

Optimization of roselle beverage formulation using response surface methodology

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Abstract

Roselle beverage as a functional drink is rich in vitamin C and anthocyanin compounds. The aim of this work is formulation of roselle beverage. In this study roselle beverage formulations were prepared. Durability and physicochemical tests were investigated. Considering that roselle beverage is effective in treating diabetes, therefore it is necessary to use other sweeteners except sugar to decrease sugar from the formulation by replacing it with stevia. In order to optimize time and temperature conditions of aqueous extraction of roselle calyx, response surface methodology was used considering features such as anthocyanin content, vitamin C content, total solids and opacity of drink. The beverage formulation was done using the response surface methodology. Results suggested 85 °C for 10 min as the best conditions for aqueous extraction. The best product considering sensory evaluation, opacity and brix was including 1% stevia, 0.08 g/l Arabic gum, 95% extract and 0.9% sugar. Pasteurization and storage conditions significantly affected the total anthocyanin contents and vitamin C content of the product. The best condition of roselle beverage formulation can be used in industrial production.

Keywords: anthocyanin, stevia, formulation, hot water extraction, response surface methodology, roselle beverage, sensory evaluation

1. Introduction

Natural substances are generally preferred over chemical ones and are generally seen as healthy. The increasing demand for natural ingredients, improving health and appearance, is also attracting beverages as the fastest growing segment on the functional food market. Functional beverages are launched as fortified water, tea, dairy or juices claiming overall nutrition, energy, anti-aging or relaxing effects (Coda *et al.*, 2011; Gruenwald, 2009; Servili *et al.*, 2011; Sorenson and Bogue, 2005).

The recently elucidated benefits of many teas has made tea extract an attractive additive to functional waters and has increased sales of the beverage itself (Dufresne and Farnworth, 2001; Gruenwald, 2009).

Hibiscus sabdariffa L. (HS) (family: Malvaceae) is an annual, erect, bushy and herbaceous sub-shrub that grows up to 2.4 m tall, with smooth or nearly smooth, cylindrical

and typically red stem. The botanical features have been excellently described by Iyare *et al.* (2010), Prenesti *et al.* (2007), Ross (2003) and Sánchez-Mendoza *et al.* (2008). In folk medicine, extracts of HS are widely believed to be effective in the treatment of a variety of ailments (Oliver, 1960; Perry, 1980; Watt and Breyer-Brandwijk, 1962).

The effectiveness of HS in the treatment of these ailments have been attributable to the various constituents of HS like flavonoids, anthocyanins, organic acids, sodium ions, vitamins A and C and iron (Appel, 2003; Adigun *et al.*, 2006; Clydesdale, 1979; Dafallah and Al-Mustafa, 1996; Duke and Francis, 1973; Fuleki and Francis, 1968; Morton, 1987).

Tsai *et al.* (2002) reported that the relationship between anthocyanin and antioxidant activity in HS was found previously and they concluded that anthocyanin is the major source of antioxidant capacity in HS.

The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-O-glucoside, delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside (Ali *et al.*, 2005; Aremu *et al.*, 2010; Nerantzaki *et al.*, 2011; Soobrattee *et al.*, 2008).

Aqueous extracts from the dry calyces of *H. sabdariffa* L., variety *sabdariffa* (ruber), contain two main anthocyanins: delphinidin-3-sambubioside (del-phinidin-3-xylosylglucoside or hibiscin) and cyanidin-3-sambubioside (cyanidin-3-xylosylglucoside or gossy-picyanin) and two minor anthocyanins, delphinidin-3-glucoside and cyanidin-3-glucoside (Duke and Francis, 1973). HS extracts have demonstrated to have a broad range of therapeutic effects (Ali *et al.*, 2005) such as hepatoprotective (Liu *et al.*, 2006), antioxidant (Olatunde and Fakoya, 2005; Ramakrishna *et al.*, 2008), anti-obesity (Alarcón-Aguilar *et al.*, 2007), anti-cholesterol (Lin *et al.*, 2007), anti-cancer (Olvera-Garcia *et al.*, 2008), inhibition of the contractility of rat bladder and uterus (Fouda *et al.*, 2007), antibacterial (Liu *et al.*, 2005) and antihypertensive (Herrera-Arellano *et al.*, 2007).

Many researches have shown that HS extracts reduce blood pressure in humans (Haji and Haji, 1999; Herrera-Arellano *et al.*, 2007) and has been postulated that the hypotensive action could be ascribed to a direct vase-relaxant effect (Adegunloye *et al.*, 1996).

In producing *zobo* drink, the extract from HS calyx is usually sweetened with a considerably large quantity of sugar due to the high concentration of ascorbic acid in extract. This research was aimed at controlling the concentration of the acid in the drink using trona, via a neutralization reaction, so that the quantity of sugar required for sweetening would be reduced.

Response surface methodology (RSM) is a statistical method for determining and simultaneously solving multivariate equations (Roy *et al.*, 2002). It usually uses an experimental design such a central composite rotatable design to fit a first- or second-order polynomial by a least significance technique. An equation is used to describe how the test variables affect the response and to determine the interrelationship among the test variables in the response. The contour plots can be used to study the response surfaces and locate the optimal parameters.

The objective of this work was to optimize the roselle beverage formulation with respect to quality attributes like extraction condition and ingredient of formulation.

2. Materials and methods

Aqueous extract preparation

The dried *H. sabdariffa* calices were coarsely ground in a mortar. The pulverized product was then taken and mixed with hot twice-distilled water (reverse osmosis) in 500 ml beakers, each containing 20 g of the crushed tea leaves per 250 ml water. The beakers were then immediately placed in a water bath at 30, 60 and 90 °C for 10, 50 and 90 min. The tea infusions were then filtered through Whatman No. 541 filter paper and the filtrate was analysed to determine the effect of extraction time and temperature for ascorbic acid, anthocyanin, total solids content, colour intensity and opacity.

Analysis of total solids content

Total solids in roselle beverage were determined according to the method of Lees (1975).

Determination of opacity

Opacity of roselle extract was determined by using spectrophotometric method as described by Penders *et al.* (1998). Light transmittance (opacity) measurements after 24 h storage at 4 °C at $\lambda = 800$ nm run were carried out using a UV/V spectrophotometer (model S2000, WPA, Cambridge, UK).

Determination of total soluble solids

The total soluble solids (brix) of samples were determined according to AOAC (2000) at room temperature (25 ± 1 °C) with a land refractometer (CETI, Kontich, Belgium).

Determination of anthocyanins

Total anthocyanins content of roselle extract was determined colorimetrically according to the procedure described by Du and Francis (1973). Specific volume of the filtered extract was diluted to 100 ml with the extracting solvent. The colour intensity was measured at wave length of 520 nm for water and citric acid solution extracts and 535 nm for acidified ethanol using a spectrophotometer (model T80+ UV/VIS, PG Instruments Ltd, Wibtoft, UK). The total anthocyanins content referred to cyanidin-3-glucoside was calculated using the following equation:

$$\text{Total anthocyanins} \left(\frac{\text{mg}}{100 \text{ g}} \right) = \frac{\text{absorbance} \times \text{dilution factor}}{\text{sample weight} \times 55.9} \times 100 \quad (1)$$

Determination of ascorbic acid content

Five ml of the extract was pipetted and transferred to a 125 ml flask. Twenty ml of water and 2 ml of a 1% soluble starch solution was added. Standardized 0.01 N solution containing 16 g potassium iodide per litre was then rapidly titrated because other substances such as glutathione and cysteine are oxidized slowly by iodine solution. Each ml of iodine is equivalent to 0.88 mg of ascorbic acid (Morris, 1958). The mg of vitamin C per ml can be calculated from the equation:

Amount of vitamin C (mg/ml) =

$$\frac{\text{burette reading of iodine solution} \times 88 \times \text{normality of iodine solution}}{\text{volume (ml) of the sample for titration}} \quad (2)$$

Sensory evaluation of roselle beverage

Roselle beverage sample were evaluated for their sensory qualities and general acceptability. A scoring test was used to determine the most preferred sample. A twenty one member test panel familiar with the beverage was requested to rate the sample using a nine-point hedonic scale (i.e. 9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely). The scores from the rating were subsequently subjected to analysis of variance (ANOVA) and means separated using response surface technique (IFT, 1981; Meilgaard *et al.*, 1991).

Determination of total titrable acidity

Total titratable acidity which was expressed as citric acid % was determined by standard (AOAC, 2000) using 0.1 N NaOH and phenolphthalein as an indicator.

Optimization procedure using response surface methodology

Optimization extraction condition

In the work hypothesis, quality attributes (opacity, total solids, vitamin and anthocyanin content) of roselle beverage were functionally related to the extraction conditions and attempts were made to fit multiple regression equations describing the responses. There were two coded independent variables in the process including extraction time (X1) and extraction temperature (X2) (Table 1). Levels of each of the independent variable were chosen for the study thus thirteen combinations including five replicates of the centre point were performed in random order, based on a central composite experimental design for four factors as shown in Table 1. The dependent variables were including opacity (Y1), total solids (Y2), vitamin C content (Y3) and anthocyanin content (Y4).

Optimization formulation

The quality attributes of roselle beverage formulation are independent of the sensory evaluation. Brix and opacity independent variables in the process were amount of stevia (X1), sugar (X2), arabic gum (X3) and extract (X4) (Table 1). Thirty combinations including six replicates of the centre point were performed in random order, based on a central composite experimental design for four factors as shown in Table 1. Range finding experiments were performed at the outset of this work in order to ascertain what extraction conditions could be applied to the roselle beverage so that the product would be acceptable to consumers on the basis of sensory properties. The dependent variables were including sensory evaluation (Y1), brix (Y2) and opacity (Y3).

Table 1. Coded independent and dependent variables in the process of optimization of extraction and formulation of roselle beverage.

	Factor	Name	Min	Max	Mean values	Response	Name
extraction	A (X1)	Temperature (°C)	30	90	60	Y1	Opacity
	B (X2)	Time (min)	10	90	50	Y2	Total solids (%)
						Y3	Vitamin C (mg/100 ml)
						Y4	Anthocyanin (mg/100 g)
formulation	A (X1)	Stevia (%)	0	2	1	Y1	Sensory evaluation
	B (X2)	Gum (g/l)	0	0.5	0.25	Y2	Brix
	C (X3)	Sugar (%)	0	2	1	Y3	Opacity
	D (X4)	Extract (%)	20	70	45		

3. Results and discussion

Extraction

Opacity

Beverage emulsions are primarily used to give opacity to clear beverages or to enhance their juice-like appearance (Taherian *et al.*, 2006), therefore decrease in opacity has good effect on beverage appearance. Effect of the different factors studied (time and temperature of infusion) on opacity are shown in Figure 1. The results show that opacity

was sharply increased with increasing time up to 60 min and then slowly increased with increasing time up to 90 min. Also opacity increased with increasing temperature up to 60 °C and then slowly increased when the temperature reached 90 °C ($P<0.05$). The results of ANOVA are shown in Supplementary Table S1. As can be seen, the quadratic model is able to describe opacity changes due to time and temperature of infusion. High correlation coefficient ($R^2=90.94$) showed the suitable correlation between this model with the experimental data. Model and correlation coefficients of the models are shown in Table 2.

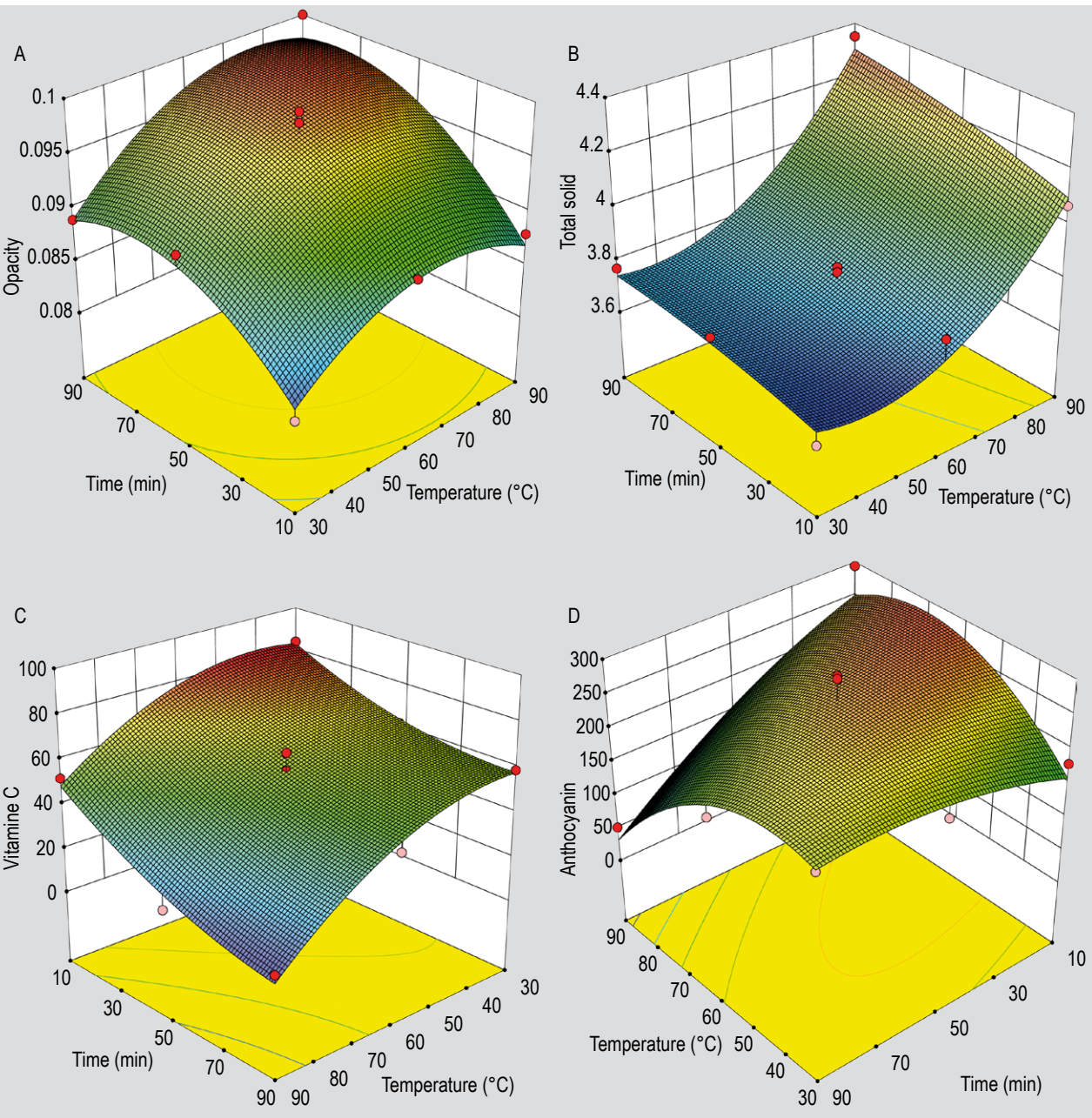


Figure 1. Variation of (A) opacity, (B) total solids, (C) vitamin C content and (D) anthocyanin content of roselle extract in relation to extraction time (min) and temperature (°C).

Table 2. Regression models for the studied response against independent variables of the process of optimization of extraction and formulation of roselle beverage.

	Response	Model	R ²
extraction	Opacity	$Y1 = 0.066567 + 0.000585(X1) + 0.000291(X2) + 1.04E-06(X1)(X2) - 4.4E-06(X1)^2 - 2.5E-06(X2)^2$	90.94
	Total solids	$Y2 = 3.959116 - 0.01719(X1) + 0.001816(X2) + 2.29E-05(X1)(X2) + 0.000204(X1)^2 - 1.4E-05(X2)^2$	96.10
	Vitamin C	$Y3 = 65.89943 + 1.249353(X1) - 0.63032(X2) - 0.00313(X1)(X2) - 0.01494(X1)^2 + 0.004095(X2)^2$	96.14
	Anthocyanin	$Y4 = -134.24 + 11.4636(X1) + 3.79648(X2) - 0.05667(X1)(X2) - 0.07812(X1)^2 - 0.01426(X2)^2$	87.15
formulation	Sensory evaluation	$Y1 = 5.338187 + 4.70731(X1) + 6.184795(X2) + 1.246199(X3) + 0.056971(X4) - 0.5(X1)(X2) - 0.375(X1)(X3) - 0.01(X1)(X4) + 2E-15(X2)(X3) + 0.02(X2)(X4) - 0.005(X3)(X4) - 1.51754(X1)^2 - 8.2807(X2)^2 - 0.51754(X3)^2 - 2.8E-05(X4)^2$	92.18
	Brix	$Y2 = 1.28856 - 0.038333(X1) + 0.091111(X2) + 1.76167(X3) + 0.093756(X4)$	93.01
	Opacity	$Y3 = 0.038328 + 0.015872(X1) - 0.035(X2) - 0.00614(X3) + 0.000733(X4) - 0.0035(X1)(X2) + 0.0005(X1)(X3) - 0.00019(X1)(X4) - 0.0015(X2)(X3) + 0.00042(X2)(X4) + 0.00004(X3)(X4) + 0.001026(X1)^2 + 0.048421(X2)^2 + 0.002526(X3)^2 - 7.6E-07(X4)^2$	96.14

Total solids

Total solids content in beverage has been observed to have a contributory effect on the overall mouth-feel of the beverage (Fasoyiro *et al.*, 2005). Variations of total solids of the roselle extract which were affected by different studied factors such as time and temperature is shown in Figure 1. As can be seen, total solids increased significantly by increasing temperature while by increasing time, total solids increased slowly ($P < 0.05$). The results of ANOVA are shown in Supplementary Table S1. As can be seen, the quadratic model with high correlation coefficient ($R^2=96.10$) selected as the best model to describe total solids changes due to increase of time and temperature and showed the suitable correlation with the experimental data. Model and coefficients of the models are cited in Table 2.

Vitamin C content

The effect of factors on vitamin C content of roselle extract is shown in Figure 1. As can be seen the vitamin C content decreased significantly ($P < 0.05$) by increase of temperature and time. Similar results were obtained from hot water extraction of roselle by Bolade *et al.* (2009). Also Bamishaiye *et al.* (2011) obtained a similar trend in vitamin C variation by boiling time on vitamin C content of three varieties of *H. sabdriffa* drink. Temperature was more effective on the vitamin C content than time. The results of ANOVA are shown in Supplementary Table S1. As can be seen, the quadratic model with high correlation coefficient ($R^2=96.14$) was the best model for prediction of vitamin C content of roselle extract against the time and temperature. Coefficients of the models are shown in Table 2.

Anthocyanin content

Effect of extraction factor on anthocyanin content is shown in Figure 1. Increasing the solid-to-solvent ratio increased the anthocyanin extraction yield. Similar results were obtained during extraction of phenolic compounds from milled berries (Cacace and Mazza, 2003), with semi-batch extraction of anthocyanin from red grape pomace (Mantell *et al.*, 2002) and during solid-liquid extraction of andrographolide from plants (Wongkittipong *et al.*, 2004).

Increasing temperature favoured extraction by enhancing solubility of anthocyanins and increasing the diffusion coefficient, which increased the extraction rate and reduced the extraction time. But the problem with raising the temperature was that all reactions were accelerated, including anthocyanin and nutritional degradation, non-enzymatic browning reactions and a decrease in the stability of anthocyanins during storage (Cissé *et al.*, 2009; Dhuique-Mayer *et al.*, 2007; Jiménez *et al.*, 2010).

As expected, the solid-to-solvent ratio had a positive effect on the anthocyanin yield but a negative effect on the anthocyanin content. Similar results about the effect of temperature and solid-to-solvent ratio on the extraction of anthocyanins compounds were also reported for grape pomace by Pinelo *et al.* (2005), for milled berries studied by Cacace and Mazza (2003) and for the extraction of andrographolide from plants studied by Wongkittipong *et al.* (2004). All of these studies also found a linear link between temperature and solid-to-solvent ratio with anthocyanin extraction yield.

Optimization of extraction

A summary of the optimization information and range of the factor that was used for optimization is shown in Table 3.

Table 3. Summary of the optimization information and range of the factor that used to optimize the extraction and formulation of roselle beverage.

	Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
extraction	Temperature	in range	30	90	1	1	3
	Time	in range	10	90	1	1	3
	Opacity	minimize	0.082	0.1	1	1	3
	Total Solids	maximize	3.6	4.35	1	1	3
	Vitamin C	maximize	12	84	1	1	4
	Anthocyanin	maximize	52	294	1	1	4
formulation	Stevia	in range	0	2	1	1	3
	Gum	in range	0	0.5	1	1	3
	Sugar	minimize	0	2	1	1	3
	Extract	in range	20	100	1	1	3
	Sensory Evaluation	maximize	6	14	1	1	5
	Brix	maximize	10	11.9	1	1	4
	Opacity	minimize	0.049	0.106	1	1	3

Minimum opacity, maximum total solids, vitamin C content and anthocyanin content were considered. The optimization was done in 5 solutions (not shown). The selected value of four factors and best results are shown in Figure 2. Lowest desirability was obtained for total solids (0.5472) and highest desirability was obtained for anthocyanin content (0.8735). The best obtained desirability for vitamin C and opacity were 0.5999 and 0.6792, respectively. Using 85 °C for 10 min in

aqueous extraction of samples resulted in the best product considering features such as anthocyanin content, vitamin C content, total solids and opacity. An optimized extraction method (RSM) was used to optimize the roselle drink.

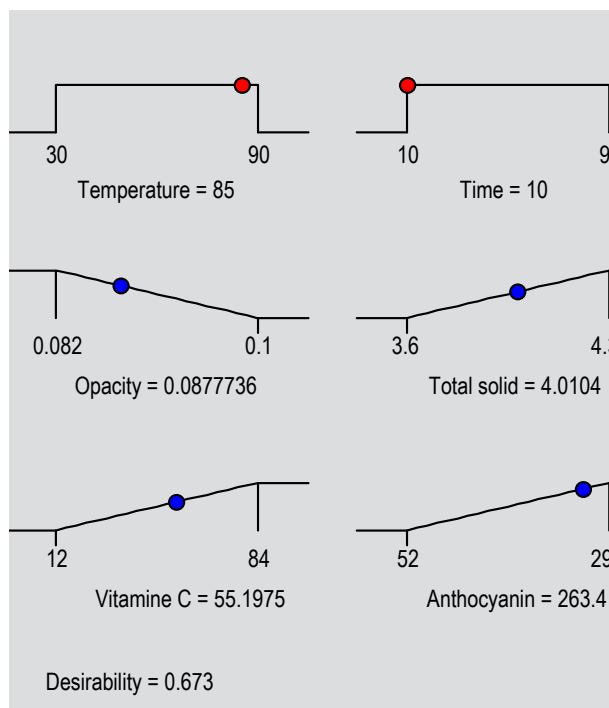
Formulation

Sensory evaluation

The changes in sensory evaluation of drink as a function of ingredients (stevia, Arabic gum, sugar and extract) are presented in Figure 3. ANOVA results are shown in Supplementary Table S2. The results show that use of 1-1.5% of stevia increase acceptability of drink. Mogra and Dashora (2009) showed that all the drinks with stevia extract were well accepted by the panel members. Best result in sensory evaluation was obtained with 1-1.5% stevia, 0.5% sugar, 0.4 gr/l Arabic gum and the highest amount of extract (70%). As shown in Supplementary Table S2, variation of the extract was the most effective in this case. A quadratic model described the effect of the factors and their interaction on the sensory evaluation. The model is shown in Table 2.

Brix

The effect of different amounts of sugar and extract on the brix value is shown in Figure 4. As can be seen, the brix value increased by increase in sugar and extract ($P < 0.05$), while different level of stevia and Arabic gum led to not significance change in the brix value of the final products. The ANOVA results (see Supplementary Table S2) show that the extract was more effective than sugar in this case. Due to high amounts of soluble solids, change in the amount

**Figure 2. Selected extraction time and temperature value and best results.**

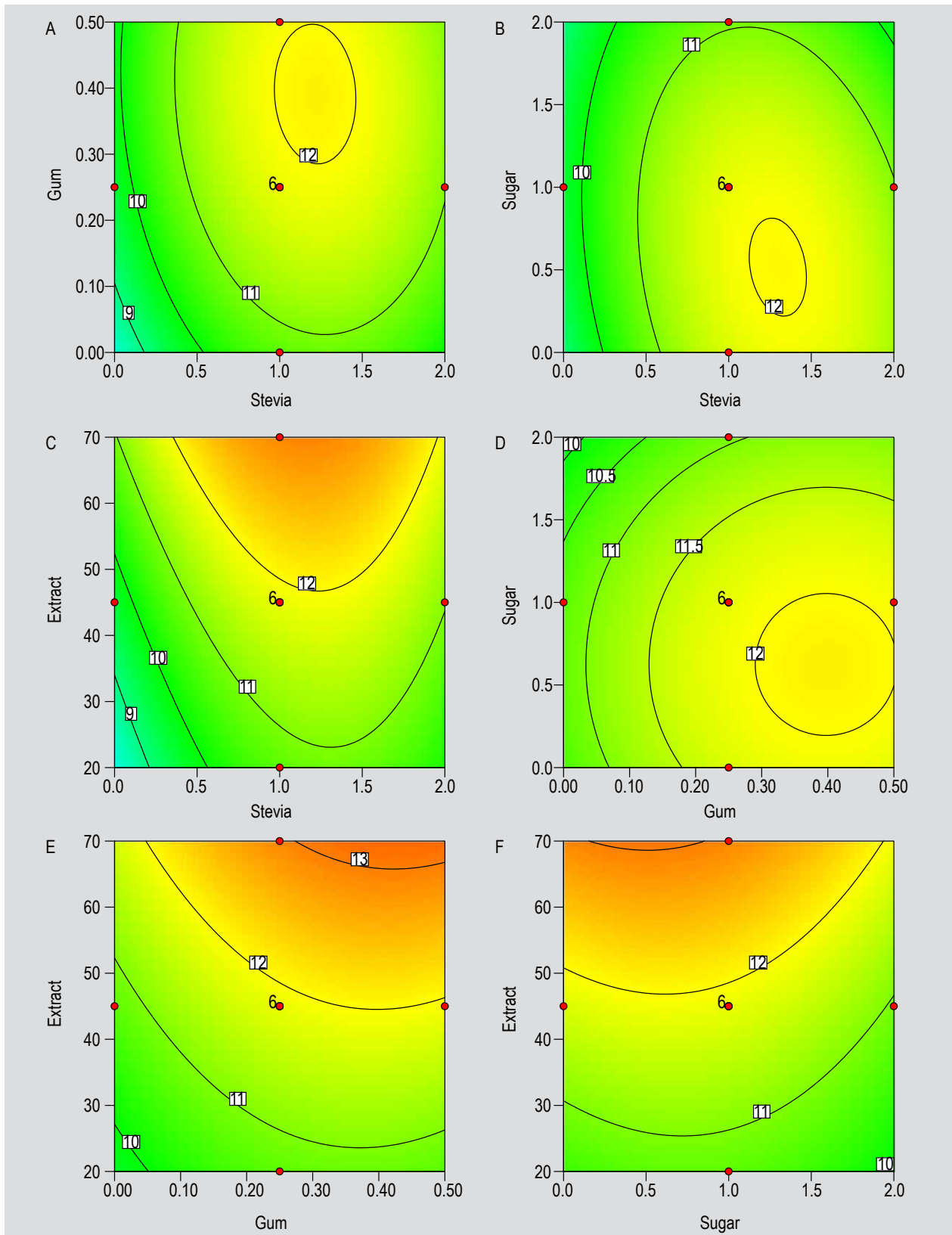


Figure 3. Variation of sensory evaluation of roselle beverage against different amount of formulation components. (A) Interaction effect of gum and stevia. (B) Interaction effect of sugar and stevia. (C) Interaction effect of extract and stevia. (D) Interaction effect of sugar and gum. (E) Interaction effect of extract and gum. (F) Interaction effect of extract and sugar of roselle beverage. Other variables are constant at mean values.

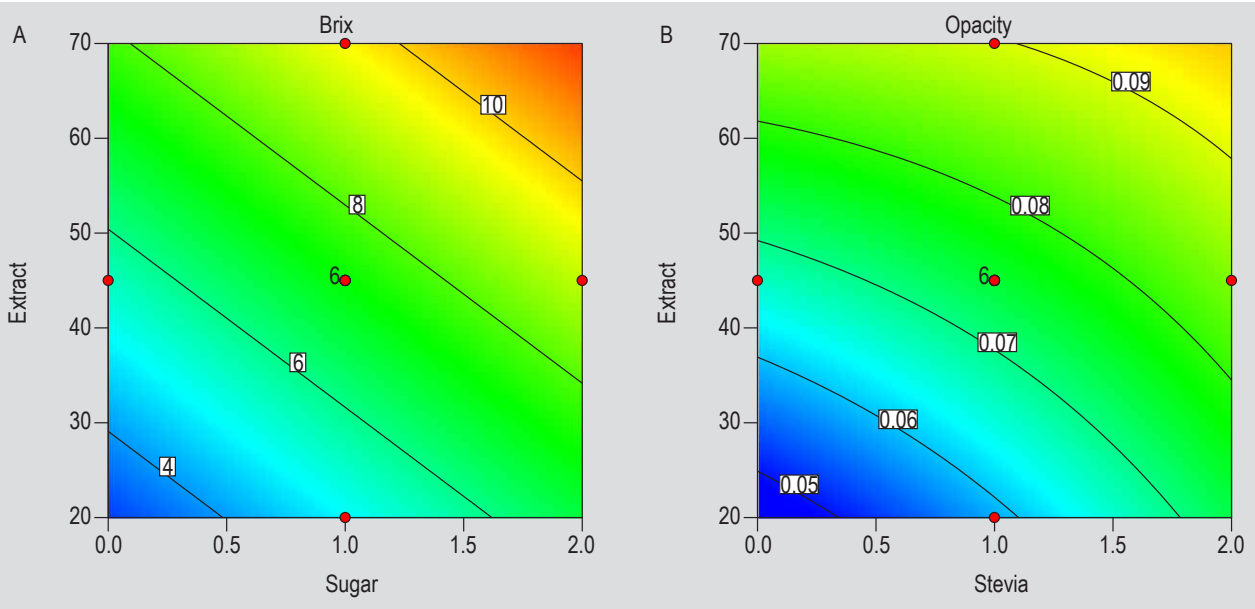


Figure 4. (A) Variation of brix of roselle beverage against different amount of extract and sugar. (B) Variation of opacity of roselle beverage against different amount of extract and stevia.

of extract had the greatest effect on changes in drink brix. A linear model described the effect of the factors and their interaction on drink brix. The model is shown in Table 2.

Opacity

Opacity of drink increased considerably by increase of stevia and extract content ($P<0.05$) and change in opacity due to the change in extract content was higher than stevia content variation (Figure 4). Arabic gum and sugar addition had no significant effect on opacity of the produced drink. The results of ANOVA are shown in Supplementary Table S2. As can be seen, the quadratic model with a high correlation coefficient selected as the best model to describe opacity of samples against the ingredients addition. The model is shown in Table 2.

Optimizing formulation

A summary of the optimization information and range of the factor that was used for optimization is shown in Table 3. Maximum sensory evaluation score, maximum brix value (higher than 10 units) and minimum opacity were considered. The optimization was done in four solutions (not shown). The selected value of four factors and best results are shown in Figure 5. The lowest desirability was obtained for opacity (0.0958) and the highest desirability was obtained for sensory evaluation (0.9106). The best result of brix desirability obtained was 0.8690.

Physicochemical properties

Pasteurization and storage conditions significantly affected the total anthocyanin contents and vitamin C content of the product. Storage at 25 °C for 60 days also significantly affected the reduction of total anthocyanin contents. Physicochemical properties of roselle drink is shown in Table 4. pH increased after pasteurization and during

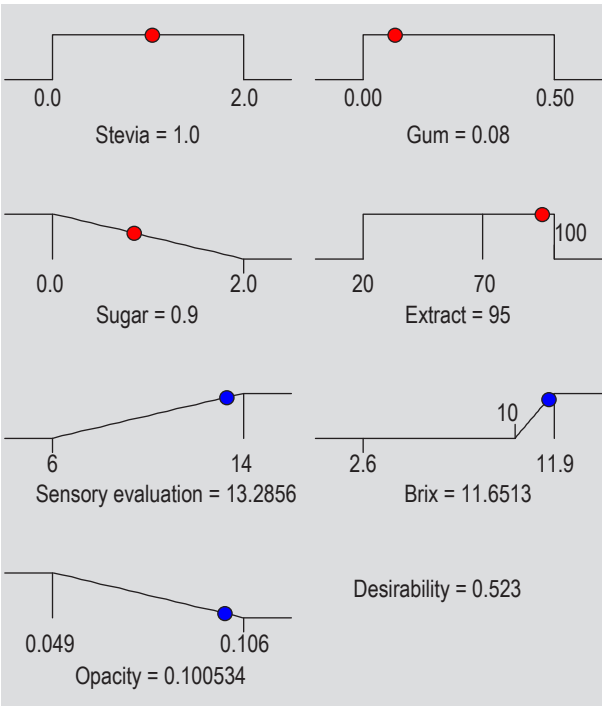


Figure 5. Selected value of four factors and best results.

Table 4. Physicochemical properties of roselle beverage before and after pasteurization and storage at 4 °C and 25 °C for 60 days.

Physicochemical properties	Before pasteurization	After pasteurization	Storage for 60 days	
			4 °C	25 °C
pH	2.61 ^b	2.76 ^a	2.76 ^a	2.77 ^a
Total acidity (malic acid) (%)	2.31 ^a	2.3 ^a	2.27 ^a	2.29 ^a
Brix	11.65 ^a	11.65 ^a	11.73 ^a	11.76 ^a
Total anthocyanin contents (mg/l concentrated extract)	251.23 ^a	239.86 ^b	190.24 ^c	93.64 ^d
Vitamin C	53.14 ^a	44.97 ^b	32.28 ^c	19.84 ^d

Values within a row with different superscript letters are significantly different from each other ($P < 0.05$).

storage time, while total acidity (malic acid %) decreased. The obtained results were in agreement with the results of Ruangsri *et al.* (2008).

4. Conclusions

Opacity of extraction sharply increased with the increase of time up to 60 min and temperature up to 60 °C at the beginning of extraction and then slowly increased. Vitamin C content decreased significantly ($P < 0.05$) by increase of temperature and time, while total solids increased. Temperature was more effective than time on the vitamin C content. Increasing temperature favoured extraction by enhancing solubility of anthocyanins and increasing the diffusion coefficient, which increased the extraction rate and reduced the extraction time. The best result for the extraction process was found to be 86 °C for 10 min.

The variation of extract was most effective in the sensory evaluation. The brix value of the final product increased by increasing sugar and extract ($P < 0.05$). Opacity of the final drink increased considerably ($P < 0.05$) by increase of stevia and extract content. Changing in opacity occurring due to the change in extract content was higher than stevia content variation. Pasteurization and storage conditions significantly affected the total anthocyanin contents and vitamin C content of the product.

Supplementary material

Supplementary material may be found online at <http://dx.doi.org/10.3920/QAS2012.0137>.

Table S1. ANOVA of the response surface quadratic model for opacity, total solid, vitamin C and anthocyanin content of roselle beverage.

Table S2. ANOVA of the response surface quadratic model for sensory evaluation, opacity and brix of roselle beverage.

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