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Optimation Of Calcium Fortification On Pineapple Juice Using Response Surface Method

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Abstract: ***The study objective was to produce the best calcium fortified pineapple juice using response surface method (RSM) of the Box-Behnken model. The optimized factors were calcium concentration (mg/100 mL), pectin concentration (%) and pH level. Total dissolved solids (^{o}Brix) were observed. The results showed quadratic equation between the response and optimized factors. Two of the best calcium fortified pineapple juice had been chosen from 20 formulations.The two formulations were calcium concentration, pectin concentration and pH level having respective values of 70.87, 0.39, 3.35 and 73.60, 0.84, 3.45. Under optimum condition, the three formulations produced the predictive values of total dissolved solid of 12.91^o Brix, 12.94^o Brix and 13.181^o Brix, respectively. Results of verification showed that the values of total dissolved solid were close to the predicted values with magnitude of 12.13^o Brix, 12.47^o Brix and 12.70^o Brix.***

Keywords: calcium, fortification, juice, pineapple, RSM

1. Introduction

Calcium is an essential macro mineral found in human body that has function as structural elements of bones and teeth as well as biological reaction catalyst [17]. Nutrition sufficiency numbers of calcium in Indonesia is 1000 mg/day. Average number of calcium intake for Indonesia people nowadays is only 250 mg/day [29]. Calcium deficiency during the growth period results in weak, easily crooked and fragile bones. Calcium deficiency for adults having 50 years of age can results in osteoporosis [1].

Food product frequently used as calcium source is milk and its processed products. However, not all people can consume milk to fulfill their calcium needs. This is due to the fact that some people suffer lactose intolerant or lactase deficiency.Human can not digest lactose without sufficient lactase so that they will suffer digestion interference such as stomach ache and diarrhea. Therefore, an effort should be done to seek other alternative product as calcium source. One of product than can be fortified with calcium is pineapple juice.

Pineapple fruit is one of well known tropical and subtropical fruit because it has specific flavor and has balance sugar and acid content [5]. Pineapple fruit is available as fresh fruit, canned fruit and juice [4]. Fruit juice is fluid extracted from consumable fruit's part. This fluid can have turbid or clear appearance depending on fruit source and may contain oil or carotenoild pigment available in fruit [27]. Based on [8], one criterium of pineapple juice quality is that it should have total dissolved solid of minimum 10 ^{o}Brix with color, flavor and general appearance not deviate from the standard of pineapple juice.

Calcium fortification on pineapple juice has opportunity (chance) to solve problem of calcium requirement deficiency. Calcium types that frequently added on fortification are milk calcium, calcium carbonate and calcium lactate [12]. Solubility property of calcium carbonate in water is highly affected by pH of solution in which solubility of calcium carbonate will increase with the decrease of pH of solution. The lower the pH value, the higher the solubility of calcium carbonate . Pineapple juice has pH value in the range of 3.2 to 4.0.Therefore, calcium fortification by using calcium carbonate is suitable for pineapple juice.

The addition of stabilizer compound is needed to maintain stability of fruit juice. One of stabilizer compound that can be used is pectin. Pectin is complex heterosaccharide polymer of D-galacturonate acid which binded with α 1,4-glycosidic either in form of free acid or acid esterified methyl group. Pectin having high metoxyl concentration (DM > 50) will soluble in cold water and can act as stabilizator on fruit juice [20]. Pectin added in fruit juice processing should totally soluble in order to prevent uneven gel formation [10]. The disadvantage of calcium carbonate is that it has very low solubility in water resulting in fruit juice having high turbidity [9]. Pectin addition is combined with pH treatment in this research in order to increase solubility of calcium carbonate in fruit juice.

The method is required to select the optimum condition for calcium fortified pineapple juice. One of method that can be used for this effort is response surface method (RSM). RSM is combination of statistical and mathematical techniques in order to develop, improve and optimize a process in which its response is affected by several factors or independent variables [14]. The advantage of RSM is capable to be used for analysis and modeling of research problem having one or more treatments [18].

2. Research Method

2.1 Materials

Materials used in this research were pineapple fruit of Queen variety obtained from Ogan Ilir District. Maturity levelof pineapple was 38 to 62 % (pineapple fruit eye was yellow), calcium carbonate ($CaCO_3$) (Merck, Germany), citric acid (Merck, Germany) and high metoxyl pectin (HM)(Cargill, Germany).

2.2 Equipments

The preparation equipment used in this research was juicer (trademark of Grand Power Juicer), whereas equipments for analysis were consisted of spectrophotometer (Jenway, seri 03133, Singapore), refractometer (Millwaukee, MA871, Europe), viscosity meter (Atago, seri VT-04, Japan), pH meter (EUTECH seri Cyberscan pH 300, Singapore), color checker (color reader seri CR10, Japan) and 80 mesh siever.

2.3 Method

The method used was consisted of four stages as follows: 1). Development of formulation and response designs; 2). Formulation; 3). Response analysis and 4). Optimation followed by verification as the proof toward prediction of optimum value of formula solution response [18].

2.4 Development step of Formulation and Response Designs

The development of formulation and response design was done by using Design Expert 8 Program to determine dependent variable and independent variables. Dependent variable is the one with the same value at each treatment because it is assumed not affecting response. Independent variablesare variables that will affect the produced response. Dependent variable in this study was pineapple juice quantity that will be fortified, whereas the independent variables were consisted of calcium concentration, pectin concentration and pH value. The determination of minimum and maximum limits for each variable can be seen in Table 1 and were determined as follows.

1) Minimum limit of calcium concentration is calcium addition with magnitude of 5% from nutrient sufficiency numbers (NSN) and maximum limit of calcium concentration is 15% from NSN which is subsequently subtracted by calcium concentration found in pineapple fruit juice.
2) Minimum limit of pectin concentration is based on pectin addition in soft drink with magnitude of 0.2%, whereas the maximum limit of pectin concentration is 1.0%.
3) Minimum pH limit of pineapple fruit is 3.2 and the maximum pH limit is 4.0. This determination is based on pH ranges of mature pineapple fruit.

Table 1: The ranges of independent variables

Component	Independent Variable	Minimum	Maximum
A	Calcium concentration (mg/100 mL)	22.8	73.6
B	Pectin Concentration (%)	0.2	1.0
C	pH	3.2	4.0

Minimum and maximum limits were entered into Design Expert 8 RSM Box-Behken Design to be randomized and subsequently produced 17 treatments that will be analyzed. Table 2 showed design expriment of pineapple juice fortification. The measured and optimized responses were consisted of total dissolved solid, viscosity and color (L^*, a^*, b^*). Total dissolved solid was measured according to [3].

Table 2: Design expriment of pineapple juice fortification

Formulation	Calcium concentration (mg/100 mL)		Pectine concentration (%)		pH	
	Code	Real	Code	Real	Code	Real
1	-1	22.8	0	0.60	-1	3.2
2	0	48.2	0	0.60	0	3.6
3	0	48.2	1	1.00	1	4.0
4	0	48.2	0	0.60	0	3.6
5	0	48.2	0	0.60	0	3.6
6	0	48.2	0	0.60	0	3.6
7	1	73.6	0	0.60	1	4.0
8	-1	22.8	1	1.00	0	3.6
9	0	48.2	-1	0.20	1	4.0
10	-1	22.8	-1	0.20	0	3.6
11	1	73.6	0	0.60	-1	3.2
12	0	48.2	0	0.60	0	3.6
13	0	48.2	1	1.00	-1	3.2
14	1	73.6	-1	0.20	0	3.6
15	0	48.2	-1	0.20	-1	3.2
16	1	73.6	1	1.00	0	3.6
17	-1	22.8	0	0.60	1	4.0

2.5 Formulation Stage

Formulation stage is fortification on pineapple fruit juice according to the design shown in Table 2. Processing of pineapple fruit juice is carried out according to the methods of [25], and [11]. Each formula of sample will be put into 100 mL pineapple fruit juice. Formulaton process is started with raw material preparation of Queen variety pineapple obtained from Ogan Ilir District which consisted of sortation, peeling, cleaning (pineapple eye disposal) and soaking in salt water (1%) for 10 minutes as well as recleaning with aquadest. The pineapple fruit flesh is subsequently crushed using juicer and the produce is sieved. Pineapple fruit juice produce is pasteurized at temperature 60^0 for 5 minutes. pH value of pineapple fruit juice is determined according to treatments followed by addition of pectin and calcium carbonate as well as homogenously mixed using stirrer for 10 minutes. Fruit juice that has been fortified was subsequently put into glass bottle with volume of 100 mL (each treatment) followed by heating at 60^0 C for 5 minutes and finally was analyzed according to the response criteria.

2.6 Response Analysis Stage

Response analysis stage is used to determine the proper model. Models available at this design are linear, 2F1 (interaction), quadratic and cubic. Model selection based on the model that give significancy and non-significancy on lack of fit was selected to analyze variables. Design Expert 8 Program also provide normal plot of residual which indicate whether residual (the difference between actual response and predicted response) follows normality line (straight line). Data points that are closer to normality line show that data is normally distributed which mean actual yield is closer to the predicted yield obtained from Design Expert 8 Program [16].

2.7 Optimation and Verification Stages

There are 3 responses to be analyzed in this stages which consisted of total dissolved solid, viscosity and color. These responses were respectively optimized by using Design Expert 8 Program. This program will do optimation according to input of variables data and measurement data. The output from optimation stage is recommendation of new formula which are considered to be optimum according to the program. The most optimum formula is the one that has maximum desirability value. Desirability value is optimation objective function value which indicate program capability to fulfill objective based on set criteria of final product. The range of desirability value is from 0 to 1.0. Desirability value closer to 1.0 show the program capability to produce the expected product is more optimum. The objective of optimation is not to obtain unity desirability value, but to determine optimum condition which bring together all objective functions [24].

After obtaining optimum process condition, the next step is verification which include processing of calcium fortified fruit juice according to 3 formulations having the highest desirability value. Verification is done with two replications. The results are compared to response variable values which are predicted by using RSM that has been equipped with prediction value for each response so that its accuracy can be seen at verification stage[2].

3. Results and Discussion

3.1 Response Analysis

Optimation is the seeking process for variable values that are considered optimum, effective and efficient to achieve the expected result. The expected result in this study was calcium fortified fruit juice having the highest total dissolved solid.Total dissolved solid from processing is one of factor that has significant role.

Based on quality standard for pineapple fruit juice according to [8], total dissolved solid in pineapple fruit juice should not be less than 10^o Brix. Most of measured components as total dissolved solid are consisted of water soluble components such as glucose, sucrose and water soluble protein.

Analysis results of processing condition effect on total dissolved solid response was shown in Table 3. Response values of total dissolved solid are in the range of 2.10^o Brix to 12.135^o Brix. The highest value was found on formula 1 and 11. These results are in accordance to the finding by [23] and are higher than the finding by [15]. [23] had reported that total dissolved solid in commercial fruit juice were in the range of 10.2^o Brix to 14.2^o Brix. [15] had reported that total dissolved solid in mix fruit juice consisting of pineapple, carrot and orange was 10^o Brix.

Model selection was based on sequential model sum of square (Table 4) and model summary was statistically shown in Table 5. This model is used to select the recommended ordo by RSM. This model showed that the significant model was quadratic ($P < 0.05$). The subsequent model selection process was based on model summary statistics.

Table 3: Total dissolved solid based on processing condition.

Formula	Calcium cconcentration (mg/100 mL)	Pectine concentration (%)	pH	Total dissolved solid (^{0}Brix)
1	22.8	0.60	3.2	12.35
2	48.2	0.60	3.6	12.20
3	48.	1.00	4.0	2.75
4	48.2	0.60	3.6	11.90
5	48.2	0.60	3.6	11.60
6	48.2	0.60	3.6	11.80
7	73.6	0.60	4.0	2.45
8	22.8	1.00	3.6	12.05
9	73.6	0.20	4.0	2.10
10	22.8	0.20	3.6	11.35
11	73.6	0.60	3.2	12.35
12	48.2	0.60	3.6	11.00
13	48.2	1.00	3.2	12.20
14	73.6	0.20	3.6	11.40
15	48.2	0.20	3.2	11.65
16	73.6	1.00	3.6	12.05
17	22.8	0.60	4.0	2.40

Table 4: The description of sequential model sum of square for optimation of total dissolved solid in calcium fortified pineapple fruit juice

Source	Sum of square	df	Mean square	F-cal.	Prob. > F	Remarks
Average	1574.1	1	1574.41			
Linear	189.48	3	63.16	9.79	0.0012	
2F1	3.750E-003	3	1.250E-003	1.490E-003	1.0000	
Quadratic	82.99	3	27.66	216.83	<0.0001	Suggested
Cubic	0.093	3	0.031	0.16	0.9211	aliased
Residual	0.80	4	0.20			

Table 5: Model summary statistic

Source	Standard of deviation	R^2	Adj. R^2	Predict. R^2	PRESS	Remarks
Linear	2.54	0.6931	0.6223	0.4365	154.04	Suggested
Linear	2.40	0.6931	0.509	-0.2487	341.36	Aliased
Quadratic	0.36	0.9967	0.9925	0.99	2.74	
Qubis	0.45	0.9971	0.9883		+	

Table 5 showed some parameters used to select the correct model. These parameters were the lowest deviation standard, the highest R-squared, the highest adjusted R-squared, the highest predicted R-squared and the lowest PRESS. Model that fulfill the recommended criteria was quadratic model.

Model summary statistic showed that quadratic model had the lowest deviation standard and the highest adjusted R^2 with magnitude of 0.9925 than that of other models. It means that calcium concentration, pectin concentration and pH variables had effect on response variance with magnitude of 99.25% and the rest of 0.75% was affected by other factors besides the studied variables.

Variance analysis results from quadratic response surface showed that quadratic model had significant effect on responses (Table 6). ANOVA calculation for response of total dissolved solid showed that pectin concentration and pH had significant effect on value of total dissolved solid. The higher the pectin concentration, the higher the total dissolved solid. Increasing pH value up to 3.6 had results in increasing

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total dissolved solid, whereas total dissolved solid would decrease above this pH value.

This trend can also be observed from the mathematical equation. The mathematical equation for response of total dissolved solid was as follows:

$$Y = 0.013X_1 + 0.32X_2 - 4.68X_3 - 0.013X_1X_2 + 0.012X_1X_3 + 0.025X_2X_3 + 0.11X_1^2 - 0.21X_2^2 - 4.42X_3^2$$

where:
Y= Total dissolved solid
X_1 = Calcium
X_2 = Pectine
X_3 = pH

Table 6: Analysis of variance for calcium carbonate fortified fruit juice

Source	Sum of square	df	Mean square	F-value	p-value Prob>F	Remarks
Model	272.48	9	30.28	237.29	<0.0001	Significant
A-calcium	1.250E-003	1	1.250E-003	9.797E-003	0.9239	
B-Pectine	0.81	1	0.81	6.37	0.0396	Significant
C-Ph	188.67	1	188.67	1478.69	<0.0001	Significant
AB	6.250E-004	1	6.250E-004	4.899E-003	0.9462	
AC	6.250E-004	1	6.250E-004	4.899E-003	0.9462	
BC	2.500E-003	1	2.500E-003	0.020	0.8926	
A^2	0.053	1	0.053	0.42	0.5387	
B^2	0.42	1	0.042	0.33	0.5836	
C^2	82.44	1	82.44	646.17	<0.0001	Significant
Residual	0.89	7	0.13			
Lack of fit	0.093	3	0.031	0.16	0.0211	Non-significant

The highest coefficient values were found on X_3, X_3^2 and X_2 which indicate that pH value had the biggest effect on total dissolved solid.

According to [6], pectin is heteropolysaccharide complex with the main chain has bonding of 1,4 α glycosidic and ramnosa, whereas its secondary chain are arabinose, galactose and xylose. [28] had added that pectin solubility in water is determined by group numbers, distribution and molecular weight of metoxyl. Solubility generally will increase with the decrease of molecular weight and the increase of methyl ester group. Pectin added to pineapple fruit juice in this case is the one that had high metoxyl group.

According to [7], pectin containing high metoxyl develops gel with sugar and acid, i.e. with sugar concentration range of 58 to 75% and pH range of 2.8 to 3.5. The gel formation is occurred through hydrogen bonding amongst free carboxyl group and amongst hydroxyl group. Water molecules and dissolved molecules are trapped in this network gap.

The gel formation mechanisms in this research was occurred on fortified pineapple fruit juice but without gel formation because sugar content of this juice was originated from pineapple fruit having sugar content of only 10.67%. This is in accordance to opinion of [22] which stated that sugar content less than 55% on pectin having high metoxyl would not produce gel.

Pectin has acid property and its colloid has negative charge due to availability of free carboxyl group. Pectin solution is stable at pH range of 2 to 4. At pH higher than 4 or less than 2, viscosity and gel strength of pectin is decrease due to pectine depolymerization. Fortified pineapple fruit juice had pH range of 3.12 to 4.77. Fortified pineapple fruit juice with pH of 3.12 had higher total dissolved solid (12.2° Brix) than that of pH of 4.77 (2.1° Brix). Pasteurization was done at 60°C for 5 minutes. Heating at acid condition can results in pectin compound degradation. This is in accordance to the opinion of [28] and [26] which stated that heating can cause pectin compound degradation in which its rate was depend on temperature, pH and oxydant ingredient concentration.Surface response of total dissolved solid for calcium fortified pineapple juice was shown in Figure 1.

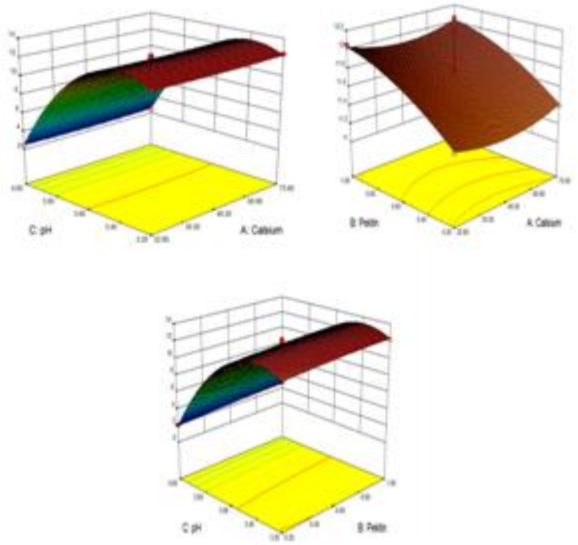

Figure 1: Surface response of total dissolved solid at optimum condition.

Optimation and Verification

Optimation is done after mathematical models are obtained for each response. Optimation is carried out to achieve the expected response (desirability). The objective of optimation is to minimize efforts required or operational cost and to maximize the expected result. Table 7 showed the optimized components, minimum and maximum limits of target, and interest level at formula optimation stage.

Table 7: The optimized response components, target, limit and interest at formula optimation stage

No.	Component	Target	Lower limit	Upper limit	Interest
1.	Calcium concentration (mg/100 mL)	Max	22.8	73.6	5(+++++)
2.	Pectine concentration (%)	Range	0.2	1.0	3(+++)
3.	pH	Range	3.2	4.0	3(+++)
4.	Total dissolved solid (°Brix)	Max	2.10	12.35	5(+++++)

Design Expert 8 Program produced 20 optimum formula solutions based on optimation process such as shown in Table 8. The proces condition with calcium concentration of 70.87 mg/100 mL, pectin of 0.39% and pH of 3.35 was recommended as optimum formula solution because it had high desirability value of 0.962. Desirability value is accuracy degree of optimal solution result. Desirability value close to 1.0 showed higher optimation accuracy value.

Table 8: Formula produced in optimation stage

No.	Calcium concentration (mg/100 mL)	Pectine concentration (%)	pH	TDS (^{0}Brix)	Desirability
1	70.87	0.39	3.35	12.91	0.962 (selected)
2	72.21	0.39	3.40	12.94	0.948
3	73.60	0.84	3.45	13.18	0.941
4	73.60	0.85	3.45	13.16	0.938
5	73.58	0.84	3.44	13.19	0.936
6	73.60	0.81	3.39	13.18	0.933
7	73.60	0.83	3.43	13.22	0.930
8	73.60	0.85	3.46	13.14	0.929
9	73.60	0.86	3.47	13.07	0.929
10	73.60	0.84	3.45	13.18	0.929
11	73.60	0.83	3.41	13.26	0.928
12	73.60	0.80	3.38	13.27	0.928
13	73.60	0.89	3.47	13.08	0.928
14	73.60	0.89	3.53	12.70	0.927
15	73.60	0.90	3.54	12.63	0.927
16	73.60	0.95	3.53	12.72	0.926
17	73.60	0.98	3.57	12.35	0.925
18	73.60	0.73	3.28	12.96	0.921
19	73.60	0.73	3.27	12.92	0.920
20	57.92	8.20	3.24	12.08	0.858

Formula produced at optimum stage is subsequently verified by taking the 3 highest values out of the existing formula. Results of verification showed that response value of total dissolved solid in Table 9 was relatively close to the value of predicted optimum formula.

Table 9: Results of verification

No.	Calcium concentration (mg/100 Ml)	Pectine concentration (%)	pH	TDS (^{0}Brix)
1.	70.87	0.39	3.35	12.13
2.	72.21	0.39	3.40	12.47
3.	73.60	0.84	3.45	12.70

The predicted optimum formula had produced total dissolved solid value of 12.91^o Brix (formula 1), 12.94^o Brix (formula 2) and 13.18^o Brix (formula 3), respectively. On the other hand, verification results showed total dissolved solid value of 12.13^o Brix (formula 1), 12.47^o Brix (formula 2) and 12.70^o Brix (formula 3), respectively.ormula 1), 12.47^o Brix (formula 2) and 12.70^o Brix (formula 3), respectively.

4. Conclusion

The conclusion obtained from first stage of this study were as follows:

1) The relationship among total dissolved solid and all factors is quadratic.
2) Optimum condition of calcium fortified pineapple juice was selected from 3 formulations out of 20 recommended formulations. These 3 formulations had calcium concentration (mg/100 mL), pectine concentration (%) and pH values asfollows: 70.87, 0.39, 3.35; 72.71, 0.39, 3.40 abd 73.60, 0.84, 3.45, respectively.
3) Prediction values of total dissolved solid at optimum condition for 3 selected formulations were 12.91 ^{0}Brix, 12.94 ^{0}Brix and 13.18 ^{0}Brix, respectively.
4) Verification results showed that total dissolved solid values were close to the predicted values with magnitudes of 12.13 ^{0}Brix, 12.47 ^{0}Brix and 12.70 ^{0}Brix, respectively.

5. Future Scope

The future scope of this research will be shelf life determination of pineapple juice fruit and calcium bioavailability measurement using in vivo method.

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