variables used in the experimental design (Table 1). The response values were the mean of the duplicate measurements. According to the analysis of variance, the regression coefficients of individual linear, quadratic, and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models.

Juice extraction: The carrot juice was prepared according to the method of (Lan *et al*., 2005), with little modifications. The carrots were washed with tap water, and peeled using Sodium hydroxide (40 g/L) at 95ºC for 1 min then washed again in tap water. This was followed by blanching in citric acid solution (60 g/L) at 95ºC for 5 min then cooled in iced water to inactivate their endogenous enzymes and soften their tissues. At the end, they were sliced and grounded with addition of distilled water 1:1 (v/w) and filtered on cheese cloth under vacuum to get fresh juice. Then, the oranges were cleaned with tap water, peeled and then orange juice was extracted using juice blender (JYL-350A, Joyoung Co., Ltd., Jinan

Table 1: Effect of temperature, pH, enzyme concentration, and time on clarity, turbidity and viscosity

S. No.	Independent variables				Dependent variables		
	Temperature °C	pH	Enzyme concentration (w/v %)	Time (min)	Clarity (660 nm)	Turbidity (NTU)	Viscosity (cPs)
	x_1	x_2	x_3	x_4	Y_1	Y_2	Y_3
1	0	-2	0	0	0.140±0.011	120.13±2.64	0.931±0.008
2	-1	-1	-1	-1	0.052±0.000	55.10±0.00	0.913±0.006
3	-1	-1	1	1	0.041±0.006	27.60±0.00	0.910±0.008
4	-1	1	1	-1	0.032±0.007	26.85±3.89	0.912±0.006
5	1	-1	-1	1	0.074±0.000	69.80±0.00	0.922±0.024
6	-2	0	0	0	0.014±0.004	14.40±4.24	0.910±0.023
7	1	1	1	1	0.040±0.004	31.84±4.16	0.909±0.000
8	0	0	0	2	0.025±0.004	15.02±2.38	0.908±0.006
9	1	1	-1	-1	0.037±0.006	24.90±0.00	0.922±0.018
10	1	1	1	-1	0.047±0.001	32.90±0.00	0.924±0.008
11	0	0	0	0	0.013±0.001	14.00±1.41	0.896±0.003
12	0	0	0	0	0.016±0.004	13.20±1.70	0.894±0.000
13	-1	-1	-1	1	0.061±0.004	52.78±2.66	0.921±0.006
14	0	0	0	0	0.015±0.001	11.71±0.44	0.888±0.008
15	1	1	-1	1	0.040±0.006	29.97±3.35	0.905±0.000
16	1	-1	1	1	0.057±0.011	47.34±9.39	0.925±0.007
17	0	0	0	-2	0.023±0.004	15.94±4.72	0.912±0.003
18	0	0	0	0	0.015±0.001	12.31±0.01	0.892±0.003
19	-1	1	1	1	0.030±0.000	21.69±3.95	0.908±0.010
20	2	0	0	0	0.041±0.010	35.90±6.65	0.923±0.007
21	0	0	0	0	0.015±0.003	11.62±1.39	0.891±0.006
22	-1	1	-1	1	0.032±0.007	23.50±1.41	0.913±0.007
23	0	0	2	0	0.017±0.004	11.02±2.66	0.901±0.004
24	-1	1	-1	-1	0.026±0.000	22.96±2.21	0.921±0.016
25	0	0	0	0	0.014±0.001	12.53±2.79	0.893±0.010
26	1	-1	1	-1	0.062±0.013	49.27±3.35	0.922±0.008
27	0	0	-2	0	0.027±0.003	26.71±8.50	0.910±0.003
28	-1	-1	1	-1	0.043±0.003	36.50±4.24	0.903±0.004
29	1	-1	-1	-1	0.070±0.004	64.18±8.17	0.921±0.007
30	0	2	0	0	0.096±0.008	72.74±0.06	0.924±0.008

Province, P.R. China). Finally, the juice obtained was mixed in proportion 70% carrot and 30% Orange (v/v) and stored at -20ºC before use.

Enzymatic treatment: For each experiment, 100 mL of juice were subjected to enzyme treatment according to the conditions cited in Table 1. The temperature was adjusted to the desired level using water bath (Blue Pard, Yiheng Technical Co., Ltd., P.R. China). The pH of the juice was adjusted with pH-meter (320-S pH meter Mettler-Toledo Instruments Co., Ltd., Shanghai, P.R. China) using citric acid or sodium hydroxide. At the end of the enzymatic hydrolysis, the enzyme was inactivated by heating the suspension at 90ºC for 5 min in a water bath. The treated juice was centrifuged at 6800×g, 30 min (GL-20.B; Shanghai Anke Co. Ltd., Shanghai, P.R. China) followed by the juice analysis.

Physico-chemical analysis: Clarity of the juice was determined by measuring the absorbance at 660 nm using a Mapada UV-Vis spectrophotometer (Model UV-1600, Mapada Corporation, P.R. China), distilled water was used as blank. The clarity was expressed in Absorbance value (Abs).

Turbidity was determined using a STZ-A_{24} turbidimeter (Wuxi Guangming Turbidimeter Factory, Wuxi, P.R. China). Distilled water was used as blank. It was expressed in Nephelometric Turbidity Units (NTU).

Relative Viscosity was determined in an Ostwald viscometer at 25ºC with distilled water as control. It was expressed in centipoises (cPs).

Determination of total carotene content: The measurement of carotenoids was carried out according to the method of (Liao *et al*., 2007) with a little modification, by measuring the A_{450} (absorbance at 450 nm) at ambient temperature by a spectrophotometer (Model UV-1600, Mapada Corporation, P.R. China). Twenty five milliliters of carrot juice were mixed with 80 ml of n-hexane/acetone (1:1, v/v) in a separation funnel. After shaking, the acetone was washed out using distilled water. The organic phase was dehydrated with anhydrous sodium sulfate. The aqueous phase was repeatedly extracted with 15 mL of n-hexane/acetone (1:1, v/v) until

it was colorless. The standard curve was drawn using the β-carotene solution at different concentrations. The total carotene content in the sample was calculated from the standard curve y = 0.43x+0.002, with R^2 = 0.9974 and expressed as mg β-carotene/ml of juice.

2-Hydroxypropyl-β and γ-Cyclodextrin treatment: The juice was added to 2-Hydroxypropyl-β-cyclodextrin or γ-Cyclodextrin in different proportion (1-5 g) per 100 mL of juice. Then, the mixture was mixed at room temperature using the magnetic stirrer for 90 min. Finally, the mixture was subjected to pectin enzyme for clarification according to the optimum conditions.

Color measurement: Juice color was measured using a spectrocolor meter (WSC-S color difference meter, Shanghai Precision and Scientific Instrument Co., Ltd., P.R. China). The ΔE index was calculated from the Hunter-Scotfield equation: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$. Where *L* represents lightness, *a* red-green, *b* represents yellow-blue and ΔE as total color difference. The deionized water was used as control.

Statistical analysis: Analysis of variance (ANOVA) was carried out by using the software SPSS 16 (Chicago IL). Significant differences ($p<0.05$) among treatments were detected using Duncan's multiple range tests. Values expressed are means ±standard deviation of triplicate measurements.

RESULTS AND DISCUSSION

Temperature, pH, enzyme concentration and time are important factors affecting the clarification efficiency of juice, such as. The experimental data obtained from the 30-run-experiment given in Table 1 were analyzed using Design-Expert 7.1.3. The analysis of variance (ANOVA) of three response variables, i.e., clarity, turbidity, and viscosity showed that experimental data had correlation coefficients (R^2) of 0.9993, 0.9994 and 0.9865, respectively with the calculated model with no significant lack of fit at $p<0.05$. That means the calculated model was able to explain 99.93, 99.94 and 98.65% of the results in the case of clarity, turbidity and viscosity respectively. The results indicated that the model used to fit the response variable was significant ($p<0.0001$) and adequate to represent the relationship between the responses and the independent variables. The effects of the independent variables and their mutual interaction on the clarity, turbidity and viscosity of blended carrot juice can also be seen on three dimensional response surface curves and contour plots shown in Fig. 1-3. Neglecting the non-significant parameters, the final predictive equations obtained were given as below:

$$Y_1 = 1.63 - 7.325E^{-3}X_1 - 0.74X_2 - 1.50X_3 - 1.533E^{-3}X_4 - 5.500E^{-4}X_1X_2 + 6.250E^{-3}X_1X_3 - 2.000E^{-5}X_1X_4 + 0.42X_2X_3 - 0.012X_3X_4 + 1.192E^{-4}X_1^2 + 0.10X_2^2 + 4.01X_3^2 + 2.104E^{-5}X_4^2 \quad (2)$$

$$Y_2 = 1623.57 - 11.65X_1 - 620.60X_2 - 2562.81X_3 - 2.36X_4 - 0.85X_1X_2 + 8.87X_1X_3 + 0.029X_1X_4 + 581.87X_2X_3 + 0.086X_2X_4 - 8.11X_3X_4 + 0.12X_1^2 + 83.60X_2^2 + 3769.27X_3^2 + 6.615E^{-3}X_4^2 \quad (3)$$

$$Y_3 = 1.93 - 0.020X_1 - 0.14X_2 - 3.26X_3 - 3.813E^{-3}X_4 - 9.250E^{-4}X_1X_2 + 0.028X_1X_3 - 3.875E^{-5}X_1X_4 - 7.875E^{-4}X_2X_4 + 2.471E^{-4}X_1^2 + 0.036X_2^2 + 8.57X_3^2 + 4.552E^{-5}X_4^2 \quad (4)$$

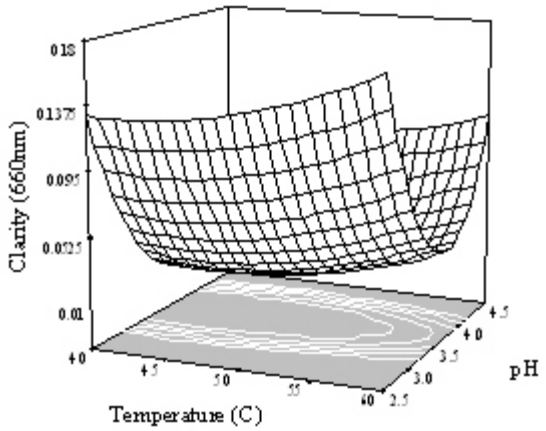
Clarity: Clarity is one of the important indices of clarified juice (Sin *et al.,* 2006). The linear effect of clarity was significantly affected by temperature, pH, and enzyme concentration (X_1, X_2, X_3) ($p<0.0001$), followed by time (X_4) ($p = 0.0081$) and the quadratic effects were positive and significant at $p<0.0001$ or $p<0.05$. The interaction parameters (X_1X_2, X_1X_3, X_1X_4, X_2X_3, and X_3X_4) for clarity were significant at the level of $p<0.05$ or $p<0.0001$ (Eq. 2).

The interaction effect between incubation temperature and pH to clarity (Fig. 1a), and it was clear that at fixed enzyme concentration and time, the clarity increased with the increase of pH from 2.5 to 3.5 and incubation temperature of 40 to 50ºC. A further increase of pH and incubation temperature over 3.5 and 50ºC, respectively, decreased the clarity. It might be explained that the pH below or above 3.5 and the higher incubation temperature denatured the enzyme.

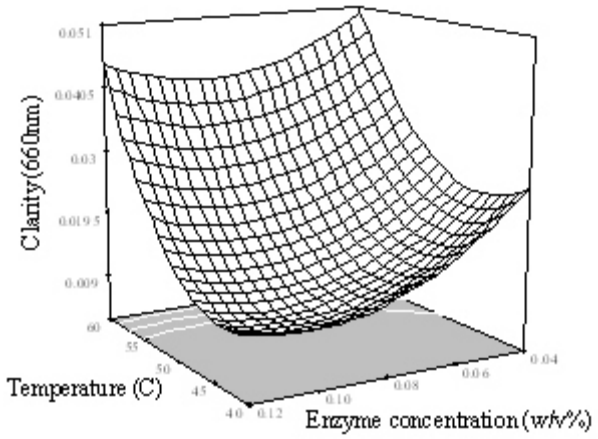
The interaction effect between enzyme concentration and incubation temperature to clarity as shown in Fig. 1b., reveals that the clarity increased with increase of enzyme concentration and a plateau in the increase was observed when the concentration reached 0.1 to 0.12%. The clarity increased slowly at the beginning when the incubation temperature increased from 40 to 50ºC. A rapid decrease in the clarity was observed when the incubation temperature increased over 50ºC.

Figure 1c, depicts the interaction effect between incubation temperature and time to clarity. As shown in Fig. 1c., the clarity increased slowly at the beginning as the incubation temperature increased up to 50ºC, then increased rapidly over 50ºC; while, the clarity increased gradually with increase of incubation time from 70 to 90 min then an increase of incubation time over 90 min showed a slow decrease of the clarity.

The effect observed between enzyme concentration and pH, to clarity, as shown in Fig. 1d, reveals that an increase enzyme concentration with pH (2.5 to 3.5) enhanced carrot-orange juice clarity; while, an increase of pH (>3.5) declined clarity. This might be explained by the



(a)



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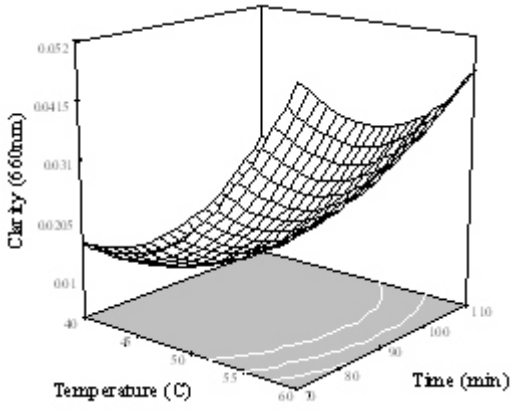
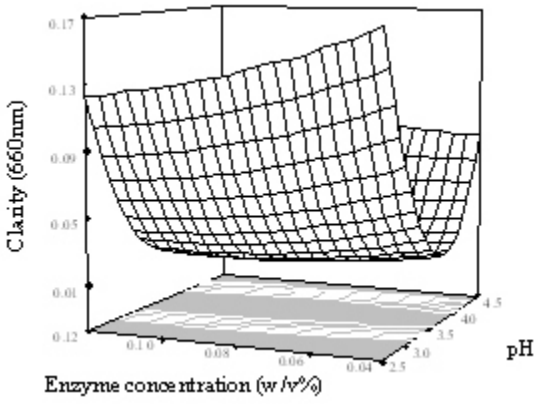
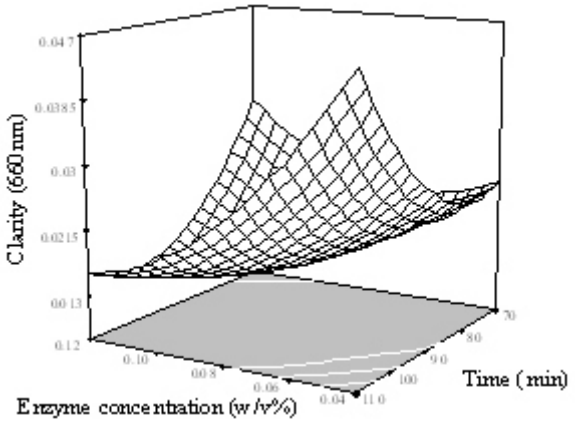


(c)



(d)



(e)

Fig. 1: Three dimension plot for turbidity of blended carrot-orange juice as function of (a) pH and incubation temperature; (b) incubation temperature and enzyme concentration; (c) enzyme concentration and pH; (d) enzyme concentration and incubation time

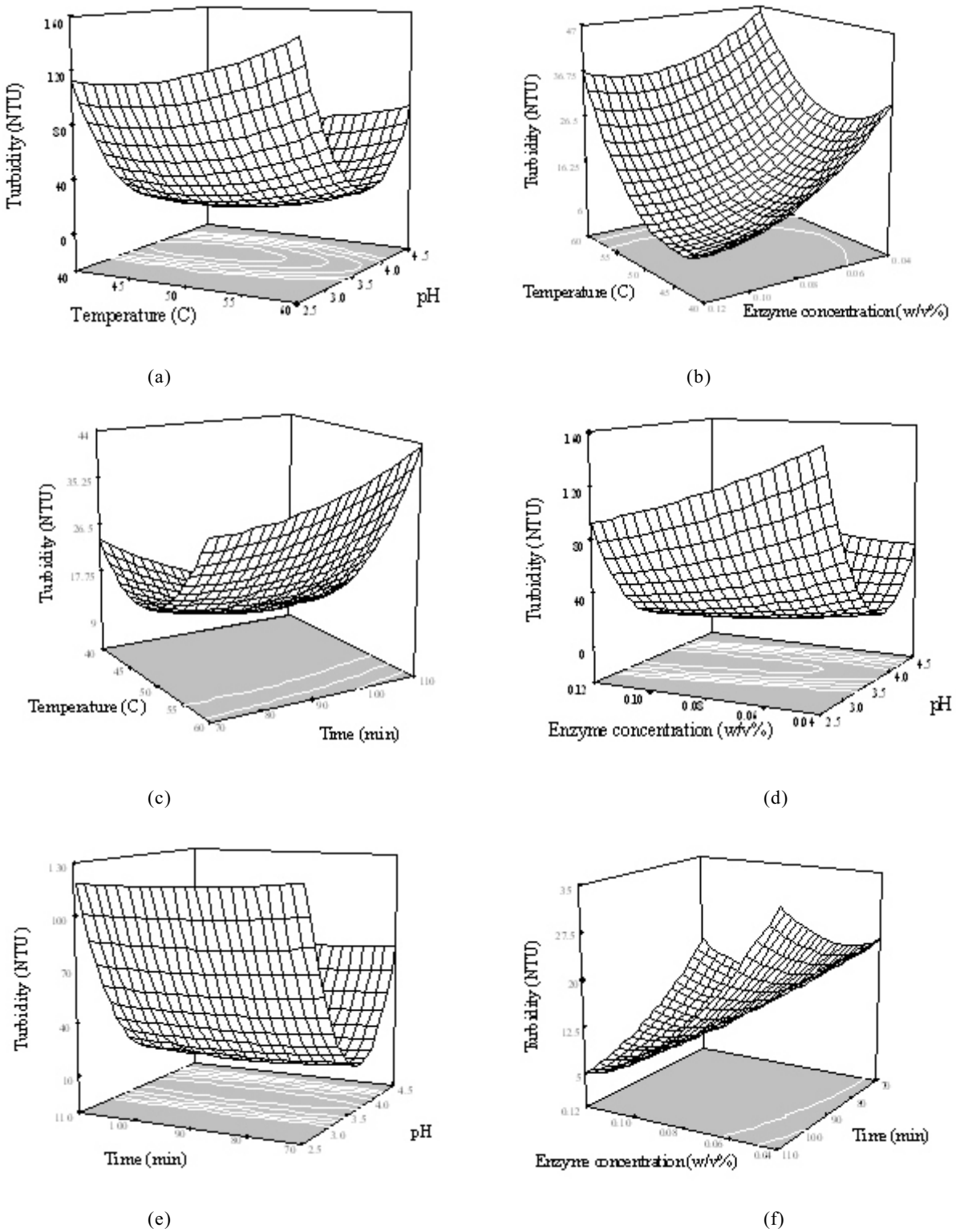


Fig. 2: Three dimension plot for turbidity of blended carrot orange juice as function of (a) enzyme concentration and incubation temperature; (b) incubation temperature and pH; (c) incubation temperature and incubation time; (d) enzyme concentration and pH; (e) incubation time and pH; (f) enzyme concentration and incubation time

denaturation of enzyme at lower and higher pH as the clarity dropped due to delayed enzyme activity.

The interaction effect between enzyme concentration and incubation time to clarity indicated in Fig. 1e, shows that the clarity increased with increase of enzyme concentration, while, the clarity increased slowly with increase of incubation time from 70 to 90 min; then decreased again rapidly afterwards.

Turbidity: In clarified fruit juices, a juice that has an unstable cloud or whose turbidity is not desirable is considered "muddy" and is unacceptable to be marketed as clear juices (Floribeth *et al.,* 1981). The turbidity was linearly affected significantly by temperature, pH, enzyme concentration and time (X_1, X_2, X_3, X_4) ($p<0.0001$), and quadratically significant at $p<0.0001$ or $p<0.05$. The interaction parameters (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, and X_3X_4) for turbidity were significant at the level of $p<0.05$ or $p<0.0001$ (Eq. 3).

The interaction effect between incubation temperature and pH to turbidity (Fig. 2a), it was observed that the turbidity decreased rapidly with increase of pH from 2.5 to 3.5; and at further increase of pH, the turbidity increased rapidly. At the same time, it was shown that the turbidity decreased slowly at the beginning as the incubation temperature increased from 40 to 50ºC, then increased slowly with a further increase of incubation temperature over 50ºC. It can be explained by the fact that lower and higher pH as well as higher incubation temperature reduced the enzyme activity in the production of clear juice.

The interaction effect between enzyme concentration and incubation temperature to turbidity (Fig. 2b), showed that the turbidity decreased with increase of enzyme concentration. This might be explained as the enzyme concentration was degrading the pectin content of juice therefore the juice turbidity was decreased (Alvarez *et al.*, 1998). While, the turbidity decreased slowly with increase of temperature from 40 to 50ºC and a further increase in temperature over 50ºC showed a rapid increase of turbidity at fixed incubation time and pH. It can be explained on basis that the optimum temperature of enzyme action favored the enzyme activity resulting in low turbidity.

Figure 2c, indicates the interaction effect between incubation temperature and incubation time to turbidity. The turbidity increased with increase of incubation time, while, the turbidity decreased slowly when the incubation temperature was increased from 40 to 50ºC. A further increase in incubation temperature resulted in rapid increase of turbidity. This might be due to that the enzyme was denatured with an increase of incubation temperature.

The interaction between enzyme concentration and pH to turbidity shown in Fig. 2d, reveals that the turbidity decreased constantly with increase of enzyme concentration. This might be explained by the degradation of pectin with the increase in enzyme concentration. Moreover, the turbidity decreased rapidly with an increase of pH from 2.5 to 3.5 then it increased afterwards. This could also be explained as both at lower and higher pH the enzyme activity was too low, leading to the juice with high turbidity.

The interaction effect between incubation time and pH to turbidity (Fig. 2e) indicated that the turbidity decreased rapidly with the increase of pH from 2.5 to 3.5 and a further increase of pH over 3.5 resulted in increase of the juice turbidity. The turbidity was kept constant as the incubation time increased. This variation of turbidity due to effect of pH can be explained by the fact that at lowest and highest pH, the enzyme was denatured and not too effective to produce clear juice.

Figure 2f, shows a plot for turbidity of blended carrot orange juice as a function of enzyme concentration and incubation time. It shows that the turbidity decreased with increase of enzyme concentration, and increases with an increase of incubation time.

Viscosity: Researchers (Carneiro *et al*., 2002; Vaillant *et al*., 2001) found that the use of enzymes leads to the drop of fruit juice viscosity, as well as improving pressability of the pulp, disintegrating the jelly structure and making it easier to obtain the fruit juices. The viscosity was linearly affected with significance by temperature, pH, enzyme concentration (X_1, X_2, X_3) ($p<0.0001$) and by time (X_4) ($p = 0.0008$); the quadratic effects were positive and significant at $p<0.0001$ or $p<0.05$. The interaction parameters (X_1X_2, X_1X_3, X_1X_4, X_2X_4) for viscosity were significant at the level $p<0.05$ or $p<0.0001$ (Eq. 4).

The interaction effect between incubation temperature and pH to viscosity as shown in Fig. 3a, indicates that increase of pH from 2.5 to 3.5 and incubation temperature from 40 to 50ºC decreased the viscosity of juice. But, the pH below or over 3.5 and the incubation temperature over 50ºC showed an increase in viscosity.

Figure 3b, presents the interaction effect between enzyme concentration and incubation temperature to viscosity. As shown in Fig. 3b, the viscosity decreased rapidly with increase of enzyme concentration and the incubation temperature to 50ºC. But there was an increase in viscosity as the incubation temperature increased over 50ºC. And at the enzyme concentration of 0.1 to 0.12%, there was not further decrease in viscosity and it reached to plateau. This might be explained by the enzyme treatment of pectin leads to reduction in its water holding capacity and the free water released to system has an effect on the viscosity. The increase in temperature during the enzyme treatment contributed to pectin cells

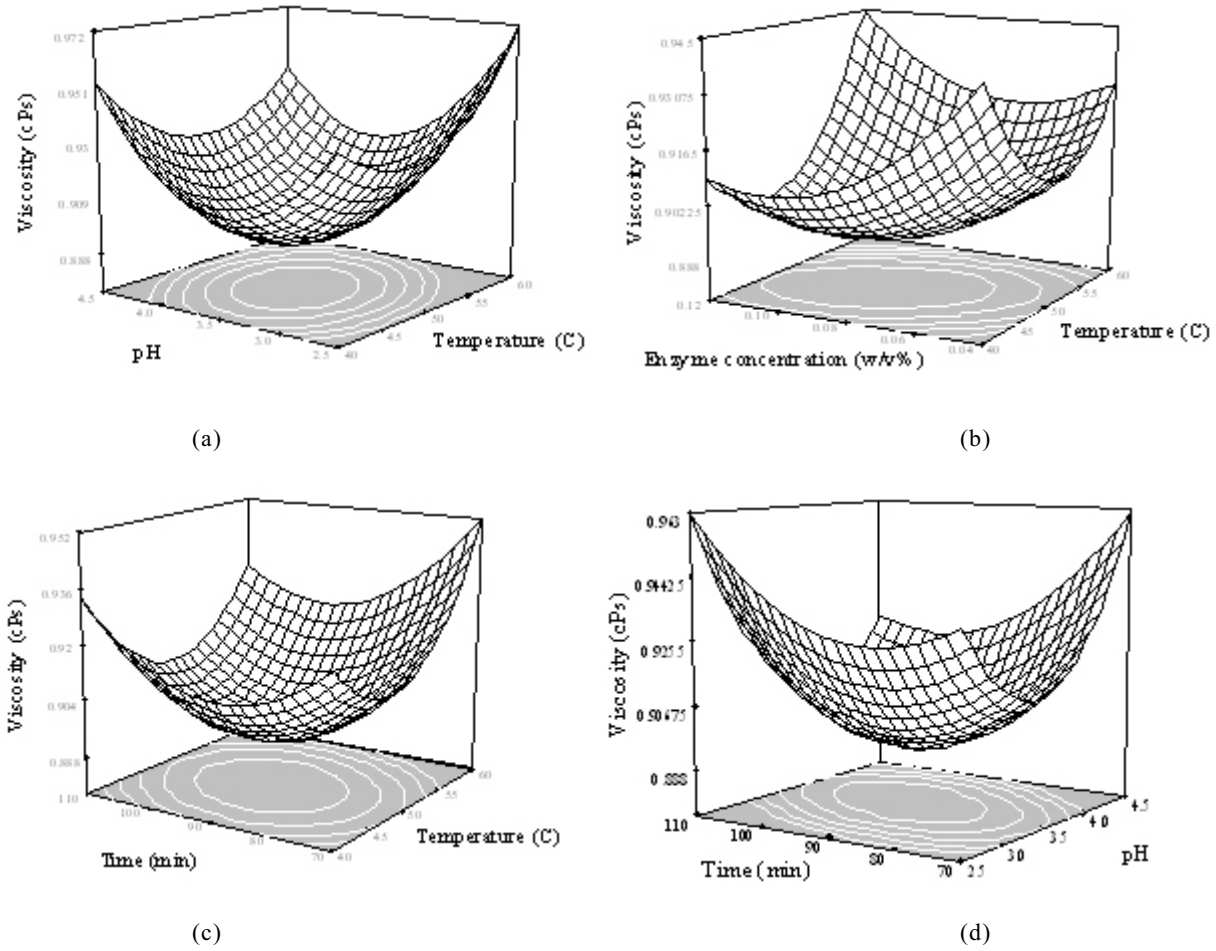


Fig. 3: Three dimension plot for viscosity of blended carrot orange juice as function of (a) enzyme concentration and incubation temperature; (b) pH and incubation temperature (c) incubation time and pH

destruction, and the enzyme activity was reduced when the incubation temperature was over 50ºC, therefore, the enzyme action on pectin cell was also reduced and this contributed to the increase in viscosity.

Figure 3c, depicts the interaction effect between incubation temperature and time to viscosity. The increase of incubation temperature from 40 to 50ºC with incubation time from 70 to 90 min decreased the viscosity. While, with increase of incubation temperature and incubation time over 50ºC and 90 min respectively re-increased the juice viscosity.

Figure 3d, reveals the interaction effect between incubation time and pH to viscosity. It was shown that increase of pH from 2.5 to 3.5 declined the viscosity. There was a constant decrease of viscosity with increase of incubation time up to 90 min. But, with an increase of pH over 3.5 and incubation time over 90 min increased the viscosity. It might be explained that the prolonged incubation time affected the enzyme activity during the juice clarification process.

Optimization: The optimum clarification conditions were determined by superimposing the contour plots of all the four responses. The final conditions would be considered optimum if the turbidity, clarity and viscosity were as low as possible. Thus, the criteria applied to obtain the optimum conditions were as follow: (a) minimum clarity at 660 nm, (b) minimum turbidity and (c) minimum viscosity. The computer generated three dimension plots for juice yield, clarity, turbidity, and viscosity (Fig. 1-3). The optimum combined conditions were found to be at 0.06% enzyme concentration; 3.6, pH, 49ºC, temperature; and 91 min, reaction time. Table 3 shows predicted responses of the dependent variables (clarity, turbidity, and viscosity) obtained using the Design Expert 7.1.3 software. The experimental responses (Table 2), were significantly related to the predicted responses given by the software, therefore, this confirmed the optimum conditions for clarification blended carrot-orange juice.

Juice characterization: The estimated total carotene content in raw juice is higher (>5 mg/mL) than that of

Table 2: Software predicted and experimental responses for the properties of blended carrot orange juice produced using pectinex Ultra-SP-L

Properties	Predicted response from software	Experimental responses
Clarity	0.0157 (Abs)	0.020±2.78 (Abs)
Turbidity	14.33 (NTU)	15.45±3.04 (NTU)
Viscosity	0.897 (cPs)	0.901±1.40 (cPs)

Table 3: Color comparison among raw juice, clear juice and the juice after addition of 2-Hydroxypropyl-β and γ-Cyclodextrin

	HP-β-CD + Juice				γ-CD + Juice			
	ΔL	Δa	Δb	ΔE	ΔL	Δa	Δb	ΔE
0%	-1.64	1.81	3.79	4.51	-1.64	1.81	3.79	4.51
1%	-4.37	5.14	5.48	8.69	-2.21	2.69	3.42	4.88
2%	-4.44	5.33	5.50	8.85	-2.74	2.74	3.99	5.56
3%	-4.47	5.88	5.55	9.24	-3.79	2.88	4.25	6.38
4%	-3.99	4.24	5.49	8.00	-3.01	2.41	3.20	5.01
5%	-3.76	3.34	5.21	7.24	-2.78	1.60	3.15	4.49

clear juice (<5 mg/mL) after enzymatic clarification. This may be explained by β-carotene exhibiting high-water insolubility causing carotene loss during the centrifugation process as carotene was attached to the precipitated particles, such as pectin, which have been hydrolyzed by pectinase enzymes in the way to make juice clear. It could also be explained to its association with the cell wall pectin that, during hydrolysis process, was destructed causing the carotene to move away during the centrifugation process.

The addition of 2-Hydroxypropyl-β-Cyclodextrin in different proportions to juice prior to enzymatic clarification improved the amount of total carotene content after the clarification process by 3.1 times than in clear juice (Fig. 4). On the other hand, the addition of γ-Cyclodextrin in different concentration to juice before the enzyme clarification process, has busted up the total carotene content by 1.7 times compared to the clear juice (Fig. 4). It might be explained that the inclusion of carotene by 2-hydroxypropyl-β and γ-Cyclodextrin into juice can improve the carotene solubility into juice, therefore, its bioavailability into juice during the enzyme clarification process.

The color attribute is one of the major problems associated with carrot juice products (Sim *et al*., 1993). Change in color of carrot juice involves the co-precipitation of color substances such as β-carotene with larger molecules or enzymatic and oxidative discoloration (Lan *et al*., 2005). From Table 3, the increase of ΔL value showed the lightening color of the juice, this was observed for clear juice with ΔL of -1.64. The addition of 2-Hydroxypropyl-β-Cyclodextrin and γ-Cyclodextrin showed a decrease of ΔL to -4.47 and -3.79, respectively with the increase concentration to 3%. That means the juice with γ-Cyclodextrin was also lighter than that with 2-Hydroxypropyl-β-Cyclodextrin. A color shift toward the positive Δ*a* and positive Δ*b* directions indicated redder and more yellow with increase of 2-Hydroxypropyl-β and γ-Cyclodextrin concentration to 3% while, the Δ*a* and Δ*b* of the clear juice presents the characteristics of green and red color. The juice with a concentration of 3% of 2-Hydroxypropyl-β-Cyclodextrin

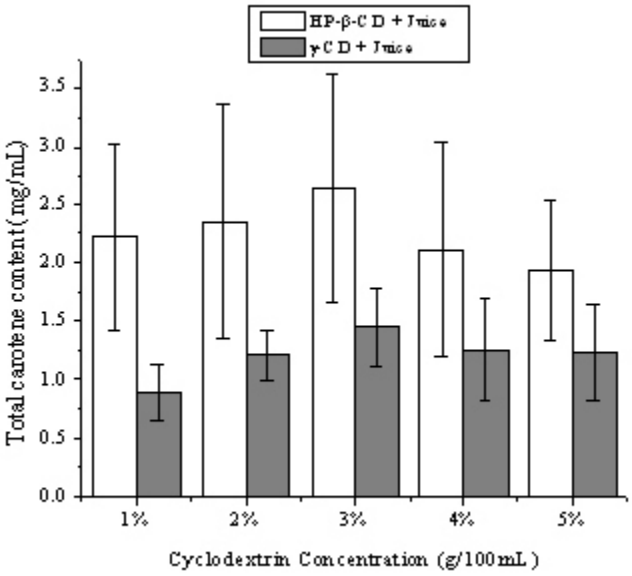


Fig. 4: Carotene content in function of 2-Hydroxypropyl-β-Cyclodextrin and γ-Cyclodextrin concentration

Table 4: Clarity comparison among clear juice and the juice after addition of 2-Hydroxypropyl-β and γ-Cyclodextrin

	HP-β-CD + Juice	γ-CD + Juice
0%	0.020±2.78	0.020±2.78
1%	0.035±3.25	0.011±0.002
2%	0.041±2.13	0.013±0.002
3%	0.042±1.59	0.015±0.003
4%	0.033±0.68	0.014±0.003
5%	0.032±2.52	0.011±0.002

with Δa and Δb values (5.88, 5.55) was more red and yellow in color compared to both clear juice and that with addition of γ-Cyclodextrin. The significant change of Δa and Δb after addition of 2-Hydroxypropyl-β and γ-Cyclodextrin which indicates the change of color to red and yellow was due to the increase of carotene content in juice compared to clear juice. The total color difference (ΔE), indicating the magnitude of the color difference was significantly increased in the juice which has been added 2-Hydroxypropyl-β and γ-Cyclodextrin concentration at the concentration 3% with the values of 9.24 and 6.38, respectively compared to the clear juice with value of 4.51.

The juice clarity after addition of 2-Hydroxypropyl-β or γ-Cyclodextrin compared to clear juice (Table 4), showed a decrease of juice clarity with increase of concentration from 1 to 3%. An increase of juice clarity was observed with increase of concentration over 3%. The lower clarity was observed with the juice containing 2-Hydroxypropyl-β-Cyclodextrin as the carotene content was higher than in juice with γ-Cyclodextrin and clear juice. Thus, the increase of carotene content by addition of cyclodextrins during clarification reduces not too much significantly the juice clarity. Thus, both carotene content and clarity can be improved with reasonable measures.

CONCLUSION

Response surface methodology was used to study the optimum conditions of the factors that affect clarification of blended carrot-orange juice using commercial enzyme (Pectinex Ultra SP-L). The optimum conditions were obtained graphically in order to obtain the desirable levels of three properties (Clarity, Turbidity and Viscosity) of blended carrot orange juice which are desirable for a clear juice. The quality assessment of the clarified compared to raw juice revealed that the carotene content was almost lost under clarification process. When addition of 2-Hydroxyproyl-β-Cyclodextrin to the juice improved more the availability of carotene content compared to the addition of γ-cyclodextrin in the juice as both increased its solubility in the clear juice.

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