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Extraction Optimization and Quality Characterization of Traditionally Prepared *Hibiscus sabdariffa* Beverage Using Response Surface Methodology

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Abstract: The effects of cold and hot water extraction on the physicochemical properties and phytonutrients content of traditionally prepared *Hibiscus sabdariffa* L.(HS) beverage using Response surface methodology was studied. The extraction with five different ratios of dry HS calyxes to water including (1:20, 1:23, 1:30, 1:37, 1:40 g/ml) were conducted at room temperature (CE) as well as in a water bath at 100°C (HE) for varied time. Total acidity ranged from 0.41 to 0.91, pH from 2.77 to 2.98, color density from 4.65 to 5.94 and hue tint from 0.56 to 1.00, respectively. The data reveals significant difference ($P<0.05$) in the total phenolics (TP), total flavonoids (TF), tannin content (TC) and DPPH among the different HS beverages. TP content ranged from 31.43 to 68.57mg Gallic acid equivalents/ml for HE7 and HE3, respectively. Wide ranges of TF were also reported and ranged from 134.00 to 250.50 mg Quercetin equivalents/ml for CE3 and CE7, respectively. The antioxidant activities measured as DPPH radical scavenging activity varied widely from 37.10to 74.11% ($P<0.05$) among cold and hot water extracts of HS beverages. Phytonutrients content of all Hibiscus drink and antioxidant capacity increased when extraction time or weight of calyxes increased. The physiochemical parameters, pH value and titrable acidity increased with increasing time for both extraction temperatures (25 and 100°C) while color density (CD) and hue tint (HU) values decreased with time in both cases. Optimal extraction conditions were identified as 10.7 hr; 1:20 ratio for cold extraction as well as 9.9 min; 1:20 for hot extraction for maximum phytonutrients content and RSA%. Experimental values for response variables at these optimal conditions match well with the predicted values. The beverage prepared at 25°C for 9.5 hr and 1:30 calyxes to water enjoyed the maximum consumer overall acceptability score (5.75).This study has implications for the beverage industry as the antioxidant and phytonutrients of HS could add value to HS currently produced and consumed.

Key words: *Hibiscus sabdariffa* L. · Extraction · Antioxidant activities · Phytonutrients content · Response surface methodology

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.), is a tropical annual shrub belongs to Malvaceae family member, is mainly grown in tropical and subtropical regions of Africa and Asia. It is an herbaceous plant cultivated for its leaves, stem, seed and calyxes [1]. The fresh or dried calyxes of *Hibiscus sabdariffa* L. are used to prepare beverages especially as tea, jelly and syrup. The water extract from the calyxes is used worldwide in the production of drinks and is consumed as hot tea (sour tea) or a cold drink [2]. Most of the hibiscus plant's economic value comes from the red calyx as an ingredient in herbal teas, although the stems are used in making rope in Africa and the seeds are expressed for the oil [3]. In Egypt the dried calyxes are prepared into a nutritious refreshing drink called in Arabic 'karkade' and are made from an extract or infusion obtained by aqueous extraction. The indigenous beverage 'karkade' is commonly believed by Egyptians to have been consumed in ancient Egypt as a preferred drink of pharaohs [4]. In folk medicine, preparations from the calyx have been used to treat hypertension and cardiac

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diseases [5, 6], inflammatory disease [7] and cancer [8]. Nowadays, the drink is becoming popular because it is easily prepared at home and affordable. It is highly associated with Ramadan 'fasting month' and is one of the favorite drinks for the *iftar* breakfast and *sahur* meal before sunrise [4]. The most commonly consumed varieties of Hibiscus in Egypt are made from the local variety. The calyxes are sun dried, stored and used year round. The extraction is typically carried out between 25°C (ambient temperature) for up to 12h and 100°C (boiling temperature) for minutes from which the pigment or flavor embedded is extracted [9]. The sharp taste of the raw extract is usually sweetened with sugar. The drink are commonly prepared cold during summer due to the high temperature that could rise up to 44°C, however during winter season the temperature is generally low and people prefer hot drinks. The drink is homemade and the difference being the amount of water and sugar added. The flowers of *Hibiscus sabdariffa* L. contain anthocyanins, flavonoids and polyphenols [10]. Studies have highlighted the role of polyphenolic acid, flavonoids and anthocyanins that may act as antioxidants or have other mechanisms contributing to the cardio protective actions [11, 12]. Available evidence also suggests that this group of phytochemicals could exhibit multiple biological effects, e.g. antioxidant-antiradical activity, anti-inflammatory action, inhibition of blood platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy and prevention of cholesterol-induced atherosclerosis [13-16]. The extractions of such bioactive compounds i.e. polyphenols from natural sources have become very important for the use of phytochemicals in the preparation of food supplements or nutraceuticals, functional food ingredients and food additives. Despite the longstanding use of 'karkade' in Egypt, the effect of the different traditional way of preparation and consequently the optimization of extraction parameters on polyphenols content is not reported to our best knowledge. Response surface methodology (RSM) is a useful optimization tool, which has been applied in research to study the effect of individual variables and their interactions on response variables. The major benefit of RSM is the ability to reduced number of experimental runs needed to arrive at optimized and statistically acceptable results. Thus, it saves time and less difficult compared with full-factorial design. It has been used extensively on the optimization of extractions of many agriculture crops [17]. Therefore, this work studies the effect of temperature, contact time and water-to-solid ratio on the phytochemical and antioxidant content of cold and hot water extracts of 'karkade'. Then response surface methodology (RSM) will be used in order to optimize the parameters for obtaining *Hibiscus sabdariffa* L. extract with both a high concentration of polyphenols and high overall likeability.

MATERIALS AND METHODS

Materials:

Samples: Sun dried intact calyxes of local cultivar of *Hibiscus sabdariffa* L. (HS) was purchased from farm located in Fayoum Governorate, Egypt. The sample was collected in July 2013 and authenticated at Department of Horticulture, National Research Centre. Calyxes free of apparent physical, insect or microbial damage were used for analysis.

Chemicals and Reagents: Reagents used for antioxidant activity determinations 2, 2-diphenyl-1-picrylhydrazyl (DPPH*) were purchased from Sigma Aldrich. All other reagents i.e. Gallic acid, rutin, vanillin, sodium carbonate, Foline Ciocalteu reagent were obtained from Sigma Aldrich, Fluka or Merck.

Methods:

Drink Preparation: Dry hibiscus calyxes was mixed with distilled water at a five different calyces to water ratios (1:20, 1:23, 1:30, 1:37, 1:40 g/ml) and maintained at 25°C (room temperature cold extraction, CE) or 100°C (hot extraction, HE) for six different times (6, 7, 8, 9, 11 and 12 hours for CE and 5, 6, 8, 9 and 10 minutes min for HE). Both liquids of CE and HE were decanted and filtered through filter paper Whatman No.1. Each extract was stored in 125ml amber bottles and labeled appropriately. Eighteen treatments (TRT's) were prepared.

Physicochemical Analysis: Total Acidity and pH: 5mls of filtered drink was shaken gently and was then tested for pH value acidity using a glass electrode pH-meter Jenway-3505. Titratable acidity (TA) was determined by titration with 0.1 M NaOH until pH 8.1 and expressed as % malic acid (g/100 ml).

Color Density and Hue Tint: Color density and hue tint were determined by measuring the absorbance (A) at 420, 520 and 700 nm for samples (200µl) using a UV-visible spectrophotometer (Jenway 6715) and calculated as described by Giusti and Wrolstad [18]:

$$\text{Color density} = (A420\text{ nm} - A700\text{ nm}) + (A520\text{ nm} - A700\text{ nm}) \quad (1)$$

Hue tint = (A420 nm-A700 nm)/(A520 nm-A700 nm) (2)

Total Phenolics Content, Tannins and Flavonoids: The total phenolics content of the calyxes extracts were determined based on the Folin-Ciocalteu (FC) method [19]. In brief, 400 µl of the sample extract was mixed with 2.0 ml of FC reagent (10 times diluted). After incubation for 5 min at room temperature, 1.6 ml of (7.5%, w/v) sodium carbonate solution was added and the solution was mixed thoroughly and incubated for 60 min at room temperature. Followed by this, absorbance was measured using a UV-visible spectrophotometer (Jenway 6715) at 765 nm. A suitable calibration curve was prepared using standard Gallic acid solution. All the results were expressed as mg Gallic acid equivalents (GAE) per gram of sample. Vanillin-HCl method [20] was employed to determine total tannins. Briefly, 1 ml of the sample extracts was treated with 5 ml of reagent mixture (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1 ratio). The color developed was read after 20 min at 500 nm using a UV-visible spectrophotometer (Jenway 6715). Suitable standard calibration curve was prepared using Catechin (20-400 µg/ml) and results were expressed as mg Catechin equivalent (CE) per 100 g dry weight of the samples, respectively. Total flavonoids in the sample extracts were determined using the aluminum chloride method as described by Liu *et al.* [21]. In brief, for 500 ml of the sample extract solution, 2.5 ml of distilled water and sodium nitrite solution (5%, w/v, 150 ml) were added to the mixture. This mixture was maintained for 5 min. followed by addition of 300 ml of aluminum chloride (10%, w/v) and again incubated for 6 min. Followed by this, 1 ml of sodium hydroxide (1 M) was added and the mixture was diluted with 550 ml of distilled water. This solution was mixed vigorously and the absorbance of the mixture was measured immediately at 510 nm using a UV-visible spectrophotometer (Jenway 6715). Results of the total flavonoids content were expressed as mg Catechin equivalents (CE) per 100 g of dry weight of the sample.

DPPH Radical Scavenging Activity: The capacity of the calyx extracts to scavenge DPPH radicals (2, 2-diphenyl-1-picrylhydrazyl) was measured based on the method described by Sanchez-Moreno *et al.* [22]. The results obtained were expressed as the percentage inhibition of DPPH based on the following formula:

Percent inhibition of DPPH= $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the DPPH solution without sample extract and A_{sample} is the absorbance of the sample with DPPH solution.

Sensory Evaluation of HS Beverage: Six HS beverage samples were evaluated for their sensory quality and overall acceptability. Those six different Hibiscus drinks had been selected among the 18 samples based on their antioxidants capacity using Response Surface Methodology (RSM). Member test panel (n=27) familiar with the beverage were asked to score the acceptability with respect to overall liking using a seven-point verbal hedonic box scale which varied from dislike extremely to like extremely [23]. The scores from the rating were subsequently subjected to analysis of variance (ANOVA) and means separated using Duncan Multiple Range test. All samples were chilled and kept in ice at a temperature of ~4°C before serving. They were then served on a tray in three digit numbered transparent plastic cups containing ~30 ml of sample. A cup of water was also provided to the panelists to cleanse their palate between evaluations.

Ethics: This study has been assessed and approved by the National Research Centre Ethics Committee. Consent was sought from panelists participating in this study. Samples were prepared according to good hygiene and manufacturing practices. Participants were informed about the study and explained that their participation was entirely voluntary, that they could stop the interview at any point and that the responses would be anonymous. A consent form was signed.

Response Surface Methodology: The calyxes to water ratio, temperature and time were the variable parameters used to optimize the extraction using response surface methodology. This methodology is commonly used for the experimental design [24, 25]. Statgraphics Plus software was used for experimental design and data treatment. After preliminary experiments, a central composite design was used to identify the relationship among three independent process variables and the dependent responses. The independent variables were calyxes to water ratio (X1) temperature (X2) (25-100°C) and times (X3) (5, 6, 8, 9, 10 min) in a hot extraction at 100°C and (6, 7, 9, 10, 11 h) at room temperature. A total of 18 assays: eight factorial, six axial and three at a central point (performed to estimate possible pure errors) was employed to optimize the tested parameters. The dependent variables were the total phenolics (Y1), tannin

Table 1: Coded independent variables in the process of optimization extraction of HS beverage.

		Independent process variables		
Experiment no.	Experiment code	Calyxes to water ratio (X1)	Extraction temperature (X2)	Extraction time (X3)
1	CE1	1:30	25°C	6h
2	CE2	1:23	25°C	7h
3	CE3	1:37	25°C	7h
4	CE4	1:30	25°C	9h
5	CE5	1:20	25°C	9h
6	CE6	1:40	25°C	9h
7	CE7	1:23	25°C	11h
8	CE8	1:37	25°C	11h
9	CE9	1:30	25°C	12h
10	HE1	1:30	100°C	5 min
11	HE2	1:23	100°C	6 min
12	HE3	1:37	100°C	6 min
13	HE4	1:30	100°C	8 min
14	HE5	1:20	100°C	8 min
15	HE6	1:40	100°C	8 min
16	HE7	1:23	100°C	9 min
17	HE8	1:37	100°C	9 min
18	HE9	1:30	100°C	10 min

CE = Cold extraction, HE = Hot extraction.

(Y2) and flavonoids (Y3) and the DPPH radical scavenging activity (Y4) in the extract. All the experiments were carried out at random to minimize systematic errors. Operating ranges and the levels of the considered variables are given in Table 1.

Statistical Analysis: Results obtained in the present study were analyzed using SPSS software (SPSS Statistics Version 17.0). One-way analysis of variance (ANOVA) was performed to evaluate the significant differences between sample means, with significant level being considered at $P < 0.05$. Mean comparisons were assessed by Duncan's test, with the values expressed as means ± standard deviations. All data presented are mean values of triplicates (n= 3), obtained from three separate runs; unless stated otherwise.

RESULTS AND DISCUSSION

The Physiochemical Properties of HS Beverages: Acidity and pH Value: The physiochemical parameters measured for the eighteen hibiscus treatments analyzed are presented in Table 2. Significant differences in pH of HS beverage found between all treatments and ranged between 2.77 and 2.98. The pH value was in accordance with previous studies on Hibiscus [26-29]. The total titratable acidity (TA) of HS from different dried calyces: water ratios, temperature and extraction durations differed significantly and ranged between 0.41 and 0.91% malic acid. Higher (TTA) in the EC2-EC5 and HE2-HE5 beverage can be a result of higher calyxes to water concentration. For the same reason, a significantly higher pH value was found in the same beverage when compared to other ratios. The general low values of TA are a reflection of low pH values of the beverage. The lower quantity of water for extraction seemed to favor greater concentration of organic acids in the beverage.

Color Density and Hue Tint: Qualitative color differences were observed between the cold and hot water hibiscus extracts. Cold extracts had a clear appearance and bright red color whereas the hot extracts presented a more opaque red color and some haze possibly associated to a higher concentration of phenolic compounds. The color intensity (optical density) of the extract ranged between 4.65 and 5.94 with CE7 (dried calyces/water ratio of 01:23 and 11h extraction duration) giving the highest color intensity while CE6 (dried calyces/water ratio of 1:40 and 9h extraction duration) gave the lowest color intensity. The color intensity in the commercial HS beverage is essentially a function of quantity and temperature of water involved in the extraction [30].

It has been observed that the color intensity in HS beverage is also favored by the low pH value as anthocyanins have little color above pH 3.5 [31]. The variation in the color intensity of HS is most probably related to the calyces/water ratios (i.e. dilution that affects the pH) and the extraction duration as seen in Figs 1 and 2. Hue tint (HT) -the ratio of A 420 nm to A 520 nm- is a measurement of color degradation in anthocyanin

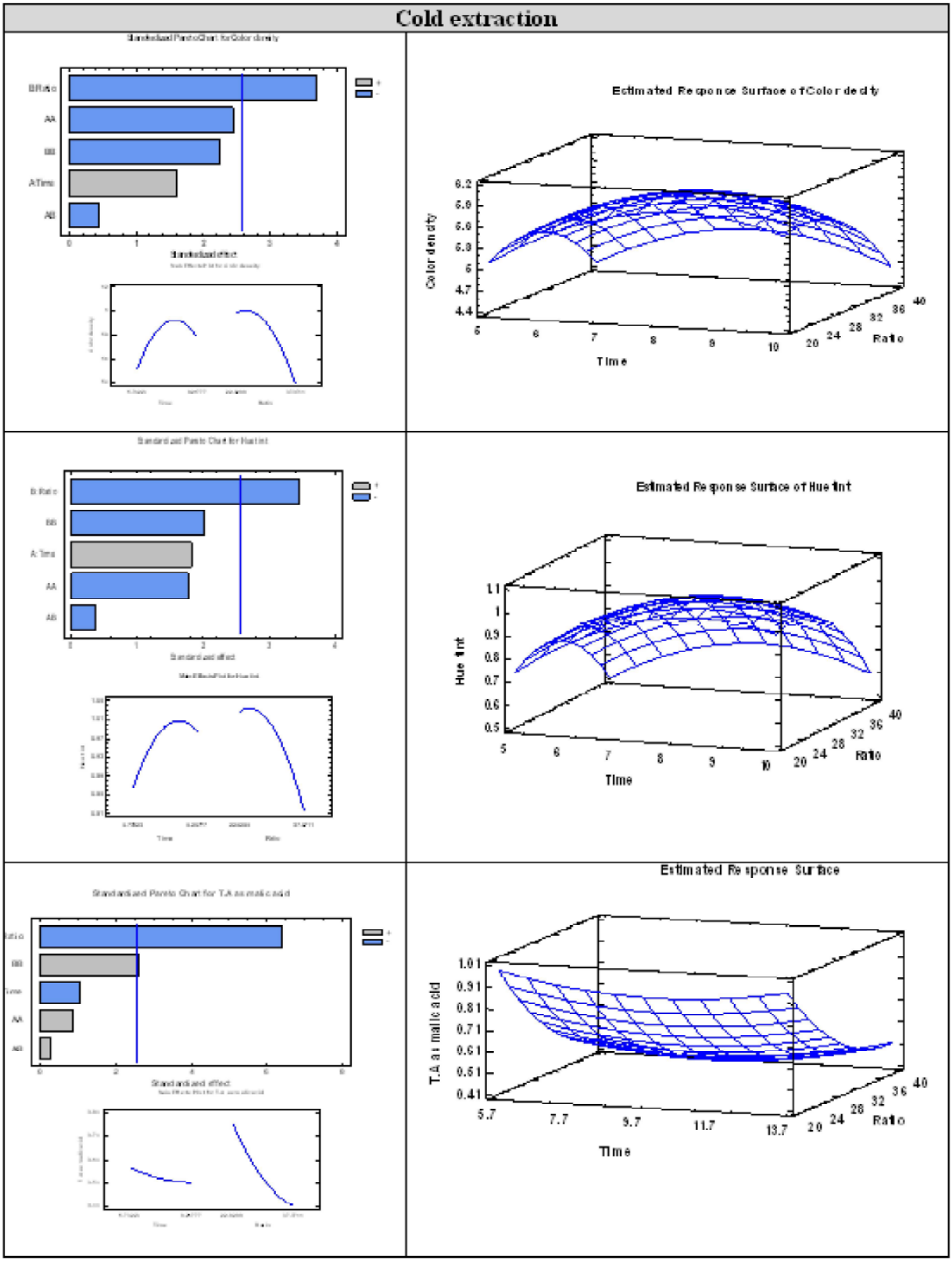

Fig. 1: Effect of COLD extraction condition on color density, hue tint, and titrable acidity.

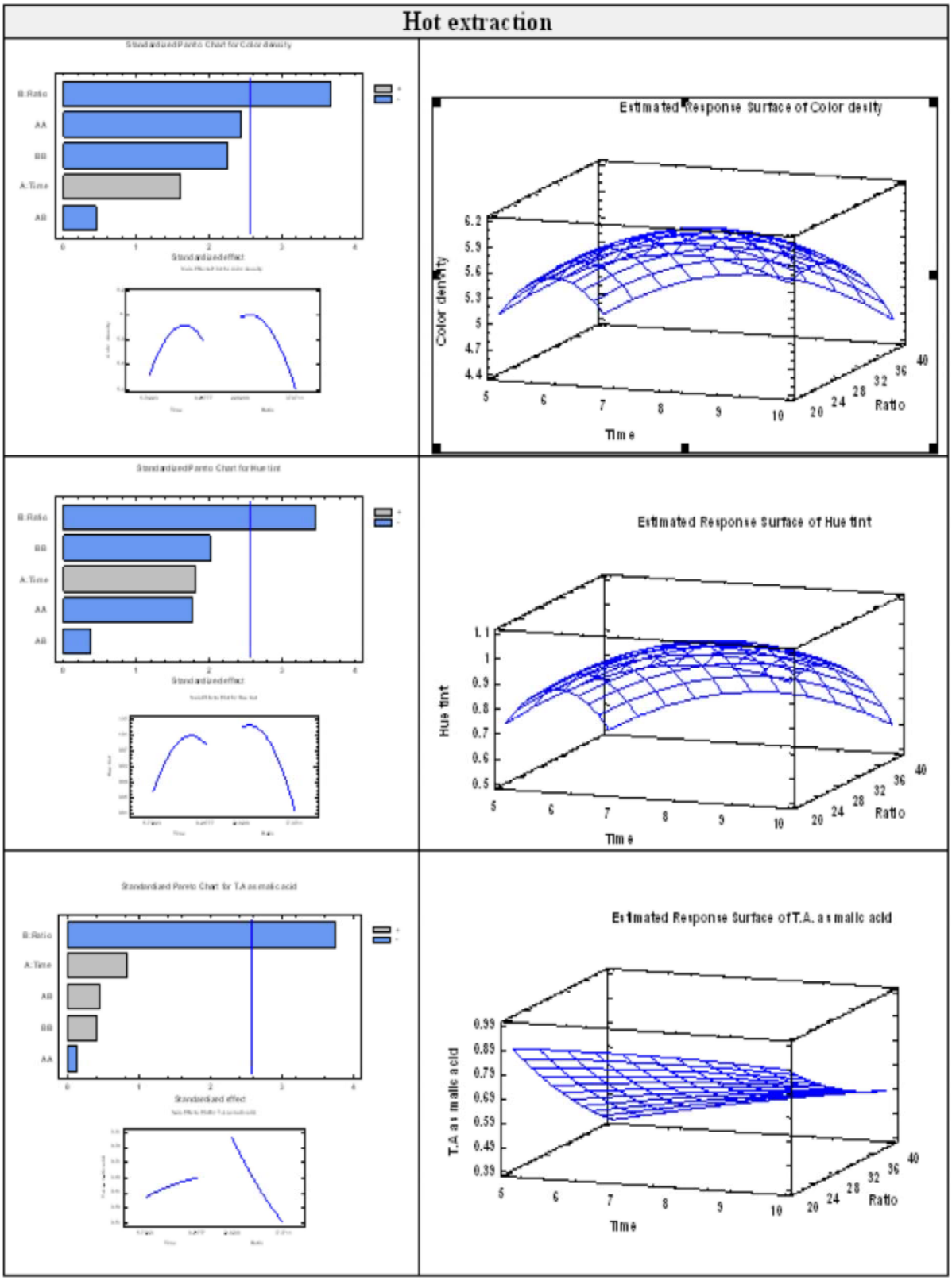

Fig. 2: Effect of HOT extraction condition on color density, hue tint, and titrable acidity

Table 2: Characteristics of HS beverage (Karkade) prepared from dried calyces/water ratios, temperatures, and extraction durations.

TRT	pH	TA1	CD2	HT3
CE1	2.95a ±0.07	0.53d±0.00	5.05f±0.00	0.69c±0.67
CE2	2.88a ±0.10	0.87a±0.01	5.91c±0.00	1.00a±1.00
CE3	2.93ab ±0.07	0.50e±0.00	4.67h±0.00	0.56d±0.83
CE4	2.95ab ±0.03	0.53d±0.00	5.25e±0.00	0.76c±1.00
CE5	2.94 ab ±0.07	0.87a±0.01	5.92b±0.00	1.00a±1.00
CE6	2.97 ab ±0.01	0.41g±0.00	4.65i±0.01	0.56d±0.66
CE7	2.84 ab ±0.04	0.76b±0.00	5.94a±0.00	1.00a±1.00
CE8	2.92 ab ± 0.03	0.42f±0.00	4.88g±0.00	0.63c±0.78
CE9	2.93 ab ± 0.00	0.60c±0.00	5.74d±0.01	0.93b±1.00
HE1	2.77ab±0.15	0.49g±0.01	4.95e±0.00	0.67d±0.00
HE2	2.79ab±0.05	0.91a±0.01	5.88a±0.00	1.00a±0.00
HE3	2.91abc± 0.01	0.60e±0.00	5.42c±0.00	0.83b±0.00
HE4	2.88abc±0.00	0.68d±0.01	5.90a±0.00	1.00a±0.00
HE5	2.95abcd±0.01	0.90a±0.01	5.86a±0.00	1.00a±0.00
HE6	2.95abcd ±0.02	0.49g ±0.04	4.93e±0.07	0.66d±0.02
HE7	2.98bcd ± 0.01	0.75c±0.01	5.90a±0.00	1.00a±0.00
HE8	2.97cd± 0.01	0.54f±0.00	5.28d±0.01	0.78c±0.00
HE9	2.98b± 0.00	0.79b±0.01	5.72b±0.00	1.00a±0.00

CE = Cold extraction, HE = Hot extraction.

1 Titrable acidity. 2 Color density. 3 Hue tint. Data represents the mean of n=3.

Values with similar letters within columns are not significantly different (Tukey's HSD, p>0.05)

Table 3: Phytochemical contents and antioxidants capacity of HS beverages.

TRT	TPC[1]mg Gallic acid equivalents/ml	TFC[2]mg Quercetin equivalents/ml	TC[3] mg tannic acid equivalent/ml	RSA[4](%)
CE1	50.50 bc±0.42	167.75ef±4.60	26.00ab±6.82	44.28e±2.24
CE2	50.75 bc±1.30	238.75abc±5.30	17.10cde±0.11	59.26b±1.98
CE3	46.13 d±3.49	134.00f±5.66	22.92cdb±0.55	37.10f±0.70
CE4	55.20 ab±1.98	207.00bcd±5.66	12.82e±0.21	51.21d±1.23
CE5	45.33 d±0.75	250.25a±27.22	17.51cde±1.27	66.84a±0.92
CE6	45.47 d±2.07	156.00f±0.71	31.74a±4.35	41.67e±0.04
CE7	45.00 d±3.58	250.50a±32.53	15.52cde±1.96	61.00b±0.04
CE8	45.88 d±3.84	205.00bcd±7.07	25.46ab±1.17	43.60e±0.31
CE9	57.40 a±4.24	228.50abc±33.23	15.48cde±0.40	56.31c±0.62
HE1	58.10c±0.98	216.25abcd±6.72	15.16de±0.35	40.68g±0.05
HE2	62.10b±0.44	214.50 abcd ±12.02	28.53ab±0.64	64.57c±2.90
HE3	68.57a±3.14	208.25bcd ±0.35	15.85cde±1.16	48.79e±0.97
HE4	40.00d±0.00	203.50cd ±0.00	23.09bc±0.33	59.07d±0.30
HE5	27.87f±0.57	209.25bcd ±2.47	31.45a±2.71	74.11a±0.48
HE6	39.06d±2.07	167.00ef±7.78	14.48e±0.24	40.40g±0.35
HE7	31.43 e±0.22	224.25abcd±18.74	26.02ab±7.40	69.24b±0.44
HE8	41.31d±0.18	190.50de±13.44	16.59cde±0.03	45.37f±0.09
HE9	40.50d±0.14	240.25ab±15.20	26.00ab±6.82	62.52c±1.32

[1]Total phenolics content, [2]Total flavonoids content, [3]Tannin content, [4]Radical scavenging activities,

Data represents the mean of n=3

Values with similar letters within columns are not significantly different (Tukey's HSD, p>0.05)

containing products. From Table 2, it can be observed that the extracts obtained with cold water have lower values than the ones obtained with hot water. This indicates that temperature had an effect on hibiscus extract's color. A higher hue tint value is associated with an increase in absorbance at 420 nm (yellow tones) in relation to that at 520 nm (red tones); this is undesirable because it is an indication of anthocyanins degradation. Generally, in nature, a diversified group of flavonoids, anthocyanins, chlorophyll, xanthones and betalains can contribute to the intense calyxes color [32]. Differences in the calyces color depend entirely on the extent of co-occurrence with other coloring or pigment compounds and factors like chemical nature of pigments, their acylation and methylation status, pH of the vacuole, accumulation of the cyanidins or pelargonidin derivatives and genetic inheritance [33].

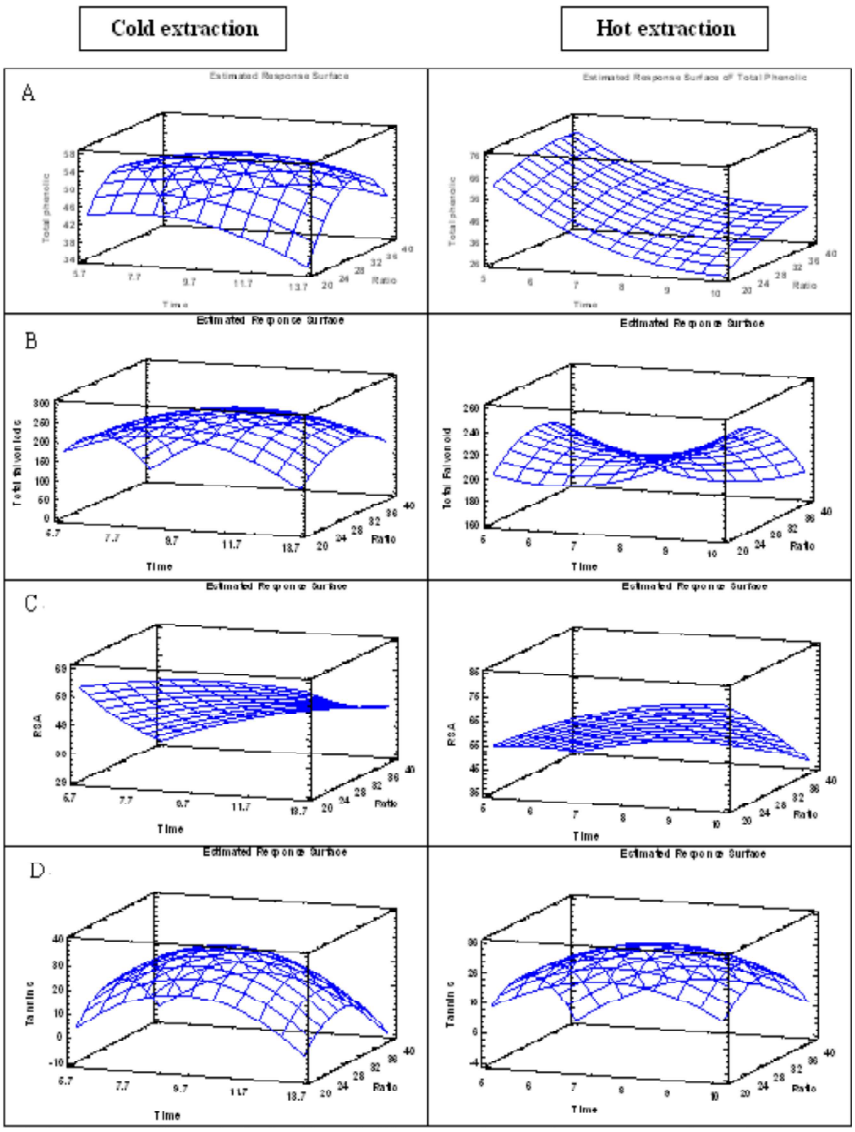

Fig. 3: Response surface graphs for the effects of solid to water ratio, extraction temperature, and extraction time on: (A) total phenolics content (X1), (B) total flavoniods (X2), (C) Radical Scavenging Activity (X3) and (D) tannin content (X4).

Phytonutrients Content and Antioxidant Capacities of HS Beverages: Profiling of phytonutrients of the eighteen hibiscus treatments was assessed by applying three different antioxidant assays namely total phenolics content, total falvonoids content, tannins content and DPPH radical scavenging activity (Table 3). The data indicated significant difference in the total phenolics (TP), total flavonoids (TF), tannins content (TC) and DPPH radical scavenging activity among the different HS beverages. Total phenolics content (TP) ranged from 31.43 to 68.57mg Gallic acid equivalents/ml for HE7 and HE3, respectively. Wide ranges of TF were also reported and ranged from 134.00 for CE3 to 250.50 mg Quercetin equivalents/ml for CE7. Tannins content (TC) ranged between 12.82 and 31.74, for CE4 and HE5, respectively. The ability of different HS extract to scavenge DPPH free radicals ranged from 37.10 to 74.11% for CE3 and HE5, respectively. The solid to water ration as well as temperature showed positive effect on phytonutrients and total antioxidants content (Fig. 3). Similar results about the temperature and solid to water ratio on the extraction of antioxidants compounds were also reported by Pinelo *et al.* [34] and for milled berries studied by Cacace and Mazza [35]. All those studies

found linear correlation between temperature and solid to water ratio with antioxidants content. The antioxidant activity of the extracts increased as temperature increased from 25°C to 100°C (Fig.3a). However, antioxidant activity within the same temperature increased by increasing extraction time.

Optimization of HS Beverage Extraction Conditions: The three-dimensional response surface plots show the interaction between the extraction parameters namely: time, temperature and water to solid ration and phytonutrients concentrations (Fig. 3). Pareto chart main effects plot of temperature and water to solid ration parameters and response surface for HS extraction are shown in Figs 1 and 2. The effect of a parameter is considered as statistically significant if the histogram cross the vertical line, translating the threshold of significance of 5%. So, according to the Figs 1 and 2 and in the field of variation of the extraction parameters, the simple effect of water to solid ration and time were statistically significant on the prepared extracts. Figure 3 shows that the higher the water to solid ratio, the lower the phytonutrients contents. Similar results were obtained with the time which has a positive effect on the antioxidants content. The greatest antioxidant activity of both cold and hot HS extract was observed from the extraction condition of 1:20 for 9h for cold extraction and 8 min for hot extraction. The results also implied that antioxidant activity did not have direct correlation with the amount of total phenolic contents.

The optimal values of the selected variables were obtained by solving the regression equation. After calculation by Design Expert software, the optimal extraction conditions of phytonutrients cold extraction were 1:30 for 9.4h; 1:27.5 for 7.9h; 1:28.7 for 9.4h; and 1:20 for 10h for TP, TF, TC and RSA respectively.

The Equation of the Fitted Model/COLD Extraction:

- TP= 144.968 - 29.3365*Time + 1.65314*Ratio + 1.37999*Time^2 + 0.074521*Time*Ratio- 0.0276475*Ratio^2
- TF= -414.28 + 71.0903*Time + 23.8927*Ratio - 5.14463*Time^2 + 1.05804*Time*Ratio - 0.622647*Ratio^2
- TC= -200.279 + 22.4425*Time + 8.83134*Ratio - 1.19321*Time^2 + 0.00201786*Time*Ratio - 0.15424*Ratio^2
- RSA = 102.675 + 2.58388*Time - 3.34116*Ratio - 0.199204*Time^2 + 0.0849107*Time*Ratio + 0.020701*Ratio^2

For hot extraction the optimal extraction conditions of phytonutrients were 1:36.6 for 5 min; 1:22.7 for 10 min; 1:27.5 for 7.7 min and 1:20 for 10 min respectively. The equation of the fitted model/HOT extraction:

- TP = 144.968 - 29.3365*Time + 1.65314*Ratio + 1.37999*Time^2 + 0.074521*Time*Ratio - 0.0276475*Ratio^2
- TF = 97.853 - 31.985*Time + 16.3106*Ratio + 3.59062*Time^2 - 0.6621*Time*Ratio - 0.213441*Ratio^2
- TC = -140.004 + 23.1906*Time + 5.98369*Ratio - 1.69495*Time^2 + 0.111743*Time*Ratio - 0.124306*Ratio^2
- RSA = -24.4763 + 23.0381*Time + 0.621632*Ratio - 0.943825*Time^2 - 0.211016*Time*Ratio 0.008964*Ratio^2

To confirm these results, tests were performed in triplicate under optimized conditions. The TP, TF, TC and RSA were 55.13±0.40 mg Gallic acid equivalents/ml, 259.65±0.70 mg Quercetin equivalents/ml and 32.21±0.31 mg tannic acid equivalent/ml and 67.14±0.97% RSA respectively for cold extraction. Meanwhile, TP, TF, TC and RSA were 69.89 ±0.40 mg Gallic acid equivalents/ml, 247.03±0.35 mg Quercetin equivalents/ml and 32.28±0.98 mg tannic acid equivalent/ml and 78.17±1.23%, respectively for cold extraction. These results clearly showed that the model fitted the experimental data and therefore optimized the phytonutrients extraction from HS beverage.

Sensory Evaluation: HS beverage sample sweetened with sugar at 10% concentration were evaluated for their sensory qualities and general acceptability. Overall likeability of the different prepared hibiscus beverages were compared using a 7-point hedonic scale. Sensory tests were performed at the National Research Centre taste panel facility using 27 panelists in each test. 55% of panelists were females and 45% male, in the 28-57 age range. Six samples with high phytonutrients content and antioxidants capacity representing hot and cold water extraction were selected for sensory evaluation. The sensory quality rating of selected HS beverages is presented in Table 4. Significant differences were detected by panelists between the six tested samples. Overall, the acceptance of the Hibiscus drinks significantly differed between the sex samples at $p<0.05$ (One-way ANOVA). All of the drinks were on average acceptable since the mean scores were greater than a score of 2.5 (neither like nor dislike). Cold extracted beverages were liked more even at high calyxes to water

Table 4: Sensory quality rating* of selected HS beverage.

Sample number	Calyex: water	Temp	Time	Sensory score
CE-a	1:20	25	5h	4.50bc ±2.03
CE-b	1:40	25	10h	5.39ab ±1.23
CE-c	1:30	25	9.5h	5.75a ±1.51
HE-d	1:20	100	5min	3.75c ±2.03
HE-e	1:40	100	10min	5.04ab ±1.60
HE-f	1:20	100	7min	2.79d ±1.47

*Mean values with similar letters within columns are not significantly different (Tukey's HSD, $p > 0.05$)

ration compared to hot extracted ones. The most liked was the sample coded CE-c (5.75^{a}±1.51) dried calyces/cold water extraction ratio of 1:30, 9.5 hr extraction duration followed by CE-b (5.39±1.23) and HE-e (5.04±1.60) where the HE-f ranked the least among tested ones. Samples CEb and HEe were not significantly different at $P<0.05$. Lower quantity of water involved in the extraction of sample CEa and HEf might have contributed to greater aroma concentration in the sample. Longer boiling time could have resulted in greater degradation of organic acids and polyphenols and lesser Hibiscus acceptability. This may indicate that thermal processing affected more the organoleptic characteristics. It may be concluded that the optimal level of cold water extraction for the beverage should be dried Calyces/cold water ratio of 1:30, at ambient temperature 25±2°C for 9.5h. The practical application of this study is that it can guarantee consistency in the product quality in terms of antioxidants capacity and sensory quality of the beverage at commercial level.

CONCLUSION

This study aimed to optimize the extraction process of phytonutrients in *Hibiscus sabdarrifa* L beverage through response surface methodology. The results revealed that both cold and hot extracted HS beverages are considered valuable, as they provide components that can contribute to health benefits. The short time high temperature extraction process of HS however, provided considerable amount of phytonutrients and antioxidant activity. The results proved also that response surface methodology is an appropriate method for optimizing the extraction of HS bioactive material. The optimal level of extraction for the HS beverage should be dried calyces/cold water ratio of 1:30 (w/v) at room temperature for 9.5 hr. Experimental values for response variables at these optimal conditions match well with the predicted values. The practical application of this study is that it can guarantee consistency in the product quality in terms of color intensity and antioxidants contents of the beverage at commercial level and would help to ascertain the potency of the crude extract from HS as potential source of natural antioxidants. Extraction process selection for industrial applications should consider sensorial preference, processing technology, time and economic considerations.

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